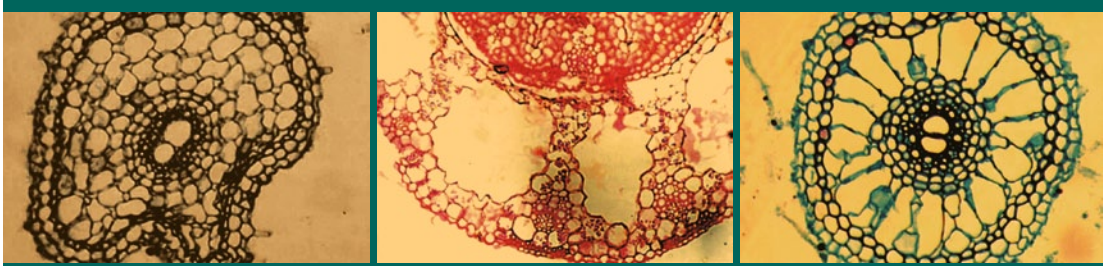
Dinesh K. Maheshwari, Meenu Saraf
Abhinav Aeron *Editors*

Bacteria in Agrobiology:



Crop Productivity

Springer

Bacteria in Agrobiology: Crop Productivity

Already published volumes:

Bacteria in Agrobiology: Disease Management
Dinesh K. Maheshwari (Ed.)

Bacteria in Agrobiology: Crop Ecosystems
Dinesh K. Maheshwari (Ed.)

Bacteria in Agrobiology: Plant Growth Responses
Dinesh K. Maheshwari (Ed.)

Bacteria in Agrobiology: Plant Nutrient Management
Dinesh K. Maheshwari (Ed.)

Bacteria in Agrobiology: Stress Management
Dinesh K. Maheshwari (Ed.)

Bacteria in Agrobiology: Plant Probiotics
Dinesh K. Maheshwari (Ed.)

Dinesh K. Maheshwari • Meenu Saraf •
Abhinav Aeron
Editors

Bacteria in Agrobiology: Crop Productivity

Springer

Editors
Dinesh K. Maheshwari
Dept. of Botany Microbiology
Gurukul Kangri University
Haridwar (Uttarakhand), India

Meenu Saraf
University School of Sciences
Dep. Microbiology and Biotechnology
Gujarat University
Ahmedabad (Gujarat), India

Abhinav Aeron
Department of Biosciences
DAV (PG) College
Muzaffarnagar (Uttar Pradesh)
India

ISBN 978-3-642-37240-7 ISBN 978-3-642-37241-4 (eBook)
DOI 10.1007/978-3-642-37241-4
Springer Heidelberg New York Dordrecht London

Library of Congress Control Number: 2013942485

Cover illustration: Optical micrograph showing cross sections of intercellular colonization rice calli and regenerated plantlets by A. *caulinodans*: CS view of root uninoculated control; magnified cross section view of leaf colonized by A. *caulinodans* in regenerated rice plant; possible sites of infection and colonization of rice root (from left to right); see also Fig. 3.1 in "Endophytic Bacteria – Perspectives and Applications in Agricultural Crop Production", Senthilkumar M, R. Anandham, M. Madhaiyan, V. Venkateswaran, Tong Min Sa, in "Bacteria in Agrobiology: Crop Ecosystems, Dinesh K. Maheshwari (Ed.)"

Background: Positive immunofluorescence micrograph showing reaction with cells of the biofertilizer strain used in autecological biogeography studies; see also Fig. 10.6 in "Beneficial Endophytic Rhizobia as Biofertilizer Inoculants for Rice and the Spatial Ecology of this Bacteria-Plant Association", Youssef Garas Yanni, Frank B. Dazzo, Mohamed I. Zidan, in "Bacteria in Agrobiology: Crop Ecosystems, Dinesh K. Maheshwari (Ed.)"

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

The damage to the ecological foundation essential for sustainable advances in productivity led to the onset of fatigue in Green Revolution. Scientific principles of soil and plant health management are being developed in order to sustain the benefits of enhanced productivity over long periods. Therefore, amazing gains and phenomenal increase have been observed in crop productivity by the use of effective microorganisms (plant growth and health-supporting bacteria) and practices. The agricultural crops which are a major source of food and nutrition are of immense importance to meet out the requirements of burgeoning human population. The productivity of the crops both in terms of quality and quantity of food is of paramount importance. Keeping in view of our immediate and long-term needs, the role of beneficial bacteria in agricultural–biological issues is envisaged.

The book entitled "*Bacteria in Agrobiology: Crop Productivity*" contains 19 chapters that cover multiple facets of contribution of the microbial attributes in addressing the crop's productivity that advance in perpetuity without accompanying ecological harm. Exploitation of endophytic, root-nodulating, and rhizospheric bacteria having beneficial plant growth-promoting and health-supporting characteristics proved significant in low-input food, forage, and nonfood crops for sustainable agricultural system. On one hand, beneficial bacteria also provide improvement in the growth of medicinal plants grown commercially, while on the other hand, also proved to be significant in adaptation of psammophytes (plants grown in sand dunes) to nutrient-limited sand dune ecosystem. Plant-associated bacteria including indigenous rhizobia and their bioformulations impart productivity enhancement in rice, banana, chickpea, and some common legumes cultivated at high altitude of western Himalayas. PGPB-mediated siderophores have indirect contribution to successful plant growth promotion in order to achieve maximum productivity, while inoculation of bacteria increasing uptake and mobilization of nutrients aiding cereal biofortification has direct contribution to the same. Other topics discussed in the book include the priming as a suitable strategy to induce plant defense responses resulting in induced systemic resistance that impart plant immunity, PGPR secreting volatile and nonvolatile substances, exopolysaccharides, PGPR adoption to heavy metal tolerance, and blending

of plant microbial remediation as one of the given cleanup processes for amalgamated chemo-remediation through rhizobacterial interactions in crop improvement.

We trust this book will be useful for researchers, teachers, students, and policy makers but also for those who are interested in the subjects of plant sciences, microbiology, phytopathology, ecology, environmental science, and agricultural sciences.

We would like to express our gratitude to all the subject experts and reviewers for their masterpiece scholarly contributions. Assistance rendered by our research scholars is thankfully acknowledged. We extend our sincere appreciation to Dr. Jutta Lindenborn of Springer for her valuable support to facilitate the completion of this book.

Makar Sankranti
January 2013

Dinesh K. Maheshwari
Meenu Saraf
Abhinav Aeron

Contents

Chapter 1
Endophytic Bacteria: A Biotechnological Potential in Agrobiology System

Paulo Teixeira Lacava and João Lúcio Azevedo

1.1 Introduction

The term endophyte is applied to microorganisms that live within plant tissues for all or part of their life cycles and cause no apparent infections or symptoms of disease (Wilson 1995; Azevedo et al. 2000; Bacon and White 2000; Saikkonen et al. 2004). Hallmann et al. (1997) describe endophytes as those organisms that can be isolated from surface-sterilized plant parts or extracted from inner tissues and that cause no damage to the host plant. In addition, Azevedo and Araújo (2007) suggested that endophytes are all microorganisms, culturable or not, that inhabit the interior of plant tissues, cause no harm to the host, and do not develop external structures. More recently, Mendes and Azevedo (2007) defined endophytic microorganisms in the same way as other authors (Hallmann et al. 1997; Azevedo et al. 2000; Azevedo and Araújo 2007) but suggested a division of endophytes in two types: type I, or endophytes that do not develop external structures, and type II, or endophytes that develop external structures.

Endophytic bacteria have been isolated from many different plant species (Lodewyckx et al. 2002; Idris et al. 2004; Rosenblueth and Martinez-Romero 2006; Barzanti et al. 2007; Sheng et al. 2008; Mastretta et al. 2009); in some cases, they may stimulate host growth through several mechanisms, including biological control, induction of systemic resistance to pathogens, nitrogen fixation, production of growth regulators, and enhancement of mineral nutrients or water uptake (Ryan et al. 2008). Additionally, the endophytic bacteria isolated from

P.T. Lacava (✉)
Center of Biological Sciences and Health, Federal University of São Carlos, Via Washington Luís Km 235, 676, São Carlos, SP 13565-905, Brazil
e-mail: ptlacava@ufscar.br

J.L. Azevedo
Department of Genetics, Escola Superior de Agricultura "Luiz de Queiroz", University of São Paulo, Av. Pádua Dias 11, PO BOX 83, 13400-970 Piracicaba, SP, Brazil

D.K. Maheshwari et al. (eds.), *Bacteria in Agrobiology: Crop Productivity*,
DOI 10.1007/978-3-642-37241-4_1,

plants that hyperaccumulate metals exhibit tolerance to high metal concentrations (Idris et al. 2004; Rajkumar et al. 2009).

There is a great deal of interest in understanding endophyte diversity and the role of endophytic bacteria in plant and bacterial ecology, evolutionary biology, and applied research, ranging from biological control to bioprospecting for genes (Azevedo et al. 2000; Araújo et al. 2008). In the past two decades, a lot of information on the role of endophytic microorganisms in nature has been collected. The ability to colonize internal host tissues has made endophytes valuable as a tool to improve crop performance. In this review, we address the major topics concerning the biotechnological potential of endophytic bacteria in agrobiology systems.

1.2 Endophytic Bacteria from Different Host Plants

The role of endophytes and their significance has been studied using many different approaches. Some of the important ones have been listed as follows.

1.2.1 Culturable Endophytic Bacteria with Agronomic Interest

Reported endophytes include both Gram-positive and Gram-negative bacteria and the classes *Alpha-*, *Beta-*, and *Gammaproteobacteria*, *Actinobacteria*, *Firmicutes*, and *Bacteroidetes* (Lodewyckx et al. 2002; Bacon and Hinton 2006). Approximately 300,000 plant species growing in unexplored areas of the earth are host to one or more endophytes (Araújo et al. 2001), and the presence of biodiverse endophytes in huge numbers plays an important role in the ecosystems with the greatest biodiversity, such as tropical and temperate rainforests (Arachevaleta et al. 1989), which are found extensively in Brazil and possess almost 20 % of its biotechnological source materials (Araújo et al. 2002).

Most studies on the occurrence of endophytic bacteria have been achieved using culture-dependent approaches. The genus *Burkholderia* has been consistently described as culturable and endophytic, and the bacteria can colonize sugarcane (Robertson et al. 2000; Caballero-Mellado et al. 2004; Mendes et al. 2007; Luvizotto et al. 2010). *Burkholderia* species are usually N_2-fixing endophytes when associated with sugarcane. Additionally, other studies have described the importance of genus *Burkholderia* in the cultivation of sugarcane (Pugsley and Oudega 1987; Perin et al. 2006; Castro-Gonzalez et al. 2011). Diazotrophic endophytic strains of *Burkholderia* have been found in banana, pineapple, and maize (Estrada et al. 2002; Weber et al. 1999).

Another genus with numerous endophytic bacteria colonizing different host plants is *Methylobacterium* (Araújo et al. 2001; Lodewyckx et al. 2002; Pirttilä et al. 2004; Idris et al. 2004, 2006; Podolich et al. 2009). Bacteria of the genus

Methylobacterium are well-studied facultative methylotrophs (Corpe 1985) and are capable of growing on one-carbon compounds (Lidstrom 1992). Sy et al. (2001) isolated *M. nodulans* showing nitrogenase activity and nodulation ability from *Crotalaria* species and assigned it to the fourth phylogenetic group of rhizobia. Members of *Methylobacterium* are ubiquitous on plant surfaces, and they are able to influence plant growth through the production of auxins or cytokinins and induce systemic resistance against diseases (Lee et al. 2006; Madhaiyan et al. 2006). Additionally, strains of *Pantoea* are found in rice and yam tubers (Omoregie et al. 1999; Verma et al. 2001). Strains of *Rhizobium* have been found within rice and maize (Gutierrez-Zamora and Martinez-Romero 2001; Tan et al. 2001; Yanni et al. 1997). Strains of *Serratia* and *Bradyrhizobium* are found in rice (Tan et al. 2001). *Azoarcus indigenes* was discovered in Kallar grass but also enters rice and sorghum easily (Egener et al. 1999; Reinhold-Hurek et al. 1993; Stein et al. 1997).

Endophytic bacteria are of agronomic interest because they can enhance plant growth and improve the nutrition of plants through nitrogen fixation and other mechanisms (Boddey et al. 2003; Sevilla et al. 2001). They are also of medical interest because some bacterial endophytes are human pathogens that cannot effectively be removed by surface sterilization (Beuchat et al. 2001; Proctor et al. 2001; Taormina et al. 1999; Weissinger and Beuchat 2000; Weissinger et al. 2001). In that way, different species or strains of enteric bacteria were found to differ greatly in their ability to colonize the interior of *Medicago sativa* (alfalfa) roots (Dong et al. 2003). *Klebsiella* species are commonly found endophytes in maize (*Zea mays*) (Fisher et al. 1992; McInroy and Kloepper 1995; Chelius and Triplett 2001), red clover (Sturz et al. 1998), grapevine (Bell et al. 1995), rice (Elbeltagy et al. 2000), sweet potato (Paula et al. 1993; Adachi et al. 2002), alfalfa (Dong et al. 2003), and soybean (Kuklinsky-Sobral et al. 2004), where they may improve plant growth via nitrogen fixation, as suggested by the dinitrogenase reductase protein of *K. pneumoniae* found within the roots of maize (Chelius and Triplett 2000). On the other hand, nitrogen-fixing bacteria that inhabit the interior of plants without causing any disease are called diazotrophic endophytes (Iniguez et al. 2004). The *K. pneumoniae* 342 (Kp342) strain is able to produce the *nif* H protein in maize (Chelius and Triplett 2000) and wheat (Iniguez et al. 2004). Kp342, originally isolated from a nitrogen-efficient maize line (Chelius and Triplett 2000), fixes N_2 and increases maize yields in the field (Riggs et al. 2001). Lacava et al. (2007a) reported the endophytic colonization of *Catharanthus roseu*s using the endophytic bacteria *K. pneumoniae*. These authors chose an appropriate strain, Kp342, labeled with the GFP gene. This strain was inoculated onto seedlings of *C. roseus*. The isolation frequency was determined 1 week after the inoculation, and the endophytic colonization of *K. pneumoniae* was observed using fluorescence microscopy. The results suggest that *C. roseus* could be used as a model plant to study endophytic bacteria.

Salmonella strains have been detected as endophytes in alfalfa (Dong et al. 2003). Outbreaks of these bacteria in alfalfa have been recorded in North America, Asia, and Europe since 1995 (Ponka et al. 1995). It has been proposed that alfalfa plants and seeds be colonized with safe bacteria to out-compete human pathogens.

For example, *Enterobacter asburiae* was found to eliminate *S. enterica* and enterohemorrhagic *Escherichia coli* from *Arabidopsis thaliana* seeds (Cooley et al. 2003). It is worrisome that there may be human or opportunistic pathogens among plant endophytes. It seems that the bacteria best adapted for living inside plants are naturally selected.

Endophytic bacteria have been isolated from a variety of plants, as reviewed by Sturz et al. (2000) and Hallmann et al. (1997). Plants harboring endophytes were reported in a recent review by Rosenblueth and Martinez-Romero (2006) of bacterial endophytes and their interactions with hosts, but, most likely, there is not a single plant species devoid of endophytes. The few examples of apparent absence of endophytes suggest that some microorganisms are not easily isolated or cultured. The diversity of endophytic bacterial species has been largely based on culture techniques. Culture-independent analysis of bacterial populations inside citrus plants also suggests that bacterial endophytic populations are much more diverse than previously realized (Araújo et al. 2002; Lacava et al. 2006). Various reports concerning endophytic bacteria in agricultural plants have demonstrated that the use of fingerprinting techniques and clone analysis can provide additional information for analyzing the community composition of endophytic bacteria (Chelius and Triplett 2001; Garbeva et al. 2001; Seghers et al. 2004; Sessitsch et al. 2004).

1.2.2 Study of Endophytic by Culture-Independent Approaches

Culture-independent molecular approaches based on 16S rRNA gene analysis, such as PCR amplification of 16S rDNAs, amplified ribosomal DNA restriction analysis (ARDRA), denaturing gradient gel electrophoresis (DGGE), and terminal restriction fragment length polymorphism (T-RFLP), have been successfully used for bacterial community analysis in a great variety of environments, including soil ecosystems (Dunbar et al. 1999), marine environments (Cottrell and Kirchman 2000), rhizospheres (Smalla et al. 2001), foods (Cocolin et al. 2002), and human intestines (Kibe et al. 2005), to overcome the limitations of culture-dependent approaches. However, these culture-independent approaches used on endophytic bacteria have met with limited success due to disturbances from chloroplast 16S rDNA and mitochondrial 18S rDNA.

Recently, Sessitsch et al. (2012) suggested a new approach to study the functional characteristics of endophytic bacteria. The authors presented the first metagenomic approach to analyze an endophytic bacterial community inside roots of rice. They asserted that assessing microbial functions is impeded by difficulties in cultivating most prokaryotes, and endophytes inside host tissues are not always amenable to biochemical or genetic analyses (Mano and Morisaki 2008; Weyens et al. 2009). From the results of Sessitsch et al. (2012), metagenome sequences were obtained from endophytic cells extracted from the roots of field-grown plants (rice). Putative functions were deduced from protein domains or similarity analyses of protein-encoding gene fragments, and this allowed insight

into the capacities of endophytic cells. Prominent features included flagella, plant-polymer-degrading enzymes, protein secretion systems, iron acquisition and storage, quorum sensing, and detoxification of reactive oxygen species. In this metagenome analysis, endophytes might be involved in the entire nitrogen cycle as protein domains involved in N_2 fixation, denitrification, and nitrification because genes involved in these cases were detected and expressed. Finally, the authors concluded that a deeper understanding of endophytic functions and mechanisms for their establishment in the endosphere could be exploited to improve agricultural management practices with respect to biocontrol, bioremediation, and plant nutrition. They suggested the metagenome approach as a method alternative to cultivation for the study of the role of bacterial endophytes that reside inside host plants.

1.3 Localization Inside of Host Plants

Endophytic bacteria appear to originate from seeds (Pleban et al. 1995; Adams and Kloepper 1996), vegetative planting material (Dong et al. 1994), rhizosphere soil (Sturz 1995; Hallmann et al. 1997; Mahaffee and Kloepper 1997), and the phylloplane (Beattie and Lindow 1995). With the exception of seed-transmitted bacteria, which are already present in the plant, potential endophytes must first colonize the root surface prior to entering the plant. The initial processes of colonization of plant tissue by endophytic bacteria can be via stoma, lenticels, areas of emergence of lateral roots, and germinating radicles (Huang 1986). Several authors have reported colonization of the secondary root emergence zone by bacterial endophytes (Reinhold and Hurek 1988; Wiehe et al. 1994; Mahaffee et al. 1997).

Various bacterial endophytes have been reported to live within cells, in intercellular spaces, or in the vascular systems of plants (Hallmann et al. 1997; James and Olivares 1998; Reinhold-Hurek and Hurek 1998; Sturz et al. 2000; Rosenblueth and Martinez-Romero 2006; Gai et al. 2009). Although endophyte populations vary in different plants according to many factors, bacterial populations are generally larger in roots and smaller in stems and leaves (Lamb et al. 1996). Additionally, the population density of endophytic bacteria found in plants depends on the plant species, genotype, and tissue; the growth stage and specialization of the bacteria; differences in colonization pathway; and mutual exclusion of different bacterial populations (Sturz et al. 1997). According to Strobel and Daisy (2003), many factors change endophytic biology, including the season, the age of the host plant, the environment, and the location.

The processes of colonization of plant tissue by endophytic bacteria are complex and include host recognition, spore germination, penetration, and colonization, and the sources of endophytic colonization are diverse, ranging from transmission via seeds (Ferreira et al. 2008) and vegetative planting material to entrance from the surrounding environment, such as the rhizosphere and phyllosphere. However, there is interest in finding bacterial strains with biological control or plant

growth-promoting capabilities. If these bacteria can be found in internal plant tissues, as they can in the rhizosphere, these bacteria may have the unique capacity to elicit beneficial effects from within the plants. As new beneficial bacterial strains are identified, delivery of these strains to specific plant tissues will be needed. To use endophytic bacteria in practical agronomic production, reliable and practical methods of inoculation must be developed. Several delivery systems have been reported for endophytic bacteria (Van Der Peer et al. 1990; Kumar and Dube 1992; Musson 1994).

In our studies, we have used culture-dependent approaches based on media culture (Fig. 1.1) and fluorescent microscopy (Fig. 1.2) to determinate the localization of endophytic bacteria in host plants. The endophytic bacterium *Methylobacterium mesophylicum* (strain SR1.6/6) in *C. roseus* and *Nicotiana clevelandii* plants was made visible by scanning electron microscopy (SEM). The highest densities were observed in the roots and hypocotyl, suggesting that these sites may be the most important points of entry for strain SR1.6/6 in both plants. Remarkably, cells adhering to the plants were immersed in a mucilagenous layer, suggesting that strain SR1.6/6 is able to form a biofilm on the root and hypocotyl surfaces of both plants (Andreote et al. 2006). Lacava et al. (2007a), using fluorescence microscopy, revealed that *Klebsiella pneumoniae* strain Kp342 colonized the xylem vessels of *Citrus sinensis* roots and branches, and it was able to colonize the xylem vessels of *C. roseus* branches and roots. Previous reports have described the ability of *K. pneumoniae* to colonize the roots and vascular tissue of plants (Dong et al. 2003). Based on isolation and fluorescence microscopy, Lacava et al. (2007b) suggested that *C. roseus* could be used as a model plant to study the interaction between endophytic bacteria and host plants. Ferreira et al. (2008) reported an endophytic bacterial community residing in *Eucalyptus* seeds and the transmission of these bacteria from seeds to seedlings. The authors suggested that endophytic bacteria can be transmitted vertically from seeds to seedlings, assuring the support of the bacterial community in the host plant. The authors evaluated the characteristics of colonization of endophytic bacteria by isolation and fluorescence microscopy. Gai et al. (2009) reported the localization of the endophytic bacterium *M. mesophilicum* in *C. roseus* and the transmission of this endophyte by *Bucephalogonia xanthophis* using isolation and fluorescence microscopy. *C. roseus* is a model plant for the study of interactions between endophytic bacteria and *Xylella fastidiosa*, the causal agent of citrus variegated chlorosis, and *B. xanthophis* is an insect vector that transmits *X. fastidiosa* to citrus plants (Hartung et al. 1994).

1.4 Endophytic Bacteria: Biotechnological Potential

A better understanding of endophytic bacteria may help to elucidate their function and potential role in developing sustainable systems of crop production (Sun et al. 2008). Bacteria interact with plants in four ways: as pathogens, symbionts, epiphytes, or endophytes. Of these four types of bacteria–plant interactions,

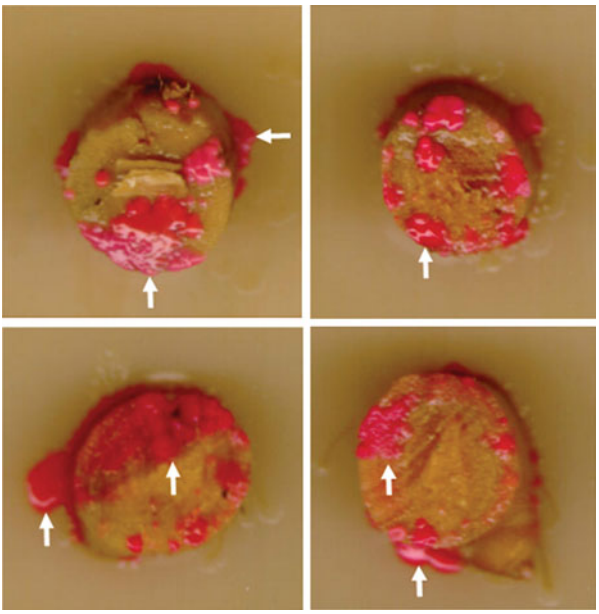


Fig. 1.1 Endophytic methylotrophic bacteria (*Methylobacterium* sp.) isolated from the branches of citrus plants. Details of endophytic growth in the *white arrows* (photos by P.T. Lacava)

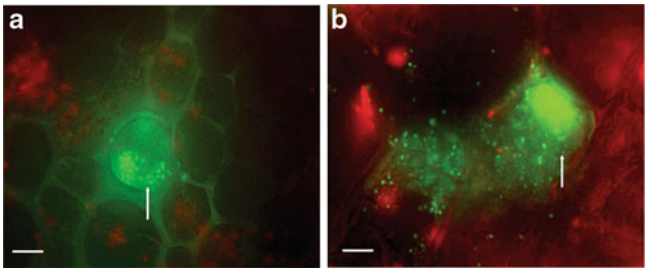


Fig. 1.2 Plant colonization by GFP-labeled *K. pneumoniae* 342 strain (**a** and **b**). Transverse sections of *Catharanthus roseus* branches showing endophytic bacterial colonies inside the xylem vessels (*arrows*) (photos by P.T. Lacava)

endophytic interactions are the least studied and least understood (Iniguez et al. 2005). Endophytic bacteria are of biotechnological and agronomic interest because they can enhance plant growth and improve the nutrition of plants, and they can also control pests and plant diseases (Boddey et al. 2003; Sevilla et al. 2001; Azevedo et al. 2000).

Endophytes may increase crop yields, remove contaminants, inhibit pathogens, and produce fixed nitrogen or novel substances (Rosenblueth and Martinez-Romero 2006). The repertoire of their effects and functions in plants has not been comprehensively defined. The challenge and goal is to be able to manage microbial communities that favor plant colonization by beneficial bacteria. This will be

possible when better knowledge of endophyte ecology and plant-endophyte molecular interactions is attained.

The endophyte–host relationship is believed to be complex and most likely varies from host to host and microorganism to microorganism (Boursnell 1950). Many experiments have been conducted to compare how endophyte-infected plants and noninfected plants behave in response to environmental stress and attack by insect and animal predators (Owen and Hundley 2004). Furthermore, endophyte-infected plants often grow faster than noninfected ones (Cheplick et al. 1989). This effect is at least in part due to the endophytes' production of phytohormones, such as indole-3-acetic acid (IAA), cytokines, and other plant growth-promoting substances (Tan and Zou 2001), and the fact that endophytes enhance the hosts' uptake of nutritional elements such as nitrogen (Reis et al. 2000) and phosphorus (Malinowski and Belesky 1999).

The search for interesting natural biological activities has been the basis for the development of various applications in biotechnology and agriculture. The microbial world, and endophytes in particular, reflects a genetic and metabolic biodiversity, which has not yet been thoroughly explored.

1.4.1 Plant Growth Promotion by Endophytic Bacteria

Plant growth promotion by bacteria in an agrobiology system consists of two levels: rhizospheric and endophytic (Bhattacharyya and Jha 2012). In endophytic relationships, growth-promoting bacteria reside within the apoplastic spaces in the host plants. There is direct evidence for the existence of endophytes in the apoplastic intercellular spaces of parenchymal tissue (Dong et al. 1997) and the xylem vessels (James et al. 2001; Araújo et al. 2002; Lacava et al. 2004). Endophyte-infected plants often grow faster than noninfected ones (Cheplick et al. 1989). The growth stimulation by endophytes can be a consequence of nitrogen fixation (Reis et al. 2000; Sevilla et al. 2001; Hurek et al. 2002; Iniguez et al. 2004), production of phytohormones, such as IAA and cytokines (Tan and Zou 2001; Lee et al. 2004), biocontrol of phytopathogens (Hallmann et al. 1997) through the production of antifungal or antibacterial agents (Rosenblueth and Martinez-Romero 2006), siderophore production (Pirttilä et al. 2004), nutrient competition and induction of acquired host resistance (Araújo et al. 2008), or enhancing the bioavailability of minerals (Sessitsch et al. 2002; Sturz et al. 2000). Several studies have indicated that endophytic colonization can also result in increased plant vigor, and it confers tolerance to biotic and abiotic stresses (Azevedo and Araújo 2003; Hallmann et al. 1997), enhanced drought tolerance (Arachevaleta et al. 1989), and improved phosphorus utilization (Verma et al. 2001; Wakelin et al. 2004).

Although the interaction between endophytic bacteria and their host plants is not fully understood, many isolates showed beneficial effects on their hosts and may play an important role in the physiology of these plants. Several bacterial

endophytes have been reported to support plant growth by providing phytohormones, low-molecular-weight compounds, or enzymes (Lambert and Joos 1989; Frommel et al. 1991; Glick et al. 1998). The colonization of ecological niches is similar to that of phytopathogens. The release of antimicrobial substances, such as antibiotics or HCN (Bangera and Thomashow 1996; Blumer and Haas 2000), the production of siderophores (O'Sullivan and O'Gara 1992), or the induction of systemic resistance to pathogens (Liu et al. 1995; Madhaiyan et al. 2004) favor them as candidates for biological control. However, many well-known plant pathogens may also be typical endophytic bacteria that normally cause no disease symptoms (Kobayashi and Palumbo 2000) but become pathogenic under certain conditions or within different host genotypes (Misaghi and Donndelinger 1990). In this context, some growth-promoting bacterial endophytes are being used in forest regeneration, agricultural crops, and phytoremediation of contaminated soil and water bodies (Jalgaonwala and Mahajan 2011).

1.4.1.1 Biological Nitrogen Fixation by Endophytic Bacteria

Tropical agriculture might be expected to be more dependent on N-fertilizers than agriculture in temperate regions because heavy rains and more rapid decomposition of organic matter cause leaching and rapid loss of N-fertilizers (Döbereiner 1997). Nitrogenous chemicals account for as much as 30 % of total crop fertilizers. However, nitrogenous fertilizers are becoming more scarce and costly. Biological nitrogen fixation (BNF) is one of the possible biological alternatives to N-fertilizers and could lead to more productive and sustainable agriculture without harming the environment (Döbereiner and Urquiaga 1992).

Nitrogen is the major limiting factor for plant growth, the application of N_2-fixing endophytic bacteria as biofertilizer has emerged as one of the most efficient and environmentally sustainable methods for increasing the growth and yield of crop plants (Singh et al. 2011). Diazotrophic endophytic bacteria provide more of fixed nitrogen than rhizospheric bacteria because the interior of plants is a more suitable niche for nitrogen fixation due to the low partial oxygen pressure (pO_2) and direct accessibility of the fixed nitrogen to the plants (James and Olivares 1998).

Diazotrophic Endophytic Bacteria from Sugarcane

Extensive research on endophytic bacteria and its beneficial effects on plant growth started with the isolation of endophytic *Gluconacetobacter diazotrophicus* from Brazilian sugarcane (James and Olivares 1998). In Brazil, the long-term continuous cultivation of sugarcane with low N-fertilizer inputs, without apparent depletion of soil-N reserves, led to the suggestion that N_2-fixing bacteria associated with the plants may be the source of agronomically significant N inputs to this crop (Boddey et al. 2003).

Many N_2-fixing bacteria are associated with sugarcane (Boddey et al. 2003). Free-living N_2-fixing bacteria belonging to the genera *Beijerinckia*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Derxia*, *Enterobacter*, and *Erwinia* appear to be frequent colonizers of sugarcane (Döbereiner and Ruschel 1958; Arias et al. 1978; Hegazi et al. 1979; Purchase 1980; Rennie et al. 1982; Graciolli et al. 1983; Seldin et al. 1984). The 1988 discovery of *Acetobacter diazotrophicus* (syn. *Gluconacetobacter diazotrophicus*), a nitrogen-fixing bacterium inhabiting the interiors of the roots, stems, and leaves of sugarcane, opened a new avenue of research into endophytic nitrogen fixation in sugarcane. Recently, many endophytic species of *Azospirillum* (Baldani et al. 1997), *Herbaspirillum* (Reis et al. 2000), *Burkholderia* (Govindarajan et al. 2006; Luvizotto et al. 2010), and *Klebsiella* (Govindarajan et al. 2007) have been isolated.

Studies of endophytes in sugarcane have focused on isolation and characterization using morphological and physiological studies of diazotrophic bacteria as well as molecular characterization of *nif* genes and 16S rRNA sequence analysis. Magnani et al. (2010) examined the diversity of endophytic bacteria in the internal tissues of sugarcane stems and leaves using molecular and biochemical methods. The strains were divided into five groups based on the 16S rRNA sequences. Group I comprised 14 representatives of the Enterobacteriaceae; group II was composed of Bacilli; group III contained one representative, a *Curtobacterium* species; group IV contained representatives of the Pseudomonadaceae family; and group V had one isolate of an uncultured bacterium. Most of the bacteria isolated from the sugarcane stem and leaf tissues belonged to Enterobacteriaceae and Pseudomonaceae, respectively, demonstrating niche specificity. Overall, these authors found the endophytic bacteria in sugarcane to be more diverse than previously reported (Magnani et al. 2010). Luvizotto et al. (2010) evaluated the ability of bacteria belonging to the genus *Burkholderia*, endophytically isolated from roots of sugarcane in Brazil, to fix atmospheric nitrogen according to Döbereiner et al. (1995). The ability to fix nitrogen was observed in 94.7 % of endophytic *Burkholderia* strains.

Diazotrophic Endophytic Bacteria from Other Crops

In the 1980s, endophytic bacteria having nitrogen-fixing activity were found in gramineous plants (Olivares et al. 1996; Reinhold-Hurek and Hurek 1998; Mano and Morisaki 2008). Some endophytic bacteria in rice plants have been reported to promote host growth. When the diazotrophic endophytes *H. seropedicae* Z67 strain (James et al. 2002), *Herbaspirillum* sp. B501 strain (Zakria et al. 2007), *Serratia marcescens* IRBG500 strain (Gyaneshwar et al. 2001), and some strains of *H. seropedicae* and *Burkholderia* spp. (Baldani et al. 2000) are inoculates on rice seedlings, the inoculated plants show a significant increase in weight compared to the controls. A significant increase in biomass and grain yield has also been recorded in greenhouse-grown rice plants inoculated with *Rhizobium leguminosarum* bv. *phaseoli* (Singh et al. 2006).

According Singh et al. (2011), six endophytic diazotrophic bacteria were isolated from surface-sterilized roots of rice variety HUR-36, which is grown with little or no nitrogen fertilizer. Out of six bacteria, one isolate, the RREM25 strain, showed an appreciable level of nitrogenase activity and was further characterized with a view to exploiting its growth-promoting activity. Based on 16S rRNA gene sequence analysis, this isolate was identified as *B. cepacia*. The diazotrophic nature of this particular isolate was confirmed by Western blot analysis of dinitrogenase reductase and amplification of *nif* H. Microscopic observation confirmed the colonization of *gfp*/*gus*A-tagged RREM25 in the intercellular spaces of the cortical as well as vascular zones of roots. Inoculation of the RREM25 strain into rice plants resulted in significant increases in plant height, dry shoot and root weights, chlorophyll content, nitrogen content, and nitrogenase activity.

Kuklinsky-Sobral et al. (2004), in a study of the isolation and characterization of bacteria associated with soybean, evaluated 75 endophytic isolates for their ability to fix atmospheric nitrogen using two methods: bacterial growth in a nitrogen-free medium (NFb medium) and PCR specific for the *nif* H gene (encodes nitrogenase protein component II). The NFb medium methodology revealed that 60 % of the analyzed endophytic isolates were able to grow in the nitrogen-free medium. These isolates belonged to α- and β-*Proteobacteria*, although the predominant groups were *Enterobacteriaceae* and *Pseudomonadaceae*. The PCR method revealed the presence of *nif* H in 21 % of the endophytic isolates, which were identified as *Acinetobacter calcoaceticus,* a *Burkholderia* sp., *Pseudomonas* spp., a *Ralstonia* sp., and species belonging to the *Enterobacteriaceae* group. In the same study, only 9 % of epiphytic isolates displayed the *nif* H gene.

The N_2-fixing ability of bacterial endophytes of ginseng (*Panax ginseng* C.A. Meyer) was screened by partial amplification of the *nif* H gene (Vendan et al. 2010). Out of 18 isolates, only two, *Stenotrophomonas maltophilia* (E-II-3 strain) and *Agrobacterium tumefaciens* (E-II-7 strain), showed amplification of the *nif* H gene. Much evidence exists for significant N_2 fixation by endophytic diazotrophs such as *Gluconacetobacter*, *Azoarcus*, and *Herbaspirillum* (Reinhold-Hurek and Hurek 1998). *A. tumefaciens* is capable of fixing nitrogen in a free-living condition (Kanvinde and Sastry 1990), and *S. maltophilia* isolated from various agricultural crops can display nitrogenase activity above 150 nmol/h/mg of protein (Park et al. 2005). Previous studies employing different *nif* H primers have also shown successful and specific amplification of *nif* H from a variety of bacteria and natural samples (Potrich et al. 2003). However, positive amplification of *nif* H in only two isolates in this study suggests the presence of host specificity or preference for diazotrophic endophytes similar to those in microbe-plant mutualisms found with *Rhizobium* and legumes (Vendan et al. 2010).

The two most widely studied genera among the diazotrophic endophytes are *Gluconacetobacter* and *Herbaspirillum*. Both of these genera were originally discovered as endophytes of sugarcane (Baldani et al. 1986; Gillis et al. 1989). Since their discovery in sugarcane, strains of *G. diazotrophicus* have been found to inhabit the interior of pineapple, sorghum, African millet, and coffee, and strains of *Herbaspirillum* have been discovered in rice, elephant grass, maiden grass, Amur

silver grass, prairie cordgrass, maize, sorghum, banana, African palm oil, and pineapple (Bastian et al. 1999; Chelius and Triplett 2001; Dong et al. 1994; Dos Reis et al. 2000; Fuentes-Ramírez et al. 2001; Gyaneshwar et al. 2002; James et al. 1997; Jiménez-Salgado et al. 1997; Kirchhof et al. 1997; Loganathan et al. 1999; Reis et al. 2000; Tapia-Hernandez et al. 2000; Weber et al. 1999).

The concept of BNF by endophytes (Döbereiner 1992) has led to investigations on the potential uses of endophytic nitrogen-fixing bacteria that colonize graminaceous plants. It has been suggested that these bacteria express their nitrogen-fixing potential better when inside plant tissues due to lower competition for nutrients and protection from the high levels of O_2 that are present on the root surface (Boddey and Döbereiner 1995).

The widespread use of synthetic fertilizers has resulted in environmental degradation, a decline in beneficial micro- and macroorganisms, and accumulation of chemical residues in the food system. For sustainable agriculture, the use of biologically derived fertilizers would be ecologically sound and economically viable alternatives. These crop-associated indigenous nitrogen fixers may be agronomically important because they could supply part of the nitrogen that the crop requires (Govindarajan et al. 2007).

1.4.1.2 Production of Indole-3-Acetic Acid by Endophytic Bacteria

It has been reported that endophytic bacteria may promote plant growth and suppress plant diseases, most likely by means similar to growth-promoting rhizobacteria (Feng et al. 2006; Vendan et al. 2010). Furthermore, plant growth promotion is often greater when it is induced by endophytes rather than by bacteria restricted to the rhizosphere and the root surface (Chanway et al. 2000). In this context, like rhizospheric bacteria, endophytic bacteria have been shown to have growth-promoting activity due to the production of phytohormones or enzymes involved in growth regulator metabolism, such as indole-3-acetic acid (IAA) (Taghavi et al. 2009). The ability to synthesize phytohormones is widely distributed among plant-associated bacteria, and 80 % of the bacteria associated with plants are able to produce IAA (Costacurta and Vanderleyden 1995; Cheryl and Glick 1996).

The physiologically most active auxin in plants is IAA, which is known to stimulate both rapid (increases in cell elongation) and long-term (cell division and differentiation) responses in plants (Cleland 1990; Hagen 1990). IAA is the most common and best characterized phytohormone. In addition to IAA, bacteria also release other compounds in the rhizosphere, like indole-3-butyric acid (IBA), Trp, and tryptophol or indole-3-ethanol (TOL) that can indirectly contribute to plant growth promotion (Lebuhn et al. 1997; El-Khawas and Adachi 1999). It has been found that bacteria synthesize IAA by way of several pathways, and the operation of more than one pathway in certain species has been proposed (Cheryl and Glick 1996). Tryptophan (Trp) is generally considered to be the IAA precursor because its addition to IAA-producing bacterial cultures promotes an increase in

IAA synthesis (Costacurta and Vanderleyden 1995). This accounts for the fact that the Trp-dependent pathways of bacterial IAA synthesis have received the most attention. For example, in *Enterobacter cloacae*, IAA is synthesized via indole-3-pyruvic acid (IPyA) (Koga et al. 1991). In *Pseudomonas syringae*, IAA biosynthesis occurs mostly from Trp via indole-3-acetamide (IAM) (Hutcheson and Kosuge 1985; Kosuge and Sanger 1987; Magie et al. 1963; Comai and Kosuge 1980), and in *P. fluorescens*, Trp, bypassing the IPyA step, is directly converted to indole-3-acetaldehyde, which is further converted to IAA (Oberhansli et al. 1991).

Many studies have described the ability of endophytic bacteria to produce phytohormones and auxins, such as IAA (Hallmann et al. 1997; Glick et al. 1998; Lodewyckx et al. 2002), and the ability to produce IAA is considered to be responsible for plant growth promotion by beneficial bacteria, such as *Azospirillum*, *Alcaligenes faecalis*, *Klebsiella*, *Enterobacter*, *Acetobacter diazotrophicus*, and *Herbaspirillum seropedicae* (Costacurta and Vanderleyden 1995). The bacterial production of IAA has been studied not merely because of its physiological effect on plants but also because of this phytohormone's role in plant–microbe interactions (Costacurta and Vanderleyden 1995). In this context, Miliūtė and Buzaitė (2011) have suggested that IAA production is a common growth-promoting trait among apple tree endophytic bacteria. These authors reported that endophytic bacteria associated with apple tree buds were isolated, characterized, and tested for their ability to produce the plant hormone IAA. Nine isolates were shown to produce IAA. The amounts of IAA produced in culture ranged from 1.2 to 2.4 μg/mL.

Recently, Vendan et al. (2010) investigated the IAA production of endophytic bacteria isolated from ginseng (*Panax ginseng* C.A. Meyer). Ginseng is one of the most important remedies in oriental medicine (Yu et al. 2003), and it is presently used as a health tonic and in adaptogenic, anti-aging, prophylactic, and restorative remedies. In general, growth of high-quality ginseng requires at least 4 years of cultivation in the shade (Cho et al. 2007; Qiu et al. 2007). According to Vendan et al. (2010), 14 of the 18 endophytic isolates produced significant amounts of IAA in nutrient broth supplemented with tryptophan as a precursor. The isolate E-I-4 (*Micrococcus luteus*) produced the highest amounts of IAA (13.93 μg/mL), followed by the isolates E-I-20 (*Lysinibacillus fusiformis*, 7.23 μg/mL), and E-I-8 (*Bacillus cereus*, 4.61 μg/mL). These results suggest the potential of endophytic bacteria to improve the production of ginseng (Vendan et al. 2010).

In our research, we have been screening endophytic bacteria for the production of IAA. Some examples of these studies are Kuklinsky-Sobral et al. (2004) and Assumpção et al. (2009). Kuklinsky-Sobral et al. (2004) isolated epiphytic and endophytic bacteria from two soybean cultivars (Foscarin and Cristalina). The isolates were identified by partial 16S rDNA sequence analysis, with most of the isolates belonging to the *Pseudomonaceae, Burkholderiacea,* and *Enterobacteriaceae* groups, and the potential of these isolates for plant growth promotion was evaluated by screening for IAA. The results indicated a higher production of IAA by endophytic (34 %) than epiphytic (21 %) isolates in this study. Additionally, the soybean-associated bacteria showing characteristics related to growth

promotion were identified as belonging to the genera *Pseudomonas, Ralstonia, Enterobacter, Pantoea,* and *Acinetobacter*.

The isolation, characterization, and identification of endophytic bacteria in soybean seeds were investigated by Assumpção et al. (2009), and the biotechnological potential of the bacteria was evaluated. The isolates that produced IAA were inoculated in soybean seeds to evaluate their ability to promote plant growth. There were 12 endophytic isolates: *Acinetobacter*, *Bacillus*, *Brevibacterium*, *Chryseobacterium*, *Citrobacter*, *Curtobacterium*, *Enterobacter*, *Methylobacterium*, *Microbacterium*, *Micromonospora*, *Pantoea*, *Paenibacillus*, *Pseudomonas*, *Ochrobactrum*, *Streptomyces,* and *Tsukamurella*. The results showed that all of the isolates synthesized IAA, and the strain 67A (57) of *Enterobacter* sp. significantly increased the dry root biomass.

1.4.1.3 Phosphate Solubilization by Endophytic Bacteria

Phosphorus is one of the major growth-limiting nutrients in plants and is often the limiting mineral nutrient for biomass production in natural ecosystems. It is only taken up in monobasic or dibasic soluble forms (Glass 1989; Zaidi et al. 2006). Phosphates applied to agricultural soils are rapidly immobilized and rendered inaccessible for plants. Due to this rapid immobilization, many agricultural soils have large reservoirs of phosphates in inaccessible forms (Rodríguez and Fraga 1999). In this scenario, phosphorus-solubilizing activity is determined by the ability of microorganisms to release metabolites such as organic acids, which through their hydroxyl and carboxyl groups chelate the cation bound to phosphate, the latter being converted to soluble forms (Sagoe et al. 1998; Pal 1998; Gyaneshwar et al. 2002; Zaida et al. 2003; Chung et al. 2005; van der Heijden et al. 2008; Khan et al. 2009).

Phosphorus is important for the plant growth and promotes root development, tillering, and early flowering and performs other functions like metabolic activities, particularly in synthesis of protein (Tanwar and Shaktawat 2003). The rhizosphere microorganisms play a significant role for P solubilization in many crops especially under P deficiencies (Khan et al. 2009). Among the soil microorganisms, phosphate-solubilizing bacteria (PSB) play an important role in solubilizing P for the plants and allowing more efficient use of P fertilizers (Gyaneshwar et al. 1998). These plant growth-promoting rhizobacteria can colonize the root surface, and some have been shown to colonize endophytically (Naher et al. 2009). The association and colonization of PSB on surface of roots involve direct competition with other rhizosphere microorganisms, while the endophytic population of PSB may give more beneficial effects to the plants due to less competition. In this context, endophyte offers several advantages over rhizobacteria, such as the endophyte is correlated more closely to plant as compared with rhizobacteria, so more effective effects may exist in complementary niches of endophyte and its host. Furthermore, the host plant provides a ready-made environment so that the endophytic bacteria

could be better protected from biotic and abiotic stresses than rhizobacteria (Newman and Reynolds 2005).

Phosphate solubilization is a common trait among endophytic bacteria. For instance, the majority of endophytic populations from peanut, legumes, sunflower, and cactus were able to solubilize mineral phosphates in plate assays (Palaniappan et al. 2010; Forchetti et al. 2007; Puente et al. 2009).

Bacterial endophytes can accelerate seedling emergence, promote plant establishment under adverse conditions, and enhance plant growth (Chanway 1997; Bent and Chanway 1998). Endophytic bacteria are believed to elicit plant growth promotion by helping plants acquire nutrients (nitrogen fixation) or chelating iron (Costa and Loper 1994), by preventing infections via antifungal or antibacterial agents, out-competing pathogens for nutrients, siderophore production, or establishing the plant's systemic resistance (van Loon et al. 1998) and by producing phytohormones, such as auxin or cytokinin (Madhaiyan et al. 2006). Also, phosphate solubilization by endophytes is an interesting component of plant growth promotion because endophytic bacteria are compatible with host plants and able to colonize the tissues of the host plants without being recognized as pathogens (Rosenblueth and Martinez-Romero 2006).

In our research group, Kuklinsky-Sobral et al. (2004) analyzed epiphytic and endophytic isolates from several growth stages and cultivars of soybean. They found that 60 % of the endobacterial isolates (mostly *Pseudomonaceae*, *Burkholderiaceae,* and *Enterobacteriaceae*) from the early plant growth stages were phosphate solubilizers compared to less than 50 % of the isolates from senescent plants. The majority of the phosphate-mobilizing isolates were also able to fix nitrogen and produce IAA. Phosphate-solubilizing bacteria also revealed other properties beneficial to plants, including the ability to grow on a nitrogen-free medium and the production of several phytohormones (Puente et al. 2009; Vendan et al. 2010). In another of our studies, Dias et al. (2009) analyzed endophytic isolates from strawberry (mostly *Bacillus subtilis* and *B. megaterium*) that were all able to solubilize calcium phosphate in plate assays. The phosphate solubilization efficiency varied markedly across isolates. The plant growth promotion capacity of the isolates correlated with their phosphate solubilization activity as well as with IAA production.

Vendan et al. (2010) examined endophytic bacterial isolates from ginseng (*Panax ginseng*) for their phosphate-solubilizing ability by detecting extracellular solubilization of precipitated tricalcium phosphate with glucose as the sole source of carbon. Half of the endophytic isolates tested showed phosphate-solubilizing activity. Based on the solubilization zone, an endophytic isolate of *Lysinibacillus fusiformis* recorded higher solubilization of mineral phosphate. In the same study, endophytic isolates of *Bacillus cereus* and *B. megaterium* also showed notable solubilization activity.

1.4.1.4 Siderophore Production by Endophytic Bacteria

Iron is a necessary cofactor for many enzymatic reactions and is an essential nutrient for virtually all organisms. In aerobic conditions, iron exists predominantly in its ferric state (Fe^{3+}) and reacts to form highly insoluble hydroxides and oxyhydroxides that are largely unavailable to plants and microorganisms. To acquire sufficient iron, siderophores produced by bacteria can bind Fe^{3+} with a high affinity to solubilize this metal for its efficient uptake. Bacterial siderophores are low-molecular-weight compounds with high Fe^{3+} chelating affinities (Sharma and Johri 2003) responsible for the solubilization and transport of this element into bacterial cells. Some bacteria produce hydroxamate-type siderophores, and others produce catecholate-types (Neilands and Nakamura 1991). In a state of iron limitation, the siderophore-producing microorganisms are also able to bind and transport the iron-siderophore complex by the expression of specific proteins (Nachin et al. 2001; Nudel et al. 2001). The production of siderophores by microorganisms is beneficial to plants because it can inhibit the growth of plant pathogens (Masclaux and Expert 1995; Nachin et al. 2001; Sharma and Johri 2003; Etchegaray et al. 2004; Siddiqui 2005).

Siderophores can also induce resistance mechanisms in the plant (Schroth and Hancook 1995). Plant growth promotion, including the prevention of the deleterious effects of phytopathogenic organisms (Sharma and Johri 2003), can be achieved by the production of siderophores (Hayat et al. 2010). Production of siderophores is a mechanism through which endophytic biocontrol agents suppress pathogens indirectly by increasing the availability of minerals to the biocontrol agent in addition to iron chelation and, thus, stimulating the biosynthesis of other antimicrobial compounds (Duffy and Defago 1999).

Endophytic bacteria colonize an ecological niche similar to that of plant pathogens, especially vascular wilt pathogens, which might favor them as potential candidates for biocontrol and growth-promoting agents (Ramamoorthy et al. 2001). Several bacterial endophytes have been reported to support plant growth by providing phytohormones, low-molecular-weight compounds, or enzymes (Lambert and Joos 1989; Frommel et al. 1991; Glick et al. 1998). Production of siderophores is another mechanism by which endophytic biocontrol agents suppress pathogens indirectly by stimulating the biosynthesis of other antimicrobial compounds by increasing availability of minerals to the biocontrol agent in addition to iron chelation (O'Sullivan and O'Gara 1992; Duffy and Defago 1999; Persello-Cartieaux et al. 2003). In this context, Vendan et al. (2010) suggested that siderophore production may be a common phenotype among endophytes (Vendan et al. 2010).

In a recent study of the diversity and potential for plant growth promotion of endophytic bacteria isolated from ginseng (*Panax ginseng* C.A. Meyer), Vendan et al. (2010) described the siderophore production by seven endophytic bacteria strains. These strains were classified as *Bacillus cereus*, *B. flexus*, *B. megaterium*,

Lysinibacillus fusiformis, *L. sphaericus*, *Microbacterium phyllosphaerae,* and *Micrococcus luteus*.

Siderophore production by endophytic bacteria has been investigated in only a few cases, mainly as a mechanism of certain bacteria to antagonize pathogenic fungi. Thus, it was observed that all the isolates from cotton roots having antagonistic activity, mainly *Pantoea* spp., excreted siderophores (Li et al. 2009). Also in rice, strains of the genera *Pseudomonas* and *Burkholderia* and two species of *Pantoea* (*P. ananatis* and *P. agglomerans*) having antagonistic activity excreted siderophores (Yang et al. 2008).

According to Verma et al. (2011), three endophytic actinobacteria strains isolated from the root tissues of *Azadirachta indica* plants were selected through tests for their potential as biocontrol and plant growth-promoting agents. It was also observed that the seed treated with the spore suspension of three selected endophytic strains of *Streptomyces* significantly promoted plant growth and antagonized the growth of *Alternaria alternata*, the causal agent of early blight disease in tomato plants. It was observed that the three selected strains prolifically produce siderophores that play a vital role in the suppression of *A. alternata*. These authors concluded that these endophytic isolates have the potential to be plant growth promoters as well as a biocontrol agent, which is a useful trait for crop production in nutrient-deficient soils.

Loaces et al. (2011) described and characterized the community of endophytic, siderophore-producing bacteria (SPB) associated with *Oryza sativa*. Less than 10 % of the endophytic bacteria produced siderophores in the roots and leaves of young plants, but most of the endophytic bacteria were siderophore-producers in mature plants. According to the results, 54 of the 109 endophytic SPB isolated from different plant tissues or growth stages from replicate plots of *O. sativa* were unique. The relative predominance of bacteria belonging to the genera *Sphingomonas*, *Pseudomonas*, *Burkholderia*, and *Enterobacter* alternated during plant growth, but the genus *Pantoea* was predominant in the roots at tillering and in the leaves at subsequent stages. *Pantoea ananatis* was the SPB permanently associated with all of the plant tissues of *O. sativa*. In the same study, the SPB and plant growth-promoting bacteria (PGPB) *Azospirillum brasilense*, *A. amazonense*, and *Herbaspirillum seropedicae* were assessed using dual culture in vitro on NFbI medium to allow the simultaneous growth of PGPB and SPB. These PGPB are considered important genera of endophytic diazotrophs (Baldani and Döbereiner 1980; Baldani et al. 2000, 2003). The results indicate that the SPB *P. ananatis* is the permanent and dominant associated species and is unable to inhibit two of the relevant plant growth-promoting bacteria, *A. brasilense* and *H. seropedicae*.

However, based on the results of our research group (Lacava et al. 2008), we suggested that in some cases the phytopathogens have the ability to use siderophores produced by endophytic bacteria. So, Lacava et al. (2008) described the production, and characterization of siderophores by endophytic bacteria in the genus *Methylobacterium* was evaluated by microbial and biochemical methods. These endophytic bacteria occupy the same niche as *X. fastidiosa,* a causal agent of

citrus variegated chlorosis (CVC), in the host plant. All strains of *Methylobacterium* spp. tested were positive in the chrome azurol S (CAS) assay for siderophore production. *Methylobacterium* spp. produce hydroxamate-type but not catechol-type siderophores. The production of siderophores by two *M. mesophilicum* isolates and one *M. extorquens* isolate was evaluated by using a CE-ESI-MS with a liquid sheath interface and IT mass analyzer. The bacterial cultures were grown in either the absence (siderophore-producing cultures) or presence (control cultures) of Fe(III) and siderophores. The results show that *M. mesophilicum* and *M. extorquens* synthesize siderophores of masses (*M*r) 1004.3 and 798.3 Da, respectively. Analysis in vitro showed that the growth of *X. fastidiosa* was stimulated by the presence of a siderophore originating from the supernatant of an endophytic *Methylobacterium mesophilicum*. If *X. fastidiosa* is able to use heterologous siderophores during its establishment inside the host plant, it may benefit from the production of siderophores by endophytic symbionts.

1.4.2 Biological Control of Insect-Pests and Plant Diseases by Endophytic Bacteria

The control of insect-pests and diseases by means of biological processes, such as the use of entomopathogenic microorganisms or those that inhibit/antagonize microorganisms pathogenic to plants, is an alternative that may help to reduce or eliminate the use of chemical products in agriculture (Azevedo et al. 2000). Agriculture by its own nature is anti-ecological, and, with the use of chemical fertilizers, insecticides, fungicides, herbicides, and antibiotics on a large scale, profound biological modifications have been occurring. Products such as insecticides and fungicides aim to control pests and phytopathogenic microorganisms. However, they are responsible for eliminating important species of insects that control other pests and microorganisms that are performing a crucial role in the environment, inhibiting the growth, and the multiplication of other microorganisms. One group of microorganisms that is affected by these anthropogenic modifications is the endophytes.

The natural and biological control of pests and diseases affecting cultivated plants has gained much attention in the past decades as a way of reducing the use of chemical products in agriculture. Biological control has been frequently used in Brazil, and it is supported by the development of basic and applied research on this field not only in our country but also in South America, as shown by several reviews (Lecuona 1996; Alves 1998; Melo and Azevedo 1998). The use of agrochemicals, although decreasing the impact of insects and phytopathogenic microorganisms, still represents a high risk for field workers and consumers.

In this part of the chapter, we will focus on examples of endophytic bacteria, especially those that may control insect-pests and plant diseases by antagonistic

effects, production of enzymes, or introduction of heterologous genes by recombinant DNA technology.

1.4.2.1 Biocontrol of Plant Diseases by Antagonistic Endophytic Bacteria

Recent studies have indicated that biological control of bacterial wilt disease could be achieved using antagonistic bacteria. Different bacterial species, namely, *Alcaligenes* spp. and *Kluyvera* spp. (Assis et al. 1998), *Pseudomonas fluorescens*, *P. alcaligenes*, *P. putida*, *Flavobacterium* spp. and *Bacillus megaterium* (Reiter et al. 2002), *B. pumilus* (Benhamou et al. 1998) and *Microbacterium* spp., *Clavibacter michiganensis*, *Curtobacterium* spp. and *B. subtilis* (Zinniel et al. 2002), have been reported as endophytes and were inhibitory to plant pathogens. Toyota and Kimura (2000) have reported the suppressive effect of some antagonistic bacteria on *Ralstonia solanacearum*. Moreover, Ciampi-Panno et al. (1989) have demonstrated the use of antagonistic microbes in the control of *R. solanacearum* under field conditions.

Ramesh et al. (2009) have suggested that Pseudomonads are the major antagonistic endophytic bacteria that suppress the bacterial wilt pathogen, *R. solanacearum*, in eggplant (*Solanum melongena* L.). Twenty-eight bacterial isolates that effectively inhibited *R. solanacearum* were characterized and identified in vitro (Ramesh et al. 2009). More than 50 % of these isolates were *P. fluorescens*. In greenhouse experiments, the plants treated with *Pseudomonas* isolates (EB9, EB67), *Enterobacter* isolates (EB44, EB89), and *Bacillus* isolates (EC4, EC13) reduced the incidence of wilt by more than 70 %. All the selected isolates reduced damping by more than 50 % and improved the growth of seedlings in the nursery stage. Large-scale field evaluations and detailed knowledge of antagonistic mechanisms could provide an effective biocontrol solution for bacterial wilt of solanaceous crops.

In our study, we suggested that the endophytic bacteria *Curtobacterium flaccumfaciens*, isolated from citrus plants (Araújo et al. 2001), can inhibit *X. fastidiosa*, a phytopathogenic bacterium that is the causal agent of citrus variegated chlorosis (CVC) (Schaad et al. 2004), both in vitro (Lacava et al. 2004) and in vivo (Lacava et al. 2007b), when inoculated in the model plant *C. roseus* (Monteiro et al. 2001). *C. roseus* has been used to study the interaction between endophytic bacteria and *X. fastidiosa* in greenhouse environments (Lacava et al. 2006; Andreote et al. 2006). To characterize the interactions of *X. fastidiosa* and the endophytic bacteria *C. flaccumfaciens* in vivo, *C. roseus* plants were inoculated separately with *C. flaccumfaciens*, *X. fastidiosa,* and both bacteria together (Lacava et al. 2007b). The number of flowers produced by the plants, the heights of the plants, and the exhibited disease symptoms were evaluated. *X. fastidiosa* induced stunting and reduced the number of flowers produced by *C. roseus*. When *C. flaccumfaciens* was inoculated together with *X. fastidiosa,* no stunting was observed. The number of flowers produced by our doubly inoculated plants was an intermediate between the number produced by the plants inoculated with either of the bacteria separately.

These data indicate that *C. flaccumfaciens*, an endophytic bacterium, interacted with *X. fastidiosa* in *C. roseus* and reduced the severity of the disease symptoms induced by *X. fastidiosa*.

The identification of biological sources for the control of plant pathogenic fungi remains an important objective for sustainable agricultural practices. In a recent project with financial support from several Brazilian agencies (Foundation of Support the Research of the State of Amazonas [FAPEAM] and the State of São Paulo Research Foundation [FAPESP—Grant/Process no. 09/53376-2]), we screened the antagonistic activity in vitro of endophytic bacteria versus *Colletotrichum* sp., the causal agent of anthracnose disease (Silva et al. 2004) of guarana (*Paullinia cupana* var. *sorbilis* [Mart.] Ducke). Fruit from guarana are of both economic and social importance in Brazil. Sodas, syrups, juices, and several pharmaceutical products are made from guarana-toasted grains (Angêlo et al. 2008). A significant decrease in the area of guarana production, particularly in the Brazilian Amazon region, can be attributed to anthracnose disease. In this study, the endophytic bacteria used in the antagonism test were isolated from guarana plants. We found some endophytic isolates from guarana with antagonism activity against *Colletotrichum* sp. in our preliminary results (Fig. 1.3).

1.4.2.2 Endophytic Actinobacteria in the Control of Phytopathogens

Endophytic actinobacteria have been isolated from a wide variety of plants, and the most frequently isolated species belong to the genera *Microbispora*, *Nocardia*, *Micromonospora,* and *Streptomyces*, the last of which is the by far the most abundantly observed (Sardi et al. 1992; Taechowisan et al. 2003). Actually, the best studied genus of actinobacteria is *Streptomyces* (Seipke et al. 2012), which has a complex developmental life cycle (Flärdh and Buttner 2009) and produces numerous secondary metabolites (Challis and Hopwood 2003).

Endophytic *Streptomyces* bacteria are not simply plant commensals but confer beneficial traits to their hosts that primarily fall into two categories: growth promotion and protection from phytopathogens. Members of the genus *Streptomyces* are prolific producers of antimicrobial compounds, and endophytic streptomycetes are no exception (Seipke et al. 2012). Numerous endophytic *Streptomyces* isolates inhibit the growth of fungal phytopathogens both in vitro and in planta, and this antibiosis has been proposed as one of the mechanisms by which endophytes suppress plant diseases (Sardi et al. 1992; Coombs and Franco 2003; Taechowisan et al. 2003; Franco et al. 2007).

Endophytic actinobacteria (Sardi et al. 1992; Coombs and Franco 2003; El-Tarabily 2003; Rosenblueth and Martinez-Romero 2006) have been isolated from within the living tissues of various plant species. These endophytes have been shown to protect plants against different plant pathogens including *Rhizoctonia solani* and *Verticillium dahliae* (Krechel et al. 2002), *Plectosporium tabacinum* (El-Tarabily 2003), *Gaeumannomyces graminis* var. *tritici* and *R. solani* (Coombs et al. 2004), *Fusarium oxysporum* (Cao et al. 2005), *Pythium aphanidermatum*

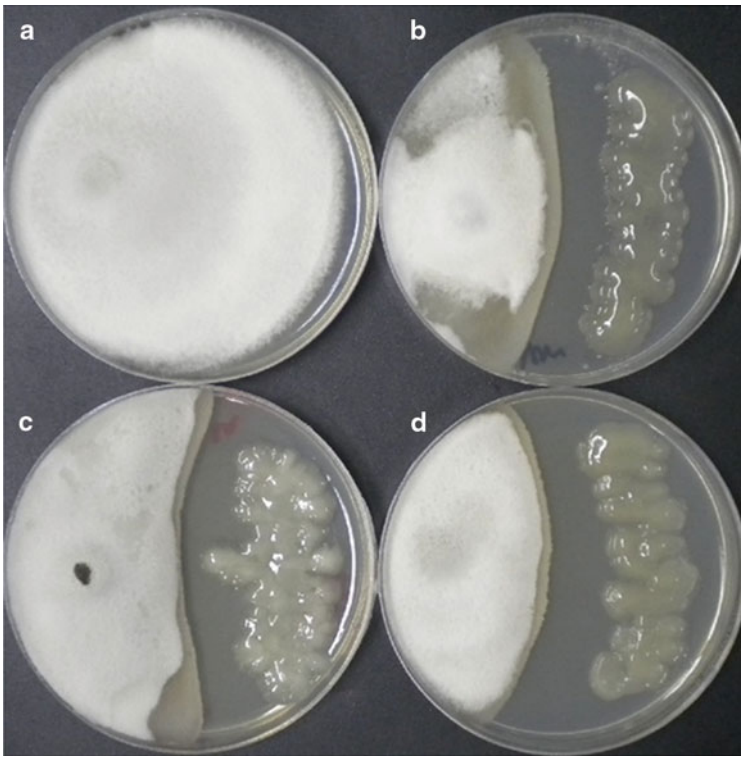


Fig. 1.3 (**a**) Growth of the phytopathogen *Colletotrichum* spp. in culture medium (control). (**b**), (**c**), and (**d**): antifungal activity of endophytic bacteria (*Bacillus* sp.) isolated from guarana (*Paullinia Cupana*) against *Colletotrichum* spp. (photos by P.T. Lacava)

(El-Tarabily 2003, El-Tarabily et al. 2009), and *Botrytis cinerea* and *Curvularia lunata* (Kafur and Khan 2011).

In our research group, Quecine et al. (2008) evaluated chitinase production by endophytic actinobacteria and the potential of this for the control of phytopathogenic fungi. Actinobacteria are used extensively in the pharmaceutical industry and agriculture owing to their great diversity of enzyme production. In this study, endophytic *Streptomyces* strains were grown on minimal medium supplemented with chitin, and chitinase production was quantified. The strains were screened for any activity towards phytopathogenic fungi with a dual-culture assay in vitro. The correlation between chitinase production and pathogen inhibition was calculated and further confirmed on *Colletotrichum sublineolum* cell walls by scanning electron microscopy. Quecine et al. (2008) report a genetic correlation between chitinase production and the biocontrol potential of endophytic actinobacteria in an antagonistic interaction with different phytopathogens, suggesting that this control could occur inside the host plant. Additionally, a genetic correlation between chitinase production and pathogen inhibition was demonstrated. Finally,

these results provide an enhanced understanding of endophytic *Streptomyces* and its potential as a biocontrol agent.

1.4.2.3 Endophytic Actinobacteria in the Control of Insect-Pests

The actinomycetes are a widely exploited group of microorganisms that can produce enzymes and antibiotics for agricultural applications such as eco-friendly crop protection. Among the actinomycetes, *Streptomyces* spp. are particularly efficient in the breakdown of chitin via chitinolytic enzymes (Bhattacharya et al. 2007; Quecine et al. 2008). During the past decade, several reports described this chitinolytic activity, and the corresponding genes responsible have been isolated and characterized (Robbins et al. 1998; Tsujibo et al. 1993; Christodoulou et al. 2001; Barboza-Corona et al. 2003; Kim et al. 2003). There is a wide variety of chitinases and a correspondingly large range of optimal temperatures and pH values for chitinase activity to determinate how well suited the chitinase is for pest control applications (Kramer and Muthukrishnan 1997). Our research group reported the partial characterization of the chitinolytic extract produced by an endophytic *Streptomyces* sp. strain (A8) (Quecine et al. 2011).

The extract produced by the A8 strain was also tested against *Anthonomus grandis* Boheman (Coleoptera: Curculionidae), the cotton boll weevil (Quecine et al. 2011). The chitinase crude extract from the A8 strain was cultured for 5 days in a minimal liquid medium supplemented with chitin. The extract was partially characterized by standard methods. The chitinolytic extract had an optimum temperature of 66 °C and an optimum pH between 4 and 9 (approximately 80 % of relative activity). We also characterized the temperature and pH stability and measured the effects of enzyme inhibitors (Figs. 1.4 and 1.5). The filtered chitinolytic extract was added to an artificial boll weevil diet. Boll weevil development from the egg stage to the adult stage was prolonged, and the percentage of adults that emerged was approximately 66 % less than on the control diet (Fig. 1.6). This study showed that the larval development of *A. grandis* was inhibited by the presence of characterized chitinolytic extract in the artificial diet. This work provides an experimental basis for using the chitinase from an endophytic *Streptomyces* sp. as an alternative to controlling the plant pest *A. grandis*. In this context, the cotton boll weevil, *A. grandis*, is major pest that affects cotton production in the Americas (Martins et al. 2007, 2008). It is typically controlled with chemical agents, but these chemicals are expensive and may disrupt predator and parasitoid populations due to their broad-spectrum activities (Burton 2006; Wolkers et al. 2006). Consequently, it is necessary to search for safer alternatives for boll weevil control. Biological and other control strategies to decrease the damage to cotton crops by the boll weevil are encouraged in integrated pest management strategies, which utilize insecticides that are more selective (Pimenta et al. 1997).

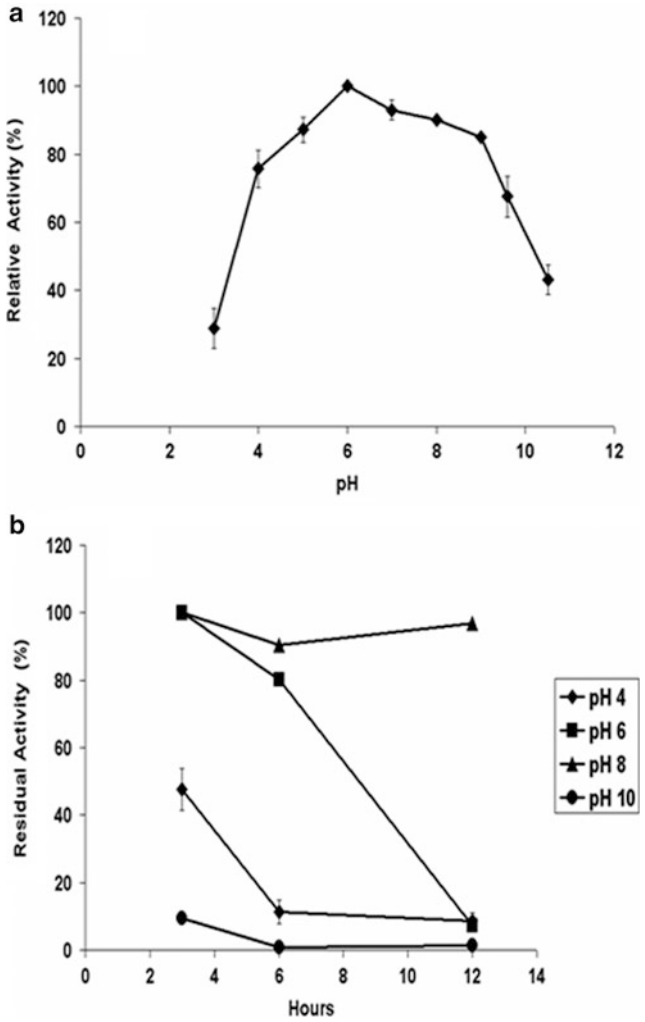


Fig. 1.4 Effects of pH on enzyme activity and stability. (**a**) Effect on activity. Chitinase activity was measured at 45 °C and at the indicated pH range (from 3 to 10.5). (**b**) Effect on stability. Chitinase extract was incubated in various buffers (100 mmol/L) at 45 °C for different periods (3, 6, and 12 h) and different pH [4 (*filled diamonds*), 6 (*filled squares*), 8 (*filled triangles*), and 10 (*filled circles*)]. Portions of the solution were withdrawn, and the residual activity was measured under the standard conditions of assay (modified Quecine et al. 2011)

1.4.2.4 The Recombinant DNA Technology and Biocontrol by Endophytic Bacteria

Recently, recombinant DNA technology has been applied to improve endophytic microorganisms, aiming to introduce new characteristics of agronomic interests, such as the biological control of insect-pests (Azevedo et al. 2000; Araújo et al.

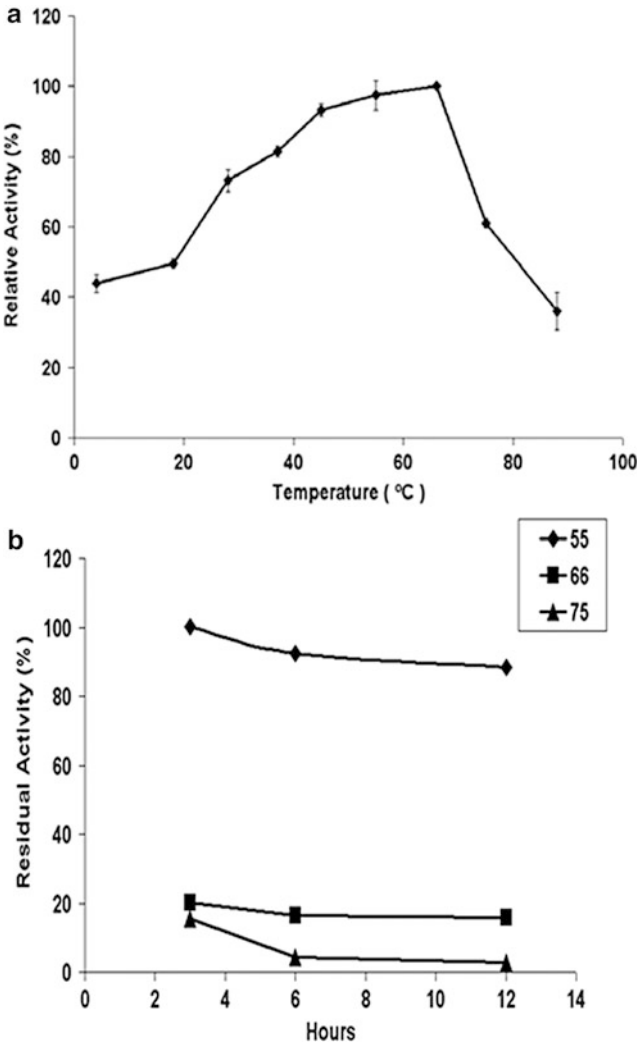


Fig. 1.5 Effects of temperature on enzyme activity and stability. (**a**) Effect on activity. Chitinase was added to the reaction mixture (100 mmol/L Tris–HCl pH 7.5, CM-chitin), and the reaction was carried out at the indicated temperatures. The maximum activity observed at 66 °C was taken as 100 %. (**b**) Effect on stability. Chitinase extract was incubated at 55 °C (*filled diamonds*), 66 °C (*filled squares*), and 75 °C (*filled triangles*) for the time indicated. Enzyme samples (0.25 μg) were withdrawn, and the residual activity was measured with CM-chitin-RBV as substrate (Loewe) (modified Quecine et al. 2011)

2008). Fahey (1988) and Fahey et al. (1991) described the first work directed at the introduction of a heterologous gene in an endophytic microorganism for the purpose of insect control. As a member of the biotechnology company Crop Genetics International, he described the major steps in the construction of an

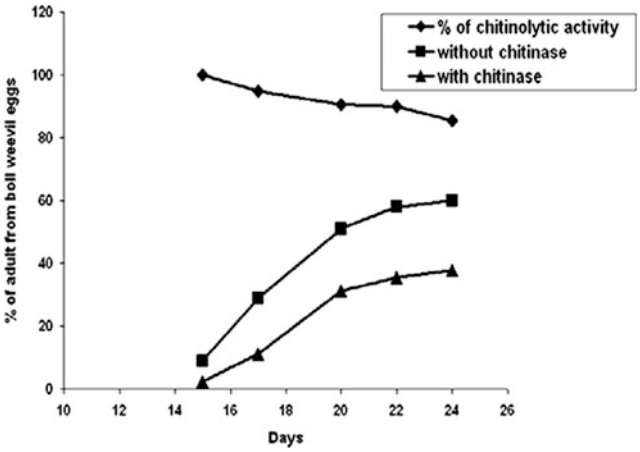


Fig. 1.6 Chitinolytic effects on boll weevil development. The percent of adult boll weevils was obtained from percent of eggs placed in the diet medium. The chitinolytic activity was the remaining activity compared. The statistical difference between the two curves and the regress equation was obtained by ANOVA using four replicates. The results for the diet without chitinase (*filled square*) equation curve $y = 5.7722x - 71.795$ (R2 = 0.9225) and the diet with chitinase (*filled triangle*) equation curve $y = 4.2335x - 59.416$ (R2 = 0.9384) differ statistically ($P > 0.05$) (modified Quecine et al. 2011)

endophytic bacterium for the purpose of insect control. This was achieved through the secretion of an insecticidal toxin in the host plant. He used the endophyte *Clavibacter xyli* subsp. *cynodontis*, a Gram-positive, xylem-inhabiting bacterium, capable of colonizing several plant species. This endophytic bacterium received a gene from another bacterium, *Bacillus thuringiensis*, which is able to produce the d-endotoxin active against insects, especially Lepidoptera and Coleoptera. Therefore, the genetically modified bacterium is able to secrete toxin inside the plant, protecting it against attacks by target insects (Azevedo et al. 2000).

Following the work of Fahey (1988), several other researchers belonging to the same company published more detailed reports describing the construction of the insect biocontrol agent. Turner et al. (1991) showed that a plasmid carrying two copies of the *B. thuringiensis* subsp. *kurstaki cryIA*(c) d-endotoxin gene and containing a genomic DNA fragment of *C. xyli* subsp. *cynodontis* could be integrated into the chromosome of *C. xyli* subsp. *cynodontis* by homologous recombination. However, the engineered bacterium exhibited insecticidal activity in artificial diets but not in planta. Lampel et al. (1994) used an improved integrative vector that, although it showed some instability, resulted in toxin production in planta.

The presence of endophytic bacteria inside the host plant may increase the plant's fitness by protecting it against pests and pathogens, improving plant growth and increasing resistance in stressful environments (Azevedo et al. 2000; Scherwinski et al. 2007). Many studies are being carried out with both natural

and genetically modified microorganisms to evaluate host colonization (Germaine et al. 2004; Ferreira et al. 2008).

Methylobacterium spp. has been described as enhancing plant systemic resistance (Madhaiyan et al. 2004), plant growth, and root formation (Senthilkumar et al. 2009). In this context, our research group decided to study the endophytic colonization of rice seedlings and *Spodoptera frugiperda* J.E. Smith larvae by the genetically modified endophytic bacterium *M. mesophilicum* in vitro (Rampelotti-Ferreira et al. 2010). The endophyte *M. mesophilicum* strain SR1.6/6 used in this work was previously isolated from *Citrus sinensis* (Araújo et al. 2002) and labeled with green fluorescent protein (*gfp*) (Gai et al. 2009). The colonization of *S. frugiperda* larvae and rice seedlings by the genetically modified endophytic bacterium *M. mesophilicum*, and also the possible transfer of this bacterium into the larva's body during consumption of the seedlings, was studied. The data obtained by bacterial reisolation and fluorescence microscopy showed that the bacteria colonized the rice seedlings and that the endophytic bacteria present in the seedlings could be acquired by the larvae. In that way, the transference of endophytic bacteria from plants to insect can be a new and important strategy in insect control using engineered endophytic bacteria.

1.5 The Potential for Bioremediation by Endophytes

Exploitation of the interactions between endophytes and plants can promote plant health and play a significant role in bioremediations.

1.5.1 Improving Phytoremediation Through Endophytic Bacteria

Metal-resistant endophytes are reported to be present in various hyperaccumulator plants growing in soils contaminated with heavy metals, and they play an important role in the survival and growth of such plants. Metal-resistant endophytes promote plant growth by various mechanisms, such as nitrogen fixation, solubilization of minerals, and production of phytohormones and siderophores (Rajkumar et al. 2009). The study of endophytic bacteria is important not only for understanding their ecological role in interaction with plants but also for their possible biotechnological applications, such as bioremediation and phytoremediation. The genetic engineering of endophytic bacteria is easier than the genetic engineering of plants, plus gene expression within endophytes might be useful as a site-monitoring tool (Araújo et al. 2008).

Metal hyperaccumulators are plants that accumulate extreme amounts of trace metals in their above-ground biomass when growing in metal-enriched habitats

(Baker et al. 2000). The interactions between endophytes and hyperaccumulator plants have attracted the attention of several investigators due to potential applications in bioremediation and the study of the composition of bacterial communities living in a contaminated natural environment (Lodewyckx et al. 2002; Idris et al. 2004).

For phytoremediation programs, engineered endophytic bacteria have been used to reduce the degradation caused by water-soluble, volatile, or organic pollutants (Barac et al. 2004; Van der Lelie et al. 2005). In some cases, it is likely that some endophytes could metabolize a pollutant if it moves through the plant's vessels (Taghavi et al. 2005). However, it is possible to improve the phytoremediation process by using recombinant endophytes modified to contain the appropriate degradation pathway (Barac et al. 2004). Additionally, some authors (Newman and Reynolds 2005; Van der Lelie et al. 2005) reinforce the idea of bacteria and phytoremediation as a new use for endophytic bacteria in plants.

In this way, work from our laboratory has indicated an interesting interaction between the soybean endophytic bacterial community and application of glyphosate herbicides before planting (Kuklinsky-Sobral et al. 2005). In this work, a culture medium with glyphosate as the sole carbon source was used to isolate endophytes, and *Pseudomonas oryzihabitans* and *Burkholderia gladioli* were the dominant species. The results from this study indicate the potential of these endophytic bacteria to be used in bioremediation programs.

The genus *Methylobacterium* (Van Aken et al. 2004) contains numerous endophytes (Sy et al. 2001; Araújo et al. 2002; Lacava et al. 2004) and is involved in the degradation of energetic compounds, such as 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (HMX), and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). The authors suggest the use of this endophyte in bioremediation and phytoremediation processes, where this type of microorganisms might be partially responsible for the degradation of environmental toxins. This hypothesis was confirmed by Siciliano et al. (2001), who observed that the addition of petroleum hydrocarbon sediment doubled the prevalence of naphthalene dioxygenase (*ndoB*)-positive endophytes in *Scirpus pungens*.

In our research group, Dourado et al. (2012) reported that *Methylobacterium* strains were isolated from mangrove samples collected from locations either contaminated or uncontaminated by oil spills. The tolerances of the strains to different heavy metals were assessed by exposing them to different concentrations of cadmium, lead, and arsenic (0.1 mM, 0.5 mM, 1 mM, 2 mM, 4 mM, and 8 mM) (Fig. 1.7). The isolates from the contaminated locations were grouped, suggesting that oil can select for microorganisms that tolerate oil components and can change the methylotrophic bacterial community. Cadmium is the most toxic heavy metal assessed in this work, followed by arsenic and lead, and two isolates of *Methylobacterium* were found to be tolerant to all three metals. These isolates have the potential to bioremediate environments contaminated by oil spills by immobilizing the heavy metals present in the oil. In the same way, Barzanti et al. (2007) isolated 83 endophytic bacteria from the roots, stems, and leaves of the Ni hyperaccumulator *Alyssum bertolonii*. They noted that despite the high

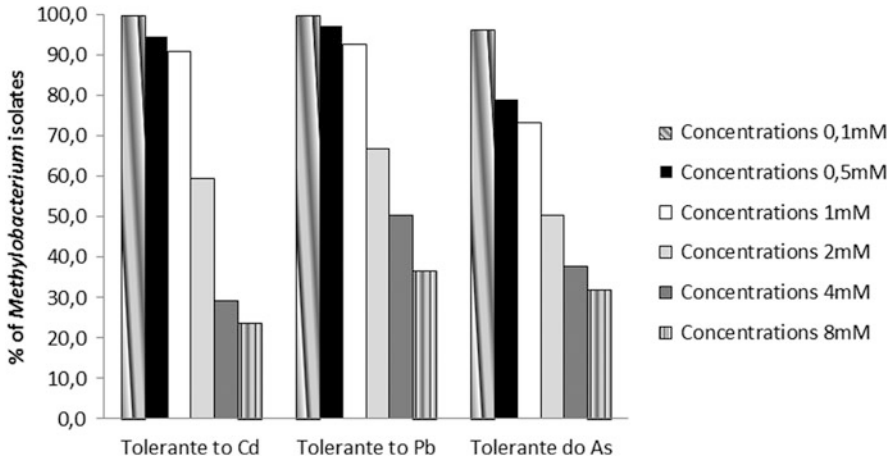


Fig. 1.7 Distribution of endophytic samples isolated from mangrove species of the *Methylobacterium* genus showing tolerance to Cd, Pb, and As at the concentrations 0.1 mM, 0.5 mM, 1 mM, 2 mM, 4 mM, and 8 mM (Dourado et al. 2012)

concentrations of heavy metals present in its tissues, *A. bertolonii* harbors an endophytic bacterial flora showing a high genetic diversity as well as a high level of resistance to heavy metals, which could potentially help plant growth and Ni hyperaccumulation.

Many experiments that involve the inoculation of endophytic bacteria (wild or engineered strains) with resistance to heavy metals have been conducted recently, including *Lupinus luteus*, which when grown on a Ni-enriched substrate and inoculated with the engineered Ni-resistant endophytic bacterium *B. cepacia* showed a significant increase (30 %) in the concentration of Ni in the roots (Lodewyckx et al. 2001). In another study, the engineered endophyte *B. cepacia* G4 (*gfp* gene) strain was reported to increase plant tolerance to toluene and decrease the transpiration of toluene to the atmosphere. Toluene is one of the four components in BTEX (benzene, toluene, ethylbenzene, and xylenes) (Germaine et al. 2004) contamination, and this has the potential to improve phytoremediation by decreasing toxicity and increasing degradation of the xenobiotic component (Barac et al. 2004). In this case, the engineered endophytic *B. cepacia* strain improved phytoremediation and promoted plant tolerance to toluene.

Sheng et al. (2008) observed that the inoculation of *Brassica napus* with Pb-resistant endophytic bacteria increased Pb uptake into the shoot from 76 % to 131 % (*Pseudomonas fluorescens*) and from 59 % to 80 % (*Microbacterium* sp.) when compared with the dead bacteria-inoculation control. Mastretta et al. (2009) found that the inoculation of *Nicotiana tabacum* with the Cd-resistant endophyte *Sanguibacter* sp. increased the concentration of Cd in shoot tissues approximately threefold when compared with the un-inoculated control. These studies suggest that it will be possible to improve the metal-extraction potential of hyperaccumulator plants by inoculating the seeds/rhizosphere with selected metal-resistant PGPB

endophytes. The works cited in this review suggest that bacteria degrading recalcitrant compounds are more abundant among endophytic populations than in the rhizosphere of the plants in contaminated sites, which could mean that endophytes have a role in metabolizing these substances (Siciliano et al. 2001; Jalgaonwala and Mahajan 2011).

Ryan et al. (2007) listed some of the advantages associated with the use of endophytic bacteria in phytoremediation of contaminated environmental soil when compared with the use of plants alone. They include the following: (1) quantitative gene expression of bacterial pollutant catabolic genes can be used to assess the efficiency of the remediation process; (2) genetic engineering of a bacterial catabolic pathway is easier to manipulate than a plant catabolic pathway; and (3) toxic pollutants taken up by the plant may be degraded in planta by endophytic degraders, reducing the toxic effects of contaminants in environmental soil on flora and fauna. However, some disadvantages associated with the use of bacteria in plant-associated bioremediation of contaminated environmental soil were suggested by the same authors (Ryan et al. 2007): (1) this technology is limited to shallow contaminants in environmental soil; (2) it is slower than traditional remediation technologies; (3) the choice of plant can mean that it is only seasonally effective; (4) it is associated with phytotoxic effects of contaminants; and (5) there is potential for the environmental contaminants or their metabolites to enter the food chain if contaminants are not completely detoxified or if the plants are consumed by local fauna.

This research field is at an early stage. The available literature demonstrating that the metal-resistant endophytic bacteria not only protect plants from metal toxicity but also enhance metal accumulation in plant tissues with concurrent stimulation of plant growth. These beneficial effects exhibited by endophytic bacteria, together with the suggested interrelationship between microbial heavy-metal tolerance and plant growth-promoting efficiency, indicate that inoculation with endophytic isolates might have significant potential to improve phytoextraction efficiency in metal-contaminated soils (Rajkumar et al. 2009).

1.6 Concluding Remarks

Endophytic bacteria are believed to elicit plant growth in many ways, including the following: helping plants acquire nutrients, e.g., via nitrogen fixation, phosphate solubilization, or iron chelation; preventing infections via antifungal or antibacterial agents; out-competing pathogens for nutrients by producing siderophores; or establishing the plant's systemic resistance and producing phytohormones. However, the effects and functions of endophytes in plants have not been comprehensively defined. The challenge and goal is to be able to manage microbial communities to favor plant colonization by beneficial bacteria. This will be possible when a better knowledge of endophyte ecology and molecular interactions is attained.

Although all of the approximately 300,000 plant species have been estimated to harbor one or more endophytes, few relationships between plants and these endophytes have been studied in detail; the legume–rhizobia symbiosis is an exception. Additionally, there remain many barriers to commercial usage of bacterial inoculants for inducing resistance, and even more studies are necessary to permit the usage of endophytes in this way. While there is a wide diversity of bacteria to be explored, supporting the idea that the most efficient resistance inducers are still to be described, genetic transformation of bacteria should also be considered a way to group important characteristics found in different strains. The combination of inducers of systemic resistance and endophytic characteristics may affect future agricultural concepts, allowing safer production with a lower impact on the environment.

References

Adachi K, Nakatani M, Mochila H (2002) Isolation of an endophytic diazotroph, *Klebsiella oxytoca*, from sweetpotato stems in Japan. Soil Sci Plant Nut 48:889–895

Adams PD, Kloepper JW (1996) Seed borne bacterial endophytes in different cotton cultivars. In: Abstract, 1996 annual meeting of American Phytopathological Society, p 97

Alves S (1998) Controle Microbiano de Insetos. Editora Fundação de Estudos Agrários Luiz de Queiroz, Piracicaba, p 1163

Andreote FD, Lacava PT, Gai CS, Araújo WL, Maccheroni W Jr, van Overbeek LS, van Elsas JD, Azevedo JL (2006) Model plants for studying the interaction between *Methylobacterium mesophilicum* and *Xylella fastidiosa*. Can J Microbiol 52:419–426

Angêlo PCS, Nunes-Silva CG, Brigido MM, Azevedo JSN, Assunção EM, Sousa ARB, Patricio FJB, Rego MM, Peixoto JCC, Oliveira WP Jr, Freitas DV, Almeida ERP, Viana AMHA, Souza AFPN, Andrade EV, Acosta POA, Batista JS, Walter MEMT, Leomil L, Anjos DAS, Coimbra RCM, Barbosa MHN, Honda E, Pereira SS, Silva A, Pereira JO, Silva ML, Marins M, Holanda FJ, Abreu RMM, Pando SC, Goncalves JFC, Carvalho ML, Leal-Mesquita ERRBP, da Silveira MA, Batista WC, Atroch AL, Franca SC, Porto JIR, Schneider MPC, Astolfi-Filho S (2008) Guarana (*Paullinia cupana* var. sorbilis), an anciently consumed stimulant from the Amazon rain forest: the seeded-fruit transcriptome. Plant Cell 27:117–124

Arachevaleta M, Bacon CW, Hoveland CS, Radcliffe DE (1989) Effect of the tall fescue endophyte on plant response to environmental stress. Agron J 81:83–90

Araújo WL, Saridakis HO, Barroso PAV, Aguilar-Vildoso CI, Azevedo JL (2001) Variability and interactions between endophytic bacteria and fungi isolated from leaf tissues of citrus rootstocks. Can J Microbiol 47:229–236

Araújo WL, Marcon J, Maccheroni W Jr, Elsas JDV, Vuurde JLV, Azevedo JL (2002) Diversity of endophytic bacterial populations and interaction with *Xylella fastidiosa* in citrus plants. Appl Environ Microbiol 68:4906–4914

Araújo WL, Lacava PT, Andreote FD, Azevedo JL (2008) Interaction between endophytes and plant host: biotechnological aspects. In: Ait Barka E, Clément C (eds) Plant-microbe interactions, vol 1. Research Signpost, Kerala, pp 1–21

Arias OE, Gatti IM, Silva DM, Ruschel AP, Vose PB (1978) Primeiras observaciones al microscopio eletronico de bacterias fijadoras de N2 en la raiz de la cana de azucar (*Saccharum officinarum* L.). Tarrialba 28:203–207

Assis SMP, Silveira EB, Mariano RLR, Menezes D (1998) Bactérias endofíticas – método de isolamento e potencial antagônico no controle da podridão negra do repolho. Summa Phytopathol 24:216–220

Assumpção LC, Lacava PT, Dias ACF, Azevedo JL, Menten JOM (2009) Diversity and biotechnological potential of endophytic bacterial community of soybean seeds. Braz J Agric Res 44:503–510

Azevedo JL, Araújo WL (2003) Genetically modified crops: environmental and human health concerns. Mutat Res 544:223–233

Azevedo JL, Araújo WL (2007) Diversity and applications of endophytic fungi isolated from tropical plants. In: Ganguli BN, Deshmukh SK (eds) Fungi: multifaceted microbes. CRC, Boca Raton, pp 189–207

Azevedo JL, Maccheroni W, Pereira JO, Araújo WL (2000) Endophytic microorganisms: a review on insect control and recent advances on tropical plants. Electr J Biotechnol 3:40–65

Bacon C, Hinton D (2006) Bacterial endophytes: the endophytic niche, its occupants, and its utility. In: Gnanamanickam S (ed) Plant-associated bacteria. Springer, Dordrecht, pp 155–194

Bacon CW, White JF Jr (2000) Microbial endophytes. Dekker, New York, p 487

Baker AJM, McGrath SP, Reeves RD, Smith JAC (2000) Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In: Terry N, Bañelos G (eds) Phytoremediation of contaminated soil and water. Lewis, Boca Raton, pp 85–108

Baldani VLD, Döbereiner J (1980) Host-plant especificity in infection of cereal with *Azospirillum* spp. Soil Biol Biochem 12:433–440

Baldani JI, Baldani VLD, Seldin L, Döbereiner J (1986) Characterization of *Herbaspirillum seropedicae* gen. nov., sp. nov., a root-associated nitrogen-fixing bacterium. Int J Syst Bacteriol 36:86–93

Baldani JI, Caruso L, Baldani VLD, Goi SR, Dobereiner J (1997) Recent advances in BNF with non-legume plants. Soil Biol Biochem 29:911–922

Baldani VLD, Baldani JI, Döbereiner J (2000) Inoculation of rice plants with the endophytic diazotrophs *Herbaspirillum seropedicae* and *Burkholderia* spp. Biol Fertil Soils 30:485–489

Baldani JI, Baldani VLD, Döbereiner J (2003) Genus *Herbaspirillum*. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM (eds) Bergey's manual of determinative bacteriology, 2nd edn. Springer, New York

Bangera MG, Thomashow LS (1996) Characterization of a genomic locus required for synthesis of the antibiotic 2, 4-diacetylphloroglucinol by the biological control agent *Pseudomonas fluorescens* Q2-87. Mol Plant Microbe Interact 9:83–90

Barac T, Taghavi S, Borremans B, Provoost A, Oeyen L, Colpaert JV, Vangronsveld J, van der Lelie D (2004) Engineered endophytic bacteria improve phytoremediation of water-soluble, volatile, organic pollutants. Nat Biotechnol 22:583–588

Barboza-Corona JE, Nieto-Mazzocco E, Velázquez-Robledo R, Salcedo-Hernandez R, Bautista M, Jiménez B, Ibarra JE (2003) Cloning, sequencing, and expression of the chitinases gene *chiA74* from *Bacillus thuringiensis*. Appl Environ Microbiol 69:1023–1029

Barzanti R, Ozino F, Bazzicalupo M, Gabbrielli R, Galardi F, Gonnelli C, Mengoni A (2007) Isolation and characterization of endophytic bacteria from the nickel hyperaccumulator plant *Alyssum bertolonii*. Microb Ecol 53:306–316

Bastian F, Rapparini F, Baraldi R, Piccoli P, Bottini R (1999) Inoculation with *Acetobacter diazotrophicus* increases glucose and fructose content in shoots of *Sorghum bicolor* (L.) Monech. Symbiosis 2:147–156

Beattie GA, Lindow SE (1995) The secret life of foliar bacterial pathogens on leaves. Annu Rev Phytopathol 33:145–172

Bell CR, Dickie GA, Harvey WLG, Chan JWYF (1995) Endophytic bacteria in grapevine. Can J Microbiol 41:46–53

Benhamou N, Kloepper JW, Tuzun S (1998) Induction of resistance against *Fusarium* wilt of tomato by combination of chitosan with an endophytic bacterial strain: ultrastructure and cytochemistry of the host response. Planta 204:153–168

Bent E, Chanway CP (1998) The growth-promoting effects of a bacterial endophyte on lodgepole pine are partially inhibited by the presence of other rhizobacteria. Can J Microbiol 44:980–988

Beuchat LR, Ward TE, Pettigrew CA (2001) Comparison of chlorine and a prototype produce wash product for effectiveness in killing *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seeds. J Food Prot 64:152–158

Bhattacharya D, Nagpure A, Gupta RK (2007) Bacterial chitinases: properties and potential. Crit Rev Biotechnol 27:21–28

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350

Blumer C, Haas D (2000) Mechanism, regulation, and ecological role of bacterial cyanide biosynthesis. Arch Microbiol 173:170–177

Boddey RM, Döbereiner J (1995) Nitrogen fixation associated with grasses and cereals recent progress and perspectives for the future. Fert Res 42:241–250

Boddey RM, Urquiaga S, Alves BJR, Reis V (2003) Endophytic nitrogen fixation in sugarcane: present knowledge and future applications. Plant Soil 252:139–149

Boursnell JG (1950) The symbiotic seed-borne fungus in the Cistaceae. I. Distribution and function of the fungus in the seedling and in the tissues of the mature plant. Ann Bot 14:217–243

Burton A (2006) Dispatches – pesticides may promote Parkinson's disease. Front Ecol Environ 4:284–289

Caballero-Mellado J, Martínez-Aguilar L, Paredes-Valdez G, Estrada-de los Santos P (2004) *Burkholderia unamae* sp. nov., an N_2-fixing rhizospheric and endophytic species. Int J Syst Evol Microbiol 54:1165–1172

Cao L, Qiu Z, You J, Tan H, Zhou S (2005) Isolation and characterization of endophytic streptomycete antagonists of *Fusarium* wilt pathogen from surface-sterilized banana roots. FEMS Microbiol Lett 247:147–152

Castro-Gonzalez R, Martinez-Aguilar L, Ramirez-Trujillo A, Estrada-de los Santos P, Caballero-Mellado J (2011) High diversity of culturable *Burkholderia* species associated with sugarcane. Plant Soil 345:155–169

Challis GL, Hopwood DA (2003) Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by Streptomyces species. Proc Natl Acad Sci USA 100:14555–14561

Chanway CP (1997) Inoculation of tree roots with plant growth promoting soil bacteria: an emerging technology for reforestation. For Sci 43:99–112

Chanway CP, Shishido M, Nairn J, Jungwirth S, Markham J, Xiao G, Holl FB (2000) Endophytic colonization and field response of hybrid spruce seedlings after inoculation with plant growth-promoting rhizobacteria. For Ecol Manage 133:81–88

Chelius MK, Triplett EW (2000) Immunolocalization of dinitrogenase are reductase produced by *Klebsiella pneumoniae* in association with *Zea mays* L. Appl Environ Microbiol 66:783–787

Chelius MK, Triplett EW (2001) The diversity of archaea and bacteria in association with the roots of *Zea mays* L. Microbiol Ecol 41:252–263

Cheplick GP, Clay K, Marks S (1989) Interactions between infection by endophytic fungi and nutrient limitation in the grasses *Lolium perenne* and *Festuca arundinacea*. New Phytol 111:89–97

Cheryl P, Glick B (1996) Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol 42:207–220

Cho KM, Hong SY, Lee SM, Kim YH, Kahng GG, Lim YP, Kim H, Yun HD (2007) Endophytic bacterial communities in ginseng and their antifungal activity against pathogens. Microb Ecol 54:341–351

Christodoulou E, Duffner F, Vorgias CE (2001) Overexpression, purification, and characterization of a thermostable chitinase (Chi40) from *Streptomyces thermoviolaceus* OPC-520. Protein Expr Purif 23:97–105

Chung H, Park M, Madhaiyan M, Seshadri S, Song J, Cho H, Sa T (2005) Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil Biol Biochem 37:1970–1974

Ciampi-Panno L, Fernandez C, Bustamante P, Andrade N, Ojeda S, Conteras A (1989) Biological control of bacterial wilt of potatoes caused by *Pseudomonas solanacearum*. Am Potato J 66:315–332

Cleland RE (1990) Auxin and cell elongation. In: Davies PJ (ed) Plant hormones and their role in plant growth and development. Kluwer Academic, Dordrecht, pp 132–148

Cocolin L, Rantsiou K, Iacumin L, Cantoni C, Comi G (2002) Direct dentification in food samples of *Listeria* spp. and *Listeria monocytogenes* by molecular methods. Appl Environ Microbiol 68:6273–6282

Comai L, Kosuge T (1980) Involvement of plasmid deoxyribonucleic acid in indoleacetic acid synthesis in *Pseudomonas savastanoi*. J Bacteriol 143:950–957

Cooley MB, Miller WG, Mandrell RE (2003) Colonization of *Arabidopsis thaliana* with *Salmonella enterica* and enterohemorrhagic *Escherichia coli* O157:H7 and competition by *Enterobacter asburiae*. Appl Environ Microbiol 69:4915–4926

Coombs JT, Franco CMM (2003) Isolation and identification of actinobacteria from surface-sterilized wheat roots. Appl Environ Microbiol 69:5603–5608

Coombs JT, Michelsen PP, Franco CMM (2004) Evaluation of endophytic actinobacteria as antagonists of *Gaeumannomyces graminis* var. *tritici* in wheat. Biol Control 29:359–366

Corpe WA (1985) A method for detecting methylotrophic bacteria on solid surfaces. J Microbiol Methods 3:215–221

Costa JM, Loper JE (1994) Characterization of siderophore production by the biological control agent *Enterobacter cloacae*. Mol Plant Microbe Interact 7:440–448

Costacurta A, Vanderleyden J (1995) Synthesis of phytohormones by plant-associated bacteria. Crit Rev Microbiol 21:1–18

Cottrell MT, Kirchman DL (2000) Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence in situ hybridization. Appl Environ Microbiol 66:5116–5122

Dias ACF, Costa FEC, Andreote FD, Lacava PT, Teixeira MA, Assumpção LC, Araújo WL, Azevedo JL, Melo IS (2009) Isolation of micro propagated strawberry endophytic bacteria and assessment of their potential for plant growth promotion. World J Microbiol Biotechnol 25:189–195

Döbereiner J (1992) History and new perspective of diazotrophs in association with non-leguminous plants. Symbiosis 13:1–13

Döbereiner J (1997) Biological nitrogen fixation in the tropics: social and economic contributions. Soil Biol Biochem 29:771–774

Döbereiner J, Ruschel AP (1958) Uma nova espécie de *Beijerinkia*. Ver Biol 1:261–272

Döbereiner J, Urquiaga S (1992) Soil biology and sustainable agriculture. An Acad Bras Sci 84:127–133

Döbereiner J, Baldani VLD, Baldani JI (1995) Como isolar e identificar bactérias diazotróficas de plantas não-leguminosas. Embrapa-SPI, Brasília, p 60

Dong Z, Canny MJ, McCully ME, Roboredo MR, Cabadilla CF, Ortega E, Rodes R (1994) A nitrogen fixing endophyte of sugarcane stems. Plant Physiol 105:1139–1147

Dong Z, McCully ME, Canny MJ (1997) Does *Acetobacter diazotrophicus* live and move in the xylem of sugarcane? Anatomical and physiological data. Ann Bot 80:147–158

Dong YM, Iniguez AL, Ahmer BMM, Triplett EW (2003) Kinetics and strain specificity of rhizosphere and endophytic colonization by enteric bacteria on seedlings of *Medicago sativa* and *Medicago truncatula*. Appl Environ Microbiol 69:1783–1790

Dos Reis FB, Reis VM, Urquiaga S, Döbereiner J (2000) Influence of nitrogen fertilisation on the population of diazotrophic bacteria *Herbaspirillum* spp. and *Acetobacter diazotrophicus* in sugarcane (*Saccharum* spp.). Plant Soil 219:153–159

Dourado MN, Ferreira A, Araújo WL, Azevedo JL, Lacava PT (2012) The diversity of endophytic methylotrophic bacteria in an oil-contaminated and an oil-free mangrove ecosystem and their tolerance to heavy metals. Biotechnol Res Int 2012:1–8

Duffy BK, Defago G (1999) Environmental factors modulating antibiotic and siderophore biosynthesis by *Pseudomonas fluorescens* biocontrol strains. Appl Environ Microbiol 65:2429–2438

Dunbar J, Takala S, Barns SM, Davis JA, Kuske CR (1999) Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning. Appl Environ Microbiol 65:1662–1669

Egener T, Hurek T, Reinhold-Hurek B (1999) Endophytic expression of *nif* genes of *Azoarcus* sp. strain BH72 in rice roots. Mol Plant Microbe Interact 12:813–819

Elbeltagy A, Nishioka K, Sato T, Suzuki H, Ye B, Hamada T, Isawa T, Mitsui H, Minamisawa K (2000) Endophytic colonization and *in planta* nitrogen fixation by *Herbaspirillum* sp. isolated from rice species. Appl Environ Microbiol 67:5285–5293

El-Khawas H, Adachi K (1999) Identification and quantification of auxins in culture media of *Azospirillum* and *Klebsiella* and their effect on rice roots. Biol Fertil Soils 28:377–381

El-Tarabily KA (2003) An endophytic chitinase-producing isolate of *Actinoplanes missouriensis*, with potential for biological control of root rot of lupine caused by *Plectosporium tabacinum*. Aust J Bot 51:257–266

El-Tarabily KA, Nassar AH, Hardy GE, Sivasithamparam K (2009) Plant growth promotion and biological control of *Pythium aphanidermatum*, a pathogen of cucumber, by endophytic actinomycetes. J Appl Microbiol 106:13–26

Estrada P, Mavingui P, Cournoyer B, Fontaine F, Balandreau J, Caballero-Mellado J (2002) A N_2-fixing endophytic *Burkholderia* sp. associated with maize plants cultivated in Mexico. Can J Microbiol 48:285–294

Etchegaray A, Silva-Stenico ME, Moon DH, Tsai SM (2004) In silico analysis of nonribosomal peptide synthetases of *Xanthomonas axonopodis* pv. *citri*: identification of putative siderophore and lipopeptide biosynthetic genes. Microbiol Res 159:425–437

Fahey JW (1988) Endophytic bacteria for the delivery of agrochemicals to plants. In: Cutle HG (ed) Biologically active natural products. American Chemical Society, Washington, DC, pp 120–128

Fahey JW, Dimock MB, Tomasino SF, Taylor JM, Carlson PS (1991) Genetically engineered endophytes as biocontrol agents: a case study in industry. In: Andrews JH, Hirano SS (eds) Microbial ecology of leaves. Springer, New York, pp 401–411

Feng Y, Shen D, Song W (2006) Rice endophyte *Pantoea agglomerans* YS19 promotes host plant growth and affects allocations of host photosynthates. J Appl Microbiol 100:938–945

Ferreira A, Quecine MC, Lacava PT, Oda S, Azevedo JL, Araújo WL (2008) Diversity of endophytic bacteria from *Eucalyptus* species seeds and colonization of seedlings by *Pantoea agglomerans*. FEMS Microbiol Lett 287:8–14

Fisher PJ, Petrini O, Scott HML (1992) The distribution of some fungal and bacterial endophytes in maize (*Zea mays* L.). New Phytol 122:299–305

Flärdh K, Buttner MJ (2009) *Streptomyces* morphogenetics: dissecting differentiation in a filamentous bacterium. Nat Rev Microbiol 7:36–49

Forchetti G, Masciarelli O, Alemano S, Alvarez D, Abdala G (2007) Endophytic bacteria in sunflower (*Helianthus annuus* L.): isolation, characterization, and production of jasmonates and abscisic acid in culture medium. Appl Microbiol Biotechnol 76:1145–1152

Franco C, Michelsen P, Percy N, Conn V, Listiana E, Moll S, Loria R, Coombs J (2007) Actinobacterial endophytes for improved crop performance. Australas Plant Pathol 36:524–531

Frommel MI, Novak J, Lazarovits G (1991) Growth enhancement and developmental modification of in vitro potato (*Solanum tuberosum* ssp. *tuberosum*) as affected by nonfluorescent *Pseudomonas* sp. Plant Physiol 96:928–936

Fuentes-Ramírez LE, Bustillos-Cristalles R, Tapia-Hernandez A, Jiménez-Salgado T, Wang ET, Martínez-Romero E, Caballero-Mellado J (2001) Novel nitrogen-fixing acetic acid bacteria *Gluconacetobacter johannae* sp. nov. and *Gluconacetobacter azotocaptans* sp. nov. associated with coffee plants. Int J Syst Evol Microbiol 51:1305–1314

Gai CS, Lacava PT, Quecine MC, Auriac MC, Lopes JRS, Araújo WL, Miller TA, Azevedo JL (2009) Transmission of *Methylobacterium mesophilicum* by *Bucephalogonia xanthophis* for paratransgenic control strategy of Citrus Variegated Chlorosis. J Microbiol 47:448–454

Garbeva P, Van Overbeek LS, Van Vuurde JWL, Van Elsas JD (2001) Analysis of endophytic bacterial communities of potato by planting and denaturing gradient gel electrophoresis (DGGE) of 16s rDNA based PCR fragments. Microb Ecol 41:369–383

Germaine K, Keogh E, Garcia-Cabellos G, Borremans B, Van der Lelie D, Barac T, Oeyen L, Vangronsveld J, Moore FP, Moore ERB, Campbell TCD, Dowling DN (2004) Colonization of poplar trees by *gfp* expressing bacterial endophytes. FEMS Microbiol Ecol 48:109–118

Gillis M, Kersters K, Hoste B, Janssen D, Kroppenstedt RM, Stephan MP, Teixera KRS, Döbereiner J, DeLey J (1989) *Acetobacter diazotrophicus* sp. nov., a nitrogen-fixing acetic acid bacterium associated with sugarcane. Int J Syst Bacteriol 39:361–364

Glass ADM (1989) Physiological mechanisms involved with genotypic differences in ion absorption and utilization. Hort Sci 24:559–564

Glick B, Penrose D, Li J (1998) A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J Theor Biol 190:63–68

Govindarajan M, Balandreau J, Muthukumarasamy R, Revathi G, Lakshminarasimhan C (2006) Improved yield of micro-propagated sugarcane following inoculation by endophytic *Burkholderia vietnamiensis*. Plant Soil 280:239–252

Govindarajan M, Kwon SW, Weon HJ (2007) Isolation, molecular characterization and growth-promoting activities of endophytic sugarcane diazotroph *Klebsiella* sp. GR9. World J Microbiol Biotechnol 23:997–1006

Graciolli LA, Freitas JR, Ruschel AP (1983) Bactérias fixadoras de nitrogênio nas raízes, caules e folhas de cana-de-acúçar (Saccharum sp.). Rev Microbiol 14:191–196

Gutierrez-Zamora ML, Martinez-Romero E (2001) Natural endophytic association between *Rhizobium etli* and maize (Zea mays L.). J Biotechnol 91:117–126

Gyaneshwar P, Naresh KG, Parekh LJ (1998) Cloning of mineral phosphate solubilizing genes from Synechocystis PCC 6803 P. Curr Sci 74:1097–1099

Gyaneshwar P, James EK, Mathan N, Reddy PM, Reinhold-Hurek B, Ladha JK (2001) Endophytic colonization of rice by a diazotrophic strain of *Serratia marcescens*. J Bacteriol 183:634–645

Gyaneshwar P, Kumar GN, Parekh LJ, Poole PS (2002) Role of soil microorganisms in improving P nutrition of plants. Plant Soil 245:83–93

Hagen G (1990) The control of gene expression by auxin. In: Davies PJ (ed) Plant hormones and their role in plant growth and development. Kluwer Academic, Dordrecht, pp 149–163

Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW (1997) Bacterial endophytes in agricultural crops. Can J Microbiol 43:895–914

Hartung JS, Beretta J, Brlansky RH, Spisso J, Lee RF (1994) Citrus variegated chlorosis bacterium: axenic culture, pathogenicity, and serological relationships with other strains of *Xylella fastidiosa*. Phytopathology 84:591–597

Hayat R, Ali S, Amara U, Khalid R, Ahmed I (2010) Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol 60:579–598

Hegazi NA, Eid M, Farag RS, Monib M (1979) Asymbiotic N2-fixation in the rhizosphere of sugarcane planted under semi-arid conditions of Egypt. Rev Ecol Biol Soil 16:23–37

Huang JS (1986) Ultrastructure of bacterial penetration in plants. Annu Rev Phytopathol 24:141–157

Hurek T, Handley LL, Reinhold-Hurek B, Piché Y (2002) *Azoarcus* grass endophytes contribute fixed nitrogen to the plant in an unculturable state. Mol Plant Microbe Interact 15:233–242

Hutcheson SW, Kosuge T (1985) Regulation of indole-3-acetic acid production in *Pseudomonas syringae* pv. *savastanoi*. *Pseudomonas syringae* pv. *savastanoi*. J Biol Chem 260:6281–6287

Idris R, Trifonova R, Puschenreiter M, Wenzel WW, Sessitsch A (2004) Bacterial communities associated with flowering plants of the Ni hyperaccumulator *Thlaspi goesingense*. Appl Microbiol Biotechnol 70:2667–2677

Idris R, Kuffner M, Bodrossy L, Puschenreiter M, Monchy S, Wenzel WW, Sessitsch A (2006) Characterization of Ni tolerant methylobacteria associated with the hyperaccumulating plant *Thlaspi goesingense* and description of *Methylobacterium goesingense* sp. nov. Syst Appl Microbiol 29:634–644

Iniguez AL, Dong Y, Triplett EW (2004) Nitrogen fixation in wheat provide by *Klebsiella pneumoniae* 342. Mol Plant Microbe Interact 17:1078–1085

Iniguez AL, Dong Y, Carter HD, Ahmer BMM, Stone JM, Triplett EW (2005) Regulation of enteric endophytic bacterial colonization by plant defense. Mol Plant Microbe Interact 18:169–178

Jalgaonwala RE, Mahajan RT (2011) A review: bacterial endophytes and their bioprospecting. J Pharm Res 4:795–799

James EK, Olivares FL (1998) Infection and colonization of sugarcane and other graminaceous plants by endophytic diazotrophs. Crit Rev Plant Sci 17:77–119

James EK, Olivares FL, Baldani JI, Döbereiner J (1997) *Herbaspirillum*, an endophytic diazotroph colonizing vascular tissue in leaves of *Sorghum bicolor* L. Moench. J Exp Bot 48:785–797

James EK, Olivares FL, de Oliveira ALM, dos Reis FB, da Silva LG, Reia VM (2001) Further observations on the interaction between sugar cane and *Gluconacetobacter diazotrophicus* under laboratory and greenhouse conditions. J Exp Bot 52:747–760

James EK, Gyaneswar P, Mathan N, Barraquio WL, Reddy PM, Iannetta PPM, Olivares FL, Ladha JK (2002) Infection and colonization of the rice seedlings by the plant growth-promoting bacterium *Herbaspirillum seropedicae* Z67. Mol Plant Microbe Interact 15:894–906

Jiménez-Salgado T, Fuentes-Ramírez LE, Tapia-Hernández A, Mascarua- Esparza MA, Martínez-Romero E, Caballero-MEllado J (1997) *Coffea arabica* L., a new host plant for *Acetobacter diazotrophicus* and isolation of the nitrogen-fixing-acetobacteria. Appl Environ Microbiol 63:3676–3683

Kafur A, Khan AB (2011) Isolation of endophytic actinomycetes from *Catharanthus roseus* (L.) G. Don leaves and their antimicrobial activity. Iran J Biotechnol 9:302–306

Kanvinde L, Sastry GRK (1990) *Agrobacterium tumefaciens* is a diazotrophic bacterium. Appl Environ Microbiol 56:2087–2092

Khan AA, Jilani G, Akhtar MS, Naqvi SMS, Rasheed M (2009) Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. J Agric Biol Sci 1:48–58

Kibe R, Sakamoto M, Yokota H, Ishikawa H, Aiba Y, Koga Y, Benno Y (2005) Movement and fixation of intestinal microbiota after administration of human feces to germfree mice. Appl Environ Microbiol 71:3171–3178

Kim KJ, Yang YJ, Kim JG (2003) Purification and characterization of chitinase from *Streptomyces* sp. M-20. J Biochem Mol Biol 36:185–189

Kirchhof G, Schloter M, Abmus B, Hartmann A (1997) Molecular microbial ecology approaches applied to diazotrophs associated with non-legumes. Soil Biol Biochem 29:853–862

Kobayashi DY, Palumbo JD (2000) Bacterial endophytes and their effects on plants and uses in agriculture. In: Bacon CW, White JF (eds) Microbial endophytes. Dekker, New York, pp 199–236

Koga J, Adachi T, Hidaka H (1991) Molecular cloning of the gene for indolepyruvate decarboxylase from Enterobacter cloacae. Mol Gen Genet 226:10–16

Kosuge T, Sanger M (1987) Indole acetic acid, its synthesis and regulation: basis for tumorigenicity in plant disease. Recent Adv Phytochem 20:147–161

Kramer KJ, Muthukrishnan S (1997) Insect chitinases: molecular biology and potential use as biopesticides. Insect Biochem Mol Biol 27:887–900

Krechel A, Faupel A, Hallmann J, Ulrich A, Berg G (2002) Potato associated bacteria and their antagonistic potential towards plant pathogenic fungi and the plant parasitic nematode *Meloidogyne incognita* (kofoid and white) chitwood. Can J Microbiol 48:772–786

Kuklinsky-Sobral J, Araújo WL, Mendes R, Geraldi IO, Pizzirani-Kleiner AA, Azevedo JL (2004) Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ Microbiol 6:1244–1251

Kuklinsky-Sobral J, Araújo WL, Mendes R, Pizzirani-Kleiner AA, Azevedo JL (2005) Isolation and characterization of endophytic bacteria from soybean (*Glycine max*) grown in soil treated with glyphosate herbicide. Plant Soil 273:91–99

Kumar BSD, Dube HC (1992) Seed bacterization with a fluorescent *Pseudomonas* for enhanced plant growth and yield and disease control. Soil Biol Biochem 26:539–542

Lacava PT, Araujo WL, Marcon J, Maccheroni W Jr, Azevedo JL (2004) Interaction between endophytic bacteria from citrus plants and the phytopathogenic bacteria *Xylella fastidiosa*, causal agent of citrus-variegated chlorosis. Lett Appl Microbiol 39:55–59

Lacava PT, Li WB, Araújo WL, Azevedo JL, Hartung JS (2006) Rapid, specific and quantitative assays for the detection of endophytic bacterium *Methylobacterium mesophilicum* in inoculated plants. J Microbiol Methods 65:535–541

Lacava PT, Araújo WL, Azevedo JL (2007a) Evaluation of endophytic colonization of Citrus sinensis and Catharanthus roseus seedlings by endophytic bacteria. J Microbiol 45:11–14

Lacava PT, Li WB, Araújo WL, Azevedo JL, Hartung JS (2007b) The endophyte *Curtobacterium flaccumfaciens* reduces symptoms caused by *Xylella fastidiosa* in *Catharanthus roseus*. J Microbiol 45:388–393

Lacava PT, Silva ME, Araújo WL, Simionato A, Carrilho E, Tsai SM, Azevedo JL (2008) Detection of siderophores in endophytic bacteria *Methylobacterium* spp. associated with *Xylella fastidiosa* subsp. *pauca*. Braz J Agric Res 43:521–528

Lamb TG, Tonkyn DW, Kluepfel DA (1996) Movement of *Pseudomonas aureofaciens* from the rhizosphere to aerial plant tissue. Can J Microbiol 42:1112–1120

Lambert B, Joos H (1989) Fundamental aspects of rhizobacterial plant growth promotion research. Trends Biotechnol 7:215–219

Lampel JS, Canter GL, Dimock MB, Kelly JL, Anderson JJ, Uratani BB, Foulke JS Jr, Turner JT (1994) Integrative cloning, expression, and stability of the cryIA(c) gene from *Bacillus thuringiensis* subsp. *kurstati* in a recombinant strain of *Clavibacter* xyli subsp. *cynodontis*. Appl Environ Microbiol 60:501–508

Lebuhn M, Heulin T, Hartmann A (1997) Production of auxin and other indolic and phenolic compounds by *Paenibacillus polymyxa* strains isolated from diferente proximity to plant roots. FEMS Microbiol Ecol 22:325–334

Lecuona RE (1996) Microorganismos patógenos empleados en el control microbiano de insectos plaga. Editora Lecuona, Castelar, p 338

Lee S, Flores-Encarnacion M, Contreras-Zentalla M, GarciaFlores L, Escamilla JE, Kennedy C (2004) Indole-3-acetic acid biosynthesis is deficient in *Gluconacetobacter diazotrophicus* strains with mutations in cytochrome c biogenesis genes. J Bacteriol 186:5384–5391

Lee HS, Madhaiyan M, Kim CW, Choi SJ, Chung KY, Sa TM (2006) Physiological enhancement of early growth of rice seedlings (*Oryza sativa* L.) by production of phytohormone of N_2-fixing methylotrophic isolates. Biol Fertil Soils 42:402–408

Li C-H, Zhao M-W, Tang C-M, Li S-P (2009) Population dynamics and identification of endophytic bacteria antagonistic toward plant-pathogenic fungi in cotton root. Microb Ecol 59:344–356

Lidstrom ME (1992) The genetics and molecular biology of methanol-utilizing bacteria. In: Murrell JC, Dalton H (eds) Methane and methanol utilizers. Plenum, New York, pp 183–206

Liu L, Kloepper JW, Tuzun S (1995) Induction of systemic resistance in cucumber against bacterial angular leaf spot by plant growth-promoting rhizobacteria. Phytopathology 85:843–847

Loaces I, Ferrando L, Fernandez Scavino A (2011) Dynamics, diversity and function of endophytic siderophore-producing bacteria in rice. Microb Ecol 61:606–618

Lodewyckx C, Taghavi S, Mergeay M, Vangronsveld J, Clijsters H, van der Lelie D (2001) The effect of recombinant heavy metal resistant endophytic bacteria on heavy metal uptake by their host plant. Int J Phytorem 3:173–187

Lodewyckx C, Vangronsveld J, Porteus F, Moore ERB, Taghavi S, Mezgeay M, Lelie DV (2002) Endophytic bacteria and their potential applications. Crit Rev Plant Sci 21:586–606

Loganathan P, Sunitha R, Prida AK, Nair S (1999) Isolation and characterization of genetically two distant group of *Acetobacter diazotrophicus* from new host plant (*Eleusine coracana* L.). J Appl Bacteriol 86:1053–1058

Luvizotto DM, Marcon J, Andreote FD, Dini-Andreote F, Neves AAC, Araújo WL, Pizzirani-Kleiner AA (2010) Genetic diversity and plant-growth related features of *Burkholderia* spp. from sugarcane roots. World J Microbiol Biotechnol 26:1829–1836

Madhaiyan M, Poonguzhali S, Senthilkumar M, Seshadri S, Chung H (2004) Growth promotion and induction of systemic resistance in rice cultivar Co-47 (*Oryza sativa* L.) by *Methylobacterium* spp. Bot Bull Acad Sin 45:315–324

Madhaiyan M, Poonguzhali S, Ryu J, Sa T (2006) Regulation of ethylene levels in canola (*Brassica campestris*) by 1-aminocyclopropane-1-carboxylate deaminase-containing *Methylobacterium fujisawaense*. Planta 224:268–278

Magie AR, Wilson EE, Kosuge T (1963) Indoleacetamide as an intermediate in the synthesis of indoleacetic acid in *Pseudomonas savastanoi*. Science 141:1281–1282

Magnani GS, Didonet CM, Cruz LM, Picheth CF, Pedrosa FO, Souza EM (2010) Diversity of endophytic bacteria in Brazilian sugarcane. Genet Mol Res 9:250–258

Mahaffee WF, Kloepper JW (1997) Temporal changes in the bacterial communities of soil, rhizosphere and endorhiza. Microb Ecol 34:210–223

Mahaffee WF, Kloepper JW, Van Vuurde JWL, Van Der Wolf JM, Van Den Brink M (1997) Endophytic colonization of *Phaseolus vulgaris* by *Pseudomonas fluorescens* strain 89B-27 and *Enterobacter asburiae* strain JM22. In: Ryder MH, Stephens PM, Bowen GD (eds) Improving plant productivity in rhizosphere bacteria. CSIRO, Melbourne, p 180

Malinowski DP, Belesky DP (1999) Endophyte infection enhances the ability of tall fescue to utilize sparingly available phosphorus. J Plant Nutr 22:835–853

Mano H, Morisaki H (2008) Endophytic bacteria in the rice plant. Microbes Environ 23:109–117

Martins ES, Praça LB, Dumas VF, Silva-Werneck JO, Sone EH, Waga IC, Berry C, Monnerat RG (2007) Characterization of *Bacillus thuringiensis* isolates toxic to cotton boll weevil (*Anthonomus grandis*). Biol Control 40:65–68

Martins ES, Aguiar RW, Martins NF, Melatti VM, Falcão R, Gomes AC, Ribeiro BM, Monnerat RG (2008) Recombinant Cry1Ia protein is highly toxic to cotton boll weevil (*Anthonomus grandis* Boheman) and fall armyworm (*Spodoptera frugiperda*). J Appl Microbiol 104:1363–1371

Masclaux C, Expert D (1995) Signalling potential of iron in plant–microbe interactions: the pathogenic switch of iron transport in *Erwinia chrysanthemi*. Plant J 7:121–128

Mastretta C, Taghavi S, van der Lelie D, Mengoni A, Galardi F, Gonnelli C, Barac T, Boulet J, Weyens N, Vangronsveld J (2009) Endophytic bacteria from seeds of *Nicotiana tabacum* can reduce cadmium phytotoxicity. Int J Phytorem 11:251–267

McInroy JA, Kloepper JW (1995) Survey of indigenous bacterial endophytes from cotton and sweet corn. Plant Soil 173:337–342

Melo IS, Azevedo JL (1998) Controle biológico I. Embrapa, Jaguariuna, 262

Mendes R, Pizzirani-Kleiner AA, Araújo WL, Raaijmakers JM (2007) Endophytic bacteria from sugarcane: genetic and biochemical characterization of *Burkholderia cepacia* complex isolates. Appl Environ Microbiol 73:7259–7267

Mendes R, Azevedo JL (2007) Valor biotecnológico de fungos endofíticos isolados de plantas de interesse econômico. In: Abstract, 5o Congresso Brasileiro de Micologia, pp 129–140

Miliūtė I, Buzaitė O (2011) IAA production and other plant growth promoting traits of endophytic bacteria from apple tree. Biologija 57:98–102

Misaghi IJ, Donndelinger CR (1990) Endophytic bacteria in symptom-free cotton plants. Phytopathology 9:808–811

Monteiro PB, Renaudin J, Jagoueix-Eveillard S, Ayres AJ, Garnier M, Bové JM (2001) *Catharanthus roseus*, an experimental host plant for the citrus strain of *Xylella fastidiosa*. Plant Dis 85:246–251

Musson G (1994) Ecology and effects of endophytic bacteria in plant. Masters thesis, Auburn University, Auburn, AL

Nachin L, El Hassouni M, Loiseau L, Expert D, Barras F (2001) SoxR-dependent response to oxidative stress and virulence of *Erwinia chrysanthemi*: the key role of SufC, an orphan ABC ATPase. Mol Microbiol 39:960–972

Naher UA, Othman R, Shamsuddin ZHJ, Saud HM, Ismail MR (2009) Growth enhancement and root colonization of rice seedlings by *Rhizobium* and *Corynebacterium* spp. Int J Agric Biol 11:586–590

Neilands JB, Nakamura K (1991) Detection, determination, isolation, characterization and regulation of microbial iron chelates. In: Winkelmann G (ed) CRC handbook of microbial iron chelates. CRC, Boca Raton, pp 1–14

Newman LA, Reynolds CM (2005) Bacteria and phytoremediation: new uses for endophytic bacteria in plants. Trends Biotechnol 23:6–8

Nudel C, Gonzalez R, Castaneda N, Mahler G, Actis LA (2001) Influence of iron on growth, production of siderophore compounds, membrane proteins, and lipase activity in *Acinetobacter calcoaceticus* BD 413. Microbiol Res 155:263–269

O'Sullivan DJ, O'Gara F (1992) Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. Microbiol Rev 56:662–676

Oberhansli T, Defago G, Haas D (1991) Indole-3-acetic-acid (IAA) synthesis in the biocontrol strain CHA0 of *Pseudomonas fluorescens* – role of tryptophan side-chain oxidase. J Gen Microbiol 137:2273–2279

Olivares FL, Baldani VLD, Reis VM, Baldani JI, Döbereiner J (1996) Occurrence of the endophytic diazotrophs *Herbaspirillum* spp. in roots, stems and leaves predominantly of Gramineae. Biol Fert Soils 21:197–200

Omoregie SN, Asemota HN, Osagie AU, Mantell S, Ahmad MH (1999) Occurrence of free-living bacteria in tubers of *Dioscorea* yams. Trop Agric 76:250–255

Owen NL, Hundley N (2004) Endophytes the chemical sintesizer inside plants. Sci Prog 87:79–99

Pal SS (1998) Interactions of an acid tolerant strain of phosphate solubilizing bacteria with a few acid tolerant crops. Plant Soil 198:169–177

Palaniappan A, Goh WH, Tey JN, Wijaya IPM, Moochhala SM, Liedberg B, Mhaisalkar SG (2010) Aligned carbon nanotubes on quartz substrate for liquid gated biosensing. Biosens Bioelectron 25:1989–1993

Park M, Kin C, Yang J, Lee H, Shin W, Kim S, Sa T (2005) Isolation and characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of Korea. Microbiol Res 160:127–133

Paula MA, Siqueira JO, Döbereiner J (1993) Ocorrência de fungos micorrízicos vesiculoarbusculares e de bactérias diazotróficas na cultura de batata-doce. Braz J Soil Sci 17:349–356

Perin L, Martínes-Aguilar L, Castro-Gonzáles R, Estrada-De-Los-Santos P, Cabellos-Avelar T, Guedes HV, Reis VM, Caballero-Mellado J (2006) Diazotrophic *Burkholderia* species associated with field-grown maize and sugarcane. Appl Environ Microbiol 72:3103–3110

Persello-Cartieaux F, Nussaume L, Robaglia C (2003) Tales from the underground: molecular plant–rhizobacteria interactions. Plant Cell Environ 26:189–199

Pimenta PFP, Modi GB, Pereira ST, Shahabuddin M, Sacks DL (1997) A novel role for the peritrophic matrix in protecting *Leishmania* from the hydrolytic activities of the sand fly midgut. Parasitology 115:359–369

Pirttilä A, Joensuu P, Pospiech H, Jalonen J, Hohtola A (2004) Bud endophytes of Scots pine produce adenine derivatives and other compounds that affect morphology and mitigate browning of callus cultures. Physiol Plantarum 121:305–312

Pleban S, Ingel F, Chet I (1995) Control of *Rhizoctonia solani* and *Sclerotium rolfsii* in the greenhouse using endophytic *Bacillus* spp. Eur J Plant Pathol 101:665–672

Podolich O, Laschevskyy V, Ovcharenko L, Kozyrovska N, Pirttila AM (2009) *Methylobacterium* sp. resides in unculturable state in potato tissues in vitro and becomes culturable after induction by *Pseudomonas fluorescens* IMGB163. J Appl Microbiol 106:728–737

Ponka A, Andersson Y, Siitonen A, de Jong B, Jahkola M, Haikala O, Kuhmonen A, Pakkala P (1995) Salmonella in alfalfa sprouts. Lancet 345:462–463

Potrich DP, Passaglia LMP, Schrank IS (2003) Partial characterization of nif genes from the bacterium *Azospirillum amazonense*. Braz J Med Biol Rev 34:1105–1113

Proctor ME, Harnacher M, Tortorello ML, Archer JR, Davis JP (2001) Multistate outbreak of *Salmonella serovar* Muenchen infections associated with alfalfa sprouts grown from seeds pretreated with calcium hypochlorite. J Clin Microbiol 39:3461–3465

Puente ME, Li CY, Bashan Y (2009) Rock-degrading endophytic bacteria in cacti. Environ Exp Bot 66:389–401

Pugsley AP, Oudega B (1987) Methods for studying colicins and their plasmids. In: Hardy KG (ed) Plasmids: a practical approach. IRL, Oxford, pp 105–161

Purchase BS (1980) Nitrogen fixation associated with sugarcane. In: Proceedings of the South African Sugar Technologists Association, pp 173–176

Qiu F, Huang Y, Sun L, Zhang X, Liu Z, Song W (2007) *Leifsonia ginsengi* sp. nov., isolated from ginseng root. Int J Syst Evol Microbiol 57:405–408

Quecine MC, Araújo WL, Marcon J, Gai CS, Azevedo JL, Pizzirani-Kleiner AA (2008) Chitinolytic activity of endophytic *Streptomyces* and potential for biocontrol. Lett Appl Microbiol 47:486–491

Quecine MC, Lacava PT, Magro SR, Parra JRP, Araújo WL, Azevedo JL, Pizzirani-Kleiner AA (2011) Partial characterization of chitinolytic extract from endophytic *Streptomyces* sp. and its effects on the boll weevil. J Agric Sci Technol 5:420–427

Rajkumar M, Ae N, Freitas H (2009) Endophytic bacteria and their potential to enhance heavy metal phytoextraction. Chemosphere 77:153–160

Ramamoorthy V, Viswanathan R, Raguchander T, Prakasan V, Samiyappan R (2001) Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. Crop Prot 20:1–11

Ramesh R, Joshi AA, Ghanekar MP (2009) *Pseudomonas*: major endophytic bacteria to suppress bacterial wilt pathogen *Ralstonia solanacearum* in the egg plant (*Solanum melongena* L.). World J Microb Biot 25:47–55

Rampelotti-Ferreira FT, Ferreira A, Vendramim JD, Lacava PT, Azevedo JL, Araújo WL (2010) Colonization of rice and *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) larvae by genetically modified endophytic *Methylobacterium mesophilicum*. Neotrop Entomol 39:308–310

Reinhold B, Hurek T (1988) Location of diazotrophs in the interior with special attention to the kallar grass association. Plant Soil 110:259–268

Reinhold-Hurek B, Hurek T (1998) Life in grasses: diazotrophic endophytes. Trends Microbiol 6:139–144

Reinhold-Hurek B, Hurek T, Gillis M, Hoste B, Vancanneyt M, Kersters K, Deley J (1993) *Azoarcus* gen. nov., nitrogen fixing proteobacteria associated with roots of Kallar grass (*Leptochloa fusca* (L.) Kunth.), and description of 2 species, *Azoarcus in digens* sp. nov. and *Azoarcus communis* sp. nov. Int J Syst Bacteriol 43:574–584

Reis VM, Baldani JI, Baldani VLD, Döbereiner J (2000) Biological dinitrogen fixation in gramineae and palm trees. Crit Rev Plant Sci 10:227–247

Reiter B, Pfeifer U, Schwab H, Sessitsch A (2002) Response of endophytic bacterial communities in potato plants to infection with *Erwinia carotovora* subsp. *atroseptica*. Appl Environ Microbiol 68:2261–2268

Rennie RJ, Freitas JRD, Ruschel AP, Vose PB (1982) Isolation and identification of N2-fixing bacteria associated with sugarcane (Saccharum sp.). Can J Microbiol 28:462–467

Riggs PJ, Chelius MK, Iniguez AL, Kaeppler SM, Triplett EW (2001) Enhanced maize productivity by inoculation with diazotrophic bacteria. Austr J Plant Physiol 28:829–836

Robbins PW, Allbright C, Benfield B (1998) Cloning and expression of a *Streptomyces plicatus* chitinase (chitinase 63) in *Escherichia coli*. J Biol Chem 263:443–447

Robertson S, Kingston G, Dart PJ, Brown S (2000) Occurrence of endophytic bacteria in Australian sugarcane. In: Abstract, 8th international symposium nitrogen fixation with non-legumes, p 140

Rodríguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Rosenblueth M, Martinez-Romero E (2006) Bacterial endophytes and their interactions with hosts. Mol Plant Microbe Interact 19:827–837

Ryan RP, Ryan DJ, Sun YC, Li F-M, Wang Y, Dowling DN (2007) An acquired efflux system is responsible for copper resistance in *Xanthomonas* strain IG-8 isolated from China. FEMS Microbiol Lett 268:40–46

Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett 278:1–9

Sagoe CI, Ando T, Kouno K, Nagaoka T (1998) Relative importance of protons and solution calcium concentration in phosphate rock dissolution by organic acids. Soil Sci Plant Nutr 44:617–625

Saikkonen K, Wäli P, Helander M, Faeth SH (2004) Evolution of endophyte–plant symbioses. Trends Plant Sci 9:275–280

Sardi P, Saracchi M, Quaroni B, Borgonovi GE, Merli S (1992) Isolation of endophytic *Streptomyces* strains from surface-sterilized roots. Appl Environ Microbiol 58:2691–2693

Schaad NW, Pastnikova E, Lacey G, Fatmi M, Chang CJ (2004) *Xylella fastidiosa* subspecies: *X. fastidiosa* subsp. piercei, subsp. nov., *X. fastidiosa* subsp. multiplex subsp. nov., and *X. fastidiosa* subsp. pauca subsp. nov. Syst Appl Microbiol 27:290–300

Scherwinski K, Wolf A, Berg G (2007) Assessing the risk of biological control agents on the indigenous microbial communities: *Serratia plymuthica* HRO-C48 and *Streptomyces* sp. HRO-71 as model bacteria. Biol Control 52:87–112

Schroth MN, Hancook GH (1995) Disease suppressive soil and root colonizing bacteria science. Soil Biol Biochem 24:539–542

Seghers D, Wittebolle L, Top EM, Verstraete W, Siciliano SD (2004) Impact of agricultural practices on the *Zea mays* L. endophytic community. Appl Environ Micobiol 70:1475–1482

Seipke RF, Kaltenpoth M, Hutchings MI (2012) *Streptomyces* as symbionts: an emerging and widespread theme? FEMS Microbiol Rev 36:862–876

Seldin L, Van Elsas JD, Penido EGC (1984) Bacillus azotofixans sp.nov., a nitrogen fixing species from Brazilian soils and grass roots. Int J Syst Bacteriol 34:451–456

Senthilkumar M, Madhaiyan M, Sundaram SP, Kannaiyan S (2009) Intercellular colonization and growth promoting effects of *Methylobacterium* sp. with plant-growth regulators on rice (*Oryza sativa* L. Cv CO-43). Microbiol Res 164:92–104

Sessitsch A, Reiter B, Pfeifer U, Wilhelm E (2002) Cultivation independent population analysis of bacterial endophytes in three potato varieties based on eubacterial and actinoymycetes specific PCR of 16S rRNA genes. FEMS Microbiol Ecol 39:23–32

Sessitsch A, Reiter B, Berg G (2004) Endophytic bacterial communities of field grown potato plants and their plant growth-promoting and antagonistic abilities. Can J Microbiol 50:239–249

Sessitsch A, Hardoim P, Döring J, Weilharter A, Krause A, Woyke T, Mitter B, Hauberg-Lotte L, Friedrich F, Rahalkar M, Hurek T, Sarkar A, Bodrossy L, van Overbeek L, Brar D, van Elsas JD, Reinhold-Hurek B (2012) Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. Mol Plant Microbe Interact 25:28–36

Sevilla M, Burris RH, Gunapala N, Kennedy C (2001) Comparison of benefit to sugarcane plant growth and N_2 incorporation following inoculation of sterile plants with *Acetobacter diazotrophicus* wild-type and *Nif*- mutant strains. Mol Plant Microbe Interact 14:358–366

Sharma A, Johri BN (2003) Growth promoting influence of siderophore-producing *Pseudomonas* strains GRP3A and PRS9 in maize (*Zea mays* L.) under iron limiting conditions. Microbiol Res 158:243–248

Sheng XF, Xia JJ, Jiang CY, He LY, Qian M (2008) Characterization of heavy metal-resistant endophytic bacteria from rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of rape. Environ Pollut 156:1164–1170

Siciliano S, Fortin N, Himoc N (2001) Selection of specific endophytic bacterial genotypes by plants in response to soil contamination. Appl Environ Microbiol 67:2469–2475

Siddiqui ZA (2005) PGPR: prospective biocontrol agents of plant pathogens. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht, pp 111–142

Silva KS, Rebouças TNH, Lemos OL, Bomfim MP, Bomfim AA, Esquivel GL, Barreto APP, José ARS, Dias NO, Tavares GM (2004) Patogenicidade causada pelo fungo *Colletotrichum gloeosporioides* (Penz) em diferentes espécies frutíferas. Rev Bras Frutic 28:131–133

Singh RK, Mishra RPM, Jaiswal HK, Kumar V, Pandey SP, Rao SB, Annapurna K (2006) Isolation and identification of natural endophytic rhizobia from rice (*Oryza sativa* L.) through rDNA PCR-RFLP and sequence analysis. Curr Microbiol 52:117–122

Singh K, Agrawal KN, Bora GC (2011) Advanced techniques for weed and crop identification for site specific weed management. Biosyst Eng 109:52–64

Smalla K, Wieland G, Buchner A, Zock A, Parzy J, Kaiser S, Roskot N, Heuer H, Berg G (2001) Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Appl Environ Microbiol 67:4742–4751

Stein T, Hayen-Schneg N, Fendrik I (1997) Contribution of BNF by *Azoarcus* sp. BH72 in *Sorghum vulgare*. Soil Biol Biochem 29:969–971

Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67:491–502

Sturz AV (1995) The role of endophytic bacteria during seed piece decay and potato tuberization. Plant Soil 175:257–263

Sturz AV, Christie BR, Matheson BG, Nowak J (1997) Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. Biol Fertil Soils 25:13–19

Sturz AV, Christie BR, Matheson BG (1998) Associations of bacterial endophyte populations from red clover and potato crops with potential for beneficial allelopathy. Can J Microbiol 44:162–167

Sturz AV, Christie BR, Nowak J (2000) Bacterial endophytes: potential role in developing sustainable systems of crop production. Crit Rev Plant Sci 19:1–30

Sun L, Qiu F, Zhang X, Dai X, Dong X, Song W (2008) Endophytic bacterial diversity in rice (*Oryza sativa* L.) roots estimated by 16S rDNA sequences analysis. Microb Ecol 55:415–424

Sy A, Giraud E, Samba R, de Lajudie P, Gillis M, Dreyfus B (2001) Certaines légumineuses du genre *Crotalaria* sont spécifiquement nodulées par une nouvelle espèce de *Methylobacterium*. Can J Microbiol 47:503–508

Taechowisan T, Peberdy JF, Lumyong S (2003) Isolation of endophytic actinomycetes from selected plants and their antifungal activity. World J Microbiol Biotechnol 19:381–385

Taghavi S, Barac T, Greenberg B, Borremans B, Vangronsveld J, van der Lelie D (2005) Appl Environ Microbiol 71:8500–8505

Taghavi S, Garafola C, Monchy S, Newman L, Hoffman A, Weyens N, Barac T, Vangronsveld J, van der Lelie D (2009) Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. Appl Environ Microbiol 75:748–757

Tan RX, Zou WX (2001) Endophytes: a rich source of functional metabolites. Nat Prod Rep 18:448–459

Tan ZY, Hurek T, Gyaneshwar P, Ladha JK, Reinhold-Hurek B (2001) Novel endophytes of rice form a taxonomically distinct subgroup of *Serratia marcescens*. Syst Appl Microbiol 24:245–251

Tanwar SPS, Shaktawat MS (2003) Influence of phosphorus sources, levels and solubilizers on yield, quality and nutrient up-take of soybean (*Glycine max*) – wheat (*Triticum aestivum*) cropping system in southern Rajasthan. Indian J Agric Sci 73:3–7

Taormina PJ, Beuchat LR, Slutsker L (1999) Infections associated with eating seed sprouts: an international concern. Emerg Infect Dis 5:626–634

Tapia-Hernandez A, Bustillo-Cristales MR, Jimenez-Salgado T, Caballero-Mellado J, Fuentes-Ramirez LE (2000) Natural endophytic occurrence of *Acetobacter diazotrophicus* in pineapple plants. Microbiol Ecol 39:49–55

Toyota K, Kimura M (2000) Suppression of *Ralstonia solanacearum* in soil following colonization by other strains of *R. solanacearum*. Soil Sci Plant Nutr 46:449–459

Tsujibo H, Minoura K, Miyamoto K, Endo H, Moriwaki M, Inamori Y (1993) Purification and properties of a thermostable chitinase from *Streptomyces thermoviolaceus* OPC-520. Appl Environ Microbiol 59:620–622

Turner JT, Lampell JS, Stearmen RS, Sundin GW, Gunyuzlu UP, Anderson JJ (1991) Stability of the d-endotoxin gene from *Bacillus thuringiensis* subsp. *kurstaki* in a recombinant strain of *Clavibacter xyli* subsp. *cynodontis*. Appl Environ Microbiol 57:3522–3528

Van Aken B, Yoon JM, Schnoor JL (2004) Biodegradation of nitro-substituted explosives 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine, and octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine by a phytosymbiotic *Methylobacterium* sp. associated with poplar tissues (*Populus deltoides* × *nigra* DN34). Appl Environ Microbiol 70:508–517

van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310

Van der Lelie D, Barac T, Taghavi S, Vangronsveld J (2005) New uses of endophytic bacteria to improve phytoremediation. Trends Biotechnol 23:8–9

Van Der Peer R, Puente HLM, LA Weger DE, Shipper B (1990) Characterization of root surface and endorhizosphere pseudomonas in relation to their colonization of roots. Appl Environ Microbiol 56:2462–2470

van Loon LC, Bakker P, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol 36:453–483

Vendan RT, Yu YJ, Lee SH, Rhee YH (2010) Diversity of endophytic bacteria in ginseng and their potential for plant growth promotion. J Microbiol 48:559–565

Verma SC, Ladha JK, Tripathi AK (2001) Evaluation of plant growth promoting and colonization ability of endophytic diazotrophs from deep water rice. J Biotechnol 91:127–141

Verma VC, Singh SK, Prakash S (2011) Bio-control and plant growth promotion potential of siderophore producing endophytic *Streptomyces* from *Azadirachta indica* A. Juss. J Basic Microb 5:550–556

Wakelin SA, Warren RA, Harvey PR, Ryder MH (2004) Phosphate solubilization by *Penicillium* spp. closely associated with wheat roots. Biol Fert Soils 40:36–43

Weber OB, Baldani VLD, Teixeira KRS, Kirchhof G, Baldani JI, Döbereiner J (1999) Isolation and characterization of diazotrophic bacteria from banana and pineapple plants. Plant Soil 210:103–113

Weissinger WR, Beuchat LR (2000) Comparison of aqueous chemical treatments to eliminate Salmonella on alfalfa seeds. J Food Prot 63:1475–1482

Weissinger WR, McWatters KH, Beuchat LR (2001) Evaluation of volatile chemical treatments for lethality to *Salmonella* on alfalfa seeds and sprouts. J Food Prot 64:442–450

Weyens N, Vander Lelie D, Taghavi S, Vangronsveld J (2009) Phytoremediation: plant-endophyte partnerships take the challenge. Curr Opin Biotechnol 20:248–254

Wiehe W, Hecht-Bucholz C, Hoflich G (1994) Electron microscopic investigations on root colonization of *Lupinus albus* and *Pisum sativum* with two associative plant growth promoting rhizobacteria, *Pseudomonas fluorescens* and *Rhizobium leguminosarum* bv. *trifolii*. Symbiosis 86:221–224

Wilson D (1995) Endophyte – the evolution of a term and clarification of its use and definition. Oikos 72:274–276

Wolkers H, van Bavel B, Ericson I, Skoglund E, Kovacs KM, Lydersen C (2006) Congener-specific accumulation and patterns of chlorinated and brominated contaminants in adult male walruses from Svalbard, Norway: indications for individual-specific prey selection. Sci Total Environ 370:70–79

Yang J-H, Liu H-X, Zhu G-M, Pan Y-L, Guo J-H (2008) Diversity analysis of antagonists from rice-associated bacteria and their application in biocontrol of rice diseases. J Appl Microbiol 104:91–104

Yanni YG, Rizk RY, Corich V, Squartini A, Ninke K, PhilipHollingsworth S, Orgambide G, deBruijn F, Stoltzfus J, Buckley D, Schmidt TM, Mateos PF, Ladha JK, Dazzo FB (1997) Natural endophytic association between *Rhizobium leguminosarum* bv. trifolii and rice roots and assessment of its potential to promote rice growth. Plant Soil 194:99–114

Yu WJ, Lee BJ, Nam SY, Yang DC, Yun YW (2003) Modulating effects of Korean ginseng saponins on ovarian function immature rats. Biol Pharm Bull 26:2574–2580

Zaida A, Khan MS, Amil MD (2003) Interactive effect of rhizotrophic microorganisms on yield and nutrient uptake of chickpea (*Cicer arietinum* L.). Eur J Agron 19:15–21

Zaidi S, Usmani S, Singh BR, Musarrat J (2006) Significance of *Bacillus subtilis* strain SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in *Brassica juncea*. Chemosphere 64:991–997

Zakria M, Njoloma J, Saeki Y, Akao S (2007) Colonization and nitrogen-fixing ability of *Herbaspirillum* sp. strain B501 gfp1 and assessment of its growth-promoting ability in cultivated rice. Microbes Environ 22:197–206

Zinniel DK, Lambrecht P, Harris NB, Feng Z, Kuczmarski D, Higley P, Ishimaru CA, Arunakumari A, Barletta RG, Vidaver AK (2002) Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. Appl Environ Microbiol 68:2198–2208

Chapter 2
Beneficial Effects of Plant Growth-Promoting Rhizobacteria on Improved Crop Production: Prospects for Developing Economies

A.O. Adesemoye and D. Egamberdieva

2.1 Introduction

Bacteria that exert beneficial effects on plant development known as plant growth-promoting rhizobacteria (PGPR) have been reported widely. One of the basic requirements for the effectiveness of PGPR is their ability to colonize hosts' rhizosphere, rhizoplane, or the root interior (Glick et al. 2007). Some inoculants enter the root interior to establish endophytic populations with adaptability to the niche and benefits to the host plants (Compant et al. 2005; Kloepper et al. 1999) while some increase root surface area, thus enhancing nutrients uptake, and in turn, induce plant productivity (Adesemoye et al. 2008a, 2009). In a review, Adesemoye and Kloepper (2009) compiled the benefits derivable from plant–PGPR interactions to include the following: improvements in seed germination rate, root development, shoot and root weights, yield, leaf area, chlorophyll content, hydraulic activity, protein content, and nutrient uptake—including phosphorus and nitrogen.

The use of beneficial microbes in agricultural production systems started long time ago, and there is increasing evidence that beneficial microbes can enhance plants' tolerance to adverse environmental stresses, which include salt stress (Egamberdieva 2008), drought stress (Zahir et al. 2008), weed infestation (Babalola 2010), nutrient deficiency, and heavy metal contaminations (Sheng 2005). The term "induced systemic tolerance" has been used to describe the capacity of PGPR to elicit tolerance to salt and drought (Yang et al. 2009). A range of salt-tolerant rhizobacteria identified so far has shown beneficial interactions with plants in

A.O. Adesemoye (✉)
Department of Microbiology, Adekunle Ajasin University, P.M.B 001, Akungba-Akoko, Ondo State, Nigeria
e-mail: semoyet@yahoo.co.uk

D. Egamberdieva
Department of Biotechnology and Microbiology, Faculty of Biology and Soil Sciences, National University of Uzbekistan, Vuzgorodok, 100174 Tashkent, Uzbekistan
e-mail: egamberdieva@yahoo.com

D.K. Maheshwari et al. (eds.), *Bacteria in Agrobiology: Crop Productivity*,
DOI 10.1007/978-3-642-37241-4_2,

stressed environments. These PGPR (e.g., *Rhizobium*, *Azospirillum*, *Pseudomonas*, *Flavobacterium*, *Arthrobacter* and *Bacillus*) utilize osmoregulation; oligotrophic, endogenous metabolism; resistance to starvation; and efficient metabolic processes to adapt under dry and saline environments (Lugtenberg et al. 2001; Egamberdiyeva and Islam 2008). The bacteria, with their physiological adaptation and genetic potential for increased tolerance to drought, increasing salt concentration, and high temperatures, could improve plant production in degraded sites (Maheshwari et al. 2012; Yang et al. 2009).

Many mechanisms have been reported for the activities of PGPR (Glick et al. 2007). Some strains produce metabolites such as hydrogen cyanide (HCN), 2, 4-diacetylphloroglucinol (DAPG) (Duffy et al. 2004); antibiotics, e.g., phenazine antibiotics (Chakraborty et al. 2009); and volatile compounds that stimulate plant growth (Ryu et al. 2003). Other strains produce siderophores and play roles in sequestering iron for plants, help in delayed senescence, biological control (Buyer et al. 1993; Kloepper et al. 1991), and produce plant hormones such as gibberellins, cytokinins, abscisic acid, and auxins, which at low concentrations influence plant physiological processes such as host's root respiration rate, metabolism, and root abundance.

Specifically, gibberellins influence seed germination, stem elongation and development, flowering, and fruit setting of plants, and auxins, especially indole acetic acid (IAA) and indole acetamide (IAM), influence root development, tissue differentiation, and responses to light and gravity. Lowering of ethylene (Saleem et al. 2007) levels in plants through the synthesis of the enzyme 1-amino-cyclopropane-1-carboxylate (ACC) deaminase that hydrolyzes the ethylene precursor ACC is another well-reported mechanism for growth promotion by PGPR (Glick et al. 2007; Shaharoona et al. 2007). The role of ACC deaminase-producing PGPR was reviewed extensively by Saraf et al. (2010).

Evidently, PGPR holds enormous prospects in improved and sustainable plant production, including enhanced plant tolerance to stress, better plant nutrient uptake and reduced use of chemical inputs. The roles of PGPR in nutrient uptake and stress management are emerging areas in agriculture that is not yet well understood; consequently, the benefits are yet to be maximized anywhere in the world. It is even less explored in many developing economies and may seem entirely new in some regions. Efforts to better understand the role of inoculants and biofertilizers in nutrient uptake and plant response to environmental stress are more compelling now that the continuous use of high amounts of chemical inputs are generating environmental problems and not sustainable.

The concept of integrated nutrient management (INM) system as proposed by Adesemoye and Kloepper (2009) relating to the use of biofertilizers in combination with chemical fertilizers to stimulate uptake of nutrients remains very important. The benefits of INM to different cropping systems have been further discussed by other authors (Joshi et al. 2006; Kumar et al. 2009a, b; Maheshwari et al. 2011). Maximizing the impacts of beneficial microbes towards enhancing the response of plants to environmental stress (Egamberdieva 2011; Glick et al. 2007) is also very important. This chapter discusses the benefits of PGPR in broad terms, but attempts

were made to present specifics about the use of PGPR to enhance plant nutrient uptake, for better plant response to environmental stress, and unexplored potentials in developing economies.

2.2 Major Crop Production Problems in Developing Regions

The Food and Agriculture Organisation (FAO) report titled "World agriculture: towards 2015/30" among others, discussed global long-term prospects for trade and sustainable development. One of the conclusions in the report was that the development of local food production in low-income countries, which depend highly on agriculture for employment and income, is the one factor that dominates all others in determining progress or failure in improving their food security. The report predicted that without the development of local food production and other related efforts, the target of halving the number of undernourished persons by no later than 2015 is far from being reached and may not be accomplished by 2030.

Socioeconomic, political, cultural, environmental factors, low technological development, bad agricultural methods and policies are major hindrances against agricultural development in many developing economies. There may be limited biological activity in response to environmental stresses such as salt and drought in certain areas resulting in low soil nutrients. In some regions, vast areas of land are highly weathered, very low in macro- and/or micronutrients or limited in arable land resources. Low level of soil fertility is a major hindrance against agriculture in some parts of Africa, South America, and many other regions, which makes productivity very low especially in locations with little or no use of fertilizers. There is continuous need for nitrogen and phosphorus, which are limiting nutrients (Graham and Vance 2000).

In arid regions of low rainfall and high evaporative demand, the causes of soil salinity are (1) cultivation of naturally saline lands, (2) rise in secondary salinity because of inflow of saline groundwater from higher plateau, and (3) increase in soluble salts concentration of water used for irrigation because of the recycling of drainage water for irrigation (Shirokova et al. 2000). Soil salinization is reducing the area that can be used for agriculture by 1–2 % every year (FAO 2002). Salinity causes a disturbance of plant–microbe interaction which is a critical ecological factor to help further plant growth in degraded ecosystems (Requena et al. 2001; Egamberdiyeva et al. 2007). As a result of soil salinization, plants are under saline or water unbalance stress and become more vulnerable to diseases, often caused by pathogenic fungi which can hardly be overcome by conventional methods of pest management (Kurth et al. 1986; Werner and Finkelstein 1995). Gratuitous use of fungicides and type of irrigation creates a strong concern regarding environmental pollution and development of fungicide resistance (Alva et al. 2000).

The benefits of resident soil microbes are hardly explored, and when commercial inoculants are used, they are usually not derived from microbes isolated locally and so may not be effective. Overall, the result is dismal agricultural productivity.

These underscore the urgent need to develop management practices and biotechnological applications that can improve soil productivity, environmental health, reduce erosion, and enhance food security. In fact, attempts to meet food needs in some regions have led to the adoption of agricultural practices capable of degrading the soil, such as high use of chemical inputs, e.g., fertilizers. Low efficiency in the uptake of fertilizer as identified by Adesemoye and Kloepper (2009) is prompting the use of high amounts of fertilizer. Consequent upon ineffective soil management is many environmental maladies, two of which Hungria and Vargas (2000) identified as nutrient depletion and soil acidification. Therefore, improvement in plant nutrient uptake is a requirement for overall reduction in fertilizer use and sustainable crop productivity.

2.3 Reported Use and Prospects of Microbes and PGPR in the African Region

Akanbi et al. (2007) compared the application of manure extract from cassava (*Manihot esculenta*) peel and Mexican sunflower (*Tithonia rotundifolia*) composts as foliar spray or liquid fertilizer with NPK in Nigeria. The authors also tested the extracts as pesticide and reported that the growth of fluted pumpkin (*Telfairia occidentalis*) plants with foliar spray of compost extracts from cassava peel and Mexican sunflower was significantly the same with those that received NPK fertilizer. Depending on the ratio of extract used, there was certain level of protection against five insect pests tested, which included leaf beetle (*Lagria villous* T.), red pumpkin beetle (*Aulacophora* spp.), cotton leaf roller (*Sylepta derogate* F.), cut worms (*Noctuidae* spp.), and green grasshopper (*Zonocerus variegatus*) (Akanbi et al. 2007).

Babalola and coworkers conducted pot experiments in Nigeria and Kenya to determine the growth effect of three different rhizobacteria (*Pseudomonas* sp. 4MKS8, *Klebsiella oxytoca* 10MKR7, and *Enterobacter sakazakii* 8MR5) on maize under *Striga hermonthica* infestation. The three bacteria were selected based on their plant growth-promoting effects (Babalola et al. 2007). Some of the treatments showed statistically significant plant growth promotion and increased agronomic characteristics of maize. The authors studied 1-amino-cyclopropane-1-carboxylic acid (ACC) deaminase gene in *Pseudomonas* sp. 4MKS8 and *Klebsiella oxytoca* 10MKR7, and *Enterobacter sakazakii* 8MR5 and found that not all plant growth-promoting rhizobacteria contain the enzyme ACC deaminase.

Ugoji et al. (2006) examined the impacts of seed coating with *Bacillus* sp. on the storage of seeds of maize (*Zea mays* L.), bean (*Phaseolus vulgaris* L.), lettuce (*Lactuca sativa* L.), and cucumber (*Cucumis sativus* L.) over a 12-month period in South Africa. One important finding was that microbial populations decreased from

month 7 to month 12 which, according to the authors, indicated protection of the seed by the applied *Bacillus* sp. against growth of pathogens and saprophytes.

In a study conducted in Nigeria, Adesemoye and Ugoji (2006) examined the effectiveness of plant growth-promotion ability of *Pseudomonas* sp. in three test crops—okra (*Abelmoschus esculentus* L.), tomato (*Lycopersicon esculentum* L.), and African spinach (*Amaranthus* sp.). The aim of the study was to determine whether inoculation method had impacts on PGPR's effectiveness. They found that tested *Pseudomonas* isolates promoted crop growth and had great potentials as PGPR in the region. The test on two methods of bacterial inoculation (soaking and coating) produced statistically similar results of plant growth enhancement. Adesemoye et al. (2008a) compared PGPR properties between *Bacillus subtilis* and *Pseudomonas aeruginosa* as representatives of their two genera. The authors reported similarities but no significant difference at $p < 0.05$ between the overall performances of *B. subtilis* and *P. aeruginosa*. It was suggested that *Bacillus* may be relatively more versatile than *Pseudomonas* as PGPR because of the ability to form endospores, which can make them retain viability for long periods either in storage or in the soil.

Jida and Assefa (2011) reported that Ethiopian soils harbor highly efficient nitrogen-fixing lentil-nodulating rhizobia. They collected 30 isolates of such rhizobia from farmers' field soils in central and northern parts of Ethiopia and selected for symbiotically efficient ones, which possess plant growth-promoting characteristics. Under glasshouse conditions, they found characteristics such as IAA production in 36.7 % and inorganic phosphate solubilization capacity in 16.7 %. Additionally, one or a combination of carbon sources and nitrogen sources utilization, tolerance to acidic or alkaline pH, metal toxicity, and antibiotics production were found in most isolates (Jida and Assefa 2011).

One study in Egypt examined tripartite interactions among bacteria (*Azospirillum brasilense*), mycorrhiza (*Glomus clarum*), and legume (*Vicia faba*) under five saline (NaCl) levels in pot cultures (Rabie and Almadini 2005). Significant effects of inoculation were reported in the plants for salinity tolerance, mycorrhizal dependency, phosphorus level, phosphatase enzymes, nodule number, nitrogen uptake, protein content, and nitrogenase enzymes. Based on the findings, the authors suggested that bacterial–AMF–legume tripartite symbioses could be a new approach to increasing the salinity tolerance of legume plants.

Galal et al. (2001) demonstrated the beneficial influence of co-inoculation of *Azospirillum lipoferum* and *Bacillus megaterium* for providing balanced nitrogen and phosphorus nutrition of wheat plants in Egypt. El-Azouni (2008) observed significant increase of dry matter, N, P uptake and yield of soybean grown in Egyptian soil inoculated with phosphate-solubilizing fungi *A. niger* and *P. italicum*. *Rhizobium leguminosarum* bv. *trifolii* was reported to colonize rice roots endophytically in the fields where rice is grown in rotation with Egyptian berseem clover (*Trifolium alexandrinum*) and can supplement 25–33 % of the recommended rate of N fertilizer for rice (Yanni et al. 1997). All these studies are evidences that PGPR have high potentials in Africa.

2.4 Reported Use and Prospects of Microbes and PGPR in the Asian Region, Including Asia Pacific and Middle East

The reduction of chemical fertilizers by using biological fertilizers based on bacteria involved in nitrogen fixation is one of the effective steps in sustainable agriculture. Owing to population growth and increasing food demand, intensive and environment-friendly agriculture such as biofertilizers and biopesticides have become the ideal model for the Asian region. According to the reports of Jee (2009), a total of 138 companies were producing hundreds of commercial products, and 23 biopesticides are now registered in Korea, and they are based on strains such as *Paenibacillus polymyxa*, *Bacillus subtilis*, *B. amyloliquefaciens*, *Paecilomyces fumosoroseus*, and *Streptomyces goshikiensis*. Quyet-Tien et al. (2010) reported regarding *P. polymyxa* KNUC265 strain, which increased plant growth of pepper and elicited both induced systemic resistance (ISR) and plant growth promotion, suggesting that it could be potentially used in improving the yield of pepper and other crops.

Meunchang et al. (2006) selected effective PGPR strains which increased plant growth and nutrient uptake of rice and indicated the possibility of producing biofertilizer for rice production in Thailand. In another work, Young et al. (2003) studied the effect of a combined treatment of multifunctional biofertilizer (mixture of *Bacillus* sp. *B. subtilis*, *B. erythropolis*, *B. pumilus*, and *P. rubiacearum*) on the growth of lettuce in Taiwan and found 25 % increase of lettuce yield over the control. In Mongolia, it was observed that *Bacillus pumilus* 8N-4 can be used as a bio-inoculant for biofertilizer production to increase the crop yield of wheat variety *Orkhon* (Hafeez et al. 2006).

Rice (*Oryza sativa*) could be described as the major food crop across the world's population, especially in Asian populations, and as noted by Kumar et al. (2011), more than 90 % of rice is produced in Asia. Rice plants require large amounts of N for their growth, development, and grain production (Sahrawat 2000). In Vietnam the application of BioGro based on various PGPR strains resulted in increase in rice growth and yield (Nguyen et al. 2003; Nguyen 2008). Mia and coworkers (2009) observed that *Rhizobium* inoculation significantly initiated more root hairs in rice seedlings. The authors also studied the effects of rhizobacterial inoculation on growth and nutrient accumulation of tissue-cultured banana plantlets under low N-fertilizer regime in Malaysia, and they found an increase in growth and yield of plant after inoculation (Mia et al. 2007). Many diseases that attack rice generate global concerns due to the popularity of the crop. However, PGPR could play very important roles in managing the diseases. For instance, PGPR has been reported exhibiting high potentials in the management of sheath blight of rice caused by *Rhizoctonia solani* AG 1-1A, particularly through combined application of PGPR with chemical fungicides in integrated disease management (IDM) systems (Kumar et al. 2011).

With the developed commercial PGPR (Ecomonas) in India, the rice sheath blight caused by *Rhizoctonia solani* reduction over the control was 37.7 % and grain

yields significantly increased (3,901 and 1,938 kg/ha) over control (2,690 and 1,550 kg/ha) (Kumar et al. 2009a). Also in India, inoculation with vesicular arbuscular mycorrhizal fungi (*Glomus mosseae*, *G. fasciculatum*, *Acaulospora laevis*, and *Gigaspora gilmorei*) resulted in increased plant height, dry weight, number of pods, and nutrient content of chickpea (Kumar et al. 2009a).

Beneficial characteristic of PGPR has been reported in Malaysia on potato (Yasmin et al. 2009). The authors screened 15 PGPR strains for indole acetic acid (IAA) production (with and without addition of the precursor L-tryptophan [L-TRP]), phosphate-solubilizing activity, nitrogen synthesis, antagonistic activity against fungal pathogens, siderophore production, and intrinsic antibiotic resistance. All isolates produced IAA and grew in N-free media, which the authors suggested was an indication of N "production."

In Indonesia, Supanjani et al. (2006) conducted experiments to evaluate whether applications of lipochitooligosaccharides (LCOs) and inoculation with rhizobia could improve the uptake of calcium into soybean (*Glycine max* [L.] Merr.) leaves by inoculating with rhizobia or application of Nod factors LCOs. Two strains of *Bradyrhizobium japonicum* reportedly increased the uptake of labeled Ca, while a *nod*C-mutant incapable of producing LCO did not. Also, rhizobia that do not normally nodulate soybean (*Rhizobium leguminosarum* and *Sinorhizobium meliloti*) did not affect calcium uptake, nor did the tetramer or pentamer of chitosan or lumichrome. However, *Rhizobium* sp. NGR234, which can nodulate certain soybean without effective N_2 fixation, did not affect calcium uptake. Based on the findings, Supanjani et al. (2006) suggested that the rhizobial symbiosis can improve early calcium uptake into soybean plants, in addition to nitrogen fixation.

The availability of K and P in arid saline soils of China is limited. In such soils having bacterial strains that are able to solubilize "unavailable" forms of K- and P-bearing minerals to bring the K and P into solution is an important approach (Ullmann et al. 1996). Sheng (2005) observed that *Bacillus edaphicus* NBT strain increased K content of cotton and rape plants by 30 % when the soil was treated with insoluble K sources. In other field experiments in China, the plant biomass, nutrient uptake, and yield of wheat were increased by phosphorus-solubilizing bacteria (PSB) *Bacillus strains* (Chen et al. 2006).

In Russian region, there are several commercially available biofertilizers and plant protectors against plant diseases caused by *Fusarium graminearum*, *F. culmorum*, and *F. avenaceum*. Effectiveness of biofertilizers based on strains *Azotobacter chroococcum, Bacillus mucilaginosus*, and *Pseudomonas fluorescence* P 469 has been tested in field trials with winter and spring wheat, spring barley, potato, and sugar beet in different soils in Central Russia (Zhigletsova et al. 2010; Kutyova et al. 2002). In early studies, Belimov et al. (1995) reported positive effect of mixed cultures of nitrogen fixers *Azospirillum lipoferum*, *Arthrobacter mysorens*, and *Agrobacterium radiobacter* on grain yield and N uptake of barley in Russia.

Hasnain and Sabri (1996) showed that inoculation of wheat with *Pseudomonas* spp. stimulated plant growth by reducing plant uptake of toxic ions and increasing the auxin content of wheat grown in Pakistan. Similar results were observed by Afzal et al. (2005) where combined inoculation of nitrogen-fixing bacteria

(*Rhizobium leguminosarum*) with PSB *Pseudomonas* sp. strain 54RB increased dry matter and yield of wheat. In 2008, Kang and coworkers showed the capacity of *Aspergillus* spp. PS 104 to solubilize rock phosphate in soil-amended medium (Kang et al. 2008). Shaharoona et al. (2007) tested several *Pseudomonas* spp. strains in the field to determine their efficacy to increase growth and yield of wheat. Nosheen et al. (2011) reported that PGPR inoculation of *A. brasilense* and *P. stutzeri* either alone or in combination with half dose of chemical fertilizers was highly effective in improving root morphology and growth in safflower.

Naveed et al. (2008) reported that application of organic fertilizer and *Pseudomonas* strains significantly improved the growth (up to 39 %) and yield of maize. They found that *P. fluorescens* significantly increased plant height (16 %), the number of grains per spike (11.7 %), and grain yield (39 %) compared to non-inoculated control. Hafeez et al. (2006) showed that biofertilizer (BioPower) gave 50–70 % savings in nitrogen fertilizer and 20 % increase in rice in Pakistan. The bacterial-based fertilizer increased the yield of wheat and maize and protected plants from fungal disease. It was reported that the PSB-plant inoculations resulted in 10–15 % increases in crop yields and P uptake in 10 out of 37 experiments in India. In another study, Tomar et al. (1996) reported the efficiency of a PSB (*Pseudomonas* sp.) on the growth and yield of gram (*Cicer arietinum*).

Similar results were observed where combined inoculation of *Rhizobium* and PSB (*Pseudomonas striata* and *Bacillus polymyxa*) led to increase in nodulation, growth, and yield of chickpea under greenhouse conditions. This was associated with increase in nitrogenase activity in nodules and phosphorous content in plants (Khurana and Sharma 2000). In other works, Verma et al. (2010) observed that chickpea inoculated with *Rhizobium leguminosarum* subsp. *ciceri* annually produced up to 176 kg N/ha as a result of significant stimulation of plant growth. Hameeda et al. (2006) reported that two P-solubilizing bacteria (*Serratia marcescens* EB-67 and *Pseudomonas* spp. CDB-35) increased the biomass of maize by 99 % and 96 %, respectively, under greenhouse conditions.

Egamberdiyeva et al. (2002) reported on the effect of a *Pseudomonas fluorescens* PsIA12 and *Pantoea agglomerans* on the growth of maize in the field, and bacterial strains were found to significantly increase root development, shoot growth, and K uptake of maize. The application of *Bradyrhizobium japonicum* enhanced the number of nodules, dry weight of plant, grain yield, and protein content in soybean grown in salinated soils of Uzbekistan (Egamberdiyeva et al. 2004). Seed inoculation of common bean (*Phaseolus vulgaris*) by *Pseudomonas chlororaphis* TSAU13 and *P. extremorientalis* TSAU20 resulted in improved root and shoot biomass in nutrient-deficient soil of Uzbekistan (Egamberdieva 2011).

Priming of seedlings with selected PGPR strains reduced *Fusarium* root rot of cucumber to as low as 10 % and showed a significant stimulatory effect on plant growth, increasing the dry weight of whole cucumber plants up to 62 % and fruit yield up to 32 % in comparison to the nonbacterized control (Egamberdieva et al. 2010). The inoculation of cotton seeds with salt-tolerant phosphate-solubilizing bacteria *Rhizobium meliloti* URM1 combined with phosphate had a significant

Table 2.1 Effects of PGPR strains on tomato cv. *Belle*) shoot length and fruit yield in salinated soil

Treatments	Plant height (cm)	%	Fruit yield (kg/m^2)	%
None	118.2 ± 3.9	100	13.9 ± 1.5	100
P. putida TSAU1	154.4 ± 4.9*	130	16.4 ± 1.6*	117
P. chlororaphis TSAU13	149.8 ± 7.1*	126	15.6 ± 1.2*	112
P. extremorientalis TSAU20	152.5 ± 7.5*	128	17.0 ± 1.2*	122

The temperature range was day 28–32 °C and night 16–18 °C
*Significantly different from the control at $P < 0.05$

Table 2.2 Effects of biological control agents on wheat growth and yield in salinated soil

Treatments	Grain yield (g/plant)	%	Biological yield (g/plant)	%
Control	19.8	100	62.2	100
TSAU20	24.0*	121	78.7*	126
TSAU1	22.4*	113	80.1*	128

Bacterial strains were *P. extremorientalis* TSAU20 and *P. putida* TSAU1
*Significantly different from the control at $P < 0.05$

stimulatory effect on total dry matter, shoot and root dry weight, yield, and P content (Egamberdiyeva et al. 2004).

The data below were obtained in recent experiments with tomato grown in salinated soil and inoculated with *P. putida* TSAU1, *P. chlororaphis* TSAU13, and *P. extremorientalis* TSAU20. The inoculants increased the growth and yield of tomato (Table 2.1).

The plant height were stimulated from 26 to 28 % after inoculation of tomato seeds with bacterial strains *P. putida* TSAU1, *P. putida* TSAU13, and *P. extremorientalis* TSAU20 compared to those in the control treatment. The yield of tomato increased up to 22 % after bacterial treatment. In wheat, traits such as grain yield and biological yield were also significantly increased by inoculation with PGPR *P. extremorientalis* TSAU20 and *P. putida* TSAU1 (Table 2.2).

As evidenced in the table, the grain yield increased after inoculation with *P. extremorientalis* TSAU20 and *P. putida* TSAU1 up to 21 % compared to non-inoculated control plants. The inoculation also increased biological yield by 28 % compared to control plants.

In Iran, *Azotobacter* in combination with PSB had been shown to increase the plant height, dry weight, and yield of maize up to 30 % over the control (Gholami et al. 2009). In another study, Khorshidi et al. (2011) showed that application of fertilizers with *Pseudomonas fluorescens* and *Azospirillum lipoferum* had a significant effect on rice yield in Iran. Rokhzadi et al. (2008) reported that combined inoculation of *Azotobacter*, *Azospirillum*, *Pseudomonas*, and *Mesorhizobium* resulted in promotion of grain yield and biomass in chickpea in Iran. In Turkey, seed inoculation of barley with N_2-fixing bacteria *P. polymyxa* RC05, *P. putida* RC06, and *R. capsulatus* RC04 increased root and shoot weight by 54 % and N uptake. *Pseudomonas* strains also increased the yield of sugar beet (Çakmakçi et al. 2001). Evidently, PGPR and biofertilizers have great potentials in agricultural productions in Asia, at least in the specific regions of isolation.

Table 2.3 Control of cotton root rot by antagonistic bacteria in two different soils

	Diseased plants	
Treatments[a]	Cambisol	Sierozem
Control, *F. oxysporum*	69 ± 5.8	76 ± 9.8
P. alcaligenes PsA15	43 ± 11.2	26 ± 10.2*
B. amyloliquefaciens BcA12	50 ± 8.2	31 ± 9.1*
B. polymyxa BcP26	48 ± 6.8	37 ± 7.2
M. phlei MbP18	39 ± 9.1*	30 ± 6.9*

*Significantly different from the negative control at $P < 0.05$

[a]Bacteria were coated on pre-germinated cotton seeds, and plants were grown under open natural conditions in pots infested with *F. oxysporum* spores (3.0×10^7 spores/kg)

2.5 Biological and Edaphic Factors That May Affect PGPR Effectiveness in Different Regions

Many countries in the world have been using bacterial fertilizers in agriculture (Dashti et al. 1997), and it is envisaged that the usage will increase but also expand to other regions. This optimism is predicated on the fact that the apathy against PGPR and biofertilizers which arose mainly from the reported variability in performance on the field is beginning to fade out. This makes it important to discuss possible factors/conditions that can affect the performance and effectiveness of PGPR and how the issues can be handled. Some of the important factors perceived to be hindering wide acceptance and use of PGPR are variability in colonization efficiency, rhizosphere competence, and field performance. Arguably, the most important factor that affects PGPR performance is colonization of the host. For instance, a strain with biological control potentials in vitro may be unable to exhibit the trait in the field if it is incapable of successful colonization of the host.

It has been discussed earlier in this chapter that plant growth stimulation and biological control of plant diseases by rhizobacteria involve one or more mechanisms which include production of phytohormones, antibiosis, parasitism, competition for nutrients and niches, and induced host resistance (Lugtenberg and Kamilova 2004; Adesemoye et al. 2009). Notably, abiotic and biotic factors may influence the different mechanisms and limit the interactions between plant and beneficial bacteria, resulting in less than acceptable performance in plant growth promotion and management of diseases (Egamberdiyeva and Hoflich 2002, 2003).

The data below exemplifies how abiotic factor (soil type) can affect the activities of PGPR. The biological control of cotton root rot caused by *F. oxysporum* using different antagonistic bacteria species showed that soil types have effects on bacterial abilities to control this root pathogen of cotton (Table 2.3).

Infestation of the soil with *F. oxysporum* resulted in an increase of the percentage of diseased plants from 69 to 76 in two different soils. Priming of seedlings with the five selected bacterial strains *P. alcaligenes* PsA15, *B. amyloliquefaciens* BcA12, *B. polymyxa* BcP26 and *M. phlei* MbP18 reduced this proportion to as low as 26 % in sierozem soil but 39 % in cambisol soil in comparison to the

non-inoculated control. Overall, the bacterial strains were more effective in sierozem soil than in cambisol soil. It is probable that the physiological adaptation of bacterial strains supported their beneficial activity much better in soil from where they were isolated.

Also, the availability level of macro- and micronutrients in soil has high effects on the performance of PGPR. According to Choudhury and Kennedy (2004), the efficiency of plant-associated N_2 fixation by diazotrophic bacteria may be hampered by a limited supply of energy and substrate. Other factors that could affect inocula success include temperature, soil type, N content, salt concentration, and moisture content. Numerous studies have shown that soil salinity decreases nodulation and dramatically reduces N_2 fixation and nitrogenase activity of nodulated legumes, as reviewed by Zahran (1999). It has been demonstrated that the performance of PGPR after inoculation into the rhizosphere is affected significantly by competition for nutrient and niches with indigenous microflora (Kamilova et al. 2006; Strigul and Kravchenko 2006).

Rashid et al. (1997) reported that response of wheat to bacterial inoculation was variable in different ecological zones of Punjab, Pakistan, ranging from 10 to 35 % increase in yield over control. The inconsistency in results might be due to many factors such as the complex interactions among hosts, rhizobacteria, pathogens, climate, and soil environment. Crop cultivars is another important factor as demonstrated in a study where inoculation of wheat with *Pseudomonas* strains improved plant growth in salinated soil of Uzbekistan at a rate that varied depending upon the wheat cultivars used (Egamberdieva 2010). It is recommended that selection for cultivars should consider bacterial inoculants so that the selected cultivar is the one that carries the trait of successful association with such bacteria. Understanding the mechanisms of growth stimulation and plant disease control by rhizobacteria and impact of abiotic factors on their interactions and beneficial effects are useful in the application of PGPR in countries with varied climatic conditions, enabling a prediction of the success of a PGPR inoculation with the specific variety of crops to be cultivated.

2.6 Unexplored Possibilities of PGPR in Developing Economies: Biofertilization and Biocontrol

It was suggested by Adesemoye and Kloepper (2009) that PGPR as biofertilizers or microbial inoculants can be important components of an integrated nutrient management system. However, the interactions among PGPR and plants are still not well understood, especially in field applications and different environments (Niranjan et al. 2005). Therefore, there is need for more studies on plant–microbe interactions and their activities in different regions and ecologies, including stressed environments, for instance, in arid and tropical regions. Availability of more information will enable the development and widespread acceptance of new

agricultural technologies, which can improve soil ecology, plant development, and resistance against diseases and pests. Akanbi et al. (2007) believed that if compost were available in nutrient-rich liquid formulations that involve the use of less quantity, and easier application, it will be more popular among farmers in Nigeria.

Cereals are major crops in many developing economies, and it has been shown by Kennedy and Tchan (1992) that biofertilizers can enhance growth, disease control and yield of cereals, but this is yet to be well explored in many parts of the developing regions of the world. Frequent rhizosphere colonizers of cereal crops and grasses include N-fixing bacteria such as *Azospirillum*, *Acetobacter*, *Azoarcus*, *Herbaspirillum* spp., and *Aeromonas* (Dobbelaere et al. 2001; Mehnaz et al. 2001). Bacteria reportedly have greater adaptability to rice ecosystems compared to fungal antagonists, and PGPR have been used vigorously in controlling rice diseases (Kumar et al. 2011). Possible benefits of PGPR on rice include biological control of diseases (especially through induced systemic resistance); better nutrient uptake—nitrogen, phosphorus, and ferric iron; enhanced seedling growth; increased yield; and sustainable use of agricultural products.

Salinity being one of the major problems in many developing countries in Asia; the use of salt-tolerant bacterial inoculants is a possible solution that can increase plant growth, induce seed germination, improve seedling emergence, and protect plants from the deleterious effects of some environmental stresses. Velagaleti and Marsh (1989) showed that the development of salt-tolerant symbioses is an absolute necessity to enable cultivation of leguminous crops in salt-affected soils. Egamberdieva and Kucharova (2009) suggested that screening and application of the enhanced potential root-colonising rhizobacteria is essential for developing sound strategies to manage the rhizosphere in such a way that it becomes more difficult for pathogens to colonise the rhizosphere; thus, these beneficial bacteria can engineer positive interactions in the rhizosphere and stimulate plant growth under saline conditions. In some locations, soils are poorly aerated and waterlogged, or well aerated but calcareous. The impact of microbial activity in the rhizosphere on roots directly on mobilization and/or immobilization or indirect effect on root morphology and/or physiology (Babalola 2010) can be utilized to manipulate nutrients uptake.

Pathogens, especially soil-borne, cause inestimable crop losses in many developing regions with more noticeable consequences in Africa. Soil suppressiveness of plant diseases (Weller and Thomshow 1993) is an important consideration that should be continuously studied for possibility of identifying and exploiting the benefits from the specific resident organisms involved. Additionally, the manipulation of the plant–microbe interactions to control quorum sensing (QS) systems in microbes to the benefit of crop production is another focus area with possible benefits awaiting exploitation. Quorum sensing (QS) in which acyl homoserine lactones are utilized is important in many plant–microbe interactions, as in *Pseudomonas aureofaciens* (Babalola 2010; Boyer et al. 2008).

Root exudates, a fraction of rhizodeposition, are rich in carbon and energy sources that affect microbial growth and development in the rhizosphere. Other fractions of rhizodeposition—lysates, mucilage, secretions, and dead cell

materials—may play some roles (Dardanelli et al. 2010; Sommers et al. 2004). These dynamics especially interactions of root exudates and PGPR activity which lead to better root growth have been previously explained by Adesemoye et al. (2009). However, more study and better understanding of the dynamics may help in better use of PGPR in crop production in developing regions, and the knowledge may have universal applications across all regions of the world—developing and developed alike.

2.7 Conclusion

One of the immediate reasoning to improve agricultural productivity and development is the use of more chemical fertilizers. However, with the resultant effects of heavy fertilizer use in many regions of the world, it is compelling to look for alternatives. Based on current events, the argument of Adesemoye et al. (2009) that the goal of reducing fertilizer usage will be to this century as what the goal of reducing pesticides was to the last century remains valid. Therefore, the integrated nutrient management (INM) system proposed in that paper, i.e., integration of microbial inoculants with less fertilizer, should be considered in many situations as it promises high crop productivity and agricultural sustainability.

The use of fungicides, bactericides, and pesticides generally continue to generate concerns, so biological control is still as relevant as it was many decades ago. The reason for the inconsistencies reported in some regions with biological control of diseases is not yet well understood though its relevance as a major limitation to widespread acceptance of biofertilizers and commercial PGPR products has been reducing as compared to almost two decades ago when an observation of inconsistencies was made by Weller and Thomshow (1993).

The complex nature of the natural soil environment is a possible explanation for the variation in effectiveness of PGPR strains or products, particularly when such products were used far away from where the microbial inoculants were originally isolated. This implies that there is high chance that commercial PGPR products made from isolates collected in a region may perform better in that region than if it was from strains collected in another region continent or country. Research should focus on identifying effective PGPR strains in each region.

In agreement with Dardanelli et al. (2010), the presence of microorganisms in the soil is critical to the maintenance of soil function, in both natural and managed agricultural soils. The microbes are involved in key processes such as soil structure formation, decomposition of organic matter, toxin removal, and the cycling of elements—carbon, nitrogen, phosphorus, potassium, and sulfur. It is also clear that beneficial microorganisms play key roles in suppressing soil-borne plant diseases and in promoting plant growth and changing the vegetation (Doran et al. 1996).

Efforts should be directed towards maximizing the identified benefits of PGPR or biofertilizers in all developing economies. If the benefits of PGPR in crop

production can be maximized, this will certainly help in the fight against hunger. Importantly, regions in developing economies may have to use more of products that are based on local isolates because as emphasized by Adesemoye et al. (2009), no microbial inoculant can be universal for all ecosystems. Rather, biofertilizers' performances may be specific as effectiveness is dependent upon factors like plant type, soil type, and many other factors.

References

Adesemoye AO, Kloepper JW (2009) Plant-microbes interactions in enhanced fertilizer use efficiency. Appl Microbiol Biotechnol 85:1–12

Adesemoye AO, Ugoji EO (2006) Evaluating *Pseudomonas aeruginosa* as plant growth-promoting rhizobacteria (PGPR) in West Africa. Arch Phytopathol Plant Protect 42(2):188–200

Adesemoye AO, Obini M, Ugoji EO (2008a) Comparison of plant growth-promotion with *Pseudomonas aeruginosa* and *Bacillus subtilis* in three vegetables. Braz J Microbiol 39:423–426

Adesemoye AO, Torbert HA, Kloepper JW (2008b) Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. Can J Microbiol 54:876–886

Adesemoye AO, Torbert HA, Kloepper JW (2009) Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58:921–929

Afzal A, Ashraf M, Asad SA, Farooq M (2005) Effect of phosphate solubilizing microorganisms on phosphorus uptake, yield and yield traits of wheat (*Triticum aestivum* L.) in rainfed area. Int J Agric Biol 7:207–209

Akanbi WB, Adebayo TA, Togun OA, Adeyeye AS, Olaniran OA (2007) The use of compost extract as foliar spray nutrient source and botanical insecticide in *Telfairia occidentalis*. World J Agric Sci 3:642–652

Alva AK, Huang B, Paramasivam S (2000) Soil pH affects copper fractionation and phytotoxicity. Soil Sci Soc Am J 64:955–962

Babalola OO (2010) Beneficial bacteria of agricultural importance. Biotechnol Lett 32:1559–1570

Babalola OO, Berner DK, Amusa NA (2007) Evaluation of some bacterial isolates as germination stimulants of *Striga hermonthica*. Afr J Agric Res 2:27–30

Belimov AA, Kojemiakov PA, Chuvarliyeva CV (1995) Interaction between barley and mixed cultures of nitrogen fixing and phosphate-solubilizing bacteria. Plant Soil 17:29–37

Boyer M, Bally R, Perrotto S, Chaintreuil C, Wisniewski-Dye F (2008) A quorum-quenching approach to identify quorum-sensing-regulated functions in *Azospirillum lipoferum*. Res Microbiol 159(9–10):699–708

Buyer JS, Kratzke MG, Sikora LJ (1993) A method for detection of pseudobactin, the siderophore produced by a plant-growth promoting *Pseudomonas* strain, in the barley rhizosphere. Appl Environ Microbiol 59:677–681

Çakmakçi R, Kantar F, Sahin F (2001) Effect of N2-fixing bacterial inoculations on yield of sugar beet and barley. J Plant Nutr Soil Sci 164:527–531

Chakraborty U, Chakraborty BN, Basnet M, Chakraborty AP (2009) Evaluation of ochrobactrum anthropi TRS-2 and its talc based formulation for enhancement of growth of tea plants and management of brown root rot disease. J Appl Microbiol 107(2):625–634

Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl Soil Ecol 34:33–41

Choudhury ATMA, Kennedy IR (2004) Prospects and potentials for systems of biological nitrogen fixation in sustainable rice production. Biol Fertil Soils 39:219–227

Compant SW, Duffy B, Nowak J, Clement C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microb 71:4951–4959

Dardanelli MS, Carletti SM, Paulucci NS, Medeot DB, Rodriguez Caceres EA, Vita FA, Bueno M, Fumero MV, Garcia MB (2010) Benefits of plant growth-promoting rhizobacteria and rhizobia in agriculture. In: Maheshwari DK (ed) Plant growth and health promoting bacteria, vol 18, Microbiology monographs. Springer, Berlin, pp 1–20

Dashti N, Zhang F, Hynes RK, Smith DL (1997) Application of plant growth-promoting rhizobacteria to soybean (*Glycine max* L. Merr.) increases protein and dry matter yield under short season conditions. Plant Soil 188:33–41

Dobbelaere S, Croonenborghs A, Thys A, Ptacek D, Vanderleyden J, Dutto P, Labandera-Gonzalez C, Caballero-Mellado J, Anguirre JF, Kapulnik Y, Brener S, Burdman S, Kadouri D, Sarig S, Okon Y (2001) Response of agronomically important crops to inoculation with *Azospirillum*. Aust J Plant Physiol 28:871–879

Doran JW, Sarrantonio M, Liebig MA (1996) Soil health and sustainability. Adv Agron 56:2–54

Duffy B, Keel C, Defago G (2004) Potential role of pathogen signaling in multitrophic plant-microbe interactions involved in disease protection. Appl Environ Microbiol 70(3):1836–1842

Egamberdieva D (2008) Plant growth promoting properties of rhizobacteria isolated from wheat and pea grown in loamy sand soil. Turk J Biol 32(1):9–15

Egamberdieva D (2010) Growth response of wheat cultivars to bacterial inoculation in calcareous soil. Plant Soil Environ 56(12):570–573

Egamberdieva D (2011) Survival of *Pseudomonas extremorientalis* TSAU20 and *P. chlororaphis* TSAU13 in the rhizosphere of common bean (*Phaseolus vulgaris*) under saline conditions. Plant Soil Environ 57(3):122–127

Egamberdieva D, Kucharova Z (2009) Selection for root colonising bacteria stimulating wheat growth in saline soils. Biol Fertil Soil 45:561–573

Egamberdieva D, Kucharova Z, Davranov K, Berg G, Makarova N, Azarova T, Chebotar V, Tikhonovich I, Kamilova F, Validov S, Lugtenberg B (2010) Bacteria able to control foot and root rot and to promote growth of cucumber in salinated soils. Biol Fertil Soil 47:197–205

Egamberdiyeva D, Hoflich G (2002) Root colonization and growth promotion of winter wheat and pea by *Cellulomonas* spp. at different temperatures. J Plant Growth Regul 38:219–224

Egamberdiyeva D, Hoflich G (2003) Influence of growth promoting bacteria on the growth of wheat at different soils and temperatures. Soil Biol Biochem 35:973–978

Egamberdiyeva D, Islam KR (2008) Salt tolerant rhizobacteria: plant growth promoting traits and physiological characterization within ecologically stressed environment. In: Ahmad I, Pichtel J, Hayat S (eds) Plant-bacteria interactions: strategies and techniques to promote plant growth. Wiley-VCH, Weinheim, pp 257–281

Egamberdiyeva D, Juraeva D, Gafurova L, Hoflich G (2002) Promotion of plant growth of maize by plant growth promoting bacteria in different soils. In: Proceeding book of 25th annual conservation tillage conference for sustainable agriculture, Alabama, 24–26 June, pp 239–244

Egamberdiyeva D, Juraeva D, Poberejskaya S, Myachina O, Teryuhova P, Seydalieva L, Aliev A (2004) Improvement of wheat and cotton growth and nutrient uptake by phosphate solubilising bacteria. In: Proceeding of 26th annual conservation tillage conference for sustainable agriculture, Auburn, pp 58–65

Egamberdiyeva D, Gafurova L, Islam KR (2007) Salinity effects on irrigated soil chemical and biological properties in the Syr Darya basin of Uzbekistan. In: Lal R, Sulaimanov M, Stewart B, Hansen D, Doraiswamy P (eds) Climate change and terrestrial C sequestration in Central Asia. Taylor-Francis, New York, pp 147–162

El-Azouni IM (2008) Effect of phosphate solubilizing fungi on growth and nutrient uptake of soybean (Glycine max L.). J Appl Sci Res 4(6):592–598

Food and Agriculture Organisation (FAO) (2002) World agriculture: towards 2015/2030. Summary report. FAO Information Division, Rome, Italy. ISBN 92-5-104761-8

Galal YG, El-Gandaour JA, El-Akel FA (2001) Stimulation of wheat growth and N fixation through Azospirillum and Rhizobium inoculation. A field trial with 15N techniques. In: Horst WJ (ed) Plant nutrition—food security and sustainability of agroecosystems. Kluwer Academic, Netherlands, pp 666–667

Gholami A, Shahsavani S, Nezarat S (2009) The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. Int J Biol Life Sci 5:35–40

Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B (2007) Promotion of plant growth by bacterial ACC deaminase. Crit Rev Plant Sci 26:227–242

Graham PH, Vance CP (2000) Nitrogen fixation in perspective: on overview of research and extension needs. Field Crops Res 65:93–106

Hafeez FY, Yasmin S, Ariani D, Mehboob-ur-Rahman ZY, Malik KA (2006) Plant growth-promoting bacteria as biofertilizer. Agron Sust Develop 26:143–150

Hameeda B, Harini G, Rupela OP, Wani SP, Reddy G (2006) Growth promotion of maize by phosphate solubilizing bacteria isolated from composts and macrofauna. Microbiol Res 163 (2):234–242

Hasnain S, Sabri AN (1996) Growth stimulation of *Triticum aestivum* seedlings under Cr-stress by nonrhizospheric *Pseudomonas* strains. In: Abstract book of 7th international symposium on nitrogen fixation with non-legumes, Faisalabad, pp 36

Hungria M, Vargas MAT (2000) Environmental factors affecting N2 fixation in grain legumes in the tropics, with an emphasis on Brazil. Field Crops Res 65:151–164

Jee H-J (2009) Current status of bio-fertilizers and bio-pesticides development, farmer's acceptance and their utilization in Korea, FFTC report

Jida M, Assefa M (2011) Phenotypic and plant growth promoting characteristics of *Rhizobium leguminosarum* bv. *viciae* from lentil growing areas of Ethiopia. Afr J Microbiol Res 5 (24):4133–4142

Joshi KK, Kumar V, Dubey RC, Maheshwari DK, Bajpai VK, Kang SC (2006) Effect of chemical fertilizer-adaptive variants, *Pseudomonas aeruginosa* GRC_2 and *Azotobacter chroococcum* AC1, on *Macrophomina phaseolina* causing charcoal rot of *Brassica juncea*. Kor J Environ Agric 25(3):228–235

Kamilova F, Kravchenko LV, Shaposhnikov AI, Azarova T, Makarova N, Lugtenberg B (2006) Organic acids, sugars, and L-tryptophan in exudates of vegetables growing on stone wool and their effects on activities of rhizosphere bacteria. Mol Plant Microbe Interact 19:250–256

Kang SC, Pandey P, Khillon R, Maheshwari DK (2008) Process of rock phosphate solubilization by *Aspergillus* spp. PS 104 in soil amended medium. J Environ Biol 29(5):743–746

Kennedy IR, Tchan YT (1992) Biological nitrogen fixation in non-leguminous field crops: recent advances. Plant Soil 141:93–118

Khorshidi YR, Ardakani MR, Ramezanpour MR, Khavazi K, Zargari K (2011) Response of yield and yield components of rice (*Oryza sativa* L.) to *Pseudomonas fluorescens* and *Azospirillum lipoferum* under different nitrogen levels. Am Eurasian J Agric Environ Sci 10(3):387–395

Khurana AS, Sharma P (2000) Effect of dual inoculation of phosphate solubilizing bacteria, *Bradyrhizobium* sp. and phosphorus on nitrogen fixation and yield of chickpea. Indian J Pulses Res 13:66–67

Kloepper JW, Zablokovicz RM, Tipping EM, Lifshitz R (1991) Plant growth promotion mediated by bacterial rhizosphere colonizers. In: Keister DL, Cregan PB (eds) The rhizosphere and plant growth. Kluwer Academic, The Netherlands, pp 315–326

Kloepper JW, Rodriguesz-Ubana R, Zehnder GW, Murphy JF, Sikora E, Fernandez D (1999) Plant root-bacterial interactions in biological control of soilborne diseases and potential extension to systemic and foliar diseases. Aust Plant Pathol 28:21–26

Kumar VK, Raju SK, Reddy MS, Kloepper JW, Lawrence KS, Groth DE, Miller ME, Sudini H, Binghai D (2009a) Evaluation of commercially available PGPR for control of rice sheath blight caused by *Rhizoctonia solani*. J Pure Appl Microbiol 3(2):485–488

Kumar S, Pandey P, Maheshwari DK (2009b) Reduction in dose of chemical fertilizers and growth enhancement of Sesame (*Sesamum indicum* L.) with application of rhizospheric competent *Pseudomonas aeruginosa* LES4. Eur J Soil Biol 45:334–340

Kumar VK, Reddy MS, Kloepper JW, Lawrence KS, Zhou XG, Groth DE, Zhang S, Sudhakara Rao R, Wang Q, Raju MRB, Krishnam R, Dilantha Fernando WG, Sudini H, Du B, Miller ME (2011) Commercial potential of microbial inoculants for sheath blight management and yield enhancement of rice. In: Maheshwari DK (ed) Bacteria in agrobiology: crop ecosystems. Springer, Berlin, pp 237–264

Kurth E, Cramer GR, Lauchli A, Epstein E (1986) Effects of NaCl and CaCl on cell enlargement and cell production in cotton roots. Plant Physiol 82:1102–1106

Kutyova TY, Durinina EP, Muravyova NE, Sheyko AV (2002) Microbal fertilizers Bamil, Omug, Ekud, Pudret their properties, influence on soil and crops. Herald of Moscow State University, Soil science series 4:40–46

Lugtenberg BJJ, Kamilova FD (2004) Rhizosphere management: microbial manipulation for biocontrol. In: Goodman RM (ed) Encyclopedia of plant and crop science. Dekker, New York, pp 1098–1101

Lugtenberg BJ, Dekkers L, Bloemberg GV (2001) Molecular determinants of rhizosphere colonization by *Pseudomonas*. Ann Rev Phytopathol 39:461–490

Maheshwari DK, Kumar S, Kumar B, Pandey P (2011) Co-inoculation of urea and DAP tolerant *Sinorhizobium meliloti* and *Pseudomonas aeruginosa* as integrated approach for growth enhancement of *Brassica juncea*. Ind J Microbiol 50(4):425–431

Maheshwari DK, Dubey RC, Aeron A, Kumar B, Kumar S, Tewari S, Arora NK (2012) Integrated approach for disease management and growth enhancement of *Sesamum indicum* L. utilizing *Azotobacter chroococcum* TRA2 and chemical fertilizer. World J Microbiol Biotechnol 28 (10):3015–3024

Mehnaz S, Mirza MS, Haurat J, Bally R, Normand P, Bano A, Malik KA (2001) Isolation and 16S rRNA sequence analysis of the beneficial bacteria from the rhizosphere of rice. Can J Microbiol 47:110–117

Meunchang S, Thongra-ar P, Sanoh S, Kewsuralikhit S, Ando S (2006) Development of rhizobacteria as a biofertilizer for rice production. International workshop on sustained management of the soil-rhizosphere system for efficient crop production and fertilizer use, 16–20 Oct 2006

Mia MAB, Shamsuddin ZH, Zakaria W, Marziah M (2007) Associative nitrogen fixation by *Azospirillum* and *Bacillus* spp. in bananas. Infomusa 16:11–15

Mia MAB, Shamsuddin ZH, Zakaria W, Marziah M (2009) The effect of rhizobacterial inoculation on growth and nutrient accumulation of tissue-cultured banana plantlets under low N-fertilizer regime. Afr J Biotechnol 8(21):5855–5866

Naveed M, Khalid M, Jones DL, Ahmad R, Zahir ZA (2008) Relative efficacy of *Pseudomonas* spp., containing ACC-deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of organic fertilizer. Pak J Bot 40:1243–1251

Nguyen TH (2008) The product BioGro and improvements in its performance. In: Kennedy IR, Choudhury ATMA, Kecskés ML, Rose MT (eds) Efficient nutrient use in rice production in Vietnam achieved using inoculant biofertilisers. Proceedings of a project (SMCN/2002/073) workshop held in Hanoi, Vietnam, 12–13 Oct 2007, pp 15–24

Nguyen TH, Deaker R, Kennedy IR, Roughley RJ (2003) The positive yield response of field grown rice to inoculation with a multi-strain biofertiliser in the Hanoi area, Vietnam. Symbiosis 35:231–245

Niranjan R, Shetty HS, Reddy MS (2005) Plant growth-promoting rhizobacteria: potential green alternative for plant productivity. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht, pp 197–216

Nosheen A, Bano A, Ullah F, Farooq U, Yasmin H, Hussain I (2011) Effect of plant growth promoting rhizobacteria on root morphology of Safflower (*Carthamus tinctorius* L.). Afr J Biotechnol 10(59):12669–12679

Quyet-Tien P, Park YM, Seul KJ, Ryu C-M, Park SH, Kim JC, Chin SY (2010) Assessment of root-associated *Paenibacillus polymyxa* groups on growth promotion and induced systemic resistance in pepper. J Microbiol Biotechnol 20(12):1605–1613

Rabie GH, Almadini AM (2005) Role of bioinoculants in development of salt-tolerance of *Vicia faba* plants under salinity stress. Afr J Biotechnol 4(3):210–222

Rashid A, Aslam M, Sajjad MR, Siddiqui G, Jami AR, Gill MA, Cheema MS, Sandhu MS, Asghar M, Nayyar MM (1997) Response of wheat to diazotrophic bacteria and nitrogen at different locations of Pakistan, Punjab area. In: Ahmad N, Hamid A (eds) Plant nutrition management for sustainable agriculture growth. NFDC, Islamabad

Requena N, Pérez-Solis E, Azcón-Aguilar C, Jeffries P, Barea JM (2001) Management of indigenous plantmicrobe symbiosis aids restoration of desertified ecosystems. Appl Environ Microbiol 67:495–498

Rokhzadi A, Asgharzadeh A, Darvish F, Nour-Muhammadi G, Majidi E (2008) Influence of plant growth promoting rhizobacteria on dry matter accumulation and yield of chickpea (C*icer arietinum* L.) under field conditions. Am Eurasian J Agric Environ Sci 3(2):253–257

Ryu CM, Farag MA, Hu CH, Reddy MS, Wie HX (2003) Bacterial volatiles promote growth of *Arabidopsis*. Proc Natl Acad Sci USA 100:4927–4932

Sahrawat KL (2000) Macro and micronutrients removed by upland and lowland rice cultivars in West Africa. Commun Soil Sci Plant Anal 31:717–723

Saleem M, Arshad M, Hussain S, Bhatti AS (2007) Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. J Ind Microbiol Biotechnol 34(10):635–648

Saraf M, Jha CK, Patel D (2010) The role of ACC deaminase producing PGPR in sustainable agriculture. In: Maheshwari DK (ed) Plant growth and health promoting bacteria, vol 18, Microbiology monographs. Springer, Berlin, pp 365–387

Shaharoona B, Jamroo GM, Zahir ZA, Arshad M, Memon KS (2007) Effectiveness of various *Pseudomonas* spp. and *Burkholderia caryophylli* containing ACC-deaminase for improving growth and yield of wheat (*Triticum aestivum* L.). J Microbiol Biotechnol 17:1300–1307

Sheng XF (2005) Growth promotion and increased potassium uptake of cotton and rape by a potassium releasing strain of *Bacillus edaphicus*. Soil Biol Biochem 37:1918–1922

Shirokova Y, Forkutsa I, Sharafutdinova N (2000) Use of electrical conductivity instead of soluble salts for soil salinity monitoring in Central Asia. Irr Drain Sys 14:199–205

Sommers E, Vanderleyden J, Srinivasan M (2004) Rhizosphere bacterial signalling: a love parade beneath our feet. Crit Rev Microbiol 30:205–240

Strigul NS, Kravchenko LV (2006) Mathematical modeling of PGPR inoculation into the rhizosphere. Env Mod Soft 21:1158–1171

Supanjani S, Habiba A, Mabooda F, Leea KD, Donnellya D, Smith DL (2006) Nod factor enhances calcium uptake by soybean. Plant Phys Biochem 44:866–872

Tomar RKS, Namdeo KN, Ranghu JS (1996) Efficacy of phosphate solubilizing bacteria-biofertilizers with phosphorus on growth and yield of gram (*Cicer arietinum*). Ind J Agron 41:412–415

Ugoji EO, Laing MD, Hunter CH (2006) An investigation of the shelf-life (storage) of *Bacillus* isolates on seeds. South Afr J Bot 72:28–33

Ullmann WJ, Kirchman DL, Welch SA, Vandevivere P (1996) Laboratory evidence for microbially mediated silicate mineral dissolution in nature. Chem Geol 132:11–17

Velagaleti RR, Marsh S (1989) Influence of host cultivars and Bradyrhizobium strains on the growth and symbiotic performance of soybean under salt stress. Plant Soil 119:133–138

Verma JP, Yadav J, Tiwari KN (2010) Application of *Rhizobium* sp. BHURC01 and plant growth promoting rhizobacteria on nodulation, plant biomass and yields of chickpea (*Cicer arietinum* L.). Int J Agric Res 5:148–156

Weller DM, Thomshow LS (1993) Use of rhizobacteria for biocontrol. Curr Opin Biotechnol 4:306–311

Werner JE, Finkelstein RR (1995) Arabidopsis mutants with reduced response to NaCl and osmotic stress. Physiol Plant 93:659–666

Yang J, Kloepper JW, Ryu C-M (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14(1):1–4

Yanni YG, Rizk RY, Corich V, Squartini A, Ninke K, Hollingsworth PS, Orgambide G, de Bruijn F, Stoltzfus J, Buckley D, Schmidt TM, Mateos PF, Ladha JK, Dazzo FB (1997) Natural endophytic association between *Rhizobium leguminosarum* by *trifolii* and rice and assessment of its potential to promote rice growth. Plant Soil 194:99–114

Yasmin F, Othman R, Sijam K, Saad MS (2009) Characterization of beneficial properties of plant growth-promoting rhizobacteria isolated from sweet potato rhizosphere. Afr J Microbiol Res 3:815–821

Young CC, Lai WA, Shen FT, Hung MH, Hung WS, Arun AB (2003) Exploring the microbial potentially to augment soil fertility in Taiwan. In: Proceedings of the 6th ESAFS international conference: soil management technology on low productivity and degraded soils, Taipei, Taiwan, pp 25–27

Zahir ZA, Munir A, Asghar HN, Shaharoona B, Arshad M (2008) Effectiveness of rhizobacteria containing ACC deaminase for growth promotion of peas (*Pisum sativum*) under drought conditions. J Microbiol Biotechnol 18:958–963

Zahran HH (1999) *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol Mol Biol Rev 63:968–989

Zhigletsova SK, Dunajtsev IA, Besaeva SG (2010) Possibility of application of microorganisms for solving problems of ecological and food safety. Agrochemical 6:83–96

Chapter 3
Role of Plant Growth-Promoting Rhizobacteria for Commercially Grown Medicinal Plants

B. Karthikeyan, U. Sakthivel, and J. Sriman Narayanan

3.1 Introduction

Over two millennia ago, the father of medicine, Hippocrates, mentioned about 400 medicinal plants and advocated, 'Let food be your medicine and let medicine be your food'. Medicinal plants constitute a segment of the flora which provides raw materials for the use of industries producing pharmaceuticals, cosmetics and fragrance flavour imparting biochemicals. Indian systems of medicine (ISM) use around 2,500 plant species belonging to more than 1,000 genera. About 800 species are used by industry of which approximately 25 % are cultivated. It is estimated that 25,000 effective phyto-based formulations are available under indigenous system of medicine, and over 7,800 manufacturing units are producing plant-derived drugs in India, as estimated by the Eximbank (Jose and Singh 2001). International market of medicinal plant-related trade is to this time US$60 billion per year, with a growth rate of 7 % annually. India exports different medicinal plants valuing at Rs. 1,200 million per annum.

The world population is likely to touch the 7.5 billion mark at the current growth rate by the year 2020, the increase is excepted mostly in the developing (or) underdeveloped countries where there is corresponding increase in the disease possibilities. One billion people, mostly in developing countries, rely on, or choose to use, medicinal plants to cover all or part of their health care needs (IUCN 1993; WHO 2002). With the progress in chemical techniques, crude drugs came to be replaced by pure chemical drugs, and the developed countries witnessed a decline in popularity of medicinal plants therapy (Malik et al. 2011).

The world scenario on use of herbal plants is rapidly changing, and several international pharmaceutical companies have now concentrated their research in

B. Karthikeyan (✉) • U. Sakthivel • J. Sriman Narayanan
Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar 608 002, Tamil Nadu, India
e-mail: balakar02@yahoo.com

D.K. Maheshwari et al. (eds.), *Bacteria in Agrobiology: Crop Productivity*,
DOI 10.1007/978-3-642-37241-4_3,

developing medicinal plants and strengthening the market production of novel drugs, nutraceuticals, nutrient and herbal dietary supplement, new biochemical flavonoid compounds, antioxidants, glycosides, etc.

In India, Tamil Nadu state is under strategic geographical location and possesses an invaluable treasure of medicinal plants holding a major share in cultivation and export of more than 50 medicinal plant species. Tamil Nadu state is a potential supplier of herbal raw material, phytochemicals, herbal medicines, essential oils, floral concentrate, plant-based natural insecticides, etc. The commercially cultivated medicinal plants include *Catharanthus roseus* (Periwinkle), *Coleus forskohlii* (Coleus), *Aloe vera* (Aloe), *Ocimum sanctum* (Ocimum) and *Withania somnifera* (Ashwagandha) (Karthikeyan et al. 2008b).

An intensive farming practice that warrants high yield and quality requires extensive use of chemical fertilisers, which are costly and create environmental problem. Moreover, the use of large quantities of chemical fertilisers not only results in high costs but also affects soil health and the productivity. In the present scenario there has been a resurgence of interest in environmental-friendly, sustainable, and organic agricultural practices use of plant growth-promoting rhizobacteria (biofertiliser) containing beneficial microorganisms are known to improve plant growth the supply of plant nutrients and releasing growth regulators and pathogen inhibitor compounds (Esitken et al. 2005).

Among different groups of plant growth-promoting rhizobacteria, nitrogen-fixing and phosphorous-solubilising/phosphate-mobilising organisms may be considered to be important since they improve plant nutrition by increasing N and P uptake by plants, and they play a significant role as plant growth-promoting rhizobacteria (PGPR) in the biofertiliser of crops (Karlidag et al. 2007; Karthikeyan et al. 2010).

The occurrence of PGPR (*Azospirillum, Azotobacter, Bacillus* and *Pseudomonas*) in the rhizosphere of medicinal plants such as *Catharanthus roseus, Coleus forskohlii, Aloe vera* and *Ocimum sanctum* has been documented earlier (Karthikeyan et al. 2008b). Hence, this chapter was focused on the role of PGPR in commercially grown medicinal plants.

3.2 Important Commercially Grown Medicinal Plants

Some of the important medicinal plants have been discussed.

3.2.1 Catharanthus roseus *(Periwinkle)*

Catharanthus roseus (L.) G.Don. Synonym is a tropical perennial plant, native to Madagascar, belonging to Apocynaceae family, where it has spread to India from Madagascar. It has got tremendous export potential and can earn foreign exchange

to the tune of several million dollars. The plant has a wide range of terpenoid indole alkaloids (TIAs), which are valued for their wide spectrum of pharmaceutical effects such as anticancer therapy (vinblastine and vincristine), hypertension, acute leukaemia and Hodgkin's diseases (ajmalicine, serpentine). The plant contains more than 100 alkaloids distributed in all parts of the plant but in different proportions. The plant has three varieties based on flower colour. The varieties are 'rosea' (pink flower), 'alba' (white flower) and 'ocellata' (white flower with pink ring).

The occurrence of diazotrophic microorganism populations of *Catharanthus roseus* was reported by Karthikeyan et al. (2008b). Among the diazotrophic microorganisms, *Azotobacter* population was recorded the highest count (12.00, 7.66×10^4 cfu g^{-1}) in both rhizosphere and non-rhizosphere, followed by *Azospirillum* (8.00, 1.50×10^4 cfu g^{-1}) and *Pseudomonas* (5.22, 3.00×10^4 cfu g^{-1}).

3.2.2 *Role of PGPR in Root Exudates of* C. roseus

Root exudates are organic substances elaborated through plant roots into the rhizosphere environment. It has got profound influence on the rhizosphere microorganisms. *C. roseus* rosea variety root exudates were collected and fractioned both qualitatively and quantitatively for their relative chemotactic activity of PGPB strains. The chemotactic response of PGPB strains was in the order of *Azospirillum lipoferum, Pseudomonas fluorescens, Azotobacter chroococcum* and *Bacillus megaterium* (Karthikeyan 2007).

3.2.3 *Collection and Fractionation of the Root Exudates of* C. roseus *Varieties*

The root exudates of the two varieties of *C. roseus*, *viz*. 'rosea' and 'alba', were collected and fractionated both qualitatively and quantitatively. The estimated quantity of the different fractions was given in Table 3.1.

Crude root exudates of rosea variety (424.8 μg $plant^{-1}$) have a cationic fraction of 170.4 μg $plant^{-1}$, an anionic fraction of 120.1 μg $plant^{-1}$ and a neutral fraction of 90.2 μg $plant^{-1}$, while the alba variety fractionation of crude root exudates (370.4 μg $plant^{-1}$) has a cationic fraction of 135.2 μg $plant^{-1}$, an anionic fractionof 110.0 μg $plant^{-1}$ and a neutral fraction of 75.5 μg $plant^{-1}$. The cationic fraction included different amino acids, anionic fraction included different organic acids and neutral fraction included different sugars. The quantity of cationic fraction was higher than that of anionic and neutral fractions, while the anionic fraction was

Table 3.1 Fractionations of the root exudates of *Catharanthus roseus* varieties

S. No.	Components of root exudates	Rosea variety Quantity (μg plant^{-1})	Alba variety Quantity (μg plant^{-1})
1.	Crude root exudates	424.8	370.4
2.	Cationic fraction	170.4	135.2
3.	Anionic fraction	120.1	110.0
4.	Neutral Fraction	90.2	75.5

Table 3.2 Qualitative and quantitative analysis of different fractions of the root exudates of *Catharanthus roseus* varieties

	Cationic fraction			Anionic fraction			Neutral fraction		
S. No.	Amino acids	Quantity in μg plant^{-1} Rosea	Alba	Organic acids	Quantity in μg plant^{-1} Rosea	Alba	Sugars	Quantity in μg plant^{-1} Rosea	Alba
1.	Aspartic acid	43.6	35.9	Malic acid	67.5	64.1	Fructose	32.2	30.00
2.	Glutamic acid	75.8	64.5	Oxalic acid	21.5	20.5	Glucose	25.5	21.00
3.	Glycine	25.5	15.5	Succinic acid	15.5	13.5	Maltose	15.0	12.5
4.	Serine	15.5	12.0	Citric acid	10.5	8.5	Ribose	10.0	7.0
5.	Proline	10.0	7.5	Glutaric acid	5.0	3.5	Arabinose	7.5	5.0

higher than neutral fraction in the root exudate of both rosea and alba varieties (Table 3.2).

Five different amino acids, *viz*. aspartic acid, glutamic acid, glycine, serine and proline, were detected in the cationic fractions. In the root exudates of *C. roseus*, rosea variety, the relative occurrence of amino acids was in the order of glutamic acid (75.8 μg plant^{-1}) > aspartic acid (43.6 μg plant^{-1}) > glycine (25.5 μg plant^{-1}) > serine (15.5 μg plant^{-1}) > and proline (10 μg plant^{-1}).

The relative occurrence of different amino acids in the root exudates of alba variety was in the order of glutamic acid (64.5 μg plant^{-1}) > aspartic (35.2 μg plant^{-1}) > glycine (15.5 μg plant^{-1}) > serine (12.0 μg plant^{-1}) > proline (7.5 μg plant^{-1}). Five organic acids, *viz*. malic acid, oxalic acid, succinic acid, citric acid and glutaric acid, were detected in the anionic fraction of both rosea and alba varieties of *C. roseus*. In *C. roseus*—rosea variety—the quantity of different organic acids present was in the order of malic acid (67.5 μg plant^{-1}) > oxalic acid (21.5 μg plant^{-1}) > succinic acid (15.5 μg plant^{-1}) > citric acid (10.5 μg plant^{-1}) and glutaric acid (5.0 μg plant^{-1}), while in the alba variety, the organic acid quantity was in the order of malic acid (64.1 μg plant^{-1}) > oxalic acid (20.5 μg plant^{-1}) > succinic acid (13.5 μg plant^{-1}) > citric acid (8.5 μg plant^{-1}) > glutaric acid (3.5 μg plant^{-1}) (Table 3.2).

Five different sugars, *viz*. fructose, glucose, maltose, ribose and arabinose, were detected in neutral fraction of the root exudates of rosea and alba varieties of *C. roseus*. In *C. roseus*—rosea variety—the quantity of different sugars present was in the order of fructose (32.2 μg plant^{-1}) > glucose (25.5 μg plant^{-1}) > maltose (15.0 μg plant^{-1}) > ribose (10.0 μg plant^{-1}) and arabinose (7.5 μg plant^{-1}).

In the neutral fraction of alba variety, the quantity of different sugars present was in the order of fructose (30.0 μg plant^{-1}) > glucose (21.0 μg plant^{-1}) > maltose (12.5 μg plant^{-1}) > ribose (7.0 μg plant^{-1}) and arabinose (5.0 μg plant^{-1}) in the root exudates (Table 3.2).

3.2.4 *Relative Chemotactic Response of PGPB Strains Towards Different Root Exudates Fractions of* C. roseus *Varieties*

The root exudates collected from the two varieties of *C. roseus, viz.* rosea and alba, were used as such (crude) as well as their fractionated compounds either singly or in combinations to study their chemotactic activity and to determine the relative chemotactic response of four selected efficient PGPB strains, *viz. A. lipoferum* CAZS-4, *A. chroococcum* CAZB-1, *B. megaterium* CPB-18 and *P. fluorescens* CPF-14 (Table 3.3).

All the four strains recorded higher RCR values to the root exudates of the variety rosea than that of alba. Further the RCR values obtained for the recombined fraction are the highest followed by crude root exudate as such and combination of fraction of any two and individual fractions.

Among the three fractions tested individually, all the four strains showed higher RCR values to anionic fraction followed by neutral and cationic fractions. The strains exerted better response to the treatment combinations of anionic + cationic, followed by anionic + neutral and cationic + neutral, whereas the recombined fractions of anionic + neutral + cationic exerted the highest chemotactic effect, and the strains tested showed higher RCR values than that of the RCR values recorded for the other treatments.

RCR value of *A. lipoferum* CAZS-4 was highest at 4.2 ± 0.18 towards recombined fractions (anionic + cationic + neutral) followed by 3.8 ± 0.24 towards crude root exudates, 3.5 ± 0.44 towards anionic + cationic, 3.2 ± 0.42 towards anionic + neutral, 3.1 ± 0.05 towards cationic + neutral, 3.0 ± 0.24 towards anionic, 2.0 ± 0.28 towards neutral and 1.5 ± 0.16 towards cationic fraction of the root exudates of rosea variety of *C. roseus*. The distilled water control recorded no chemotactic activity.

The RCR value of *A. chroococcum* CAZB-1 was highest at 3.0 ± 0.98 towards recombined fractions followed by 2.5 ± 0.12 towards crude root exudates, 2.2 towards anionic + cationic, 2.0 ± 0.48 towards anionic + neutral, 1.8 ± 0.67 towards cationic + neutral, 1.6 ± 0.88 towards anionic, 1.5 ± 0.44 towards neutral and 1.0 ± 0.42 towards cationic fraction of the root exudates of rosea variety of *C. roseus*.

For the root exudates of rosea variety of *C. roseus*, the RCR values of *B. megaterium* CPB-18 were highest at 1.1 ± 0.90 towards recombined fractions followed by 1.0 ± 0.18 towards crude root exudates, 0.9 ± 0.44 towards anionic ± cationic, 0.8 ± 0.74 towards anionic + neutral, 0.7 ± 0.50 towards cationic + neutral,

Table 3.3 Relative chemotactic response (RCR) of PGPB strains towards different root exudate fractions of *Catharanthus roseus* varieties

		RCR to root exudate fractions of Rosea variety				RCR to root exudate fractions of Alba variety			
		PGPB strains[a]				PGPB strains[a]			
S. No.	Fraction of root exudates	CAZS-4[b]	CAZB-1[c]	CPB-18[d]	CPF-14[e]	CAZS-4[b]	CAZB-1[c]	CPB-18[d]	CPF-14[e]
1.	Crude	3.8 ± 0.24	2.5 ± 0.12	1.0 ± 0.18	3.0 ± 0.12	3.0 ± 0.22	2.1 ± 0.49	0.9 ± 0.66	2.8 ± 0.94
2.	Anionic	3.0 ± 0.24	1.6 ± 0.88	0.5 ± 0.26	2.5 ± 0.24	1.8 ± 0.16	1.6 ± 0.26	0.4 ± 0.84	1.8 ± 0.78
3.	Cationic	1.5 ± 0.16	1.0 ± 0.42	0.3 ± 0.68	1.2 ± 0.16	1.2 ± 0.44	0.9 ± 0.10	0.1 ± 0.22	1.0 ± 0.77
4.	Neutral	2.0 ± 0.28	1.5 ± 0.44	0.4 ± 0.37	1.7 ± 0.44	1.7 ± 0.14	1.2 ± 0.14	0.2 ± 0.12	1.3 ± 0.94
5.	Anionic + cationic	3.5 ± 0.44	2.2 ± 0.77	0.9 ± 0.44	3.2 ± 0.66	2.7 ± 0.78	1.9 ± 0.78	0.8 ± 0.42	2.5 ± 0.62
6.	Anionic + neutral	3.2 ± 0.42	2.0 ± 0.48	0.8 ± 0.74	2.8 ± 0.66	2.5 ± 0.46	1.8 ± 0.22	0.7 ± 0.72	2.3 ± 0.14
7.	Cationic + neutral	3.1 ± 0.05	1.8 ± 0.67	0.7 ± 0.50	2.4 ± 0.88	2.2 ± 0.17	1.7 ± 0.62	0.6 ± 0.10	2.0 ± 0.24
8.	Anionic + cationic + neutral	4.2 ± 0.18	3.0 ± 0.98	1.1 ± 0.90	3.6 ± 0.44	3.5 ± 0.88	2.8 ± 0.86	1.0 ± 0.66	3.2 ± 0.10
9.	Control	0.01	0.01	–	0.01	0.01	0.01	–	0.01

[a]At 1×10^6 CFU ml^{-1} inoculant level
[b]*A. lipoferum* CAZS-4
[c]*A. chroococcum* CAZB-1
[d]*B. megaterium* CPB-18
[e]*P. fluorescens* CPF-14

0.5 ± 0.26 towards anionic, 0.4 ± 0.37 towards neutral and 0.3 ± 0.68 towards cationic fraction.

The RCR value of *P. fluorescens* CPF-14 was highest at 3.6 ± 0.44 towards recombined fractions followed by 3.2 ± 0.66 towards anionic + cationic, 2.8 ± 0.66 towards anionic + neutral, 2.4 ± 0.88 towards cationic + neutral, 2.5 ± 0.24 towards anionic, 1.7 ± 0.16 towards neutral and 1.2 ± 0.16 towards cationic fractions of the root exudates of rosea variety of *C. roseus*.

Chemotactic response to all the four strains although higher towards recombined fractions than the other fractions and the RCR values obtained were in the order of 4.2 ± 0.18 for *A. lipoferum* CAZS-4, 3.6 ± 0.44 for *P. fluorescens* CPF-14, 3.0 ± 0.98 for *A. chroococcum* CAZB-1 and 1.1 ± 0.90 for *B. megaterium* CPB-18.

For the root exudates of alba variety of *C. roseus,* the RCR value of *A. lipoferum* CAZS-4 was highest at 3.5 ± 0.88 towards recombined fractions followed by 3.0 ± 0.22 towards crude root exudates, 2.7 ± 0.78 towards anionic + cationic, 2.5 ± 0.46 towards anionic + neutral, 1.7 ± 0.14 towards neutral, 2.2 ± 0.17 towards cationic + neutral, 1.3 ± 0.16 towards anionic and 1.2 ± 0.44 towards cationic.

For the root exudates of alba variety of *C. roseus,* the RCR value of *A. chroococcum* CAZB-1 was highest at 2.8 ± 0.86 towards recombined fraction followed by 2.1 ± 0.49 towards crude root exudates, 1.9 ± 0.78 towards anionic + cationic, 1.8 ± 0.22 towards anionic + neutral, 1.7 ± 0.62 towards cationic + neutral, 1.6 ± 0.26 towards anionic, 1.2 ± 0.14 towards neutral and 0.9 ± 0.10 towards cationic.

For the root exudates of alba variety of *C. roseus*, the RCR value of *B. megaterium* CPB-18 was highest at 1.0 ± 0.66 towards recombined fraction followed by 0.9 ± 0.66 towards crude root exudates, 0.8 ± 0.42 towards anionic + cationic, 0.7 ± 0.72 towards anionic + neutral, 0.6 ± 0.10 towards cationic + neutral, 0.4 ± 0.84 towards anionic, 0.2 ± 0.12 towards neutral and 0.2 ± 0.22 towards cationic.

For the root exudates of alba variety of *C. roseus*, the RCR value of *P. fluorescens* CPF-14 was highest at 3.2 ± 0.10 towards recombined fractions, followed by 2.8 ± 0.94 towards crude root exudates, 2.5 ± 0.62 towards anionic + cationic, 2.3 ± 0.14 towards anionic + neutral, 2.0 ± 0.24 towards cationic + neutral, 1.8 ± 0.78 towards anionic, 1.3 ± 0.9 towards neutral and 1.0 ± 0.77 towards cationic. In the recombined fraction of the alba variety, the RCR values obtained were in the following order: that of *A. lipoferum* CAZS-4 was 3.5 ± 0.88, that of *P. fluorescens* CPF-14 was 3.2 ± 0.10, that of *A. chroococcum* CAZB-1 was 2.8 ± 0.86 and that of *B. megaterium* CPB-18 was 1.0 ± 0.66.

The ability of *Pseudomonas* sp. and *Azospirillum* sp. to detect low concentrations of components of root exudates in soils (Lopezdevictoria and Lovell 1993). Reinhold et al. (1985) reported that the optimum concentrations for amino acids, organic acids and sugars were 40 mM for the highest chemotactic attraction of *A. brasilense* and *A. lipoferum*. Root exudates modulate the interaction between plants and PGPR; reported by Deweert et al. (2002) under certain conditions, many compounds present in the root exudates stimulate a positive chemotactic response

in bacteria (Somers et al. 2004). The bacterial diversity was found to increase with the stages of plant growth gradually from seedling up to maturation stage and then eventually followed a decline with only transient changes (Kumar et al. 2011).

3.2.5 *Role of Plant Growth-Promoting Bacteria in Improvement of Alkaloid Content in* C. roseus

Plant growth-promoting rhizobacteria (PGPR) represent a wide variety of soil bacteria which, when grown in association with a host plant, result in stimulation of growth of their host. A study was conducted on *C. roseus* for the effect of seed treatments with native diazotrophs on its seedling growth and antioxidant enzyme activity. The seed treatment with native isolates of *Azospirillum* and *Azotobacter* increased the germination percentage, root length, shoot length and vigour index of *C. roseus*. The maximum germination percentage (70 %) was recorded in *Azotobacter* treatment followed by *Azospirillum* (66 %). The native isolate *Azotobacter* and *Azospirillum* significantly increased the germination rate in *C. roseus* which was 70 % as against 35 % recorded by untreated control. The treatments with diazotrophs resulted in significantly higher dry matter than control (Karthikeyan et al. 2007). There was a significant increase in superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT) antioxidant enzymes under *Azotobacter* and *Azospirillum* treatments. SOD activity directly modulates the amount of ROS. It catalyses the dismutation of superoxide anion radical (O_2) with great efficiency resulting in the production of H_2O_2 and O_2. The changes in SOD activity under *Azotobacter* and *Azospirillum* treatments can be also a consequence of an altered synthesis and accumulation of less active enzymes and/or of a higher turnover of SOD. Karthikeyan et al. (2007) also reported that the *Pseudomonas fluorescens*-treated plants increases the plant height, root length and ajmalicine content of *C. roseus*, which is due to the production of growth hormones by PGPR (Jaleel et al. 2007).

3.2.6 *Effect of PGPB Consortium Inoculation on the Ajmalicine Content in the Roots of* C. roseus *Varieties by TLC*

The results on the root ajmalicine (alkaloid) content of both rosea and alba varieties of *C. roseus* were presented in Table 3.4. The inoculation with plant growth-promoting bacteria significantly increased the alkaloid content of *C. roseus* varieties. The ajmalicine content of roots of *C. roseus* ranged from 0.500 to 1.120 mg g^{-1} of root in both the varieties with various treatments as measured by thin layer chromatography (TLC).

Table 3.4 Effect of PGPB consortium inoculation on the ajmalicine content of *Catharanthus rosea* varieties

Treatments	Ajmalicine content of Rosea variety[a]				Ajmalicine content of Alba variety[a]			
	90 DAS	120 DAS	150 DAS	180 DAS	90 DAS	120 DAS	150 DAS	180 DAS
T_1—*Azospirillum*	0.610	0.712	0.800	1.000	0.600	0.695	0.758	0.875
T_2—*Azotobacter*	0.535	0.615	0.720	0.900	0.510	0.600	0.720	0.790
T_3—*Bacillus*	0.500	0.585	0.625	0.750	0.450	0.510	0.600	0.700
T_4—*Pseudomonas*	0.585	0.695	0.770	0.950	0.500	0.610	0.720	0.800
T_5—consortium ($T_1 + T_2 + T_3 + T_4$)	0.650	0.785	0.825	1.120	0.620	0.750	0.825	0.925
T_6—uninoculated control	0.500	0.625	0.700	0.800	0.400	0.550	0.625	0.700
SEd	0.004	0.005	0.017	0.022	0.005	0.017	0.017	0.020
CD ($P = 0.05$)	0.009	0.011	0.034	0.045	0.010	0.034	0.035	0.041

[a]mg g^{-1} root dry weight

The consortium-inoculated treatment recorded the maximum alkaloid content of 1.120 mg g^{-1} in 'rosea' variety followed by the single inoculant treatment of *A. lipoferum* CAZS-4, 1.000 mg g^{-1}; *P. fluorescens* CPF-14, 0.950 mg g^{-1}; *A. chroococcum* CAZB-1, 0.900 mg g^{-1}; and *B. megaterium* CPB-18, 0.750 mg g^{-1}.

In the alba variety also, consortium inoculant recorded maximum ajmalicine content of 0.925 mg g^{-1} on 180 DAS followed by the single inoculant treatments of T_1, T_4, T_2 and T_3, while the uninoculated control recorded least ajmalicine content in both the varieties.

Karthikeyan et al. (2010) reported that in *C. roseus* plants treated with triple combined application of PGPR (*Azotobacter* + *Pseudomonas* + *Bacillus*), there was a significant increase in plant height (41.79 %), root length (60.02 %), root girth (109.09 %) and ajmalicine alkaloid content (179.41 %) compared with control. Likewise nutrient content (N, P, K) was also increased in triple combination followed by double combination of PGPR.

3.3 Other Medicinal Plants

Many other medicinal plants are used extensively in alternative medicinal systems and are indigenous in origin. Two of them have been highlighted herein.

3.3.1 Coleus forskohlii

Coleus forskohlii is an important indigenous medicinal plant in India. It has been used in traditional ayurvedic medicine for curing various disorders, and this is the only source of diterpenoid forskolin. Forskolin is used for the treatment of eczema, asthma psoriasis, cardiovascular disorders and hypertension, where decreased

intracellular cAMP level is believed to be a major factor in the development of the disease process (Kavitha et al. 2010).

The occurrence of PGPR organisms in Coleus and Ashwagandha was reported by Karthikeyan (2007). In Coleus and Ashwagandha plant *Azotobacter* population recorded maximum (5.68×10^4, 2.49×10^6) followed by *Azospirillum* and *Pseudomonas* population. Priya (2010) reported the occurrence of plant growth-promoting rhizobacteria in the rhizosphere soil of *Coleus forskohlii*. The highest occurrence of PGPR in the rhizosphere soil of Coleus is *Azospirillum*, *Pseudomonas*, *Azotobacter* and *Bacillus*.

Priya (2010) also reported that the combined inoculation of PGPR significantly increased the forskolin alkaloid content *Coleus forskohlii*. The combined inoculant treatment recorded increased in forskolin content of 0.93 % while compared to uninoculated control (0.23 %) and it is reported the per cent of disease index (PDI) occurrence of root rot disease in *Coleus forskohlii*. The combined PGPR (*Azospirillum* + *Azotobacter* + *Pseudomonas* + *Bacillus*) recorded the lowest disease index in *Coleus forskohlii*.

3.3.2 Withania somnifera *(Ashwagandha)*

Ashwagandha (*Withania somnifera*) is an important medicinal plant. Withanin and Somniferine are important in Ayurvedic and Unani preparations. The dried roots of the plant are used in the treatment of nervous and sexual disorders (Rajasekar and Elango 2011).

The occurrences of PGPR in the rhizosphere soil of *Withania somnifera* were reported by Karthikeyan et al. (2008a, b). Among the PGPR, *Azotobacter* recorded maximum population (2.49×10^4 g of soil) followed by *Azospirillum* and *Pseudomonas*. He also reported the growth-promoting traits of strains. Rajasekar and Elango (2011) also reported that the PGPR consortium significantly increased the plant height, root length and alkaloid content of *W. somnifera*.

3.4 Conclusions

The considerable efforts towards understanding the ecology and management of PGPR have been directed, yet their development as inoculants remains a considerable challenge. Research in last decade has opened up new horizons for commercially grown medicinal plants. Exploration and identification of traits involved in the ability of certain bacteria to establish themselves into the rhizosphere at levels sufficient to exert effects on plant growth effectively complete with the indigenous microflora co-operatively interact with other beneficial members of rhizospheric biota and understand the mechanisms that occur between plants and bacteria are also required.

Medicinal plants have become potential research areas in the developing countries. Further research needs to be carried out on the role of PGPR, while production of alkaloid time in the rhizosphere soil and mechanisms were thoroughly studied under natural environments.

References

Deweert S, Vermeiren H, Mulders IH, Kuiper I, Hendrick N, Bloomberg GV (2002) Flagella-driven chemotaxis towards exudates components in an important trait for tomato root colonization by *Pseudomonas fluorescens*. Mol Plant Microbe Interact 15:1173–1180

Esitken A, Ercisli S, Karlidag H, Sahin F (2005) Potential use of plant growth promoting rhizobacteria (PGPR) in organic apricot production. In: Proceedings of the international scientific conference of environmentally friendly fruit growing, Tartu- Estonia, 7–9 Sept, pp 90–97

IUCN (1993) Guidelines on the conservation of medicinal plants. IUCN, Gland

Jaleel MA, Sankar P, Kishore Kumar B, Gopi A, Somasundaram R, Panneerselvam R (2007) *Pseudomonas fluorescens* enhances biomass yield and ajmalicine production in *Catharanthus roseus* under water deficit stress. Coll Surf B Biointer 60:7–11

Jose CS, Singh HP (2001) Development of medicinal and aromatic plants. A national perspective paper. In: The national seminar on transfer of technology of medicinal and aromatic crops, Lucknow, 20–22 Feb, pp 1–12

Karlidag H, Esitken A, Turan M, Sahin F (2007) Effect of root inoculation of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient element contents of apple. Sci Hortic 114:16–20

Karthikeyan B (2007) Studies on the development of biofertilizer consortium for commercially grown medicinal plants. Ph.D. Thesis, Annamalai University, Annamalai Nagar

Karthikeyan B, Jaleel CA, Gopi R, Deiveekasundaram M (2007) Alternations in seedling vigour and antioxidant enzyme activities in *Catharanthus roseus* under seed priming with native diazotrophs. J Zhejiang Univ Sci B 8(7):453–457

Karthikeyan B, Jaleel CA, Changxing Z, Joe MM, Sriman Narayanan J, Deiveekasundaram M (2008a) The effect of AM fungi and phosphorus level on the biomass yield and ajmalicine production in *Catharanthus roseus*. EurAsia J Bio sci 2&3:26–33

Karthikeyan B, Jaleel CA, Lakshmananan G, Deiveekasundaram M (2008b) Studies on the microbial biodiversity of some commercially important medicinal plants. Coll Surf B Biointer 62:143–145

Karthikeyan B, Joe MM, Jaleel CA, Deiveekasundaram M (2010) Effect of root inoculation with plant growth promoting rhizobacteria (PGPR) on plant growth, alkaloid content and nutrient control of *Catharanthus roseus* (L.) G. Don. Nat Croat 19(1):205–212

Kavitha C, Rajamani K, Vadivel E (2010) *Coleus forskohlii*: a comprehensive review on morphology, phytochemistry and pharmacological aspects. J Med Plant Res 4(4):278–285

Kumar A, Prakash A, Johri BN (2011) *Bacillus* as PGPR in crop ecosystem. In: Maheshwari DK (ed) Bacteria in agrobiology: crop ecosystems. Springer, Berlin, pp 37–59. doi:10.1007/978-3-642-18357-7-2

Lopezdevictoria G, Lovell GR (1993) Chemotaxis of *Azospirillum* spp. to aromatic compounds. Appl Environ Microbiol 59:2451–2955

Malik AA, Suryapani S, Ahmad J (2011) Chemical Vs organic cultivation of medicinal and aromatic plants; the choice is clear. Int J Med Arom Plants 1(1):5–13

Priya (2010) Studies on the development of plant growth promoting rhizobacteria for the improvement of *Coleus forskohlii* and control of root rot disease. M. Phil, Thesis. Annamlalai University, Annnamalai Nagar

Rajasekar S, Elango R (2011) Effect of microbial consortium on plant growth and improvement of alkaloid content in *Withania somnifera* (Ashwagandha). Curr Bot 2(8):27–30

Reinhold B, Hurek T, Fendrik I (1985) Strain specific chemotaxis of *Azospirillum* spp. J Bacteriol 162:190–915

Somers E, Vandirleyden J, Srinivasan M (2004) Rhizosphere bacterial signalling: a love parade beneath our feet. Crit Rev Microbiol 30(4):205–240

WHO (2002) WHO Traditional medicine strategy 2002–2005 (on line) Geneva. http://www.who.int/medicines/library/trm/trm_strat_eng

Chapter 4
Rhizosphere Bacteria from Coastal Sand Dunes and Their Applications in Agriculture

Aureen Godinho and Saroj Bhosle

4.1 Introduction

Coastal sand dunes are a nutrient-limited ecosystem. Their types and vegetation vary from place to place.

4.1.1 Coastal Sand Dune Ecosystem

The word sand dune reflects the images of vast amount of shifting sand barren to plants and hostile to human habitation. Sand dunes are generally of two types. The first type is the extremely dry interior deserts such as Sahara in Africa or Rajasthan in India, and the other type is known as the coastal sand dunes which occur along the coasts of the Atlantic, Pacific, North America, and Australia. In Asia the coastal dunes occur in Japan, India, and several other countries (Desai and Untawale 2002; Boorman 1977; Carter 1998).

4.1.1.1 Sand Dune Vegetation

Vegetation plays a dominant role in determining the size, shape, and stability of fore dunes (Fig. 4.1). The aerial parts of the vegetation obstruct the wind and absorb wind energy. Wind velocity near vegetation is thus reduced below that needed for sand transport and hence the sand deposit around the vegetation. A characteristic of dune vegetation, particularly the grasses growing under these conditions, is its ability to produce upright stems and new roots in response to sand covering.

A. Godinho (✉) • S. Bhosle
Department of Microbiology, Goa University, Taleigao Plateau, Goa 403206, India
e-mail: aureengomes@gmail.com; sarojbhosle@yahoo.co.in

D.K. Maheshwari et al. (eds.), *Bacteria in Agrobiology: Crop Productivity*,
DOI 10.1007/978-3-642-37241-4_4,

Fig. 4.1 Beach grasses and shrubs growing on coastal sand dunes

The development of vegetation cover on newly formed dunes, if undisturbed, creates conditions which suit the colonization and growth of a wider range of plant species. Dead plants and litter from these plants add humus to the sand. The accumulation of humus results in improved moisture- and nutrient-holding capacity of developing dune soils. Thus, with lower surface temperature and increased moisture and nutrient content, the sand is able to support a great variety of plants (Desai and Untawale 2002).

4.2 Rhizosphere as a Site of Plant: Microbe Interactions

The rhizosphere is the portion of the soil under the direct influence of the roots of higher plants. It is considered the most intense ecological habitat in soil in which microorganisms are in direct contact with plant roots. The root system of all higher plants is associated with a distinct, diverse community of metabolically active soil microbiota that carries out biochemical transformations. Rhizosphere microorganisms may have specific associations with plants through which they exert their influence on plant growth. The production of biologically active metabolites, particularly the plant growth regulators by rhizosphere microbiota, is considered one of the most important mechanisms of action through which the rhizosphere microbiota affect plant growth directly after being taken up by the plant or indirectly by modifying the rhizosphere environment. The plant rhizosphere is a dynamic environment in which many factors may affect the structure and species composition of the microbial communities that colonize the roots. Microbial communities associated with the rhizosphere also vary depending on the plant species, soil type, and cultural practices such as crop rotation or tillage (Frankenberger and Arshad 1995; Davison 1988).

Bacteria can form close associations with roots within the root tissue itself, on the root surface (rhizoplane), and within the soil immediately adjacent to the root (rhizosphere). Inhabitants of these sites rely heavily for their energy supply on organic substances provided by the roots, and their growth is therefore related intimately to the metabolic activity of the plants involved (Gaskins et al. 1985). While many bacteria found in soil are bound to the surface of soil particles and are found in soil aggregates, a number of soil bacteria interact specifically with the

roots of plants. In fact, the concentration of bacteria(per gram of soil) that is found around the roots of the plants (i.e., in the rhizosphere) is generally much greater than the bacterial density, or concentration, that is found in the zone around the roots and can be used to support bacterial growth and metabolism (Glick 1995; Alexander 1977). The rhizobacteria respond to plant signals, exchange nutrients with plant cells, suffer damage due to plant defense responses, and colonize or even evade root tissues, creating pathologies or symbiosis as compared to the bacteria present in bulk soil. Mucigel provides the immediate environment for rhizobacteria; it consists of plant mucilage, bacterial exopolymers, and soil particles. Plant roots sheathed with mucigel have higher relative water content than do bare roots, and thus mucigel protects the root and associated microflora from dehydration (Miller and Wood 1996).

The constituents of root exudates play an important role in selecting and enriching the types of bacteria. Depending on the ability of the bacteria to utilize these as sources of energy, the bacterial community develops in the rhizosphere. Plant root exudate components serve as a source of carbon substrate for microbial growth; in addition they also contain chemical molecules that promote chemotaxis of microbes to the rhizosphere. Root exudates are supplemented in maintaining a steady concentration of flavonoids and mineral nutrients in the rhizosphere by the compounds released from the decomposition of organic matter such as dead roots and fallen leaves (Dakora and Phillips 2002). Thus, depending on the nature and concentrations of organic constituents of exudates, and the corresponding ability of the bacteria to utilize these as sources of energy, the bacterial community develops in the rhizosphere. Bacteria living in the soil are called free-living as they do not depend on root exudates for their survival, while rhizospheric bacterial communities have efficient systems for uptake and catabolism of organic compounds present in root exudates. Several bacteria have the ability to attach to the root surfaces (rhizoplane) allowing these to derive maximum benefit from root exudates. Some of these are more specialized, as they possess the ability to penetrate inside the root tissues (endophytes) and have direct access to organic compounds present in the apoplast. It is also known that some of the Plant growth-promoting rhizobacteria (PGPR) strains can colonize inside plant tissues, and bacterial strains that naturally exist in healthy plant tissues are referred to as "endophytes." Hallmann et al. (1997) defined endophytic bacteria as "bacteria that can be isolated from surface disinfested plant tissue or extracted from within the plant, and that do not visibly harm the plant." Most of the endophytes reported previously were isolated by maceration of surface-sterile plant tissues. Various endophytes have been isolated from agronomic crops and prairie plants (Halmann 1997; Weller 1988), and many of them have been utilized as microbial inoculants to control plant pathogens and promote plant growth. By occupying this privileged endophytic location, bacteria do not have to face competition from their counterparts as encountered in the rhizosphere or in soil. Such bacteria which influence the plant growth either directly or indirectly are termed as plant growth-promoting bacteria (PGPB). They inhabit majority of healthy and symptomless plants, in various tissues, seeds, roots, stems, and leaves (Johri 2006). Plants benefit

extensively by harboring these endophytic microbes; they promote plant growth (Compant et al. 2005) and confer enhanced resistance to various pathogens by producing antibiotics. Endophytes also produce unusual secondary metabolites of plant importance. It has been suggested that the presence of a mutualistic endophyte acts as a "biological trigger" to activate the stress response system more rapidly and strongly than nonmutualistic plants (Bandara et al. 2006).

4.3 Bacteria Associated with Sand Dune Vegetation

Little is known about the bacterial communities associated with the plants inhabiting sand dune ecosystems. Accelerated coastal erosion threatens private and public property in many areas of the world. Lost sand is replaced with material of compatible physical properties which is shaped to the desired beach profile and planted with pioneer species, such as *Ipomoea* and *Spinifex*, to enhance beach stability and begin the dune-building process. The major factors limiting establishment and early, vigorous growth of dune plants in the face of environmental extremes are infertility and the poor moisture-holding capacity of coarse replenishment materials. Rhizosphere microorganisms may allow beach grasses to overcome these environmental extremes (Will and Sylvia 1990). Plants are known to alter the composition of microbial communities associated with their roots (Grayston et al. 1996; Marschner et al. 2001). Plant roots in the soil represent a four-dimensional region, in space and time, of profuse activity relative to the bulk soil, revolving around pH, nutrient, redox potential, and exudate gradients changing as distance from the root increases (Marschner 1995). This region of gradients in chemical and physical factors strongly influenced by the presence of plant roots and characterized by high rates of microbial population and activity is referred to as the rhizosphere.

In 1904, Hiltner first defined the rhizosphere as ". . . that zone of soil in which the microflora are influenced by plant roots" (Kang and Mills 2004). This rhizosphere effect is primarily due to the influx of mineral nutrients to the plant roots through mass flow and diffusion, alongside the efflux and accumulation of plant root exudates. Microbial communities in the rhizosphere are primarily plant driven, responding with respect to density, composition, and activity to the abundance and diversity of plant-derived exudates, eventually leading to plant species-specific microflora. A substantial portion of the root exudates consist of carbon and energy sources readily available for microbial growth; by now it is clear that plant roots excrete amino acids, proteins, sugars, organic acids, vitamins, and other bacterium-beneficial substances affecting growth, development, and physiology of a microbial population. Low molecular weight plant-derived exudates, mainly amino acids, organic acids, and sugars commonly found in most plants, are rapidly utilized by microorganisms. In addition, high molecular weight root mucilage, consisting of approximately 95 % sugars and 5 % amino acids in the form of heteropolysaccharides and glycoproteins, also serve as a source of energy for rhizosphere bacteria (Somers et al. 2004). The exact composition of the exudates is determined by many

factors, including species and nutritional status of the plant, soil structure, and micronutrient status (Marschner 1995). Depending on the composition of the exudates secreted by a given plants' roots, that plant may be able to alter the physical and chemical properties of the soil, inhibit the propagation or growth of another plant species, withstand underground herbivory, enhance the possibilities and success of symbiotic relationships, and dictate, to some extent, the soil microbial community in the rhizosphere. In fact, most rhizosphere bacteria and fungi are highly dependent on associations with plants that are clearly regulated by root exudates (Bais et al. 2004), and in the rhizosphere numbers of microorganisms can reach 10^{10}–10^{12} organisms g^{-1} soil (Forster 1979). Plant–microbe symbioses have been exploited in programs of sand dune restoration. Plant-associated bacteria may increase the ability of plants to utilize nutrients from the soil by increasing root development, nitrate uptake, or solubilizing phosphorus and to control soil-borne pathogens (Smith and Read 1997; Whipps 2001).

In order to understand the effects of plant–bacteria interactions, it is essential to study the bacterial diversity associated with plants, and there have actually been a number of studies characterizing the structures and functions of rhizosphere and root bacterial communities (Hallmann et al. 1997; Mahaffee and Kloepper 1997; Maloney et al. 1997; Germida et al. 1998). Plants are known to alter the composition of microbial communities associated with their roots (Grayston et al. 1996; Marschner et al. 2001). Plant communities in sand dunes are controlled by the interaction between biotic and physicochemical components of the sand matrix (Read 1989). Interactions with microbes appear crucial in obtaining inorganic nutrients or growth-influencing substances. In addition, human activities may also be an important factor, as they will certainly affect the vegetation as well as plant–microbe interactions.

Dalton et al. (2004) suggested that the nitrogen-fixing bacteria isolated from the rhizosphere and root of *Ammophila arenaria* may contribute to the prolific success of these plants in nutrient-poor sand. Despite the important role played by bacterial diversity in sand dune plant communities, little is known on the distribution and abundance of root or rhizosphere associated bacteria. Park et al. (2005) first reported on the diversity of culturable bacteria associated with the two major sand dune plant species, *Calystegia soldanella* (beach morning glory) and *Elymus mollis* (wild rye), which are found as the dominant plant species along the coastal sand dune areas in Tae-An, Chungnam Province. While in another study carried out by Lee et al. (2006), bacterial diversity in the rhizosphere of beach morning glory (*Calystegia soldanella*) and wild rye (*Elymus mollis*), two of the major plant species inhabiting the coastal sand dune in Tae-An, Korea, was studied by the analysis of community 16S rRNA gene clones.

In our studies the seasonal variation of rhizosphere and endophytic bacteria associated with *Ipomoea pes-caprae* and *Spinifex littoreus* was studied. Based on the cultural, physiological, and biochemical characteristics, it was observed that among the neutrophiles, majority of the isolates belonged to *Bacillus* genus, while among the alkaliphiles, the majority of the isolates were gram-positive irregular rods belonging to genera such as *Brochothrix*, *Cellulomonas*, *Microbacterium*, and

Brevibacterium. Zinniel et al. (2002) identified *Cellulomonas, Clavibacter, Curtobacterium*, and *Microbacterium* as the most promising colonizing strains with four agronomic crop species. Karp and Nelson (2004) reported that the sand and soil root zones were dominated largely by gram-positive species, e.g., *Arthrobacter, Bacillus*, and *Microbacterium* species, as also observed during the present study. Soil rhizosphere communities consisted almost entirely of *Actinobacterium*, *Arthrobacter*, and *Bacillus* isolates, whereas sand root zones contained clones of a few gram-negative genera such as *Aminobacter*, *Chelatobacter*, *Ensifer*, and *Pseudomonas*. Smit et al. (2001) studied the bacterial diversity and dynamics in Lovinkhoeve soil samples the most dominant bacterial genera detected by plating appeared to be *Micrococcus* and *Arthrobacter*. These genera are often found in various soils, such as those of wheat fields, deciduous woodlands, grasslands, and sand dunes. Tiago et al. (2004) investigated the bacterial diversity in a nonsaline alkaline environment and reported that the majority of the isolates were related to *Microbacteriaceae* family members, while another set of isolates represented populations related to different species in the lineage of the *Micrococcaceae*, namely, *Micrococcus luteus*, *Citrococcus muralis*, and *Rothia dentocariosa*, and others were related to various species of the genera *Kocuria* and *Nesterenkonia*. Overall, it was observed that endophytic bacteria counts were higher than rhizosphere bacterial counts among the different bacterial groups. Interestingly, the total viable counts in unvegetated areas of sand dunes were lower than the vegetated areas as seen from the analysis of the samples collected from unvegetated area.

4.4 Plant Growth-Promoting Rhizobacteria

PGPR are naturally occurring, free-living soil bacteria that are capable of colonizing roots and enhancing plant growth when added to seeds or roots (Kloepper and Schroth 1978; Frankenberger and Arshad 1995). There are several ways in which plant growth-promoting bacteria can directly facilitate the proliferation of their host plants. They may fix atmospheric nitrogen and supply it to plants; solubilize minerals such as phosphorus; produce siderophores, which can solubilize and sequester iron and provide it to plants; and synthesize phytohormones, including auxins, cytokinins, and gibberellins, which can enhance various stages of plant growth. Indirect promotion of plant growth occurs when these bacteria decrease or prevent some of the deleterious effects of a pathogenic organism by any one or more of several different mechanisms including improving growth-restricting conditions either via production of antagonistic substances or by inducing resistance against plant pathogens (Kloepper 1993; Tilak et al. 2005). For example, production of antibiotics can interfere directly with growth and activity of deleterious soil microorganisms (Glick and Bashan 1997), whereas induction of resistance in the plant increases the plants defense capacity (VanLoon et al. 1998). In addition,

bacteria may reduce stresses resulting from the presence of toxic wastes by sequestering heavy metals or degrading organic pollutants.

There are 20 different biocontrol PGPR strains commercially available in the market at present. Biocontrol of plant pathogens is achieved through antibiotic synthesis; secretion of iron-binding siderophores to obtain soluble iron from the soil and provide it to a plant, thereby depriving fungal pathogens in the vicinity of soluble iron; production of low molecular weight metabolites such as hydrogen cyanide with antifungal activity; and production of enzymes including chitinase, β-1,3-glucanase, protease, or lipase which can lyse some fungal cells, outcompeting phytopathogens for nutrients and niches on the root surface (Penrose and Glick 2003). A particular bacterium may promote plant growth and development using any one, or more, of these mechanisms. For example, following seed germination a PGPR may lower the plants ethylene concentration thereby decreasing the ethylene inhibition of seedling root length. Once the seedling has depleted the resources that are contained within the seed, the same PGPR may help to provide the plant with iron and phosphorus from the soil. The impact of the mechanisms by which the bacteria provides a compound or nutrient such as fixed N, P, or Fe to the plant varies considerably depending upon the soil composition. Thus PGPR often have little or no measurable effect on plant growth when the plants are cultivated in nutrient-rich soil and grown under optimal conditions.

Further root-associated bacteria capable of fixing nitrogen occur regularly in diverse soils which vary widely in nitrogen content. Common genera capable of fixing nitrogen include *Azospirillum, Azotobacter, Bacillus, Clostridium*, *Derxia*, and *Klebsiella*. These are commonly designated "free-living" bacteria, since they are able to exist in the soil and reduce nitrogen without entering into symbiotic association with plants (Gaskins et al. 1985). Denitrification which transforms reduced nitrogen compounds into gaseous nitrogen allows return of nitrogen to the atmosphere from the soil. *Alcaligenes*, *Bacillus*, and *Pseudomonas* spp. are common types of denitrifying bacteria. The removal of soil nitrogen by denitrifying bacteria is normally considered detrimental to crop production, because in most instances nitrogen is the element which most severely limits plant growth. However, these bacteria are useful since they prevent nitrogen compounds from accumulating to toxic levels, particularly in poorly drained areas. Also, denitrification activity beneath the root zone is beneficial, since it reduces the nitrate load in groundwater. Denitrification tends to maintain a balance between soil and atmospheric nitrogen (Gaskins et al. 1985). Also the mechanism most often invoked to explain the various effects of plant growth-promoting bacteria on plants is the production of phytohormones most notably auxin. Auxins are a class of PGPR known to stimulate both rapid (e.g., increases in cell elongation) and long-term (e.g., cell division and differentiation) responses in plants. Diverse soil microorganisms including bacteria, filamentous fungi, and yeasts are capable of producing physiologically active quantities of auxins and which have pronounced effects on plant growth and development. L-Tryptophan (L-TRP) is considered as a physiological precursor of auxin biosynthesis in both higher plants and

microorganisms (Arshad and Frankenberger 1998). Since plants as well as plant growth-promoting bacteria can synthesize indoleacetic acid (IAA), it is important when assessing the consequences of treating a plant with a plant growth-promoting bacterium to distinguish between the bacterial stimulation of plant auxin synthesis on the one hand and auxin that is synthesized by the bacterium on the other. The level of auxin produced by a bacterium in the rhizosphere determines its effect on the host plant; high levels induce developmental abnormalities and stimulate formation of lateral and adventitious roots, while low levels promote root elongation (Van Loon and Glick 2004).

A number of different bacteria considered to be PGPR include *Azotobacter* spp., *Azospirillum* spp., *Pseudomonads, Acetobacter* spp., *Burkholderia* spp., *Bacillus*, *Alcaligenes*, *Klebsiella*, *Enterobacter*, *Herbaspirillum*, and *Xanthomonas* (Glick 1995). A number of bacterial species associated with the plant rhizosphere belonging to genera *Azospirillum*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, *Rhizobium*, and *Serratia* are able to exert a beneficial effect on plant growth (Tilak et al. 2005).

The PGPR play a significant role in supporting growth of plants. These bacteria possess traits which help in either improving the availability of the nutrients or inhibiting the pathogenic bacteria. The availability of nutrients is facilitated by production of siderophores, exopolysaccharides (EPS), and polyhydroxyalkanoates (PHAs).

4.5 Significant Plant Growth-Promoting Metabolites Produced by Sand Dune Rhizobacteria

Many scientists have evaluated the efficiency of isolated PGPR. Some of their significant mechanisms have been enlisted below.

4.5.1 ACC Deaminase

Ethylene, which is produced in almost all plants, mediates a range of plant responses and developmental steps. Ethylene is involved in seed germination, tissue differentiation, formation of root and shoot primordia, root elongation, lateral bud development, flowering initiation, anthocyanin synthesis, flower opening and senescence, fruit ripening and degreening, production of volatile organic compounds responsible for aroma formation in fruits, storage product hydrolysis, leaf and fruit abscission, and the response of plants to biotic and abiotic stresses (Frankenberger and Arshad 1995). In some instances ethylene is stimulatory, while in others it is inhibitory. The increased level of ethylene formed in response to trauma inflicted by temperature extremes, water stress, ultraviolet light, chemicals,

mechanical wounding, insect damage, and disease can be both the cause of some of the symptoms of stress (e.g., onset of epinastic curvature and formation of aerenchyma) and the inducer of responses, which will enhance survival of the plant under adverse conditions (e.g., cell wall strengthening, production of phytoalexins, and synthesis of defensive proteins).

1-Aminocyclopropane-1-carboxylate (ACC), the cyclopropanoid amino acid, is a precursor in the biosynthetic pathway of the plant hormone ethylene. Plant growth-promoting soil bacteria have been found to contain ACC deaminase (ACCD), a PLP-dependent enzyme that converts ACC to a ketobutyrate and ammonium. Introduction of ACCD in higher plants by gene modification technology reduced the production of ethylene and delayed ripening of fruits. *Pseudomonas putida* UW4, a novel ACCD-containing bacterium, has been shown to promote plant growth under different environmental stresses including flooding, drought, and the presence of heavy metals and phytopathogens. The possibility of a close mutualistic relationship between the plants and the soil bacteria has been suggested and the role of ACCD in ensuring low levels of ethylene at critical stages of root growth has been proposed by Hontzeas et al. 2004a, b). The enzyme ACC deaminase is important as this enzyme can cleave the plant ethylene precursor ACC and thereby lowers the level of ethylene in a developing or stressed plant. A burst of ethylene is required to break seed dormancy for many plants, but following germination a sustained high level of ethylene would inhibit root elongation. PGPR that contain the enzyme ACC deaminase when bound to the seed coat of a developing seedling act as a mechanism for ensuring that the ethylene level does not become elevated to the point where crucial root growth is impaired. By facilitating the formation of longer roots, these bacteria may enhance the survival of some seedlings especially during the first few days after the seeds are planted.

Thus, plant growth-promoting bacteria are supplied with a unique additional source of nitrogen in the form of ACC that enables them to proliferate under conditions in which other soil bacteria may not flourish, for instance, when nitrogen availability is low and competition for nutrients is intense. As a result of lowering the ACC level within the plant, either the endogenous level or the IAA-stimulated level, the amount of ethylene in the plant is also reduced. Plant growth-promoting bacteria that possess the enzyme ACC deaminase and are bound to seeds or roots of seedlings can reduce the amount of plant ethylene and the extent of its inhibition on root elongation. Thus, these plants should have longer roots and possibly longer shoots as well, stem elongation is also inhibited by ethylene, except in ethylene-resistant plants (Van Loon and Glick 2004).

4.5.2 Auxins

One of the direct mechanisms by which PGPR promote plant growth is by production of plant growth regulators or phytohormones (Glick 1995). Frankenberger and Arshad (1995) have discussed in detail the role of auxins, cytokinins, gibberellins,

ethylene, and abscisic acids (ABA) which, when applied to plants, help in increasing plant yield and growth. Microbial production of individual phytohormones such as auxins and cytokinins has been reviewed by various authors over the last 20 years (Pilet et al. 1979; Hartmann et al. 1983; Fallik and Okon 1989; Barbieri and Galli 1993; Patten and Glick 1996, 2002). Auxins are a class of plant hormones and one of the most common and well characterized is indoleacetic acid (IAA), which is known to stimulate both rapid (e.g., increases in cell elongation) and long-term (e.g., cell division and differentiation) responses in plants (Glick 1995). Some of the plant responses to auxin are as follows: (a) cell enlargement, (b) cell division, (c) root initiation, (d) root growth inhibition, (e) increased growth rate, (f) phototropism, (g) geotropism, and (h) apical dominance (Frankenberger and Arshad 1995; Leveau and Lindow 2005). Most notably, exogenous auxin production by bacteria has been associated with altered growth of the roots of plants on which they were inoculated. While many plant growth-promoting bacteria, which stimulate the growth of roots, can produce at least small amounts of the auxin indole-3-acetic acid (IAA), high IAA producers are inhibitory to root growth (Lindow et al. 1998). Bacterial IAA producers (BIPs) have the potential to interfere with any of these processes by input of IAA into the plant's auxin pool (Leveau and Lindow 2005).

IAA is a common product of L-tryptophan metabolism by several microorganisms including PGPR. Promotion of root growth is one of the major markers by which the beneficial effect of plant growth-promoting bacteria is measured. Rapid establishment of roots, whether by elongation of primary roots or by proliferation of lateral and adventitious roots, is advantageous for young seedlings as it increases their ability to anchor themselves to the soil and to obtain water and nutrients from their environment, thus enhancing their chances for survival (Patten and Glick 2002).

Bacteria belonging to the genera *Azospirillum*, *Pseudomonas*, *Xanthomonas*, and *Rhizobium* as well as *Alcaligenes faecalis*, *Enterobacter cloacae*, *Acetobacter diazotrophicus*, and *Bradyrhizobium japonicum* have been shown to produce auxins which help in stimulating plant growth (Patten and Glick 1996).

4.5.3 Hydrogen Cyanide

Cyanide is a potential inhibitor of enzymes involved in major plant metabolic processes including respiration, CO_2 and nitrate assimilation, and carbohydrate metabolism and may also bind with the protein plastocyanin to block photosynthetic electron transport (Grossman 1996). HCN is a potent inhibitor of cytochrome c oxidase and of several other metalloenzymes—some of them involved in respiratory processes. HCN biosynthesis is catalyzed by HCN synthase, from glycine, with stoichiometric production of CO_2. HCN affects sensitive organisms by inhibiting the synthesis of ATP mediated by cytochrome oxidase and is highly toxic to all aerobic microorganisms at picomolar concentrations (Pal and McSpadden 2006). No role is known for HCN in primary bacterial metabolism, and it is generally

considered as a secondary metabolite (Blumer and Haas 2000). HCN-producing bacteria can help plants in their defense against fungal pathogens (Voisard et al. 1989; Blumer and Haas 2000). This property was predominantly described among *Pseudomonas* strains (Kremer and Souissi 2001). Therefore depending on the target organisms, HCN-producing microorganisms are regarded as harmful when they impair plant health and beneficial when they suppress unwanted components of the microbial community (Bellis and Ercolani 2001).

Hydrogen cyanide production is a physiological activity which is energetically dependent on the availability of organic carbon sources and low oxygen pressure, i.e., conditions which commonly prevail in the rhizosphere (Tarnawski et al. 2006). It has been shown that cyanide released by *Pseudomonas fluorescens* suppresses the growth of microorganisms (e.g., phytopathogenic bacteria and fungi) sharing the same ecological niche (e.g., the rhizosphere), thereby acting as a biocontrol metabolite (Voisard et al. 1989). Hence, cyanide production would increase the biological fitness by providing cyanogenic species with a selective advantage over competitors (Haas and Défago 2005; Voisard et al. 1989). The production of HCN by certain fluorescent pseudomonads is believed to be involved in the suppression of root pathogens. *P. fluorescens* CHA0 produces antibiotics, siderophores, and HCN, but suppression of black rot of tobacco caused by *Thielaviopsis basicola* appeared to be primarily due to HCN production (Pal and McSpadden 2006).

4.5.4 Ammonia

Biological N_2-fixation (BNF) by soil microorganisms is considered one of the major mechanisms by which plants benefit from the association of micropartners. One of the benefits that diazotrophic microorganisms provide to plants is fixed nitrogen in exchange for fixed carbon released as root exudates (Glick 1995). Many of the PGPR described to date are free-living diazotrophs that can convert molecular nitrogen into ammonia in a free state by virtue of the nitrogenase enzyme complex (Postgate 1982; Saikia and Jain 2007). Raj Kumar and Lakshmanan (1995) suggested that ammonia excretion seems to be the result of nitrogenase activity in symbiotic associations where relatively large amounts of atmospheric N reach the plant as ammonia released by the bacteroids. By contrast, most of the ammonia produced in PGPB by the nitrogenase-catalyzed N_2 fixation would be assimilated by the rhizobacteria through the glutamine synthetase/glutamate synthase (GS/GOGAT) pathway. Also plant growth-promoting bacteria contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase and this enzyme can cleave the ethylene precursor ACC to α-ketobutyrate and ammonia and thereby lower the level of ethylene in developing or stressed plants (Hontzeas et al. 2004a, b).

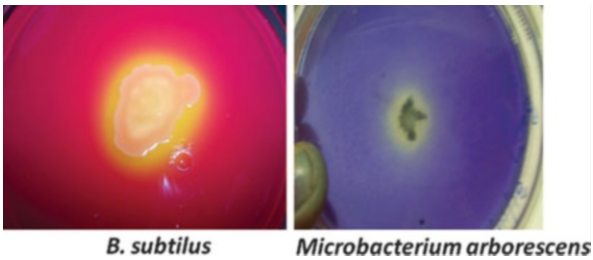


Fig. 4.2 Plate assays of P solubilizers *Bacillus subtilis* (Pikovskaya medium incorporated with *phenol red dye*) and *Microbacterium arborescens* (Pikovskaya medium incorporated with *bromothymol blue dye*) isolated from rhizosphere of coastal sand dune plants

4.5.5 Phosphate Solubilization

Bacteria isolated from the rhizosphere are capable of increasing availability of phosphorus to plants either by mineralization of organic phosphate or by solubilization of inorganic phosphate by production of acids (Lifshitz et al. 1987). These bacteria referred to as phosphobacteria and have been considered to have potential use as bioinoculants.

Many soil microorganisms are able to solubilize "unavailable" forms of calcium-bound P through their metabolic activity by excreting organic acids which either directly dissolve rock phosphate or chelate calcium ions to bring P into solution (Fig. 4.2). The production of microbial metabolites results in a decrease in soil pH, which probably plays a major role in solubilization. Besides changes in pH, chelation by organic acids which bind phosphate anions also brings about phosphate in soil solution. Soil inoculation with phosphate solubilizing bacteria has been shown to improve solubilization of fixed soil P and applied phosphates resulting in higher crop yields (Nautiyal et al. 2000).

4.5.6 Exopolysaccharide

Exopolysaccharide is a term first used by Sutherland to describe high molecular weight carbohydrate polymers produced by marine bacteria. EPS can be found as capsular material that closely surrounds a bacterial cell or as a dispersed slime in the surrounding environment with no obvious association to any one particular cell (Sutherland 1982; Decho 1990). In the natural environment bacteria occur mostly in aggregates whose structural and functional integrity is based on the presence of a matrix of extracellular polymeric substance. Thus EPS production seems to be important for their survival (Sutherland 1982).

Production of exopolymeric substances especially EPS by bacteria is one of the mechanisms to overcome desiccation. The rate of drying within the colony microenvironment is slower with EPS and helps increase bacterial survival by increasing

Fig. 4.3 Viscous exopolymer produced by sand dune rhizobacteria *M. arborescens*

the time available for metabolic adjustment. Further an EPS matrix provides another advantage to bacteria living within it as decreasing water content of soil restricts diffusion of nutrients to microorganisms. Polysaccharides being hygroscopic maintain higher water content in the colony microenvironment than in the bulk soil as water potential declines. This increase in water content could increase nutrient availability within the bacterial colony. Roberson and Firestone (1992) revealed that bacteria respond to desiccation by channeling energy and nutrients into polysaccharide production. Soil is an extremely heterogeneous environment, and wetting and drying may not proceed uniformly throughout it, and any microbial processes in soil depend on this heterogeneity. Godinho and Bhosle (2009) studied the aggregation of sanddune soils by exopolysaccharide-producing *Microbacterium arborescens*, a sand dune rhizobacterial isolate (Fig. 4.3). It was observed that the rhizosphere and endophytic bacteria associated with the sand dune plants may be playing an important role in aiding in the survival of these plants in the sand dunes. Coastal sand dunes is a previously unexplored habitat for EPS-producing bacteria. These exopolymeric substances might be involved in ecological roles, protecting the cells against dessication especially in nutrient-limited environments such as the coastal sand dunes more so in the extreme conditions of pH. Such polysaccharides may be helping the bacteria to adhere to solid substrates and survive during nutrient limited conditions.

4.5.7 Siderophores

Iron is the fourth most abundant element on earth, but in the presence of oxygen and at neutral pH, it is not sufficiently available to microbes due to the rapid oxidation of Fe^{+2} to Fe^{+3} and the formation of ferric hydroxides and oxyhydroxide polymers (Neilands 1995). Concentration of free iron in soil under these conditions is as low as 10^{-17M}, which is much less than that required for optimal growth of soil microflora (Guerinot et al. 1990). A large number of proteins require iron for their activity, which underlines the importance of iron for living organisms. The iron that is present in proteins can exist in several different forms: heme, iron–sulfur, iron–nickel, di-iron, and mononuclear iron (Andrews 1998).

Iron is made biologically available by iron-chelating compounds called siderophores that are synthesized and secreted by many bacteria and fungi under conditions of iron limitation (Fig. 4.4) (Neilands 1995). Siderophores are water-soluble, low molecular weight molecules that are secreted by bacteria and fungi. The term

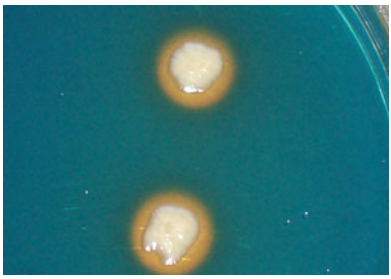


Fig. 4.4 The *yellow orange halo* surrounding the bacterial colony is indicative of the production of an Fe-binding compound such as siderophore, which removes Fe(III) from the Fe(III)–CAS–HDTMA complex in the plate and turns the *blue dye* to *yellow color*. The bacteria were isolated from the rhizosphere of sand dune plants

siderophore stands for "iron carriers" or "iron bearers" in Greek. This is an appropriate term because the siderophore binds iron with an extremely high affinity and is specifically recognized by a corresponding outer membrane receptor protein, which in turn actively transports the complex into the periplasm of the cell (Braun and Braun 2002; Gomez and Sansom 2003) or which imparts specificity of uptake and works in association with periplasmic iron-binding proteins and cytoplasmic membrane-associated proteins (Gomez and Sansom 2003). The molecular weights of siderophores range from approximately 600 to 1,500 Da, and because passive diffusion does not occur for molecules greater than 600 Da, siderophores must be actively transported (Ishimaru 1993). The role of these compounds is to scavenge iron from the environment and to make the mineral, which is almost always essentially present available to the microbial cell (Neilands 1995). There are more than 500 different types of siderophores produced by bacteria, yeasts, and fungi. Siderophores are produced and secreted only when the amount of iron is low in the growth environment. The genes involved in siderophore production regulate siderophore production based on the concentration of iron in the environment. That is, siderophore production is shut off when iron is present at sufficient concentration and vice versa.

Siderophores specifically bind to ferric ion with high affinity. The binding power of the siderophore for iron has a stability constant range from 10^{22} to 10^{50} (Ratledge and Dover 2000). This range is sufficiently high for the removal of iron attached to molecules like ferritin and transferrin by siderophore, but not high enough for the removal of iron present in heme proteins. Siderophore molecules display considerable structural variation but can be classified as either hydroxamates or catechols. Structurally, 20 siderophores are ring- or semiring-shaped structures containing oxygen atoms. Siderophores show high affinity for ferric ion, since the oxygen atoms present can form coordination bonds with a single Fe(III) ion (Neilands 1995). The production of siderophores has been reported in aerobic and facultative anaerobic microbes, but their production has not yet been reported in strict anaerobes, lactic acid bacteria, or in higher organisms such as plants and animals. The main function of siderophores is involved in the high affinity acquisition and receptor-dependent transport of ferric ion. Siderophores are also associated with growth or germination factors and virulence factors.

In gram-negative bacteria, Fe^{+3} siderophores bind to highly specific receptor proteins and are then transported into the cytoplasm (Faraldo-Gomez and Sansom 2003), while in gram-positive bacteria, which lack an outer membrane, the receptors are binding proteins that are anchored to the cytoplasmic membrane by a covalently linked lipid. A periplasmic transport protein and several inner membrane-associated proteins complete the transport of iron into the cell. This arrangement of proteins from periplasm to cytoplasm is similar to other bacterial periplasmic protein-dependent systems, termed ABC transporters (for ATP-binding cassette-type transport), which transport amino acids, peptides, and sugars into the cell (Braun and Killman 1999; Clarke et al. 2001; Fatht and Kolter 1993).

4.5.8 Resting Bodies

Bacteria have also evolved numerous mechanisms of resistance to stress conditions and nutrient limitations. For example, many microorganisms have an inherent ability to form resting stages (e.g., cysts and spores). Even without the formation of such elaborately differentiated cells, bacteria enter starvation-induced programs that allow them to survive long periods of nongrowth and to restart growth when nutrients become available again. This often leads to the formation of metabolically less active cells that are more resistant to a wide range of environmental stresses. This adaptation to starvation conditions is often accompanied by a change in cell size as well as the induction of genes and the stabilization of proteins that are essential for long-term survival. The best-studied examples of starvation–survival in nondifferentiating bacteria are *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio* sp. strain S14, which show qualitative similarities in their survival responses (Madison and Huisman 1999).

4.5.9 Nutrient Availability

Nutrients may become available locally, for example, in decaying plant and animal material or via plant roots, which are one of the major sites of carbon input into soil. The rhizosphere therefore is a soil region with a transiently high availability of carbon in a form readily available to soil bacteria. Soil bacteria that have evolved in close association with plants, such as rhizobia and pseudomonads, benefit from being able to quickly escape the starvation state and colonize the plant root. The accumulation of intracellular storage polymers is another bacterial strategy that increases survival in a changing environment. Poly(3-hydroxyalkanoates) (PHAs) are accumulated as discrete granules to levels as high as 90 % of the cell dry weight and are generally believed to play a role as a sink for carbon and reducing equivalents. The bacterial origin of PHAs makes these polyesters a natural material,

and microorganisms have evolved ability to degrade these macromolecules (Madison and Huisman 1999).

In bacteria, PHAs constitute a major carbon and energy storage material, which accumulates when a carbon source is provided in excess and another nutrient (such as nitrogen, sulfur, phosphate, iron, magnesium, potassium, or oxygen) is limiting. The polymerization of soluble intermediates into insoluble molecules does not change the osmotic state of the cell, thereby avoiding leakage of these nutrient-rich compounds out of the cell. In addition, PHA-producing bacteria have the advantage of nutrient storage at a relatively low maintenance cost and with a secured return of energy (Berlanga et al. 2006). PHAs produced by these bacteria are important due to their biodegradability, water resistance, and oxygen permeability. Their applications are varied; they are used for all sorts of biodegradable packaging materials (Thakor et al. 2006).

In our study since the bacteria were isolated from coastal ecosystem, we evaluated their growth-promoting ability under a similar ecosystem. Eggplant was selected as a model plant as it is popularly grown in Goa. The four sand dune bacterial isolates chosen for the study were *B. subtilis, M. arborescens, K. rosea*, and *B. subtilis* sp. *MF-4* shows good ACC deaminase activity, HCN production, IAA and siderophore production, and phosphate solubilization. Results of pot studies indicated that *K. rosea* and *B. subtilis* increased shoot length and weight of the plants consistently up to 44 DAS. However *Bacillus* sp. *MF-A4* increased the growth significantly from 37 DAS after sowing, while *M. arborescens* was effective in the latter stages (at 44DAS). The study confirmed the bioprospects of using the sand dune bacteria as biofertilizers for agricultural crops (Godinho et al. 2010).

4.6 Concluding Remarks and Future Perspectives

This chapter contributes significantly to the knowledge of the wide occurrence of effective PGPR bacteria associated with sand dune vegetation in the ecosystem. A large number of bacteria are associated with rhizosphere and as endophytes with vegetation growing on coastal sand dunes. Such organisms are shown to play a role in promoting growth of plants by making the soils available with nutrients. Plant growth-promoting characteristics of promising isolates studied indicated that native plant growth-promoting microorganisms with properties such as phosphate solubilization, disease control potential, and rhizosphere colonization would seem ideal for selection as a suitable bioinoculant. The cultures were found to produce siderophores, solubilize inorganic phosphates, ammonia, hydrogen cyanide, and indole-3-acetic acid. All these metabolites are important for plant growth promotion. They were all found to utilize ACC as a sole source of nitrogen further confirming the presence of ACC deaminase enzyme.

Plant growth-promoting sand dune rhizobacteria therefore present an alternative to the use of chemicals for plant growth enhancement in many different applications. This research work has demonstrated that sand dune rhizobacteria

could have an important role in agriculture and horticulture in improving crop productivity. Among the four sand dune bacterial isolates, *B. subtilis*, *K. rosea*, *and M. arborescens* were found to have a significant effect on plant growth promotion of eggplant, an agriculturally important crop.

Acknowledgment The authors are grateful to Ministry of Earth Sciences for the financial assistance provided to carry out this research work.

References

Alexander M (1977) Introduction to soil microbiology. Wiley, New York

Andrews SC (1998) Iron storage in bacteria. Adv Microb Physiol 40:281–351

Arshad M, Frankenberger WT Jr (1998) Plant growth-regulating substances in the rhizosphere: microbial production and functions. Adv Agron 62:45–151

Bais HP, Park SW, Weir TL, Callaway RM, Vivanco JM (2004) How plants communicate using the underground information superhighway. Trends Plant Sci 9(1):26–32

Bandara WM, Seneviratne MSG, Kulasooriya SA (2006) Interactions among endophytic bacteria and fungi: effects and potentials. J Biosci 31(5):645–650

Barbieri P, Galli E (1993) Effect on wheat root development of inoculation with an *Azospirillum brasilense* mutant with altered indole-3-acetic acid production. Res Microbiol 144:69–75

Berlanga M, Montero MT, Hernández-Borrell J, Guerrero R (2006) Rapid spectrofluorometric screening of poly-hydroxyalkanoate producing bacteria from microbial mats. Int Microbiol 9:95–102

Blumer C, Haas D (2000) Mechanism, regulation, and ecological role of bacterial cyanide biosynthesis. Arch Microbiol 173:170–177

Boorman AL (1977) Sand dunes. In: Barnes KSR (ed) The coastline. Wiley, New York, pp 161–197

Braun V, Braun M (2002) Active transport of iron and siderophore antibiotics. Curr Opin Microbiol 5:194–201

Braun V, Killman H (1999) Bacterial solutions to the iron-supply problem. Trends Biochem Sci 24:104–109

Carter RWG (1998) "Coastal dunes". Coastal environments: an introduction to the physical, ecological and cultural systems of coastlines. Academic, London

Clarke TE, Tari LW, Vogel HJ (2001) Structural biology of bacterial iron uptake systems. Curr Top Med Chem 1:7–30

Compant S, Nowak BJ, Clément C, Ait Barka E (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959

Dakora FD, Phillips DA (2002) Root exudates as mediators of mineral acquisition in low nutrient environments. Plant and Soil 245:35–47

Dalton DA, Kramer S, Azios N, Fusaro S, Cahill E, Kennedy C (2004) Endophytic nitrogen fixation in dune grasses (*Ammophila arenaria* and *Elymus mollis*) from Oregon. FEMS Microbiol Ecol 49:469–479

Davison J (1988) Plant beneficial bacteria. Biotechnology 6:282–286

De Bellis P, Ercolani GL (2001) Growth interactions during bacterial colonization of seedling rootlets. Appl Environ Microbiol 67(4):1945–1948

Decho AW (1990) Microbial exopolymer secretions in ocean environment, their roles in food webs and marine processes. Oceanog Mar Biol Ann Rev 28:73–153

Desai KN, Untawale AG (2002) Sand dune vegetation of Goa; conservation and management. Botanical Society of Goa

Fallik E, Okon Y (1989) Identification and quantification of IAA and IBA in *Azospirillum brasilense* inoculated maize roots. Soil Biol Biochem 21:147–153

Faraldo-Gómez JD, Sansom MS (2003) Acquisition of siderophores in gram negative bacteria. Nat Rev Mol Cell Biol 4:105–116

Fatht MJ, Kolter R (1993) ABC transporters: bacterial exporters. Microbiol Rev 53(4):995–1017

Forster SM (1979) Microbial aggregation of sand in an embryo dune system. Soil Biol Biochem 11:537–543

Frankenberger WT Jr, Arshad M (1995) Phytohormones in soil: microbial production and function. Dekker, New York, p 503

Gaskins MH, Albrecht SL, Hubbell DH (1985) Rhizosphere bacteria and their use to increase plant productivity: a review. Agric Ecosyst Environ 12:99–116

Germida JJ, Siciliano SD, Freitas JR, Seib AM (1998) Diversity of root-associated bacteria associated with field grown canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.). FEMS Microbiol Ecol 26:43–50

Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117

Glick BR, Bashan Y (1997) Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of phytopathogens. Biotechnol Adv 15:353–378

Godinho AL, Bhosle S (2009) Sand aggregation by exopolysaccharide-producing microbacterium arborescens–AGSB. Curr Microbiol 58(6):616–621

Godinho A, Ramesh R, Bhosle S (2010) Bacteria from sand dunes of Goa promoting growth in eggplant. World J Agric Sci 6(5):555–564

Grayston SJ, Vaughan D, Jones D (1996) Rhizosphere carbon flow in trees, in comparison to annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Appl Soil Ecol 5(1):29–56

Grossman K (1996) A role for cyanide, derived from ethylene biosynthesis, in the development of stress symptoms. Physiol Planta 97:772–775

Guerinot ML, Meidl EJ, Plessner O (1990) Citrate as a siderophore in *Bradyrhizobium japonicum*. J Bacteriol 172:3298–3303

Haas D, Défago G (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3:307–319

Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW (1997) Bacteria endophytes in agricultural crops. Can J Microbiol 43:895–914

Hartmann A, Singh M, Klingmüller W (1983) Isolation and characterization of *Azospirillum* mutants excreting high amounts of indoleacetic acid. Can J Microbiol 29:916–923

Hiltner L (1904) Über neuere Erfahrungen und Probleme auf dem Gebiete der Bodenbakteriologie unter besonderer Berücksichtigung der Gründüngung und Brache. Arb DLG 98:59–78

Hontzeas N, Richardson AO, Belimov A, Safronova V, Abu-Omar MM, Glick BR (2004a) Evidence for horizontal transfer of 1-aminocyclopropane-1-carboxylate deaminase genes. Appl Environ Microbiol 71(11):7556–7558

Hontzeas N, Zoidakis J, Glick BR, Bu-Omar AMM (2004b) Expression and characterization of 1-aminocyclopropane-1-carboxylate deaminase from the rhizobacterium *Pseudomonas putida* UW4: a key enzyme in bacterial plant growth promotion. Biochim Biophys Acta 1703:11–19

Ishimaru CA (1993) Biochemical and genetic analysis of siderophores produced by plant-associated *Pseudomonas* and *Erwinia* species. In: Barton LB, Hemming BC (eds) Iron chelation in plants and soil microorganisms. Academic, New York

Johri BN (2006) Endophytes to the rescue of plants. Curr Sci 90:1315–1316

Kang S, Mills AL (2004) Soil microbial community structure changes following disturbance of the overlying plant community. Soil Sci 169(1):55–65

Karp MA, Nelson EB (2004) Bacterial communities associated creeping bentgrass in soil and sand rootzones. USGA Turfgrass Environ Res Online 3(24):1–19

Kloepper JW (1993) Plant growth-promoting rhizobacteria as biological control agents. In: Metting FB (ed) Soil microbial ecology–applications in agricultural and environmental management. Dekker, NewYork, pp 255–274

Kloepper JW, Schroth MN (1978) Plant growth promoting rhizobacteria on radishes. In: Proceedings of the 4th international conference on plant pathogenic bacteria. Station de Pathologic Vegetal et Phytobacteriologi, vol 2. Angers, France, pp 879–882

Kremer RJ, Souissi T (2001) Cyanide production by rhizobacteria and potential for suppression of weed seedling growth. Curr Microbiol 43:182–186

Lee MS, Do JO, Park MS, Jung S, Lee KH, Bae KS, Park SJ, Kim SB (2006) Dominance of *Lysobacter* sp. In the rhizosphere of two coastal sand dune plant species, *Calystegia soldanella* and *Elymus mollis*. Antonie Van Leeuwenhoek 90:19–27

Leveau JHJ, Lindow SE (2005) Utilization of the plant hormone indole-3-acetic acid for growth by *Pseudomonas putida* strain 1290. Appl Environ Microbiol 71(5):2345–2371

Lifshitz R, Kloepper JM, Kozlowski M, Simonson C, Carlso J, Tipping EM, Zaleska I (1987) Growth promotion of canola (rapeseed) seedlings by a strain of *Pseudomonas putida* under gnotobiotic conditions. Can J Microbiol 33:390

Lindow SE, Desurmont C, Elkins R, McGourty G, Clark E, Brandl MT (1998) Occurrence of indole-3-acetic acid producing bacteria on pear trees and their association with fruit russet. Phytopathology 88:1149–1157

Madison LL, Huisman GW (1999) Metabolic engineering of poly(3-hydroxyalkanoates): from DNA to plastic. Microbiol Mol Biol Rev 63(1):21–53

Mahaffee WF, Kloepper JW (1997) Bacterial communities of the rhizosphere and endorhiza associated with field-grown cucumber plants inoculated with a plant growth-promoting rhizobacterium or its genetically modified derivative. Can J Microbiol 43:344–353

Maloney PE, Van Bruggen AHC, Hu S (1997) Bacterial community structure in relation to the carbon environment in lettuce and tomato rhizospheres and bulk soil. Microb Ecol 34:109–117

Marschner H (1995) Mineral nutrition of higher plants. Academic Press International, San Diego, CA

Marschner P, Yang CH, Lieberei R, Crowley DE (2001) Soil plant specific effects on bacterial community composition in the rhizosphere. Soil Biol Biochem 33:1437–1445

Miller KJ, Wood JM (1996) Osmoadaptation by rhizosphere bacteria. Annu Rev Microbiol 50:101–136

Nautiyal BP, Prakash V, Nautiyal MC (2000) Structure and diversity pattern along an altitudinal gradient in an alpine meadow of Madhyamaheshwar, Garhwal Himalaya, India. Indian J Environ Sci 4:39–48

Neilands JB (1995) Siderophores: structure and function of microbial iron transport compounds. J Biol Chem 270:26723–26726

Pal KK, McSpadden G (2006) Biological control of plant pathogens. The Plant Health Instructor

Park MS, Jung S, Lee MS, Kim KO, Do JO, Lee KH, Kim SB, Bae KS (2005) Isolation and characterization of bacteria associated with two sand dune plant species, *Calystegia soldanella* and *Elymus mollis*. J Microbiol 43(3):219–227

Patten C, Glick BR (1996) Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol 42:207–220

Patten CL, Glick BR (2002) Regulation of indoleacetic acid production in *Pseudomonas putida* GR12-2 by tryptophan and stationary-phase sigma factorRpoS. Can J Microbiol 48:635–642

Penrose DM, Glick BR (2003) Methods for isolating and characterizing ACC deaminase containing plant growth promoting bacteria. Physiol Plant 18:10–15

Pilet PE, Elliott MC, Moloney MM (1979) Endogenous and exogenous auxin in the control of root growth. Planta 146:405–408

Postgate JR (1982) Biological nitrogen fixation: fundamentals. Philos Trans R Soc Lond B 296:375–385

Raj Kumar K, Lakshmanan M (1995) Influence of temperature on the survival and nitrogen fixing ability of *Azotobacter chroococcum* beij. Indian J Microbiol 35:25–30

Ratledge C, Dover LG (2000) Iron Metabolism in Pathogenic Bacteria. Annu Rev Microbiol 54:881–941

Read DJ (1989) Mycorrhizas and nutrient cycling in sand dune ecosystems. In: Gimmingham CH, Ritchie W, Willetts BB, Willis AJ (eds) Coastal sand dunes. Proceedings of the Royal Society of Edinburgh, Edinburgh, vol 96, pp 89–110

Roberson EB, Firestone MK (1992) Relationship between desiccation and exopolysaccharide production in a soil *Pseudomonas* sp. Appl Environ Microbiol 58:1284–1291

Saikia SP, Jain V (2007) Biological nitrogen fixation with non-legumes: an achievable target or a dogma? Curr Sci 92(3)

Smit E, Leeflang P, Gommans S, Broek J, Mil S, Wernars K (2001) Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. Appl Environ Microbiol 67(5):2284–2291

Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic, San Diego, CA

Somers E, Vanderleyden J, Srinivasan M (2004) Rhizosphere bacterial signalling: a love parade beneath our feet. Crit Rev Microbiol 30:205–240

Sutherland IW (1982) Biosynthesis of microbial exopolysaccharides. Adv Microb Physiol 23:79–150

Tarnawski S, Hamelin J, Jossi M, Aragno M, Fromin N (2006) Phenotypic structure of Pseudomonas populations is altered under elevated pCO2 in the rhizosphere of perennial grasses. Soil Biol Biochem 38:1193–1201

Thakor N, Trivedi U, Patel KC (2006) Microbiological and biotechnological aspects of biodegradable plastics: polyhydroxyalkanoates. Indian J Biotechnol 5(2):137–147

Tiago I, Chung A, Ver´ssimo A (2004) Bacterial diversity in a non saline alkaline environment: heterotrophic aerobic populations. Appl Environ Microbiol 70:7378–7387

Tilak KVBR, Ranganayaki N, Pal KK, De R, Saxena AK, Nautiyal CS, Mittal S, Tripathi AK, Johri BN (2005) Diversity of plant growth and soil health supporting bacteria. Curr Sci 89:136–150

Van Loon LC, Glick BR (2004) Increased plant fitness by rhizobacteria. In: Sandermann H (ed) Molecular ecotoxicology of plants, ecological studies, vol 170., pp 177–205

VanLoon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol 36:453–483

Voisard C, Keel C, Haas D, Défago G (1989) *Cyanide production by Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. EMBO J 8:351–358

Weller DM (1988) Biological control of soil borne plant pathogens in the rhizosphere with bacteria. Annu Rev Phytopathol 26:379–407

Whipps JM (2001) Microbial interaction and biocontrol in the rhizosphere. J Exp Bot 52:487–511

Will ME, Sylvia DM (1990) Interaction of rhizosphere bacteria, fertilizer, and vesicular-arbuscular mycorrhizal fungi with sea oats. Appl Environ Microbiol 56(7):2073–2079

Zinniel DK, Lambrecht P, Harris NB, Feng Z, Kuczmarski D, Higley P, Ishimaur CA, Arunakumari A, Barletta RG, Vidaver AK (2002) Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. Appl Environ Microbiol 68 (5):2198–2208

Chapter 5
Plant-Associated Bacteria in Nitrogen Nutrition in Crops, with Special Reference to Rice and Banana

Md. Abdul Baset Mia, Md. Motaher Hossain, Zulkifli Haji Shamsuddin, and M. Tofazzal Islam

5.1 Introduction

Nitrogen is a key component of many biomolecules such as nucleic acids and proteins—the two most important polymers of life. The requirement of nitrogen for life is enormous. Depending on the life form, two to twenty atoms of nitrogen are needed for every 100 atoms of carbon incorporated into cells (Sterner and Elser 2002). Despite the paramount importance of nitrogen in living organisms, N_2 is basically inert. Therefore, fixed inorganic nitrogen usually in ionic forms [most commonly nitrate (NO_3^-) and ammonium (NH_4^+) ions] limits primary productivity in both terrestrial and marine ecosystems (Falkowski et al. 2008). Obviously, the availability of fixed inorganic nitrogen is the most limiting factor for crop productivity. Global agriculture has to adjust with the increasing demands of nitrogen nutrition in crop plants to ensure food security of increasing population of the world in the twenty-first century. The limited nitrogen availability for crop plants has long been overcome through applications of synthetic nitrogen-rich fertilizers such as urea. In fact, the increased use of chemical fertilizers has revolutionized crop yield and food production worldwide. However, it causes the largest human interference

M.A.B. Mia
Department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh

M.M. Hossain
Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh

Z.H. Shamsuddin
Department of Land Management, Faculty of Agriculture, University Putra Malaysia, Serdang, Selangor, Malaysia

M.T. Islam (✉)
Department of Biotechnology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh
e-mail: tofazzalislam@yahoo.com

D.K. Maheshwari et al. (eds.), *Bacteria in Agrobiology: Crop Productivity*,
DOI 10.1007/978-3-642-37241-4_5,

in the nitrogen cycle, which has prompted concerns regarding the substantial economic cost and environmental pollution due to increased emissions of nitrogen oxides, soil acidification, and eutrophication in the aquatic environment (Dixon and Kahn 2004). The environmental cost due to application of inorganic fixed N_2 is about $100 billion per year including the cost of global industry and environmental nitrogen pollution (Beatty and Good 2011). Therefore, growing calls emerged in recent days for global action to address the source of nitrogen pollution and to assess the way forward in reducing the dependence on synthetic inorganic nitrogen fertilizer in agriculture.

Most nitrogen exists in the atmosphere as N_2 gas, and hence, utilization of this natural source is considered as a viable option for nitrogen nutrition in plants. However, the ability to fix atmospheric nitrogen is restricted to prokaryotes in the bacterial and archaeal domains, so-called diazotrophs that convert atmospheric nitrogen into ammonia (Canfield et al. 2010). Nitrogen-fixing bacteria include diverse phylogenetic groups such as green sulfur bacteria, actinomycetes, cyanobacteria, and all subdivisions of the Proteobacteria. However, in Archaea, nitrogen fixation is mainly restricted to methanogens (Dixon and Kahn 2004). The nitrogen-fixing ability in bacteria also contains a wide range of physiologies including aerobic (e.g., *Azotobacter*), facultatively anaerobic (e.g., *Klebsiella*) or anaerobic (e.g., *Clostridium*) heterotrophs, anoxygenic (e.g., *Rhodobacter*) or oxygenic (e.g., *Anabaena*) phototrophs, and chemolithotrophs (e.g., *Leptospirillum ferrooxidans*) (Dixon and Kahn 2004; Kneip et al. 2007; Canfield et al. 2010). Interestingly, diazotrophs are found in a wide variety of habitats including free-living in soils and water, associative symbioses with grasses, symbiotic associations in termite guts, actinorhizal associations with woody plants, cyanobacterial symbiosis with various plants, and root–nodule symbioses with legumes (Dixon and Kahn 2004; Kneip et al. 2007).

Biological nitrogen fixation (BNF) by variety of symbiotic, associative, and free-living microorganisms has tremendous importance to the environment and to world agriculture. Nitrogen fixation is considered as one of the key steps of the nitrogen cycle as it replenishes the overall nitrogen content of the biosphere and compensates for the losses that are incurred due to denitrification. The fixed N_2 that is provided by BNF is less prone to leaching and volatilization as it is utilized in situ. Therefore, this biological process contributes as an important and sustainable input into agriculture (Dixon and Kahn 2004). Root nodule symbiosis of N_2-fixing bacteria provides legumes with enhanced capacity to obtain fixed N_2 (Quispel 1974). The discovery of symbiosis between N_2-fixing bacteria and legumes raises the eventual question of whether such a relationship is possible for nonlegume plants (Mia and Shamsuddin 2010a). More research is needed to find molecular mechanisms of BNF in non-legume crop species based on our understanding of nitrogen fixation biology in legumes (Godfray et al. 2010). Recently, Markmann et al. (2008) have found that several genes, including the so-called symbiosis receptor kinase (SYMRK) gene, are involved in a genetic program that links arbuscular mycorrhiza and one form of bacterial nodule symbiosis. And the analysis of SYMRK in several species of plant provided the striking evidence that most

plants have a short version of SMYRK, which is required for AM symbiosis, while a longer variant was found only in plants involved in the symbiotic relationships with nitrogen-fixing bacteria. This finding can be considered as an important step toward understanding the evolution of nitrogen fixation in plants, and even whether plants that do not form symbiosis with nitrogen-fixing bacteria could be engineered to do so, thus increasing their N nutrition to ensure higher productivity.

Nitrogen is highly mobile in the plant system, deficiency symptoms quickly develop due to shortage of its supply, and therefore, frequent supply is beneficial for the crop growth and development. Like other non-legumes, rice and banana suffer from a mismatch of its N demand. Biological N_2 fixation (BNF) technology can play an important role in substituting for commercially available N-fertilizer use in these crops. Making crop plants capable of fixing their own nitrogen via a close interaction with diazotrophic bacteria may be used as an alternative strategy for solving nitrogen nutrition in economically important crops like rice and banana. Therefore, plant-associative and endophytic bacteria either in roots or shoots could be targeted as the potential inoculants of N_2 fixation in rice and banana plants (Mia et al. 2010). Although providing nitrogen nutrition to rice and banana through BNF is a novel approach, however, its potential has a considerable payoff in terms of increasing the production of these crops that not only help resource-poor farmers for reducing cost of production but also significantly reduce environmental pollution (Cassman et al. 1998; Ladha et al. 1997).

A large body of literature indicates that both associative and endophytic bacteria isolated from rice and bananas have potentials for nitrogen nutrition in host plants (Table 5.1). Although several good reviews have recently been published on diazotrophic bacteria in nitrogen nutrition in plants (Beatty and Good 2011; Doty 2011; Borriss 2011), however, very few of them have been focused on the roles of plant-associative bacteria in nitrogen nutrition with practical examples in major crop plants like rice and banana. In this chapter, we attempt to review current knowledge on potentials for nitrogen nutrition in crop plants by the application of plant-associative bacteria. This review covers bioassay methods for isolation, screening, and identification of nitrogen fixing bacteria; their root colonization ability; and effects of bacteria on nitrogen nutrition in plants. Current knowledge of molecular mechanisms of nitrogen nutrition of plants by diazotrophic bacteria and biotechnological approaches for exploiting these mechanisms for better nitrogen management in crop production is also discussed, with special reference to rice and banana.

5.2 Isolation, Screening, and Identification of Bacteria

Bacteria from diverse taxonomic groups have been found to inhabit the rhizosphere and endorhizosphere of rice and banana (Jha et al. 2009). The association between these diazotrophs and plant roots is one of the potentials for increasing N nutrition (Rao and Adhya 1994). Several lines of evidence suggest that diazotrophic bacteria have been isolated from diverse environmental origins ranging from soils, water,

Table 5.1 List of associative and endophytic N_2-fixing bacteria that promote growth and productivity of rice, banana, and other monocotyledonous plants

Species	Type	Test crop	References
Acetobacter diazotrophicus	Associative and endophytic	Rice and sugarcane	Anssens et al. (1989), Boddey et al. (1995)
Azospirillum brasilense	Associative and endophytic	Rice and wheat	Baldani et al. (1993), Tarrand et al. (1978)
A. lipoferum	Associative	Rice	Baldani and Dobereiner (1980), Ladha et al. (1982), Tarrand et al. (1978), Reinhold et al. (1987)
A. amazonense	Associative	Rice	Pereira et al. (1988), Magalhaes et al. (1983)
A. halopraeferens	Associative	Kallar grass rice	James (2000), Magalhaes et al. (1983)
A. irakense	Associative and endophytic	Rice	Khamas et al. (1989), Vemeiren et al. (1998)
A. doebereinerae	Associative	C_4 grasses	Eckert et al. (2001)
A. oryzae	Associative	Rice	Xie and Yokota (2005)
Azoarcus spp.	Associative and endophytic	Rice	Engelhard et al. (2000)
Azorhizobium caulinodans	Associative	Rice	Van Nieuwenhove et al. (2000)
Bacillus spp.	Associative and endophytic	Rice and banana	Rao et al. (1998)
Bacillus pumilus	Endophytic	Rice	Bacilico-Jimenz et al. (2003)
Burkholderia sp.	Endophytic	Rice	Baldani et al. (1997)
Enterobacter spp.	Endophytic	Rice	Triplett (1996), Stoltzfus et al. (1997)
Herbaspirillum seropedicae	Associative and endophytic	Rice and sugarcane	Gyaneshwar et al. (2002), Baldani et al. (1986)
Klebsiella spp. *Klebsiella planticola*	Associative	Rice	Triplett (1996), Stoltzfus et al. (1997), Ladha et al. (1983)
Methylococcus spp.	Associative	Rice	Rao et al. (1998)
Methylosinus spp.	Associative	Rice	Rao et al. (1998)
Pantoea sp.	Endophytic	Rice	Mano and Morisaki (2008)
Pseudomonas spp.	Associative	Rice	You and Zhou (1989), You et al. (1991), Vemeiren et al. (1998)
Rhizobium leguminosarum bv. *trifolii*	Endophytic	Rice	Yanni et al. (1997)
Serratia marcescens	Endophytic	Rice	Gyaneshwar et al. (2001)
A. amazonense	Associative	Banana	Magalhaes et al. (1983)
A. brasilense	Associative	Banana	Tarrand et al. (1978), Mia et al. (2007)
A. lipoferum	Associative	Banana	Reinhold et al. (1987)

Bacillus sphaericus	Associative and endophytic	Banana	Mia et al. (2007)
H. seropedicae	Associative	Sugarcane and banana	Weber et al. (1999)
Herbaspirillum spp.	Associative	Sugarcane and banana	Weber et al. (1999)
Burkholderia spp.	Associative	Banana and rice	Weber et al. (1999), Naher et al. (2009), Doty (2011)

rhizosphere, rhizoplane, and inside the plant tissues. For example, *Herbaspirillum frisigense* has been isolated as diazotrophic bacterium from two C_4 grasses, *Miscanthus sinensis* and *Pennisetum purpureum* (Jha et al. 2009). Similarly, various diazotrophs, related to *Azospirillum amazonense*, *A. lipoferum*, *Burkholderia* sp., and a group of similar to the genus *Herbaspirillum* sp. have been isolated from rhizosphere of banana and pineapple (Weber et al. 1999). On the other hand, some other genera of diazotrophic bacteria have been isolated from the internal tissue (endophytes) of many poaceaeous shoot and roots which includes *Burkholderia* sp. (Hartmann et al. 1995) and *Acetobacter diazotrophicus* (Cavalcante and Dobereiner 1988; Dobereiner et al. 1993). *Azospirillum brasilense* and two groups of *Herbaspirillum*-like bacteria have been isolated from banana roots (Weber et al. 1999).

Some convenient methods have been established for isolation of bacteria from the rhizosphere, rhizoplane, and internal tissues of plants (Islam et al. 2007; Islam 2011; Islam and Hossain 2012, 2013). Generally, isolation of bacteria from roots, rhizoplane, and rhizosphere is done by using dilution plate or streak culture methods on suitable agar medium (Muthukumarasamy et al. 2007; Islam et al. 2007). The potential of isolates as diazotrophic is generally assessed by in vitro screening through various semisolid nitrogen-free media (Dobereiner 1995).

The candidate isolates selected from the in vitro screening are further tested by assessing the in situ acetylene reduction assay (ARA) that indirectly measures the nitrogen fixing ability of the bacteria through estimating nitrogenase enzyme activity based on electron flux (Danso 1985). The assessment of ARA can be performed in pot culture using soil or other growing media wherein field condition to confirm the BNF potential in association with the test crops can be performed by ^{15}N isotopic dilution technique (Boddey et al. 1996). Recently, metagenomic analysis has been applied to investigate diazotrophic endophytic bacteria recalcitrant to cultivate in the culture media (Sessitsch et al. 2012). This approach will help provide a deeper understanding of endophytic functions and mechanisms for their establishment in the endosphere which could be exploited to improve agricultural management practices with respect to nitrogen nutrition in crop plants.

To confirm the phylogenic affiliation of elite bacterial isolate, classical methods based on morphological, physiological, and biochemical features have been used (Prayitno and Rolfe 2010). However, morphological, physiological, and biochemical tests have sometimes been found insufficient for accurate identification of the bacteria. Therefore, molecular techniques such as gene sequencing have widely been used as an acceptable method for proper identification of the bacteria (Magalhaes et al. 2001). Nowadays, 16S rRNA gene sequencing has been found useful for identification and studying microbial ecology of N_2-fixing bacteria (Reis et al. 2004; Islam et al. 2005, 2007; Fürnkranz et al. 2008). The extent of divergence in the sequence of this gene provides an estimate of the phylogenetic distance existing between different species (Igual et al. 2001).

A simplified diagrammatic scheme has been presented below (Fig. 5.1) to show steps of isolation, screening, and identification of associated diazotrophic bacteria:

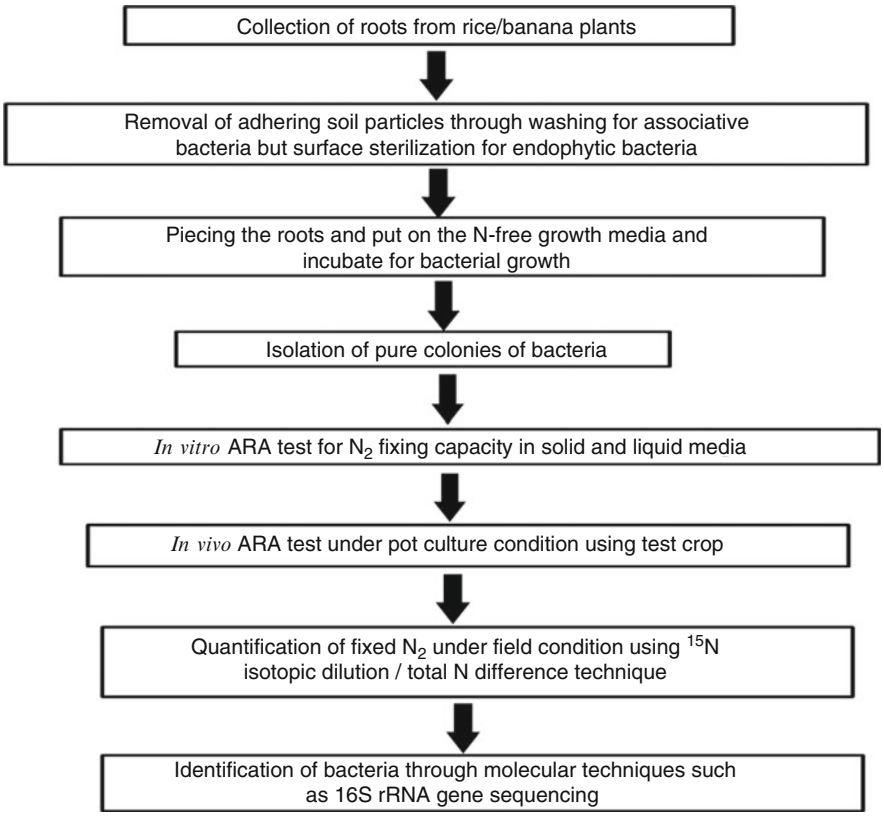


Fig. 5.1 A simplified schematic representation of the isolation, screening, and identification of associative and endophytic bacteria from roots

5.3 Root Colonization Ability of Bacteria

Root colonization either associative or endophytic is precondition for successful establishment of beneficial bacteria on plants (Suslow 1982; Islam et al. 2005, 2007). The process of root colonization of bacteria is known to have several stages, namely, the movement of microbes to the plant root guided by host signal (e.g., flavonoid), adsorption or invasion to the roots, and specific complex molecular interactions between the bacterium and the host plant, leading to induction of bacterial gene expression (Brimecombe et al. 2001). The attachment of bacteria to the host plant roots is essential for the establishment of an efficient and persistent beneficial association between host and bacteria (Brudman et al. 2000). This is owing to the following reasons: (1) bacteria should be attached to the root epidermal cells and if not, nutrient and bioenhancing substances excreted from the bacteria will not be utilized by the host plants; (2) without a protected attachment, water may wash the bacteria away from the rhizosphere to pass away in the

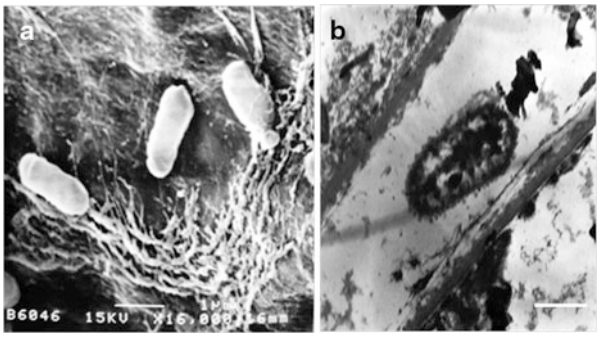


Fig. 5.2 Root colonization of associative bacteria. (**a**) Root surface of banana by *Azospirillum brasilense*. (**b**) Endophytic colonization by *A. brasilense* in banana roots. White error bar in (**b**) is 1 μm (adapted from Mia and Shamsuddin 2010b)

surrounding, nutrient-deficient soil; and (3) association sites on roots with no attached beneficial bacteria are susceptible to other aggressive and possibly non-beneficial microorganisms (Bashan and Holguin 1997). Therefore, in compatible host, epiphytic bacteria attach actively and then form a stable biofilm on the surface of root (Islam et al. 2005, 2007; Islam 2010).

On the other hand, endophytic bacteria colonize inside the tissues of the roots, stems, and leaves of different plants where they face less competition from other microorganisms for carbon sources and excrete part of their fixed N_2 directly into the host cell as NH_4^+ ion (Baldani et al. 2000; Mia and Shamsuddin 2010b). Several endophytic bacteria, namely, *Pantoea, Methylobacterium, Azospirillum, Herbaspirillum, Burkholderia*, and *Rhizobium*, have been observed colonized inside the rice plants (Mano and Morisaki 2008). Bacterial strains of *A. brasilense* and *B. sphaericus* successfully formed colonies on the banana roots (Fig. 5.2) (Mia et al. 2010). Although endophytic bacteria have been recognized to originate from outside of the plant body, they enter the plant system through stomata, lenticels, cracks of epiblema, epidermis of shoot, entry of lateral roots, and emerging radicles (Huang 1986). Electron micrographs from the root colonization study in rice and bananas clearly demonstrated that the bacteria can colonize the root surface efficiently, and more bacteria were found in the root hair propagation zone than the root hair itself, which is free from bacterial cells (Mia et al. 1999, 2010). In general, bacteria colonize just behind the root cap to have steady supply of root exudates (Islam et al. 2005). However, root caps of many plants have been found free from bacterial colonization (Foster and Bowen 1982). Several lines of evidence suggest that the associative bacteria that colonize rice roots are also found in the seeds (Sundaram and Klucas 1988). Similarly, bacteria, those found in the apoplastic site of banana roots, exert direct effect on intercellular space of roots which developed through schizogenously (Mia et al. 1999). Within the apoplastic region they find micro-niches to fix N_2 without having any competition with other organisms. The entry of bacteria inside the cortex was done likely through crack of epiblema or apoplastically by the activity of cellulase enzyme as the cell wall of young root was mainly composed of cellulose (Esau 2002).

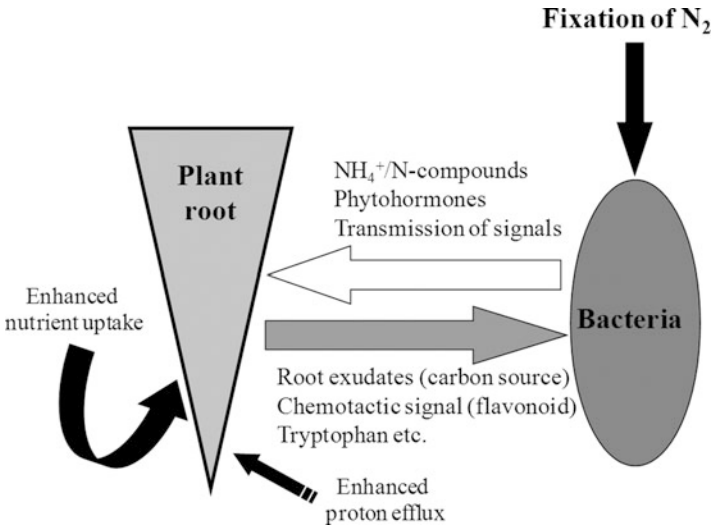


Fig. 5.3 A simplified schematic representation of the beneficial interactions of associative and endophytic bacteria with plant roots

5.4 Effects of Bacteria on Nitrogen Nutrition in Rice and Banana

In symbiotic association, the bacterium undergoes a highly specific interaction with the plant, involving several stages of developmental process and complex signal exchanges between the bacterium and plant (Sprent 1989). The plants supply simple carbon compounds to the bacteria as root exudates, and in return, the bacteria convert nitrogen (N_2) from air and excrete part of their fixed N_2 directly into the host cell as NH_4^+ ion for the use of plant host. The endophytic bacteria also enhance plant growth by doing bioenhancing activity through production of phytohormones. The complex interaction of associative and endophytic bacteria with host plants can be schematically presented in Fig. 5.3.

5.4.1 Nitrogen Fixation by Bacteria in Association with Rice and Banana Root

Nitrogen fixation was the first mechanism anticipated to explain improved plant growth following inoculation with associative beneficial bacteria. This was largely due to an increase in N-compound and nitrogenase enzyme activity in inoculated plants (Bashan and Holguin 1997). Researches using N-balance, ^{15}N isotope dilution, and ^{15}N enrichment studies have offered strong proof that some nonlegumes, especially rice, sugarcane, grasses, and bananas, can obtain at least part of their

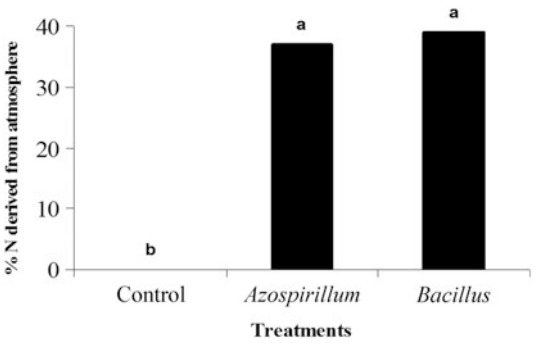


Fig. 5.4 Nitrogen fixation in banana plantlets (40 day old) by the associative bacteria *Azospirillum* sp. SP7 and *Bacillus* sp. UPMB10 (adapted from Mia et al. 2007). *Bars* showing same letter do not differ significantly at 5 % level of significance

N-needs through BNF process (Chalk 1991; Urquiaga et al. 1992; Shrestha and Ladha 1996; Malik et al. 1997; James and Olivares 1998; James 2000; Mia et al. 2007; Mano and Morisaki 2008). Recent evidence of significant BNF contribution in economically important poaceaeous crops, particularly rice (Shrestha and Ladha 1996), banana (Mia et al. 2007; Mia and Shamsuddin 2009a), sugarcane (Urquiaga et al. 1992), and forage grasses, such as kallar grass (Malik et al. 1997), has created tremendous interest on N_2 fixation by associative and endophytic bacteria. The association of the N_2-fixing bacteria *Azospirillum* spp. with roots of graminaceous plants has shown considerable rate of N_2 fixation (Nur et al. 1980; Van-Berkum and Bohlool 1980; Watanabe and Lin 1983; Charylulu et al. 1985; El-Komy et al. 1998). Using the ^{15}N isotope dilution technique, Baldani et al. (1997) and Mia et al. (2007) demonstrated that N_2-fixing bacteria could accumulate about 20 kg N ha^{-1} $year^{-1}$ in *Paspalum notatum* cv. batatais. Results of Mia et al. (2007) on associative bacterial inoculation on tissue-cultured banana plantlets indicated that *A. brasilense* and *B. sphaericus* can fix around 33–37 % of required N through BNF process (Fig. 5.4).

5.4.2 *Release of Fixed N_2 from Bacteria to Plant Cell*

The quantity of transferable fixed N_2 by rhizobacteria to their host plants greatly varies among the genotypes of bacteria (Kapulnik et al. 1985; Boddey et al. 1986; Kucey 1988). Studies using the ^{15}N isotope dilution technique indicated that most of the fixed N_2 remained below ground, probably still bound to bacterial cells, and contributed very little to growth of the upper plant parts (Nayak et al. 1986; Boddey and Dobereiner 1988). The limited N supply from N_2 fixation is probably because free-living diazotrophs release very small portion of the fixed N_2 to the environment. On the other hand, endophytic bacteria have greater advantages over free-living rhizobacteria because there is no chance of washing out the fixed N_2 as the bacteria inhabit inside the host tissue. However, the releasing NH_4^+ from endophytic bacteria is distinct from legume–*Rhizobium* symbiotic system. The bacteria

Table 5.2 The N, P, K, Ca, and Mg concentration in shoot of bananas inoculated with *Azospirillum* sp. SP7 and *Bacillus* sp. UPMB10 (adapted from Mia et al. 2010)

	Nutrient concentration in shoot (%)				
Treatments	N	P	K	Ca	Mg
$N_{33\ \%}$ (control)	0.89c	0.46b	3.8b	0.52b	0.10b
$N_{33\ \%}$ + *Azospirillum*	1.15b	0.65a	4.2a	0.60a	0.14a
$N_{33\ \%}$ + *Bacillus*	1.36a	0.48b	4.5a	0.54b	0.11b

Note: Values in a column having same letter(s) are not significantly different at 5 % level of probability

may secrete the fixed N_2 as NH_4^+; however, mechanism of NH_4^+ excretion varies among the genotypes of bacteria (Mia and Shamsuddin 2010b). In endophytic system, the exact mechanism of releasing NH_4^+ from bacterial cytoplasm has not been clearly understood. However, once the NH_4^+ comes out from the bacterial cells to the apoplastic region, it is easily transported to the host cell via passive or active transport.

5.4.3 Stimulation of Membrane ATPase and Enhanced Uptake of Nutrient

Enhanced mineral uptake in inoculated nonlegumes was proposed as a possible mechanism of plant growth enhancement by plant growth-promoting associative bacteria where the major elements involved were suggested to be N, Ca, and Mg in the roots only (Mia et al. 2005, 2009). The N was incorporated from atmospheric N_2, and other elements such as P, K, Ca, and Mg also play a key role in this plant–bacterium interaction. Shamsuddin et al. (1999) found increased amounts of P and K uptake in banana plants inoculated with rhizobacteria. Similarly, banana plant inoculated with *Azospirillum* showed higher content of N, P, K, Ca, and Mg in shoot biomass, while plant treated with *Bacillus* had only the higher content of N and K compared to that with untreated plant (Table 5.2) (Mia et al. 2010). Mixed culture of *Azospirillum, Azotobacter*, and inorganic N-fertilizer resulted in taller plants, number of leaves, and girth of bananas (Wange and Patil 1994). Inoculation of *A. brasilense* increased the dry weight, plant height, P absorption, and lipid content in oil seed (Bashan et al. 2000).

The bacteria secrete the signaling substances, which is perceived by the root plasma membrane and stimulate the membrane-bound ATPase enzyme (Bashan and Holguin 1997). The stimulation of ATPase enzyme induces the proton efflux which resulted in the uptake of nutrient ions especially the cations. This is of importance since mineral uptake is usually closely related to membrane activity. *A. brasilense* inoculation can affect membrane activity and proton efflux of wheat root which requires high metabolic activity of both participants in the plant–bacteria association and may be involved in increasing mineral uptake of *Azospirillum*–inoculated plants (Bashan 1990). Inoculation with *A. brasilense*

Table 5.3 Altered ionic ratio of inoculated rice tissue due to application of associative and endophytic bacteria

	Ionic ratio			
Treatments	P/N	K/N	Ca/N	Mg/N
Control	0.45b	1.10b	0.08a	0.08a
Rhizobium sp.	0.45b	0.93c	0.10a	0.09a
Azorhizobium sp.	0.46b	1.20b	0.08a	0.07ab
Rhizobium sp.	0.54a	1.04c	0.07ab	0.07ab
Rhizobium sp.	0.46b	1.22b	0.07ab	0.07ab
Bacillus sp.	0.41c	0.98c	0.06b	0.06b
Burkholderia sp.	0.48b	1.37a	0.08a	0.08a

Note: Values in a column having same letter(s) are not significantly different at 5 % level of probability

increased proton efflux in their roots and changed the phospholipid content in cowpea plant membranes (Bashan et al. 1990, 1992).

5.4.4 *Influence of Bacterial N Supply on Ionic Balance in Plant Tissue*

Inoculation of associative and endophytic bacteria in rice and bananas has been shown to alter the tissue ionic ratio of P/N, K/N, Ca/N, and Mg/N. Our experimental results indicated that *B. sphaericus* significantly diluted the P, K, Ca, and Mg concentration in the tissue of rice seedlings indicating a higher incorporation of N which might come from fixed N_2 or added N from enhanced uptake (Table 5.3).

5.4.5 *Plant Growth and Development*

Bacteria that can increase plant growth and productivity have been known as plant growth-promoting bacteria (PGPB) for over a century. Plant growth promotion and development are facilitated by plant association with endophytic and associative bacteria through the synthesis of plant growth hormones (Amer and Utkhede 2000), N_2 fixation (Cakmakci et al. 2001; Islam 2011), solubilization of insoluble nutrient element (Islam et al. 2007; Islam and Hossain 2012), and increase uptake of other nutrients (Sahin et al. 2004).

5.4.5.1 Root Growth and Hair Formation

Increased root growth and activity were suggested in the late 1970s as a possible mechanism by which beneficial bacteria affects plant growth (Fallik et al. 1994)

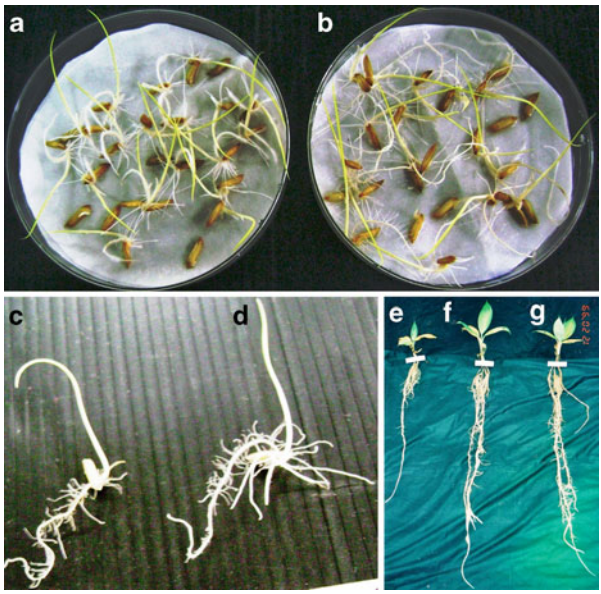


Fig. 5.5 Effects of bacterial inoculation on root growth of rice and banana seedlings. (**b**) and (**c**) Germinated rice seedlings showing less root hair (control); (**a**) and (**d**) *Rhizobium* sp. UPMR29 inoculated germinated rice seedlings showing profuse root hairs (adapted from Mia and Shamsuddin 2010b); (**e**) Control banana seedling with less developed roots; *Azospirillum brasilense* SP7 (**f**) and *Bacillus sphaericus* UMPB10 (**g**) inoculated tissue-cultured banana plantlets showing greater root growth

where higher number of root hairs increased the surface area of a root system. In emerging rice seedlings, abundant root hair formation and enhanced plumule and radicle growth were observed by the associative and endophytic bacterial inoculation (Fig. 5.5a–d) (Mia and Shamsuddin 2009b, 2010b). However, in inoculated tissue-cultured banana plantlets, the increased root growth occurred almost in all dimensions, such as production of primary and secondary roots, longer roots, and higher root mass (Fig. 5.5e–g) (Mia et al. 2009). The higher number of secondary root initiation in *A. brasilense* inoculated plants could be due to the presence of more bacterial cells and their beneficial interaction in the hair formation zone *vis-a-vis* the zone of secondary root formation. This interaction might stimulate pericycle to produce more lateral roots as pericycle is the site of lateral root formation. The parenchymatous tissue of pericycle achieved meristematic activity by bacterial activity. The possible route of bacterial movement from cortex to pericycle is apoplastic in nature via intercellular space especially in young roots. Since the casparian strip of endodermis is not well developed in very young roots, the endophytic bacteria may get extra benefits for their easy traveling (Esau 2002). This is supported by Bilal et al. (1993) and Mia et al. (2010) who found that more bacterial cells were found in the root hair zone, in an area around the point of lateral roots emergence of grasses.

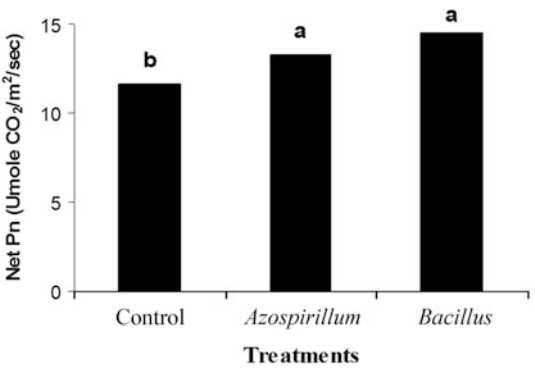


Fig. 5.6 Net photosynthesis (Pn) in young expanded leaf of bananas inoculated with associative bacteria *Azospirillum brasilense* SP7 and *Bacillus sphaericus* UPMB10 (adapted from Mia et al. 2010). *Bars* showing same letter do not differ significantly at 5 % level of significance

It is also thought that the associative and endophytic bacteria have the potential to synthesize plant hormone such as cytokinins, gibberellins, and auxin for stimulation of root growth (Tien et al. 1979; Molla et al. 2001). Associative and endophytic bacterial inoculation in rice and bananas has been shown to enhance cell division in the root meristematic zone (Levanony and Bashan 1989), while in maize it increased diameter and length of lateral roots (Hartmann et al. 1983), and promoted root hair development and branching which caused polarization and differentiation of root cortex cells (Kapulnik and Okon 1983).

5.4.5.2 Enhancement of Net Photosynthesis

Bacterial inoculation has been found to increase the photosynthetic rate of the host plants. Inoculation of tissue-cultured banana plantlets with *A. brasilense* and *B. sphaericus* significantly increased the net photosynthesis (Fig. 5.6) (Mia et al. 2010). Similarly *Azospirillum* and rhizobacterial inoculation increased the photosynthetic rate of oil palm seedlings (Amir et al. 2001). The increased photosynthetic activity (25 %) was due to more water and nutrient absorption. Higher leaf nutrient concentration increased the leaf photosynthesis capacity (Amax), carboxylation efficiency (Vcmax), and RuBP generation capacity (Jmax) across the plant kingdom (Field and Mooney 1986). Photosynthetic capacity of N_2-fixing bacteria was higher compared to N user, since the former needed more photosynthate to meet the higher demand by diazotrophs during the N_2-fixing process (Quilici and Medina 1998). Strong sink strength of inoculated roots has been shown to induce an increase in source leaf photosynthesis (De Veau et al. 1990). Inoculation of associative bacteria also increased stomatal conductance by reducing proline accumulation (Shamsuddin et al. 1999).

Fig. 5.7 Associative bacteria *(Bacillus sphaericus)* inoculation resulted in early flowering in banana (adapted from Mia 2002)

5.4.5.3 Shoot Growth and Early Flowering

Growth-promoting effects of associative bacterial inoculation are largely consequential changing the morphophysiology state of plants, which may contribute to the enhancement of shoot growth and earliness of plant reproductive activity. Murty and Ladha (1988) found that inoculation of *Azospirillum lipoferum* to rice roots caused significant increases in shoot fresh and dry weights but had no effect on root surface area. The inoculation process also increased P content through P solubilization (Bashan et al. 2000). Bacterial inoculation of banana plant effectively increased the plant height, leaf number, leaf area, and leaf chlorophyll content. Moreover, bacterial inoculation together with 33 % N-fertilizer application has been shown to produce an equivalent total dry matter as the 100 % N-applied control plants, an indication of the beneficial bioenhancing effect of bacteria through higher photosynthetic activity and more nutrient (P, K, Ca, and Mg) uptake (Mia et al. 2005, 2009). Inoculation of bacterial strain *B. sphaericus* also enhanced the earliness of flower initiation in bananas as shown the Fig. 5.7 (right plant).

5.5 Molecular Basis of Biological Nitrogen Fixation in Plants

The components of the nitrogen acquisition pathway interact in multiple and complex ways. The *Rhizobium*–legume symbiosis model had attracted series of studies ever since Beijerinck's demonstration that bacterium caused nodule formation (Quispel 1974). In the last four decades, considerable progress has been made in elucidating the underlying molecular mechanism of nitrogen fixation

(Long 2001). However, the regulatory mechanisms involved in nonlegumes are still insufficiently resolved. Herein, we discuss the molecular basis of signal transduction, transcriptional, and post translational regulation of biological nitrogen fixation in plants.

5.5.1 Nod *Factor Signals in Biological Nitrogen Fixation*

The early steps of the nitrogen-fixing symbiosis between plants belonging to the family Leguminosae and soil bacteria (e.g., *Rhizobium*) are mediated by an exchange of chemical signals between the two partners. Much progress has been made toward the characterization of biochemical signals during biological nitrogen fixation in legumes (Stougaard 2001; Geurts and Bisseling 2002; Trevaskis et al. 2002). Perception of flavonoid signal (e.g., luteolin) that exudes from legume roots triggers the production of rhizobial lipochitooligosaccharide (Nod factor, NF) signals with strain-specific substitutions (D'Haeze and Holsters 2002). Recognition of NFs by compatible LysM-type plant receptors induces the formation and deformation of root hairs, intra- and extracellular alkalinization, membrane potential depolarization, changes in ion fluxes, early nodulin gene expression (ENOD), and formation of nodule primordia (D'Haeze and Holsters 2002; Capoen et al. 2010). The extensive cell division and the production of cellulose microfibrils by the plant lead to the formation of root nodules in which differentiated bacteria (bacteroids) fix nitrogen for their host (Broughton et al. 2003). In most legumes, infection occurs in susceptible root hair cells that curl to form a compartment for a bacterial microcolony from which an intracellular infection thread guides the rhizobia to the base of the root hair and, subsequently, through layers of cortical cells toward the nodule primordium cells. There, rhizobia are engulfed by the plant plasma membrane and differentiate into nitrogen-fixing bacteroids, thus forming a new organelle called the symbiosome (Jones et al. 2007). The formation of symbiosomes is presumed to represent a major step in the evolution of legume–nodule symbiosis, because symbiosomes facilitate the exchange of metabolites between the two symbionts. Within these symbiosomes, membrane-bound vesicular compartments, rhizobia are supplied with energy derived from plant photosynthates and in return supply the plant with biologically fixed nitrogen, usually as ammonia. This minimizes or eliminates the need for inputs of synthetic nitrogen fertilizers (Cocking et al. 2005).

Some of the plant genes involved (synonymously called the "common SYM" or the "does not make infection" [DMI] genes) also are required for symbiosome formation. The DMI2 gene mediates Nod factor perception and transduction leading to rhizobial infection, not only in root epidermal cells but also during nodule development (Bersoult et al. 2005; Limpens et al. 2005). Application of NF stimulated biomass accumulation and changes in plant structure and morphology support a view of NFs as "hormone-like" molecules (Souleimanov et al. 2002). It is becoming increasingly apparent that the genes necessary for nitrogen fixation in

many diazotrophs have common structures and functions. However, the mechanisms by which cellular nitrogen levels are sensed and nitrogen signals are transmitted can vary considerably among different nitrogen-fixing bacteria. From the biodiversity studies of NFs, it appears that their structures belong with the phylogenetic evolution of plants, rather than that of bacteria, suggesting a coevolution of symbiotic bacteria with their host plant (Promé 1999).

Some preliminary and indirect observations indicate that similar molecules seem to exist in nonlegume plants. Since no true nodule formation has been demonstrated in nonlegumes like rice, still, some morphological responses to rhizobial inoculation have been documented in rice. *Rhizobium*-produced plant hormones can favor the growth of short and thick roots (Reddy et al. 1997), and root hair deformation, a process associated with early bacterial infection, has also been demonstrated (Reddy et al. 2000). Interestingly, rice appears capable of perceiving NFs coded for bacterial nod genes, and several homologues to legume early nodulin genes (*ENODs*) are present in rice (Reddy et al. 1999). Similarly, the NF elicited positive effects on corn, another non-host species, increasing the total length (Souleimanov et al. 2002). This suggests that the perception of NF might be conserved among a wide variety of plants. Moreover, the promoter activity of rice *ENOD40* in soybean revealed that its tissue-specific expression was identical to that of the endogenous soybean promoter, indicating that key regulatory features of these genes may be conserved in rice (Reddy et al. 2000). A partial explanation for this may lie in the fact that rice possesses the capacity to form symbiotic associations with underground fungi (mycorrhizae) and that there appear to be substantial similarities at the genetic level between the formation of such mycorrhizal associations and the formation of symbioses with diazotrophs (Hirsch and Kapulnik 1998). The *Rhizobium* sp. NF signals show strong resemblance to lipochitooligosaccharides produced by mycorrhizal fungi (Maillet et al. 2011). The NF-signaling cascade has been genetically dissected (reviewed by Kouchi et al. 2010). These studies showed that several signaling components have been recruited from the network that is also essential for endomycorrhizal symbiosis. Genes that are essential for NF-induced signaling of mycorrhiza as well as *Rhizobium* sp. form a common symbiotic signaling pathway. Nod factor signaling also requires several transcription factors. Legume GRAS (GAI, RGA, SCR)-type transcription factors nodulation signaling pathway1 (NSP1) and NSP2 are essential for *Rhizobium* Nod factor-induced nodulation (Kaló et al. 2005; Smit et al. 2005; Heckmann et al. 2006; Murakami et al. 2006). Although both transcription factors are not essential for mycorrhizal symbiosis, it was recently found that an NSP2-dependent signaling pathway facilitates mycorrhizal root colonization (Maillet et al. 2011). However, legume NSP1 and NSP2 can be functionally replaced by nonlegume orthologs, including rice NSP1 and NSP2, indicating that both proteins are functionally conserved in higher plants. NSP1 and NSP2 are indispensable for strigolactone (SL) biosynthesis in the legume *Medicago truncatula* and in rice (Liu et al. 2011).

Recently, using novel inoculation conditions with very low numbers of bacteria, the cells of root meristems of maize, rice, wheat, and other major nonlegume crops, such as oilseed rape and tomato, can be intracellularly colonized by the

non-rhizobial, non-nodulating, nitrogen-fixing bacterium *Gluconacetobacter diazotrophicus* that naturally occurs in sugarcane (Cocking et al. 2005). A successful nitrogen-fixing symbiosis requires the accommodation of rhizobial bacteria as new organelle-like structures, called symbiosomes, inside the cells of their legume hosts (Ovchinnikova et al. 2011). *G. diazotrophicus* expressing nitrogen-fixing (*nif* H) genes was found in symbiosome-like compartments in the cytoplasm of cells of the root meristems of cereals and nonlegume crop species, somewhat similar to the intracellular symbiosome colonization of legume nodule cells by rhizobia (Cocking et al. 2005).

5.5.2 Molecular Nitrogen Fixation and Nitrogenase Function

During biological nitrogen fixation (BNF), molecular nitrogen is reduced in multiple electron transfer reactions, resulting in the synthesis of ammonia and the release of hydrogen (Kim and Rees 1992). Ammonium is then used for the subsequent synthesis of biomolecules. This reduction of molecular nitrogen to ammonium is catalyzed in all nitrogen-fixing organisms via the nitrogenase enzyme complex. Nitrogenase catalyzes the conversion of N_2 to NH_4^+, as represented by the following equation:

$$N_2 + 10H^+ + 8e^- + \text{nMgATP} \rightarrow 2NH_4^+ + H_2 + \text{nMgADP} + \text{NPi}\,(n \geq 16)$$

This reaction shows that nitrogen fixation is very expensive in biological energy equivalents, requiring large amounts of both reducing power and high-energy phosphate (ATP). Obligate proton reduction occurs during nitrogenase catalysis, with a minimum of 1 mol of H_2 produced per mol of N_2 reduced (Simpson and Burris 1984). The proportion of electrons allocated to proton reduction increases under conditions of limiting electron flux, further increasing the consumption of MgATP (Burgess and Lowe 1996). Thus nitrogen-fixing microorganisms tightly control both the synthesis and activity of nitrogenase to avoid the unnecessary consumption of energy (Schmitz et al. 2002). In addition to protons, nitrogenase can reduce several other alternative substrates, which resemble N_2 on the basis of double or triple bonds in their structures. Acetylene has proven to be a particularly useful substrate in nitrogenase research because the reduction product, ethylene, is easily quantified by gas chromatography. Because acetylene and ethylene are both permeable to the bacterial envelope, nitrogenase activity may be measured in vivo as well as in vitro by the acetylene reduction assay method. Reduction of all substrates, except protons, can also be inhibited by CO, suggesting that proton reduction occurs by a slightly different pathway (Burgess and Lowe 1996).

The nitrogenase complex is comprised of two main functional subunits (Hageman and Burris 1978). The smaller dimeric component, known as the iron (Fe) protein, functions as an ATP-dependent electron donor to the larger

heterotetrameric component, known as the molybdenum–iron (MoFe) protein, which contains the enzyme catalytic site. The structural components of these subunits are the Nif (nitrogen fixation) proteins NifH (γ2 homodimeric azoferredoxin) and NifD/K (α2β2 heterotetrameric molybdoferredoxin). Basically three types of nitrogenases are known based on the composition of their metal centers: iron and molybdenum (Fe/Mo), iron and vanadium (Fe/V), or iron only (Fe) (Bishop and Premakumar 1992). The most common form is the Fe/Mo type highly conserved in sequence and structure throughout nitrogen-fixing bacteria (Schrock 2006). Under conditions of molybdenum depletion, some organisms—for example, *Azotobacter vinelandii* and *Rhodobacter capsulatus*—induce the synthesis of alternative nitrogenases containing vanadium–iron or iron–iron cofactors (Eady 1996).

Each Fe protein dimer can bind two nucleotide molecules, at sites distal from the redox-active center [Fe_4S_4] directly involved with electron transfer to MoFe protein active site (Burgess and Lowe 1996). Binding of MgATP at these sites causes a conformational change in Fe protein. The two subunits (α and β) rotate toward each other, extruding the [Fe_4S_4] cluster toward the protein surface (and surmised interaction with the P-clusters of MoFe protein) by 4 Å (Schindelin et al. 1997). This conformational change is thought to be a key step in the catalytic cycle of nitrogenase. Although there is little apparent variation in the sequences and structures of nitrogenases, there appear to be almost as many nitrogenase-regulating schemes as there are nitrogen-fixing species (Halbleib and Ludden 2000).

5.5.3 *Transcriptional Regulation of Nitrogen Fixation*

Nitrogen-fixing microorganisms colonize a wide variety of habitats and can be found free-living in soils and water, in association with grasses, or in root–nodule symbioses with legumes. Consequently, they have evolved sophisticated regulatory networks that respond to multiple environmental cues (Dixon and Kahn 2004). Biological nitrogen fixation is highly controlled at the transcriptional and posttranscriptional level by regulatory networks that respond to the availability of fixed nitrogen. Regulation of the nitrogen fixation gene (*nif*) expression has been most extensively studied in diazotrophic proteobacteria (Arcondeguy et al. 2001). In all diazotrophic proteobacteria examined, the σ54-dependent transcriptional activator NifA is required for expression of all the nitrogen-fixing (*nif*) genes (Schmitz et al. 2002). NifA expression and activity is regulated in response to the environmental signals of molecular oxygen and combined nitrogen. Because the nitrogenase components are oxygen labile, it is advantageous for bacteria to repress transcription when oxygen levels are high (Halbleib and Ludden 2000). The *nifA* gene is cotranscribed with *nifL*, which encodes a redox- and nitrogen-responsive regulatory flavoprotein (NifL). NifL acts as a negative regulator of NifA, effectively adding another level of regulation in response to oxygen and fixed nitrogen (Halbleib and Ludden 2000). Indeed, NifA and NifL form an atypical two-component sensor–regulator system, and NifL modulates

the activity of NifA by direct protein–protein interaction (Martínez-Argudo et al. 2004). NifA proteins are structurally similar to each other. This enabled the description of NifA as a multidomain protein (Studholme and Dixon 2003 and references therein). Different regulatory mechanisms at the transcriptional level have been documented in several diazotrophs, such as in the α-Proteobacteria *Azospirillum brasilense*, which lacks NifL (Arsene et al. 1996), suggesting that nitrogenase transcriptional control mechanisms must be elucidated separately for any given diazotroph (Halbleib and Ludden 2000).

Because of the metabolically demanding nature of nitrogen fixation, an additional layer of nitrogenase regulation is present in a few free-living diazotrophs. To prevent unproductive nitrogen fixation during energy-limiting or nitrogen-sufficient conditions, the nitrogenase complex is rapidly, reversibly inactivated by ADP-ribosylation of Fe protein. The ADP-ribosylation of Fe protein is carried out by NAD1-dependent enzyme, or NADH dinitrogenase reductase ADP-ribosyltransferase (DraT) and dinitrogenase reductase glycohydrolase (DraG) (Ludden and Roberts 1989). DraT/DraG-mediated posttranslational regulation of the nitrogenase Fe protein by ADP-ribosylation has been previously described for a few diazotrophic bacteria belonging to the class α-proteobacteria. However, recently Oetjen and Reinhold-Hurek (2009) for the first time presented the DraT/DraG system of a β-proteobacterium, *Azoarcus* sp. strain BH72, a diazotrophic grass endophyte. This suggests that the DraT/DraG system might be operating in a wider range of proteobacterial diazotrophs than previously suspected, albeit with some functional distinctions. The availability of diazotrophic genome data will provide more insights into the gene regulatory network and the underlying molecular mechanisms of nitrogen fixation and contribute to our understanding of the evolution of nitrogen-fixing bacteria.

5.5.4 *Transgenic Rice Harboring Putative Nitrogen-Fixing Gene*

There has been much interest in exploring the feasibility of transferring symbiotic nitrogen fixation capability to important cereals such as rice. Engineering of NF gene in rice genome to enter into nitrogen-fixing symbiosis with rhizobia akin to that in legumes is still a big challenge to the scientists. Rice is shown to harbor at least partial genetic makeup in its genome for interacting with rhizobia (Reddy et al. 2000, 2002). However, rice varieties have a very low capacity to induce nod genes in rhizobia, presumably because of a lack of ability to synthesize nod gene-inducing flavonoids (Reddy et al. 2000; Rolfe et al. 2000). Hence, isoflavone production may pave the way for rice plants to enter into a symbiotic relationship with rhizobia. The key enzyme that redirects phenylpropanoid pathway intermediates from flavonoids to isoflavonoids is the isoflavone synthase (IFS) (Jung et al. 2000). In an effort to develop a rice variety possessing the ability to induce nodulation (nod) genes in rhizobia, Sreevidya et al. (2006) incorporated the IFS gene from soybean into rice

(*Oryza sativa* L. cv. Murasaki R86) under the control of the 35S promoter. The presence of IFS gene in transgenic rice led to the expression of isoflavone synthase conferring rice plants with the ability to produce flavonoids that were able to induce nod gene expression, albeit to varied degrees. These results suggest that the isoflavone synthase enzyme is functionally active in rice. Incorporation of *nif* genes essential to nitrogenase activity into the rice genome and limiting *nif* expression to root plastids have been suggested to be one of the most suitable approaches (Britto and Kronzucker 2004). This is because plastidic genetics most closely resembles that of N-fixing prokaryotes (Whitfeld and Bottomley 1983), and the root plastids do not contain photosynthetically produced oxygen (Britto and Kronzucker 2004). Another approach might be the expression of the oxygen-tolerant nitrogenase in rice found in the bacterium *Streptomyces thermoautotrophicus* (Ribbe et al. 1997). Although these approaches are, in principle, realizable, it will likely require huge intensive research before a useful product makes it to the field trial stage. At present, optimizing associations between rice and naturally colonizing endophytic bacteria to enhance rice nitrogen status may be more promising.

5.6 Conclusion and Future Perspective

Nitrogen is an essential nutrient element for plant growth and development. However, it is unavailable in its most prevalent form as atmospheric nitrogen which is estimated to be more than 30,000 tons ha^{-1}. Crop production instead commonly depends upon industrially produced nitrogen fertilizers (more than 100 million tons annually), and overuse of these chemicals has led to worldwide ecological problems. Global efforts to address N-related environmental hazards and ecological impacts in an integrated manner are currently in progress. These increasing efforts have led to the development of innovative research areas related to utilization of atmospheric nitrogen by high N input crops including rice and banana. The use of associative and endophytic diazotrophic bacteria for increasing N nutrition in these nonlegumes has been a long-standing goal. Reviews of recent advances indicate that several species of associative and endophytic diazotrophs such as *Acetobacter, Azoarcus, Azospirillum*, *Bacillus, Burkholderia*, *Herbaspirillum, Klebsiella, Rhizobium,* and *Pseudomonas* are able to form close associations with these plants and can fix appreciable amounts of nitrogen within the rhizosphere of the host plants. This symbiotic association exerts beneficial effects on root–shoot growth, nutrient uptake, dry matter yield, fruit quality, and other traits of crops. Better understanding on underlying molecular mechanism of N nutrition of plants by the help of diazotrophic bacteria and molecular tools for incorporating N_2 fixing gene into plant or the associated bacterium is needed for development of sustainable technology for agriculture alternative to synthetic fertilizers. Another approach that has received less attention because of their current inefficiency in nitrogen yields is the engineering of free-living microbes that fix nitrogen. The implementation of any of these nitrogen technologies will depend on our knowledge base of biological

nitrogen fixation. Recent advances in understanding nitrogen fixation biology indicate that application of genetic engineering tools for enhancement of nitrogen fixation may be less difficult than originally thought. A technology that economically eliminates or lessens the need for commercial nitrogen fertilizer could be invaluable both in developed and developing countries.

Acknowledgment The authors are thankful to the Ministry of Science, Technology and Innovation, Malaysia (IRPA Grants 51070 and 51386), RMC of University Putra Malaysia, and the World Bank (sub-project CP # 2071 of Higher Education Quality Enhancement Project (HEQEP) of University Grants Commission, Bangladesh to the Department of Biotechnology of BSMRAU in Bangladesh) for funding the work.

References

Amer AG, Utkhede RS (2000) Development of formulation of biological agents for management of root rot of lettuce and cucumber. Can J Microbiol 46:809–816

Amir HG, Shamsuddin ZH, Halimi MS, Ramlan MF, Marziah M (2001) Effects of *Azospirillum* inoculation on N_2 fixation and growth of oil palm plantlets at nursery stage. J Oil Palm Res 13:42–49

Anssens DJ, Kroppenstedt RM, Stephan MP, Teixeira KRS, Dobereiner J, De Ley J (1989) *Acetobacter diazotrophicus* sp. nov., a nitrogen-fixing acetic acid bacterium associated with sugarcane. Int J Syst Evol Microbiol 39:361–364

Arcondeguy T, Jack R, Merrick M (2001) P(II) signal transduction proteins, pivotal players in microbial nitrogen control. Microbiol Mol Biol Rev 65:80–105

Arsene F, Kaminski PA, Elmerich C (1996) Modulation of *NifA* activity by PII in *Azospirillum brasilense*: evidence for a regulatory role of the *NifA* N-terminal domain. J Bacteriol 178:4830–4838

Bacilico-Jimenz M, Aguiler S, Ventura-zapta E, Perez-Campos E, Bouquelet S, Zenteno E (2003) Chemical characterization of root exudates from rice (*Oryza sativa)* and their effects on the chemotactic response of endophytic bacteria. Plant Soil 249:271–277

Baldani JI, Dobereiner J (1980) Host plant specificity in the infection of cereals with *Azospirillum* spp. Soil Biol Biochem 12:433–439

Baldani JI, Baldani VLD, Seldin L, Dobereiner J (1986) Characterization of *Herbaspirillum seropedicae* gen nov., a root associated nitrogen fixing bacterium. Int J Syst Bacteriol 36:86–93

Baldani VLD, James EK, Baldani JI, Dobereiner J (1993) Colonization of rice by the nitrogen fixing bacteria *Herbaspirillum* spp. and *Azospirillum brasilense*. In: Palacios R, Mora J, Newton WE (eds) New horizons in nitrogen fixation. Kluwer Academic, Dordrecht

Baldani JI, Caruso L, Baldani VLD, Goi SR, Dobereiner J (1997) Recent advances in BNF with non-legume plants. Soil Biol Biochem 29:911–922

Baldani VLD, Baldani JI, Dobereiner J (2000) Inoculation of rice plants with the endophytic diazotrophs *Herbaspirillum seropedicae* and *Burkholderia* spp. Biol Fertil Soils 30:485–489

Bashan Y (1990) Short exposure to *A. brasilense* Cd inoculation enhanced proton efflux of intact wheat root. Can J Microbiol 36:419–425

Bashan Y, Holguin G (1997) *Azospirillum*/plant relationship: environmental and physiological advances (1990–1996). Can J Microbiol 43:103–121

Bashan Y, Harrison SK, Whit-Moyer RE (1990) Enhanced growth of wheat and soybean plants inoculated with *A. brasilense* is not necessarily due to general enhancement of mineral uptake. Appl Environ Microbiol 56:769–775

Bashan Y, Aclarz-Melendez L, Toledo G (1992) Response of soybean and cowpea root membranes to inoculation with *A. brasilense*. Symbiosis 13:217–228

Bashan Y, Moreno M, Troyo E (2000) Growth promotion of seawater-irrigated oilseed halophyte *Salicornia bigelovii* inoculated with mangrove rhizosphere bacteria and halotolerant *Azospirillum* spp. Biol Fertil Soils 32:265–272

Beatty PH, Good AG (2011) Future prospects for cereals that fix nitrogen. Science 333:416

Bersoult A, Camut S, Perhald A, Kereszt A, Kiss GB, Cullimore JV (2005) Expression of the *Medicago truncatula* DMI2 gene suggests roles of the symbiotic nodulation receptor kinase in nodules and during early nodule development. Mol Plant Microbe Interact 18:869–876

Bilal R, Rasul G, Arshad M, Malik KA (1993) Attachment, colonization and proliferation of *Azospirillum brasilense* and *Enterobacter* spp. on root surface of grasses. World J Microbiol Biotechnol 9:63–69

Bishop PE, Premakumar R (1992) Alternative nitrogen fixation systems. In: Stacey G, Burris RH, Evans DJ (eds) Biological nitrogen fixation. Chapman & Hall, New York, pp 736–762

Boddey RM, Dobereiner J (1988) Nitrogen fixation associated with grasses and cereals: recent results and perspective for future research. Plant Soil 108:53–65

Boddey RM, Baldani VLD, Baldani JI, Dobereiner J (1986) Effect of inoculation of *Azospirillum* spp. on nitrogen accumulation by field-grown wheat. Plant Soil 95:109–121

Boddey RM, de Oliveira OC, Urquiaga S, Reis VM, Olovares FL, Baldani VLD, Dobereiner J (1995) Biological nitrogen fixation associated with sugarcane and rice: contribution and prospects for improvements. Plant Soil 174:195–209

Boddey RM, Alves BJR, Urquiaga S (1996) Evaluation of biological nitrogen fixation associated with non-legumes. In: Malik KA et al (eds) Nitrogen fixation with non-legumes. Kluwer Academic, London, pp 287–306

Borriss R (2011) Use of plant-associated *Bacillus* strains as biofertilizers and biocontrol agents in Agriculture. In: Maheshwari DK (ed) Bacteria in agrobiology: plant growth responses. Springer, Berlin, pp 41–76

Brimecombe JM, De Leij FA, Lynch JM (2001) The effect of root exudates on rhizosphere microbial population. In: Pinton R, Varanini Z, Nannipieri P (eds) The rhizosphere. Dekker, New York, pp 95–140

Britto DT, Kronzucker HJ (2004) Bioengineering nitrogen acquisition in rice: can novel initiatives in rice genomics and physiology contribute to global food security? Bioessays 26:683–692

Broughton WJ, Zhang F, Perret X, Staehelin C (2003) Signals exchanged between legumes and *Rhizobium*: agricultural uses and perspectives. Plant Soil 252:129–137

Brudman S, Okon Y, Jurkevitch E (2000) Surface characteristics of *Azospirillum brasilense* in relation to cell aggregation and attachment to plant roots. Crit Rev Microbiol 26(2):91–110

Burgess BK, Lowe DJ (1996) Mechanism of molybdenum nitrogenase. Chem Rev 96:2983–3011

Cakmakci R, Kantar F, Sahin F (2001) Effect of N_2-fixing bacterial inoculations on yield of sugar beet and barley. J Plant Nutr Soil Sci 164:527–531

Canfield DE, Glazer AN, Falkowski PG (2010) Evolution & future of earth's N cycle. Science 330:192–196

Capoen W, Goormachtig S, Holsters M (2010) Water-tolerant legume nodulation. J Exp Bot 61:1251–1255

Cassman KG, Peng S, Olk DC, Ladha JK, Rechardt W, Doberman A, Singh U (1998) Opportunities for increased nitrogen use efficiency from improved resource management in irrigated rice systems. Field Crop Res 56:7–39

Cavalcante VA, Dobereiner J (1988) A new acid-tolerant nitrogen-fixing bacterium associated with sugarcane. Plant Soil 108:23–31

Chalk PM (1991) The contribution of associative and symbiotic nitrogen fixation to the nitrogen nutrition of non-legumes. Plant Soil 132:29–39

Charylulu PBBN, Fourcassie F, Barbouche AK, Rondro-Harisoa L, Mar ANN, Weinhard P, Marie R, Balandreau J (1985) Field inoculation of rice using *in vitro* selected bacterial and plant genotypes. In: Klingmuller WP (ed) *Azospirillum* III genetics, physiology and ecology. Springer, Berlin, pp 163–179

Cocking EC, Stone PJ, Davey MR (2005) Symbiosome-like intracellular colonization of cereals and other crop plants by nitrogen-fixing bacteria for reduced inputs of synthetic nitrogen fertilizers. Sci China C Life Sci 48:888–896

D'Haeze W, Holsters M (2002) Nod factor structures, responses, perception during initiation of nodule development. Glycobiology 12:79–105

Danso SKA (1985) Methods of estimating biological nitrogen fixation. In: Ssali H, Keya SO (eds) Biological nitrogen fixation in Africa. Nirobi, MIRCEN, pp 213–244

De Veau EJ, Robinson JM, Warmbrodt RD, van Berkum P (1990) Photosynthesis and photosynthate partitioning in N_2-fixing soybeans. Plant Physiol 94:259–267

Dixon R, Kahn D (2004) Genetic regulation of biological nitrogen fixation. Nat Rev Microbiol 2:621–631

Dobereiner J (1995) Isolation and identification of aerobic nitrogen fixing bacteria from soil and plants. In: Alef K, Nanniperi P (eds) Methods in applied soil microbiology and biochemistry. Academic, London, pp 134–141

Dobereiner J, Reis VM, Paula MA, Olivers F (1993) Endophytic diazotrophs in sugarcane cereal and tuber plants. In: Palacios R, Mora J, Newton WE (eds) New horizons in nitrogen fixation. Kluwer Academic, Dordrecht, pp 671–676

Doty SL (2011) Nitrogen-fixing endophytic bacteria for improved plant growth. In: Maheshwari DK (ed) Bacteria in agrobiology: plant growth responses. Springer, Berlin, pp 183–199

Eady RR (1996) Structure–function relationships of alternative nitrogenases. Chem Rev 96:3013–3030

Eckert B, Weber OB, Kirchhof G, Halbritter A, Stoffels M, Hartmann A (2001) *Azospirillum doebereinerae* sp.nov., a nitrogen-fixing bacterium associated with the C_4 grass Miscanthus. Int J Syst Evol Microbiol 51(1):17–26

El-Komy HMA, Moharram TMM, Safwat MSA (1998) Effects of *Azospirillum* inoculation on growth and N_2 fixation of maize subjected to different levels of FYM using ^{15}N-dilution method. In: Malik KA (ed) Nitrogen fixation with non-legumes. Kluwer Academic, London, pp 49–59

Engelhard M, Hurek T, Reinhold-Hurek B (2000) Preferential occurrence of diazotrophic endophyte, *Azoarcus* sp. in wild rice species and land races of *Oryza sativa* in comparison with modern races. Environ Microbiol 2:131–141

Esau K (2002) Anatomy of seed plants. Wiley, New York

Falkowski PG, Fenchel T, Delong EF (2008) The microbial engines that drive Earth's biogeochemical cycles. Science 320(5879):1034–1039

Fallik E, Sarig S, Okon Y (1994) Morphology and physiology of plant roots associated with *Azospirillum*. In: Okon Y (ed) *Azospirillum*/plant associations. CRC, Boca Raton, FL, pp 77–85

Field C, Mooney HA (1986) The photosynthesis-nitrogen relationship in wild plants. In: Girmish TJ (ed) On the economy of plant form and function. Cambridge University Press, Cambridge, pp 25–55

Foster RC, Bowen GD (1982) Plant surfaces and bacterial growth. In: Lacy GH (ed) The rhizosphere, phytopathogenic prokaryotes. Academic, New York, pp 159–185

Fürnkranz M, Wanek W, Richter A, Abell G, Rasche F, Sessitsch A (2008) Nitrogen fixation by phyllosphere bacteria associated with higher plants and their colonizing epiphytes of a tropical lowland rainforest of Costa Rica. ISME J 2:561–570

Geurts R, Bisseling T (2002) *Rhizobium* Nod factor perception and signaling. Plant Cell 14: S239–S249

Godfray HC, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food security: the challenge of feeding 9 billion people. Science 327:812

Gyaneshwar P, James EK, Mathan N, Reddy PM, Reinhold-Hurek B, Ladha JK (2001) Endophytic colonization of rice by a diazotrophic strain of *Serratia marcescens*. J Bacteriol 183:2634–2645

Gyaneshwar P, James EK, Reddy PM (2002) *Herbaspirillum* colonization increases growth and nitrogen accumulation in aluminium-tolerant rice varieties. New Phytol 154(1):131–145

Hageman RV, Burris RH (1978) Dinitrogenase and nitrogenase reductase associate and dissociate with each catalytic cycle. Proc Natl Acad Sci USA 75:2699–2702

Halbleib CM, Ludden PW (2000) Regulation of biological nitrogen fixation. J Nutr 130:1081–1084

Hartmann A, Singh M, Klingmuller W (1983) Isolation and characterization of *Azospirillum* mutants excreting high amounts of indoleacetic acid. Can J Microbiol 29:916–923

Hartmann A, Baldani JI, Kirchhof G, Assumus B, Hutzler P, Springer N, Ludwig GW, Baldani VL, Dobereiner J (1995) Taxonomic and ecological studies of diazotrophic rhizosphere bacteria phylogenic probes. In: Fendrik I, Del Gallo M, Vanderleyden J, Zamaroczy M (eds) *Azospirillum* VI and related microorganisms: genetic, physiology and ecology. Springer, New York, pp 416–427

Heckmann AB, Lombardo F, Miwa H, Perry JA, Bunnewell S, Parniske M, Wang TL, Downie JA (2006) *Lotus japonicus* nodulation requires two GRAS domain regulators, one of which is functionally conserved in a non-legume. Plant Physiol 142:1739–1750

Hirsch AM, Kapulnik Y (1998) Signal transduction pathways in mycorrhizal associations: comparisons with the *Rhizobium*-legume symbiosis. Fungal Genet Biol 23:205–212

Huang JS (1986) Ultrastructure of bacterial penetration in plants. Annu Rev Phytopathol 24:141–157

Igual JM, Valverde A, Cervantes E (2001) Phosphate-solubilizing bacteria as inoculants for agriculture: use of updated molecular techniques in their study. Agronomie 21(6–7):561–568

Islam MT (2010) Mode of antagonism of a biocontrol bacterium *Lysobacter* sp. SB-K88 toward a damping-off pathogen *Aphanomyces cochlioides*. World J Microbiol Biotechnol 26:629–637

Islam MT (2011) Potentials for biological control of plant diseases by *Lysobacter* spp., with special reference to strain SB-K88. In: Maheswari DK (ed) Bacteria in agrobiology: plant probiotics. Springer, Berlin, pp 335–363

Islam MT, Hossain MM (2012) Plant probiotics in phosphorus nutrition in plants, with special reference to rice. In: Maheswari DK (ed) Bacteria in agrobiology: plant probiotics. Springer, Berlin, pp 325–363

Islam MT, Hossain MM (2013) Biological control of peronosporomycete phytopathogens by antagonistic bacteria. In: Maheshwari DK (ed) Bacteria in agrobiology: plant disease management. Springer, Berlin, pp. 167–218

Islam MT, Hashidoko Y, Deora A, Ito T, Tahara S (2005) Suppression of damping-off disease in host plants by the rhizoplane bacterium Lysobacter sp. strain SB-K88 is linked to plant colonization and antibiosis against soil borne Peronosporomycetes. Appl Environ Microbiol 71:3786–3796

Islam MT, Deora A, Hashidoko Y, Rahman A, Ito T, Tahara S (2007) Isolation and identification of potential phosphate solubilizing bacteria from the rhizoplane of *Oryza sativa* L. cv. BR29 of Bangladesh. Z Naturforsch 62c:103–110

James EK (2000) Nitrogen fixation in endophytic and associative symbiosis. Field Crops Res 65:197–209

James EK, Olivares FL (1998) Infection and colonization of sugarcane and other graminaceous plants by endophytic diazotrophs. Crit Rev Plant Sci 17:77–119

Jha B, Thakur MC, Gontia I, Albrecht V, Stoffels M, Schmid M, Harmann A (2009) Isolation, partial identification and application of diazotrophic bacteria from traditional Indian rice cultivars. Eur J Soil Biol 45:62–72

Jones KM, Kabayashi H, Davies BW, Taga ME, Walker GC (2007) How rhizobial symbionts invade plants: the *Sinorhizobium–Medicago* model. Nat Rev Microbiol 5:619–633

Jung W, Yu O, Lau SC, O'Keefe DP, Odell J, Fader G, McGonigle B (2000) Identification and expression of isoflavone synthase, the key enzyme for biosynthesis of isoflavones in legumes. Nat Biotechnol 18:208–212

Kaló P et al (2005) Nodulation signaling in legumes requires NSP2, a member of the GRAS family of transcriptional regulators. Science 308:1786–1789

Kapulnik Y, Okon Y (1983) Benefits of *Azospirillum* inoculation on wheat: effects on root development, mineral uptake, nitrogen fixation and crop yield. In: Klingmuller W (ed) *Azospirillum* II. Brikhauser Verlag, Stuttgart, pp 163–170

Kapulnik Y, Gafny R, Okon Y (1985) Effect of *Azospirillum* spp. inoculation on root development and NO_3^- uptake in wheat (*Triticum aestivum* cv. Miriam) in hydroponics systems. Can J Bot 63:627–631

Khamas KM, Agero E, Grimont PAD, Kaiser P (1989) *Azospirillum irakense* sp. nov., a nitrogen fixing bacterium associated with rice roots and rhizosphere soil. Res Microbiol 140:679–693

Kim J, Rees DC (1992) Structural models for the metal centers in the nitrogenase molybdenum-iron protein. Science 257:1677–1682

Kneip C, Lockhart P, Maier U-G (2007) Nitrogen fixation in eukaryotes – new models for symbiosis. BMC Evol Biol 7:55

Kouchi H, Imaizumi-Anraku H, Hayashi M, Hakoyama T, Nakagawa T, Umehara Y, Suganuma N, Kawaguchi M (2010) How many peas in a pod? Legume genes responsible for mutualistic symbioses underground. Plant Cell Physiol 51:1381–1397

Kucey RMN (1988) Alteration of size of wheat root system and nitrogen fixation by associative nitrogen fixing bacteria measured under field conditions. Can J Microbiol 34:735–739

Ladha JK, Barraquio WL, Watanabe I (1982) Immunological techniques to identify *Azospirillum* associated with wetland rice. Can J Microbiol 28:478–485

Ladha JK, Barraquio WL, Watanabe I (1983) Isolation and identification of nitrogen-fixing *Enterobacter cloacae* and *Klebsiella planticola* associated with rice plants. Can J Microbiol 29:1301–1308

Ladha JK, de Bruijin FJ, Malik KA (1997) Assessing opportunities for nitrogen fixation in rice: a frontier project. Plant Soil 194:1–10

Levanony H, Bashan Y (1989) Enhancement of cell division in wheat root tips and growth of root elongation zone induced by *Azospirillum brasilense* Cd. Can J Bot 67:2213–2216

Limpens E, Mirabella R, Fedorova E, Franken C, Franssen H, Bisseling T, Geurts R (2005) Formation of organelle-like N2-fixing symbiosomes in legume root nodules is controlled by DMI2. Proc Natl Acad Sci USA 102:10375–10380

Liu W, Kohlen W, Lillo A, Camp R, Ivanov S, Hartog M, Limpens E, Jamil M, Smaczniak C, Kaufmann K, Yang WC, Hooiveld GJEJ, Charnikhova T, Bouwmeester HJ, Bisseling T, Geurts R (2011) Strigolactone biosynthesis in *Medicagotruncatula* and rice requires the symbiotic GRAS-type transcription factors NSP1 and NSP2. Plant Cell 23(10):3853–3865

Long SR (2001) Genes and signals in the *Rhizobium*-legume symbiosis. Plant Physiol 125:69–72

Ludden PW, Roberts GP (1989) Regulation of nitrogenase activity by reversible ADP ribosylation. Curr Top Cell Regul 30:23–56

Magalhaes FM, Baldani JI, Souto SM, Kuykendall JR, Dobereiner J (1983) A new acid tolerant *Azospirillum* species. An Acad Bras Cienc 55:417–430

Magalhaes CL, de Souza EM, Weber OB, Baldani JI, Dobereiner J, Peddrosa FO (2001) 16S ribosomal DNA characterization of nitrogen-fixing bacteria isolated from banana (*Musa* spp.) and pineapple (*Ananas comosus* (L) Merril). Appl Environ Microbiol 67:2375–2379

Maillet F et al (2011) Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. Nature 469:58–63

Malik KA, Rakhshanda B, Mehnaz S, Rasul G, Mirza MS, Ali S (1997) Association of nitrogen-fixing plant-growth promoting rhizobacteria (PGPR) with kallar grass and rice. Plant Soil 194:37–44

Mano H, Morisaki H (2008) Endophytic bacteria in the rice plant. Microbes Environ 23 (2):109–117

Markmann K, Giczey G, Parniske M (2008) Functional adaptation of a plant receptor-kinase paved the way for the evolution of intracellular root symbioses with bacteria. PLoS Biol 6:e68

Martínez-Argudo I, Little R, Shearer N, Johnson P, Dixon R (2004) The NifL-NifA system: a multidomain transcriptional regulatory complex that integrates environmental signals. J Bacteriol 186:601–610

Mia MAB (2002) Beneficial effects of rhizobacterial inoculation on nutrient uptake, growth and yield of banana (*Musa* spp.). Ph.D. thesis, Faculty of Agriculture, UPM, Malaysia

Mia MAB, Shamsuddin ZH (2009a) Use of plant growth promoting bacteria in banana: a new insight for sustainable banana production. Int J Agric Biol 13(3):459–467

Mia MAB, Shamsuddin ZH (2009b) Enhanced emergence and vigour seedling production of rice through plant growth promoting bacterial inoculation. Res J Seed Sci 2:96–104

Mia MAB, Shamsuddin ZH (2010a) *Rhizobium* as a crop enhancer and biofertilizer for increased cereal production. Afr J Biotechnol 9:6001–6009

Mia MAB, Shamsuddin ZH (2010b) Nitrogen fixation and transportation by rhizobacteria: a scenario of rice and bananas. Int J Bot 6(3):235–242

Mia MAB, Shamsuddin ZH, Zakaria W, Marziah M (1999) External and internal root colonization of *Azospirillum brasilense* on tissue-cultured banana plantlets. In: Vidyadaran MK, Tengku ASTM (eds) Proceedings of the eighth scientific conference of Electron Microscopy Society of Malaysia. Genting Highlands, Pahang, Malaysia, pp 173–174

Mia MAB, Shamsuddin ZH, Zakaria W, Marziah M (2005) High-yielding and quality banana production through plant growth promoting rhizobacterial inoculation. Fruits 60:179–185

Mia MAB, Shamsuddin ZH, Zakaria W, Marziah M (2007) Associative nitrogen fixation by *Azospirillum* and *Bacillus* spp. in bananas. Infomusa 16:11–15

Mia MAB, Shamsuddin ZH, Zakaria W, Marziah M (2009) The effect of rhizobacterial inoculation on growth and nutrient accumulation of tissue-cultured banana plantlets under low N-fertilizer regime. Afr J Biotechnol 8:5855–5866

Mia MAB, Shamsuddin ZH, Zakaria W, Marziah M (2010) Rhizobacteria as bioenhancer for growth and yield of banana (*Musa* spp. cv. "Berangan"). Sci Hort 126:80–87

Molla AH, Shamsuddin ZH, Halimi MS (2001) Mechanism of root growth and promotion of nodulation in vegetable soybean by *Azospirillum brasilense*. Commun Soil Sci Plant Anal 32 (13&14):2177–2187

Murakami Y, Miwa H, Imaizumi-Anraku H, Kouchi H, Downie JA, Kawaguchi M, Kawasaki S (2006) Positional cloning identifies *Lotus japonicus* NSP2, a putative transcription factor of the GRAS family, required for NIN and ENOD40 gene expression in nodule initiation. DNA Res 13:255–265

Murty MG, Ladha JK (1988) Influence of *Azospirillum* inoculation on the mineral uptake and growth of rice under hydroponics conditions. Plant Soil 108:281–285

Muthukumarasamy R, Kang UG, Park KD, Jeon WT, Cho CY, Kwon SW, Song J, Roh DH, Revathi G (2007) Enumeration, isolation and identification of diazotroph from Korean wetland rice varieties grown with long-term application of N and compost and their short-term effect on rice plants. J Appl Microbiol 102:981–991

Naher UA, Radziah O, Shamsuddin ZH, Halimi MS, Razi MI (2009) Isolation of diazotrophs from different soils of tanjongkarang rice growing area of Malaysia. Int J Agric Biol 11:547–552

Nayak DN, Ladha JK, Watanabe I (1986) The effect of marker *Azospirillum lipoferum* inoculated into rice its effect on growth, yield and N_2 fixation of plants studied by acetylene reduction, $^{15}N_2$ feeding and ^{15}N–dilution studies. Biol Fertil Soils 2:7–14

Nur J, Okon Y, Henis Y (1980) An increase in nitrogen content of *Setaria italica* and *Zea mays* inoculated with *Azospirillum*. Can J Microbiol 26:482–485

Oetjen J, Reinhold-Hurek B (2009) Characterization of the DraT/DraG-system for posttranslational regulation of nitrogenase in the endophytic beta-proteobacterium *Azoarcus* sp. BH72. J Bacteriol 191:3726–3735

Ovchinnikova E, Etienne-Pascal J, Chabaud M et al (2011) IPDC controls the formation of nitrogen-fixing symbiosomes in pea and *Medicago* spp. Mol Plant Microbe Interact 24:1333–1344

Pereira JAR, Cavalcante VA, Baldani JI, Dobereiner J (1988) Field inoculation of sorghum and rice with *Azospirillum* spp. and *Herbaspirillum seropedicae*. Plant Soil 110:269–274

Prayitno J, Rolfe B (2010) Characterization of endophytic diazotroph bacteria isolated from rice. Hayati J Biosci 17(2):73–78

Promé JC (1999) Nod factors, chemical signal exchange between bacteria and leguminous plants in nitrogen fixing symbiosis. Ann Pharm Fr 57:240–245

Quilici A, Medina E (1998) Photosynthesis-nitrogen relationships in pioneer plants of disturbed tropical montane forest sites. Photosynthetica 35:525–534

Quispel ANN (1974) The biology of nitrogen fixation. North-Holland Press, Amsterdam

Rao VR, Adhya TK (1994) Bacterial nitrogen fixation associated with rice ecosystem. In: Kannayan S (ed) Rice management biotechnology. Associated Publishing Company, New Delhi, pp 321–327

Rao VR, Ramakrishnan B, Adhya TK, Kanungo PK, Nayak DN (1998) Review: current status and future prospects of associative nitrogen fixation in rice. World J Microbiol Biotechnol 14:621–633

Reddy PM, Ladha JK, So R, Hernandez R, Dazzo FB et al (1997) Rhizobial communication with rice: induction of phenotypic changes, mode of invasion and extent of colonization in roots. Plant Soil 194:81–98

Reddy PM, Aggarwal RK, Ramos MC, Ladha JK, Brar DS et al (1999) Widespread occurrence of the homologues of the early (ENOD) nodulin genes in *Oryza* species and related grasses. Biochem Biophys Res Commun 258:148–154

Reddy PM, Ladha JK, Kouchi H, Stacey G, Hernandez-Oane RJ et al (2000) Realizing the genetic predisposition of rice for symbiotic nitrogen fixation. In: Ladha JK, Reddy PM (eds) The quest for nitrogen fixation in rice. International Rice Research Institute, Los Banos, Philippines, pp 241–261

Reddy PM, James EK, Ladha JK (2002) Nitrogen fixation in rice. In: Leigh GJ (ed) Nitrogen fixation at the millennium. Elsevier, Amsterdam, pp 421–445

Reinhold B, Hurek T, Fendrik I, Pot B, Gillis M, Kresters K, Trielemans S, De Ley J (1987) *Azospirillum halopraeferens* sp. nov., a nitrogen fixing organism associated with roots of kallar grass (*Leptochloa fusca* Kunth.). Int J Syst Bacteriol 37:43–51

Reis VM, Estrada-de los Santos P, Tenorio-Salgado S, Vogel J, Stofels M, Guyon S, Mavingui P, Baldani VLD, Schmid M, Baldani JI, Balandreau J, Hartmann A, Caballero-Mellado J (2004) *Burkholderia tropica* sp. nov., a novel nitrogen-fixing, plant associated bacterium. Int J Syst Evol Microbiol 54:2155–2162

Ribbe M, Gadkari D, Meyer O (1997) N_2 fixation by *Streptomyces thermoautotrophicus* involves a molybdenum-dinitrogenase and a manganese-superoxide oxidoreductase that couple N_2 reduction to the oxidation of superoxide produced from O_2 by a molybdenum-CO dehydrogenase. J Biol Chem 272:26627–26633

Rolfe BG, Mathesius U, Prayinto J, Perrine F, Weinman JJ, Stefaniak J, Djordjevic M, Guerreiro N, Dazzo FB (2000) *Rhizobium* nodulation and interaction with legumes and nonlegumes. In: Ladha JK, Reddy PM (eds) The quest for nitrogen fixation in rice. International Rice Research Institute, Makati, Philippines, pp 291–309

Sahin F, Cakmakci R, Kantar F (2004) Sugar beet and barley yields in relation to inoculation with N2-fixing and phosphate solubilizing bacteria. Plant Soil 265:123–129

Schindelin H, Kisker C, Schlessman JL, Howard JB, Rees DC (1997) Structure of ADP x AlF4–stabilized nitrogenase complex and its implications for signal transduction. Nature 387:370–376

Schmitz RA, Klopprogge K, Grabbe R (2002) Regulation of nitrogen fixation in *Klebsiella pneumoniae*: nifL, transducing two environmental signals to the nif transcriptional activator NifA. J Mol Microbiol Biotechnol 4:235–242

Schrock RR (2006) Reduction of dinitrogen. Proc Natl Acad Sci USA 103:17087

Sessitsch A, Hardoim P, Döring J, Weilharter A et al (2012) Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. Mol Plant Microbe Interact 25:28–36

Shamsuddin ZH, Amir HG, Mia MAB, Halimi MS, Zakaria W, Marziah M (1999) Symbiotic and associative N_2 fixation with vegetable soybean, oil palm and bananas. In: Yoshida T, Seki T, Pornchai M, Ebora RV, Sukara E, Ismail MAKM (eds) Biotechnology for sustainable utilization of biological resources in the tropics, vol 14. International Center for Biotechnology, Osaka University, Osaka, Japan, pp 102–118

Shrestha RK, Ladha JK (1996) Genotypic variation in promotion of rice nitrogen fixation as determined by nitrogen ^{15}N dilution. Soil Sci Soc Am J 60:1815–1821

Simpson FB, Burris RH (1984) A nitrogen pressure of 50 atmospheres does not prevent evolution of hydrogen by nitrogenase. Science 224:1095–1097

Smit P, Raedts J, Portyanko V, Debelle´ F, Gough C, Bisseling T, Geurts R (2005) NSP1 of the GRAS protein family is essential for rhizobial Nod factor-induced transcription. Science 308:1789–1791

Souleimanov A, Prithiviraj B, Smith DL (2002) The major nod factor of *Bradyrhizobium japonicum* promotes early growth of soybean and corn. J Exp Bot 53:1929–1934

Sprent J (1989) Which steps are essential for formation of functional legume nodules? New Phytol 111:129–153

Sreevidya VS, Srinivasa Rao C, Sullia SB, Ladha JK et al (2006) Metabolic engineering of rice with soybean isoflavone synthase for promoting nodulation gene expression in rhizobia. J Exp Bot 57:1957–1969

Sterner RW, Elser JJ (2002) Ecological stoichiometry: the biology of the elements from molecules to the biosphere. Princeton University Press, Princeton, NJ

Stoltzfus JR, So R, Malarvizhi PP, Ladha JK, De Bruijn FJ (1997) Isolation of endophytic bacteria from rice and assessment of their potential for supplying rice with biologically fixed nitrogen. Plant Soil 194:25–36

Stougaard J (2001) Genetics and genomics of root symbiosis. Curr Opinion Plant Biol 4:328–335

Studholme DJ, Dixon R (2003) Domain architectures of σ54-dependent transcriptional activators. J Bacteriol 185:1757–1767

Sundaram S, Klucas RV (1988) Characterization of Azospirilla isolated from seeds and roots of turf grass. Can J Microbiol 34:212–217

Suslow TV (1982) Role of root-colonizing bacteria in plant growth. In: Mount MS, Lacy GH (eds) Phytopathogenic prokaryotes, vol 1. Academic, New York, pp 186–223

Tarrand JJ, Krieg NR, Dobereiner J (1978) A taxonomic study of the *Spirillum lipoferum* group, with description of new genus, *Azospirillum* gen. nov. and *A. brasilense* sp. nov. Can J Microbiol 24:967–980

Tien TM, Diem HG, Gaskins MH, Hubbe DH (1979) Plant growth substances produced by *Azospirillum brasilense* and their effects on the growth of pearl millet (*Pennisetum americanum* L.). Appl Environ Microbiol 37:1016–1024

Trevaskis B, Colebatch G, Desbrosses G, Wandrey M, Wienkoop S et al (2002) Differentiation of plant cells during symbiotic nitrogen fixation. Comp Funct Genomics 3:151–157

Triplett EW (1996) Diazotrophic endophyte: progress and prospects for nitrogen fixation in monocots. Plant Soil 186:29–38

Urquiaga S, Cruiz KHS, Boddey RM (1992) Contribution of nitrogen fixation to sugarcane: nitrogen-15 and nitrogen balance estimates. Soil Sci Soc Am J 56:105–114

Van Nieuwenhove C, Van Holm L, Kulasooriya SA, Vlassak K (2000) Establishment of *Azorhizobium caulinodans* on the rhizosphere of wetland rice (*Oryza sativa* L.). Biol Fertil Soils 31:143–149

Van-Berkum P, Bohlool BB (1980) Evaluation of nitrogen fixation by bacteria in association with roots of tropical grasses. Microbiol Rev 44:491–517

Vemeiren H, Vanderleyden J, Hai W (1998) Colonization and nifH expression on rice roots by *Alcaligenes faecalis* A15. In: Malik KA, Mirza MA, Ladha JK (eds) Nitrogen fixation with non-legumes. Kluwer Academic, Dordrect, pp 287–305

Wange SS, Patil PI (1994) Effect of combined inoculation of *Azotobacter*, *Azospirillum* with chemical nitrogen on Basarai banana. Madras Agric J 81:163–165

Watanabe I, Lin C (1983) Response of wetland rice to inoculation with *Azospirillum lipoferum* and *Pseudomonas* sp. Soil Sci Plant Nutr 30:117–124

Weber OB, Baldani VLD, Teixeira KRS, Kirchhof G, Baldani JI, Dobereiner J (1999) Isolation and characterization of diazotrophic bacteria from banana and pineapple plants. Plant Soil 210:103–113

Whitfeld PR, Bottomley W (1983) Organization and structure of chloroplast genes. Annu Rev Plant Physiol 34:297–310

Xie CH, Yokota A (2005) *Azospirillum oryzae* sp. nov., a nitrogen fixing bacterium isolated from the root of rice plant *Oryza sativa*. Int J Syst Evol Microbiol 55:1435–1438

Yanni YG, Rizk RY, Corich V, SquartiniA NK, Philip-Hollingsworth S, Orgambide G, deBruijin FD, Stoltzfus J, Buckley D, Schmidit TM, Mateos PF, Ladha JK, Dazzo FB (1997) Natural endophytic associations between *Rhizobium leguminosarum* bv. *trifolii* and rice roots and assessment of its potential to promote rice growth. Plant Soil 194:99–114

You C, Zhou F (1989) Non-nodular endorhizospheric nitrogen fixation in wetland rice. Can J Microbiol 35:403–408

You CB, Song W, Wang HX, Li JP, Lin M, Hai WL (1991) Association of *Alcaligenes faecalis* with wetland rice. Plant Soil 137:81–85

Chapter 6
Potential of Rhizobia in Productivity Enhancement of *Macrotyloma uniflorum* L. and *Phaseolus vulgaris* L. Cultivated in the Western Himalaya

Dinesh K. Maheshwari, Mohit Agarwal, Shrivardhan Dheeman, and Meenu Saraf

6.1 Introduction

High altitude regions like the mighty Himalayas are prone to freezing and desiccation and characterized by dramatic seasonal change in physical, chemical and biological consistency including property of soil. Less crop production is foreseen in hilly or mountain area due to freezing and desiccation including soil texture incompatibility due to rocks and stones. In high altitude regions farmers are interested to produce high crop yield using fertilizers in fields. Intensive agricultural practices, rapid industrialization and an increase imbalance uses of nutrient supply leads to decline in soil productivity and soil degradation. Soil a habitat and substratum for plants with nutritional source of them affect the health and growth of plant as well as the productivity of crop planted.

In the Himalayan climatic condition where temperature are crucial determinant for microbial growth as well as the growth of plant, their health and productivity cold-tolerant rhizospheric microflora can be used to induce for crop enhancement of legume in the western Himalaya. These are characterized to retain their activity in suboptimal temperature conditions. Psychrotolerant rhizobia which can grow over a wide temperature range from 4 to 42 °C and usually grow optimally at temperature above 20 °C, are extremely important, since they have survive and retain their functionality in low-temperature area such as Himalaya mountain ranges in India.

D.K. Maheshwari (✉) • M. Agarwal • S. Dheeman
Department of Botany and Microbiology, Gurukul Kangri University, Haridwar 249404, Uttarakhand, India
e-mail: maheshwaridk@gmail.com

M. Saraf
Department of Microbiology and Biotechnology, University School of Sciences, Gujarat University, Ahmedabad 380009, Gujarat, India

D.K. Maheshwari et al. (eds.), *Bacteria in Agrobiology: Crop Productivity*,
DOI 10.1007/978-3-642-37241-4_6,

Crop plants of commercial importance that are severally restricted by a variety of environmental factors, including drought, salinity, and low temperature, have a great significant effect in reducing agricultural productivity around the world. In addition, natural distribution of plants species is determined by their ability to survive freezing events. In higher altitude, especially in the western Himalaya, low temperature is one of the major determinants for crop productivity, whereas in some plant species from temperate climates, winter survival is greatly influenced by the plant's cold acclimatization ability.

The concept of crop productivity emerges with the concurrent development of major scientific pathway involved green revolution during 1960s. From the beginning of period until today, the crop productivity is a major issue of Indian agricultural economy. Our food demand remains parallel to the population increase; accordingly, the same pattern is expected; and by the end of 2020, population will be ~2 billion (approximately). To eradicate the challenges food requirements of the burgeoning population and plateauing productivity of agricultural lands can only be met by a second green revolution or ever green revolution. Declining crop productivity mainly results due to unsuitable agricultural practices and galloping rate of population. Pertaining to massive population pressure, increase in food grain production is an uphill task in today's world. Thus, introduction of evergreen revolution proved to be an advantageous task in achieving higher productivity. Lessons drawn from the green revolution lead to productivity enhancement, conservation and improvement of soil, availability of water, biodiversity, atmosphere, renewable energy sources, etc. A system of agriculture evolved that involves sustainable management of natural resources and progressive enhancement of soil quality, biodiversity, and productivity.

Legumes seeds are widely recognized as an important source of food and feed proteins (Duranti and Guis 1997) and have become very important in human nutrition and as a feed for domestic animals (Egli 1998; Cummings et al. 2001). Additionally, the increasing interest in low-input sustainable agriculture systems on cropping farms from an economic, managerial, and environmental standpoint opens the door for continued interest in grain legumes (White 1989). The importance of legumes to these systems is not only for their nitrogen (N)-fixing capabilities but also their ability in breaking the cycles of diseases and pests affecting the other crops (White 1989; Cummings et al. 2001).

In the past few decades, field and greenhouse inoculation studies with plant growth-promoting rhizobacteria (PGPR) have shown that these microorganisms are able to enhance yield of agriculturally important crops grown under climatic conditions and different soils (Okon and Labandera-Gonzalez 1994). During last decades different genera involved in plant growth promotion have been widely applied and now well established and used commercially worldwide for sustainable agriculture, silviculture, horticulture, and environmental remediation (Kloepper et al. 1989; Jeffries et al. 2003; Reed and Glick 2005; Fravel 2005; Aeron et al. 2011). Involvement of different PGPR-mediated positive factors such as indoleacetic acid (Park et al. 2005; Mordukhova et al. 1991; Gupta et al. 1999; Kumar et al. 2005), gibberellic acid (Mahmoud et al. 1984), and cytokinins (Tien

et al. 1979; Garcia de Salamone et al. 2001) impart significant effects on different crops. Growth regulators like IAA and cytokinin producing PGPR observed in growth promotion of various agricultural crops, i.e., *Sesamum indicum* L., *Trifolium repens*, *Arachis hypogea* L., *Cajanus cajan*, *Trigonella foenum*, *Mucuna pruriens*, *Pinus roxburghii*, *Mimosa pudica*, *Meloidogyne incognita,* etc. (Noel et al. 1996; Hirsch et al. 1997; Kumar et al. 2005).

PGPR competitively colonize plant root, stimulate plant growth, and reduce plant disease (Kloepper and Schorth 1978). Genetic improvement of PGPR enhances plant by colonizing in rhizosphere and enhancing effectiveness of PGP attributes parallel. It might be due to one or more associated trait associated in plant growth promotion (Bloemberg and Lugtenberg 2001; Glick et al. 1995).

PGPR are most commonly used in agriculture, and their application in various crops resulted in an average increase (~ 20–40 %) in yield across multiple crops all over the world when various reports were combined over last decade. In general, PGPR carried plant growth benefit owing to the increase in pre-emergence of seedling, seed germination rates, root growth, leaf area, chlorophyll, proteins, and hydraulic activity, fluid movement within the plant besides tolerance to drought, low temperature, delayed leaf senescence, disease resistance, and finally enhanced grain size and crop yield.

Fabaceae which is the second largest family of flowering plant comprises of about 750 genera and more than 18,000 species, and among them, only 15–20 % have been explored for rhizobial diversity. Legume of economic importance is grown in India under various agroclimatic conditions, and the presence of native rhizobia has, therefore, been anticipated. In order to tap the vast diversity of rhizobia in the country, it is important to screen legumes that are wild or are found in rare habitats.

Besides pulses (chief agricultural legumes), there are certain important medicinal plants beneficial to mankind. Some are of the wild and native species of the sub-Himalayan region of Uttarakhand. These species are used in traditional and folk system of medicines since Vedic ages. A few of these plant of the family Leguminosae are *Abrus precatorius* L. (Ratti), *Acacia concinna* L. (Shikakai), *Acacia catechu* L. (Kattha), *Acacia nilotica* L., *Astragalus condolleanus* L., (Rudravanti), *Canavalia ensiformis* L. (Jack bean), *Canavalia gladiate* L. (Sword bean), *Clitoria ternatea* L. (Aprajita), *Crotalaria juncea* L. (Sunn-hemp), *Dalbergia sissoo* L. (Sheesham), *Glycine max* L. (Barg var.) (Bhatt), *Glycyrrhiza Glabra* L. (Mulethi), *Macrotyloma Uniflorum* L. (Gahat), *Melilotus officinalis* L., *Mimosa hamata* L., *Mimosa himalayensis* L., *Mimosa pudica* L. (Lajwanti), *Mucuna pruriens* L. (Kaunch), *Phaseolus vulgaris* L. (Cheema), *Prosopis spicigera* L. (Shami), *Psoralea corylifolia* L. (Bagchi), *Sesbania rostrata* L., *Sesbania sesban* L. (Dhaincha), *Tamarindus indica* L. (Imli), *Tephrosia purpurea* L., *Trigonella foenum-graecum* L. (Methi), *Vicia angustifolia* L., etc.

6.2 PGPR in Crop Production

The bacteria useful to plants are characterized in two general types: bacteria forming a symbiotic relationship with the plant and another the free-living ones found in the soil but comprise near or even within (inside) the plant tissues (Kloepper et al. 1988; Frommel et al. 1991). Beneficial free-living soil bacteria that enhance plant growth are usually referred to as "plant growth promoting rhizobacteria" (Kloepper and Schorth 1978) or yield-increasing bacteria (YIB) (Paio et al. 1992; Tang 1994). PGPR originally defined (Kloepper and Schorth 1978) as root-colonizing bacteria (rhizobacteria) that cause either growth promotion or biological control of plant diseases. The mechanisms of plant growth promoting by nonpathogenic, plant-associated bacteria have not been completely elucidated but are important mechanisms are categorized into the direct and indirect plant growth-promoting mechanisms (Glick et al. 1995). The direct plant growth-promoting (PGP) mechanisms include solubilization of minerals such as phosphorus (Malboobi et al. 2009), production of siderophore that solubilize and sequester iron (Kloepper et al. 1980), or production of plant growth regulators (hormones) that induce growth and yield of plants (Mordukhova et al. 1991; Glick 1995; Gupta et al. 1999; Garcia et al. 2001; Ma et al. 2002) and enhance plant growth at various stages of development, whereas indirect plant growth promotion occurs when PGPR promote plant growth by improving growth-restricting conditions by secreting antagonistic substances or indirectly by inducing systemic resistance to pathogens (Glick et al. 1995, 1999). In PGPR, a broad range of metabolites such as cyanogens mainly hydrogen cyanide (HCN) (Flaishman et al. 1996), antibiotics (Shanahan et al. 1992; Haas and Defago 2005), lytic enzymes (Chitinase, β-1, 3-glucanase, protease) (Lim et al. 1991; Renwick et al. 1991; Arora et al. 2001), and toxins have been reported (Fig. 6.1).

The PGPR are defined by three intrinsic characteristics: (1) They must be able to colonize the root; (2) they must survive and multiply in the microhabitats associated with the root surface, in competition in other microbiota, at least for the time needed to expressed their plant promotion/protection activities; and (3) they must promote the plant growth (Barea et al. 2005). The PGPR known to participate in many important ecosystem processes were first used for agriculture purposes in the former Soviet Union and India and are now being tested worldwide (Lucy et al. 2004). These rhizospheric beneficial bacteria enhance plant growth and control the phytopathogens by indirect and indirect mean, but the specific mechanism by which PGPB promote plant growth is not fully understood (Glick 1995; Ahmad et al. 2008). These bacteria can improve plant development by nitrogen fixation; phytohormones and siderophore production; solubilization of phosphorus, zinc, and potassium; elevation of the stress by secreting the ACC deaminase enzyme; and disease control by suppressing or killing of phytopathogens.

PGPR can be divided in to two groups according to their relationship with the plant: symbiotic bacteria and free-living rhizobacteria (Khan 2005). Agricultural manipulation of symbiotic and free-living PGPR has become a significant

Direct PGP activities	Indirect PGP activities
1. Atmospheric Nitrogen Fixation	1. Antibiosis
2. Production of plant hormones	2. Iron scavenging
3. Enhanced iron availability	3. Competition for nutrients/niche
4. Phosphorus solubilization	4. Parasitism and predation

Fig. 6.1 Plant growth-promoting activities of PGPR

component of modern agricultural practice in many countries (Bashan and Holguin 1998). For this purpose, the most successful plant-bacteria relationships have been those involving symbiotic rhizobia and free-living, non-leguminous bacteria such as *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, and *Pseudomonas* (Dobereiner and Pedroza 1987). On the other hand, PGPR includes various genera, namely, *Achromobacter*, *Acidovorax*, *Acetobacter*, *Acinetobacter*, *Azoarcus*, *Azomonas*, *Azospirillum*, *Actinoplanes*, *Agrobacterium* (*Rhizobium radiobacter*), *Alcaligenes*, *Arthrobacter*, *Azotobacter*, *Azorhizobium*, *Bacillus*, *Beijerinckia*, *Bradyrhizobium*, *Burkholderia*, *Cellulomonas*, *Chryseobacterium*, *Delftia*, *Enterobacter*, *Erwinia, Flavobacterium, Gluconacetobacter*, *Herbaspirillum*, *Klebsiella*, *Ochrobacterium*, *Mesorhizobium*, *Methylobacterium*, *Methylovorus*, *Micromonospora*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Rhodococcus*, *Rhizobium, Serratia*, *Stenotrophomonas*, *Streptomyces*, *Sinorhizobium*, *Variovorax*, and *Xanthomonas*.

6.3 Rhizobia

Symbiotic nitrogen-fixing, Gram-negative bacteria having caliber to invade roots of leguminous plants are referred to as rhizobia. The rhizosphere is the region where rhizobia are heavily populated, and it makes a first line of defense of plant pathogenic fungi attack (Weller 1988). Most important of rhizobia in crop

productivity is that it induces the formation of plant membrane structure called nodules by infecting host plant. These nodules have significant role in nitrogen fixation into a useful symbiosis with legume plants; this process is called BNF (bacterial nitrogen fixation).

Rhizobia are able to fix N_2 in the symbiotic and free-living relationship with host plant. Rhizobia can combine nitrogen gas from air to nitrogenous compound that plant can utilize as a direct nitrogen source. Rhizobia are the best-known plant symbiotic bacteria and have importance in atmospheric nitrogen fixation, which enhance the plant growth promotion and biomass yield. Currently, rhizobia have been established both as bio-fertilizers and biopesticides. Rhizobia do not form endospores and bear a single polar flagellum or two to six peritrichous flagella. Uneven Gram staining is frequently encountered with rhizobia, depending on the age of the culture. Cell from a young culture and bacteroids usually show even Gram staining, while older and longer cells give a banded appearance with unstained area. These unstained areas have been identified to be large granules of polymeric beta-hydroxybutyric acid (PHBA). Rhizobia are predominantly aerobic chemoorganotrophs and are relatively easy to culture. They grow well in the presence of oxygen and utilize relatively simple carbohydrates and amino compounds. Rhizobia are facultative symbionts that can live either as member of the natural soil microbial community or symbiotically in root nodules of the host legumes.

6.4 Taxonomy

Continuous evolving taxonomy of rhizobia parallel to the evolvement of molecular biology and polyphasic approach that leads regular increases in the number of root-nodulating symbiotic microorganisms. Mystery factors behind this phenomenon are symbiotic genes which may be responsible for the cluster or disperse through conjugation with high frequency on the plasmid as well as chromosomes.

A taxonomy given will help to explore current status of rhizobia. Rhizobia belong to α- and β-proteobacteria and consist of 95 species which are distributed in 13 genera, namely, *Azorhizobium*, *Bradyrhizobium*, *Burkholderia*, *Cupriavidus*, *Devosia*, *Ensifer*, *Herbaspirillum*, *Mesorhizobium*, *Methylobacterium*, *Ochrobacterium*, *Phyllobacterium*, *Shinella*, and *Rhizobium* (Table 6.1).

6.5 Rhizobia in Crop Productivity

In the rhizospheric vicinity, Rhizobia are recognized as predominating microorganisms to induce plant crop growth in symbiotic relation, where rhizobia induce root nodulation in plant and fix nitrogen content for plant growth and health; on the other hand, plants provide microhabitat for rhizobia. Common bean is a

Table 6.1 Current taxonomy of Rhizobia and related species (www.rhizobia.co.nz/taxonomy/rhizobia.html)

Genera	Species	Hosts name	References
Rhizobium (30 spp.)	*R. alamii*	*Medicago ruthenica*	Berge et al. (2009)
	R. alkalisoli	*Caragna intermedia*	Lu et al. (2009b)
	R. cellulosilyticum	*Sawdust of Populus alba (nodulates)*	Garcia-Fraile et al. (2007)
	R. daejeonense	*Cyanide treatment bioreactor*	Quan et al. (2005)
	R. endophyticum	*Phaseolus vulgaris*	Lopez-Lopez et al. (2010)
	R. etli	*Phaseolus vulgaris*	Segovia et al. (1993)
	R. faba	*Vicia faba*	Tian et al. (2008)
	R. galegae	*Galega orientalis*	Lindstrom (1988)
	R. gallicum	*Phaseolus vulgaris*	Amarger et al. (1997)
	R. giardinii	*Phaseolus vulgaris*	Amarger et al. (1997)
	R. hainanense	*Desmodium sinuatum*	Chen et al. (1997)
	R. herbae		Ren et al. (2011a)
	R. huautlense	*Sesbania herbacea*	Wang et al. (1998)
	R. indigoferae	*Indigofera* spp.	Wei et al. (2002)
	R. leguminosarum	*Pisum sativum*	Frank (1889)
	R. loessense (R. huanglingense)	*Astragalus complanatus*	Wei et al. (2003)
		Astragalus scobwerrimus	
		Astragalus chrysopterus	
	R. lusitanum	*Phaseolus vulgaris*	Valverde et al. (2006)
		Macroptilium atropurpureum	
		Leucaena leucocephala	
	R. mesosinicum	*Albizia* sp.	Lin et al. (2009)
	R. miluonense	*Kummerowia* sp.	Gu et al. (2008)
		Dalbergia sp.	
		Lespedeza chinensis	
	R. mongolense	*Medicago ruthenica*	Van Berkum et al. (1998)
	R. multihospitium	*Multiple legume species*	Han et al. (2008b)
	R. oryzae	*Oryza alta*	Peng et al. (2008)
	R. phaseoli	*Phaseolus vulgaris*	Ramirez-Bahena et al. (2008)
	R. pisi	*Pisum sativum*	Ramirez-Bahena et al. (2008)
	R. sullae (R. hedysari)	*Hedysarum coronarium*	Squartini et al. (2002)
	R. tropici	*Phaseolus vulgaris*	Martinez-Romero et al. (1991)
	R. tubonense	*Oxytropis glabra*	Zhang et al. (2011)

(continued)

Table 6.1 (continued)

Genera	Species	Hosts name	References
	R. undicola (Allorhizobium undicola)	*Neptunia natans*	De Lajudie et al. (1998)
	R. vignae		Ren et al. (2011b)
	R. yanglingense	*Gueldenstaedita multiflora*	Tan et al. (2001)
		Amphicarpaea trisperma, Coronilla varia	
Mesorhizobium (21 spp.)	*M. albiziae*	*Albizia kalkora*	Wang et al. (2007)
	M. alhagi		Chen et al. (2009)
	M. amorphae	*Amorpha fructose*	Wang et al. (1999b)
	M. australicum	*Biserrula pelecinus*	Nandasena et al. (2009)
	M. camelthorni		Chen et al. (2011)
	M. caraganae	*Caragana* spp.	Guan et al. (2008)
	M. chacoense	*Prosopis alba*	Velazquez et al. (2001)
	M. ciceri (Rhizobium ciceri)	*Cicer arietinum*	Nour et al. (1994)
	M. gobiense	*Wild desert legumes*	Han et al. (2008a)
	M. huakuii (Rhizobium huakii)	*Astragalus sinicus*	Jarvis et al. (1997)
	M. loti (Rhizobium loti)	*Lotus corniculatus*	Jarvis et al. (1982, 1997), Nour et al. (1995)
	M. mediterraneum (Rhizobium mediterraneum)	*Cicer arietinum*	Nour et al. (1995) Jarvis et al. (1997)
	M. metallidurans		Vidal et al. (2009)
	M. opportunistum		Nandasena et al. (2009)
	M. plurifarium	*Acacia senegal*	
	M. robiniae		Zhou et al. (2010)
	M. shangrilense		Lu et al. (2009a)
	M. septentrionale	*Astragalus adsurgens*	
	M. tarimense	*Wild desert legumes*	Han et al. (2008a)
	M. temperatum	*Astragalus adsurgens*	
	M. tianshanense (Rhizobium tianshanense)	*Glycyrrhiza pallidiflora*	Jarvis et al. (1997)
Ensifer (formerly Sinorhizobium) (17 spp.)	*E. abri*	*Abrus precatorius*	Ogasawara et al. (2003)
	E. americanum	*Abrus acatlensis*	Toledo et al. (2004)
	E. arboris	*Prosopis chilensis*	Nick et al. (1999)
	E. fredii (Rhizobium fredii)	*Glycine soja*	Chen et al. (1988)
	E. garamanticus		Merabet et al. (2010)
	E. indiaensis	*Sesbania sesban*	Ogasawara et al. (2003)
	E. kostiense	*Acacia senegal*	Nick et al. (1999)

(continued)

Table 6.1 (continued)

Genera	Species	Hosts name	References
	E. kummerowiae	*Kummerowia stipulacea*	Wei et al. (2002)
	E. medicae	*Medicago* spp.	Rome et al. (1996)
	E. meliloti (Rhizobium meliloti)	*Medicago sativa*	de Lajudie et al. (1994)
	E. mexicanus	*Acacia angustissima (Mill.) Kuntze*	Lloret et al. (2007)
	E. morelense (Ensifer adhaerens)	*Leucaena leucocephala*	Martens et al. (2007)
	E. numidicus		Merabet et al. (2010)
	E. saheli	*Sesbania cannabina*	de Lajudie et al. (1994)
	E. sojae	*Glycine max*	Li et al. (2011)
	E. terangae	*Acacia laeta*	de Lajudie et al. (1994)
Bradyrhizobium (8 spp.)	*B. canariense*		
	B. denitrificans		
	B. elkanii	*Glycine max*	Kuykendall et al. (1992)
	B. iriomotense	*Entada koshunensis*	Islam et al. (2008)
	B. japonicum (Rhizobium japonicum)	*Leguminous plants*	Jordan (1982)
	B. jicamae	*Pachyrhizus erosus*	Ramírez-Bahena et al. (2009)
	B. liaoningense	*Glycine max, Glycine soja*	
	B. pachyrhizi	*Pachyrhizus erosus*	Ramírez-Bahena et al. (2009)
	B. yuanmingense	*Lespedeza*	Yao et al. (2002)
Azorhizobium (02 spp.)	*A. caulinodans*	*Sesbania rostrata*	Dreyfus et al. (1988)
	A. doebereinerae (Azorhizobium johannae)	*Sesbania virgata (Caz.) Pers*	
Methylobacterium (01 spp.)	*M. nodulans*	*Crotalaria podocarpa*	Jourand et al. (2004)
Burkholderia (07 spp.)	*B. caribensis*	*Tropical mimosoid woody legumes*	Vandamme et al. (2000)
	B. cepacia	*Tropical mimosoid woody legumes*	Vandamme et al. (2002, 2007)
	B. mimosarum	*Mimosa* spp.	Chen et al. (2006)
	B. nodosa	*Mimosa bimucronata*	Chen et al. (2007)
		Mimosa scabrella	
	B. phymatum	*Tropical mimosoid woody legumes*	Vandamme et al. (2000)
	B. sabiae	*Mimosa caesalpiniifolia*	Chen et al. (2008)
	B. tuberum	*Tropical mimosoid woody legumes*	Vandamme et al. (2000), Moulin et al. (2001)

(continued)

Table 6.1 (continued)

Genera	Species	Hosts name	References
Cupriavidus (01 spp.)	*C. taiwanensis*	*Mimosa pudica*	Chen et al. (2001)
		Mimosa diplotricha	Chen et al. (2003)
Devosia (01 spp.)	*D. neptuniae*	*Neptunia natans*	Rivas et al. (2003)
Herbaspirillum (01 spp.)	*H. lusitanum*	*Phaseolus vulgaris*	Valverde et al. (2003)
Ochrobacterium (02 spp.)	*O. cytisi*	*Cytisus scoparius*	Zurdo-Piñeiro et al. (2007)
	O. lupine	*Lupinus albus*	Trujillo et al. (2005)
Phyllobacterium (03 spp.)	*P. trifoli*	*Trifolium pratense*	Valverde et al. (2003)
	P. ifriqiyense	*Lathyrus numidicus*	Mantelin et al. (2006)
		Astragalus algerianus	
	P. leguminum	*Astragalus algerianus*	Mantelin et al. (2006)
		Argyrolobium uniflorum	
Shinella (01 spp.)	*S. kummerowia*	*Kummerowia stipulacea*	Lin et al. (2008)

promiscuous host plant since the capability of nodulate *P. vulgaris* effectively is present in a genetically heterogeneous group of bacteria originating from all over the world (Laguerre et al. 1993). Historically, *P. vulgaris* was the first legume in which Rhizobium symbiosis was identified (Taylor et al. 1983), and the original microsymbiont of *P. vulgaris* is *Rhizobium etli* (Segovia et al. 1993). Root-nodulating bacteria are of extreme importance in legumes. They provide them with the advantageous factor in relation to nitrogen fixation besides other's such as scavenging of phytopathogens. Several workers reported different species of rhizobia nodulate *P. vulgaris* such as *R. etli* (D'Haeze et al. 2007), *R. gallicum* (Shamseldin et al. 2005), *R. giardinii* bv. *giardinii* (Mhamdi et al. 2002), *R. leguminosarum* (Mhamdi et al. 2002; Garcia-Fraile et al. 2010), *R. tropici* (Gressent et al. 2002), and *E. meliloti* bv. *mediterranense* (Zurdo-Piñeiro et al. 2009).

Rhizobia strains were able to increase seed yield enhancement by various PGP mechanism invariable climatic and soil conditions (Epping et al. 1994; Mostasso et al. 2002; Asadi et al. 2005; Mnasri et al. 2007a; Gutierrez and Barraquio 2010). Rhizobia strains are able to increase seed yield, pods per plant, seeds per pod, weight of seed per plant, seed protein yield, total dry matter, etc. (Deshwal et al. 2003a; Mazen et al. 2008). Khalequzzaman and Hossain (2008) reported maximum yield reduction of foot rot disease by *S. sclerotium* in bean on application of rhizobia. The effect of rhizobia on the growth and nutrition uptake of various crop plants has been discussed by Kloepper et al. (2007), and Glick et al. (2007).

The major concern of rhizobia is maximum yield production utilizing its essential PGP attributes for major leguminous crop of the Himalayan diversity,

namely, *M. uniflorum* and *P. vulgaris*. Similarly, *Rhizobium leguminosarum* br. *trifoli* E11 and E12 increased grain yield of rice in field inoculation experiment (Yanni et al. 1997). Huang and Erickson (2007) tested the effectiveness of *R. leguminosarum* for improving growth and yield of pea and lentil. They found improved seedling growth, nodule biomass, and shoot and root biomass in peas. The effect of different methods of rhizobia inoculation on yield, root nodulation, and seed protein contents of two lentil varieties and improvement in nodulation was observed in peanut by inoculation with *Rhizobium* species (Ahmad et al. 2008; Dey et al. 2004). Pandey et al. (2004) reported that four isolates from legume plants of the sub-Himalayan region having nitrogen-fixing ability were confirmed by molecular technique and amplification of 781 bp *nif*H fragment. Another separate study on the enhancement of pulse crop in the Northeast Himalayan region done by Choudhury and Azad (2005) reported that rhizobia are more effective for the enhancement of pulse crop production by observing almost all growth parameters, namely, nodule number and nodule dry weight, root and shoot biomass, nitrogenase activity, N and P content of plant, and chlorophyll content enhancement. Similar results of yield enhancement due to *Rhizobium* inoculation were reported by Subba Rao (1993) and Tilak (1992).

6.5.1 Nitrogen Fixation

Rhizobium is the most extensively and longest exploited PGPR for their ability to fix N_2 in legume host including high altitude crop *Macrotyloma uniflorum* L. and *Phaseolus vulgaris* L. Nitrogen is abundant in atmosphere (78 %) in the air of gaseous form measuring about 8,000 pounds nitrogen in the air over every area at land. The available N_2 or fixed nitrogen utilized by the plant for their growth and maintenance and further for their biomass production. Nitrogen as an essential plant nutrient is being used annually about 42 million tons on global scale for the great production of cereal crop. Symbiotic association of N_2-fixing microorganisms with legumes converts atmospheric elemental nitrogen (N_2) into ammonia (NH_3) (Shiferaw et al. 2004). Reduction of nitrogen gas to required ammonia involves enzymatic reaction carried over by the majority of PGPR including rhizobia. Rhizobia form intimate symbiotic relationship with legume by responding chemotactically to signaling molecule flavonoid, which induce the expression of nodulation in rhizobia (Lhuissier et al. 2001; Dakora 2003; Matiru and Dakora 2004). In relation to crop productivity inoculation of compatible rhizobial strain significantly produce substantial amounts of nitrogen resulting symbiosis interaction (Deshwal et al. 2006). Rhizobia were found to enhance nodulation, dry weight of nodules, nitrogen fixation, and yield legume plants. Rhizobial inoculation of *P. vulgaris* showed significant difference in nitrogen content for studied parameters (Mohammed Ahmed et al. 2009). It was observed that legume inoculations with rhizobia had higher concentration of hemoglobin, nitrogenase activity, and N-fixing efficacy and thus form a greater symbiotic relation to enhance the content of

nitrogen in the rhizosphere for plant growth promotion. Lee et al. (2005) reported that inoculation of rhizobia increases growth and yield of plants under nitrogen-producing condition.

Rhizobia are soil bacteria capable of forming a nitrogen-fixing symbiosis with leguminous plants. To form an effective symbiosis, rhizobia require several classes of specific genes. These include *nod* genes, which encode the production of Nod factors, which stimulate the plants to produce symbiotic nodules. The *nod* genes are found in all rhizobia and code for Nod factor which are responsible for nodule formation (Lindström et al. 1995). These specific genes, which produce the nitrogen-fixing nitrogenase enzymes and nodulation genes, which encode the production of Nod factors, stimulate the plants to produce symbiotic nodules. *Nif*H gene codes for Fe-protein subunit of nitrogenase enzyme and for dinitrogenase reductase (Dean and Jacobson 1992). Nitrogen fixation ability and nodulation ability of rhizobia can be confirmed by the amplification of *nif*H and *nod*C fragments. *R. leguminosarum* and *E. meliloti* nodulated their host plant horse gram which further substantiate the presence of *nod*C genes. *nod*C gene was also recently used to study the nodulation gene diversity of soil rhizobial population (Sarita et al. 2005). The nitrogenase activity of the rhizobia is usually confirmed by the amplification of some specific *nif* genes such as *nif*H (Young and Haukka 1996; Pandey et al. 2004). Recently, Leelahawonge et al. (2010) reported two major symbiotic genes, *nif*H and *nod*C to confirm the symbiotic properties of *Indigofera tinctoria* symbionts at the molecular level. Both *nif*H and *nod*C are well conserved among symbiotic nitrogen-fixing bacteria (Zhang et al. 2000; Leelahawonge et al. 2010). Many workers reported the presence of *nod* and *nif* genes in different species of *Rhizobium* (Estrella et al. 2009; Wang et al. 2009) and *Ensifer* (Pandey et al. 2004; Kumar et al. 2006; Wang et al. 2009).

6.5.2 Plant Growth Hormone (IAA) Production

As stated, growth substances of bacterial origin accounts for induction of plant growth development (Tien et al. 1979; Fulchieir et al. 1993; Teale et al. 2006). Such chemical substances are released by rhizospheric bacteria and later absorbed by roots (Libbert and Silhengst 1970). These are mainly composed of indoleacetic acid (IAA) (Gupta et al. 1999), gibberellic acid (Mahmoud et al. 1984), cytokinins (Tien et al. 1979; Garcia et al. 2001), and ethylene (Arshad and Frankenberger 1991; Glick et al. 1995; Ma et al. 2002). IAA and cytokinins produced by PGPR reported to play a significant role in growth promotion of both leguminous and non-leguminous plants (Noel et al. 1996; Hirsch et al. 1997; Patten and Glick 2002). The plant growth-promoting effect is tentatively attributed to production of auxin commonly produced by soil bacteria. In general, biosynthesis of IAA uses tryptophan (Trp) as a precursor, and several pathways for conversion of Trp into IAA have been described (Costacurta and Vanderleyden 1995; Baca and Elmerich 2007). IAA is a common product of L-tryptophan metabolism of soil fungi and plant

Table 6.2 Different rhizobial isolates from selected legumes showing the IAA activity

S.No.	Genera	Legume	References
1.	*Rhizobium* spp.	*Mimosa pudica*	Roy and Basu (1989)
2.	*R. meliloti* RMP1-12	*Arachis hypogaea*	Arora et al. (2001)
3.	*Rhizobium* spp.	*Dalbergia lanceolaria*	Ghosh and Basu (2002)
4.	Rhizobia MPR1-4	*Mucuna pruriens*	Kumar et al. (2006)
5.	*Rhizobium* spp.	*Sesbania sesban*	Sridevi and Mallaiah (2007a, b)
6.	*Rhizobium* spp.	*Phaseolus mungo*	Ghosh et al. (2008)
7.	*Rhizobium* spp. HGR3 and HGR8	*Macrotyloma uniflorum*	Prabhavati and Mallaiah (2008)
8.	*Rhizobium* DASA 57053, 57065, 57076, 57010, 57027	*Indigofera tinctoria*	Pongsilp and Nuntagij (2009)
9.	*Sinorhizobium* DASA 57015, 68012	*Derris elliptica*	Pongsilp and Nuntagij (2009)
10.	*Rhizobium* sp. BHURC01	*Cicer arietinum*	Verma et al. (2010)
11.	*Sinorhizobium* spp. KCC1-8	*Cajanus cajan*	Dubey et al. (2010)
12.	*Rhizobium* spp.	*Vigna radiata*	Zahir et al. (2010)
13.	*R. leguminosarum* $LSI_{19,}$ $LSI_{23,}$ LSI_{30}	*Lens culinaris*	Mehboob et al. (2011)
14.	*Rhizobium* spp.	*Arachis hypogaea, Cicer arietinum, Melilotus, Trigonella*	Sahasrabudhe (2011)

growth-promoting bacteria (Lynch and Bragg 1985); hence, several rhizobacteria are reported to produce indole-3-acetic acid (IAA) in culture media especially in the presence of tryptophan (Frankenberger and Arshad 1990; Gamalero and Glick 2011). The production of IAA by rhizobia in different legumes has been shown in Table 6.2.

Indole 3-acetic acid is a growth-stimulating hormone which increases plant root length, further enhancing the root surface to absorb nutrient from the surrounding soil for plant growth promotion. So, the bacteria produced IAA secrete in the soil which is immobilized by the seed during germination. Whereas the IAA is itself also produced by the plant at various times of age, bacterial origin IAA promotes the growth of plant directly by secreting such hormones.

6.5.3 Solubilizing Rock/Soil Phosphate

Agricultural production remains highly reliant on the application of phosphatic (P) fertilizers derived from phosphate rock. Due to increasing demand and dwindling stocks, it is predicted that current global reserves of phosphate rock may be depleted

within 50–100 years (Cordell et al. 2009). Furthermore, continued agricultural expansion had led to co-saturation of many ecosystems with both N and P, resulting in the degradation of terrestrial, freshwater, and marine resources (Tilman et al. 2001). This concern has highlighted the imperative need to better understand the plant–soil–microbial P cycle, with an aim of reducing our reliance on mineral fertilizers. It is, therefore, the need of the hour to harness microorganism that could support P cycling in agroecosystems. It is established that majority of soil microbes have the potential to enhance the rate of organic P (P_o) or inorganic P (P_i) cycling by solubilizing insoluble organically bound and mineral-bound phosphorus.

Phosphorus (P) is second only to N in terms of quantitative requirement for crop plants (Goldstein 1986; Feng et al. 2004; Fernandez et al. 2007; Takahashi and Anwar 2007; Gamalero and Glick 2011). It is found in soil, plant, and microorganisms in both organic and inorganic forms. Soil contains phosphorus in insoluble form complexed with cations like iron, aluminum, and calcium. However, the total P content in an average soil is 0.05 % (w/w), and only a very small fraction (~0.1 %) of the total P present in the soil is available to the plants because of its chemical fixation and low solubility (Stevenson and Cole 1999; Illmer and Schinner 1995). Phosphorus may be added for enhancing fertility to soil either as chemical fertilizers or as leaf litter, plant residues, or animal remains. However, 75 % of phosphate fertilizer applied to soil are rapidly immobilized and thus become unavailable to plants (Rodriguez and Fraga 1999). Therefore, P deficiency is a major constraint to crop production, and under such conditions, the microorganisms especially PGPR offer a biological rescue system capable of solubilizing the insoluble inorganic P (Esitken 2011). Phosphate-solubilizing microorganisms (PSMs) are ubiquitous, and their numbers vary from soil to soil. In soil, P-solubilizing bacteria constitute 1–50 % and fungi 0.5–0.1 % of the total respective population. The majority of the phosphate-solubilizing microbes (PSMs) solubilize Ca–P complexes, and few can solubilize Fe–P and Al–P (Banik and Dey 1983; Kucey et al. 1989).

Seed or soil inoculation with phosphate-solubilizing bacteria such as *Azotobacter*, *Bacillus*, *Clostridium*, fluorescent pseudomonads, and rhizobia solubilized inorganic phosphorus (Gupta et al. 1999, 2001a, b, 2002; Arora et al. 2001; Deshwal et al. 2003a, b; Kumar et al. 2010, 2011; Singh et al. 2010); such bacteria improve solubilization of fixed soil phosphorus and applied phosphates resulting in higher crop yield (Abd-Alla 1994; Yadav and Dadarwal 1997).

Rhizobia are, perhaps, the most promising group of PSB on account of their efficient ability for solubilizing insoluble inorganic phosphate compounds (Halder et al. 1990; Barea et al. 2005). Several workers have demonstrated that phosphate-solubilizing strains of Rhizobium and Bradyrhizobium increased growth and P content of non-leguminous as well as leguminous plants (Chabot et al. 1996, 1998; Antoun et al. 1998). Rhizobia with the potential to solubilize soil P and also with the ability to promote the growth of non-legumes had been reported (Halder and Chakrobartty 1993). In fact, a P-solubilizing strain of *R. leguminosarum* bv. *trifolii* have been reported to stimulate the growth of non-legumes members of Poaceae and Brassicaceae (Höflich et al. 1995). Phosphate

Table 6.3 Selected Rhizobia solubilizing phosphate

S.No.	Rhizobia	Host plant	References
1.	*R. leguminosarum* P31, R1	Quebec soil	Chabot et al. (1996)
2.	*R. leguminosarum* ARPV02	*Phaseolus vulgaris*	Abril et al. (2003)
3.	*Rhizobium* MPR1-5	*Mucuna pruriens*	Kumar et al. (2006)
4.	*R. leguminosarum* AGR-7, DPE-12, ELD-15, SBO-3, SGA-15	*Trifolium repens*	Vargas et al. (2009)
5.	*Rhizobium* sp. BHURC01	*Cicer arietinum*	Verma et al. (2010)
6.	*Sinorhizobium* spp. KCC1-KCC8	*Cajanus cajan*	Dubey et al. (2010)
7.	*R. leguminosarum* $LSI_{19,}$ LSI_{23}	*Lens culinaris*	Mehboob et al. (2011)

solubilization by rhizobia in leguminous and non-leguminous plant association reported by several workers is mentioned (Table 6.3).

The ability to solubilize Ca–P complexes had been attributed to the nature of PSMs that reduced the pH of their surroundings, either by the release of organic acid due to exchange of phosphates by acid anion, chelate both Fe and Al ions associated with phosphate (Katznelson and Bose 1959; Bajpai and Sundra Rao 1971; Bardia and Gaur 1972; Moghimi et al. 1978). PSMs produced organic acids such as acetate, lactate, isovaleric, oxalic oxalate, tartrate, succinate, citrate, gluconate, ketogluconate, and glycolate (Duff and Webley 1959; Banik and Dey 1982; Cunningham and Kuiack 1992; Omar 1998; Whitelaw et al. 1999; Rodriguez and Fraga 1999; Thakuria et al. 2004; Puente et al. 2004; Alikhani et al. 2006; Rodriguez et al. 2006; Saraf et al. 2011). Kang et al. (2002) reported decrease in pH and production of citrate responsible for P-solubilization activity as evidenced by addition of NaOH. It indicated that P-solubilizing activity was mainly due to lowering of the pH of the media due to bacterial mediated acid production (Halder and Chakrobartty 1993; de Werra et al. 2009; Berg and Zachow 2011; Osorio 2011). However, acidification did not seem to be the only mechanism of solubilization, as the ability to reduce the pH in some cases did not correlate with the ability to solubilize mineral phosphates (Subba Rao 1982).

6.5.4 Siderophore Production

Iron is the fourth most abundant element found in the Earth's crust but present in the highly insoluble form of ferric hydroxide (Fe^{3+}) and thus unavailable to microorganisms and plants. Some bacteria have developed iron uptake systems (Neilands and Nakamura 1991). These systems involved a siderophore—an

iron-binding legend—and an uptake protein needed to transport iron into the cell. Actually, siderophores are low molecular weight (~ 400–1,000 Da) iron-chelating compounds that bind Fe^{3+} (ferric iron) with high affinity (Crosa and Walsh 2002; Siddiqui 2006; Saraf et al. 2011) and transport it back to the cell, making it available for the microbial cells (Neilands and Leong 1986; Briat 1992). The secreted siderophore molecules with high affinity (kd = 10^{-20}–10^{-50}) for iron bind most of the Fe^{3+} that is available in the rhizosphere, thus preventing the pathogens present in the immediate vicinity from proliferation because of lack of iron (O'Sullivan and O'Gara 1992). Bacterial antagonists can prevent the proliferation of fungal phytopathogens by producing siderophores that bind most of the Fe^{3+} in the rhizosphere (Aeron et al. 2011; Esitken 2011). The resulting lack of the iron prevents such fungal pathogens from proliferating in this immediate vicinity. Earlier, Kloepper et al. (1988) stated that the production of siderophores that chelate and thereby scavenge the ferric iron in the rhizosphere may result in growth inhibition of other microorganisms whose affinity for iron is lower. It has been suggested that the ability to produce specific siderophores and/or to utilize a broad spectrum of siderophores may contribute to the root colonization ability of biocontrol strains (Aeron et al. 2011). In addition, siderophores also mediated the iron uptake by plant roots in iron-limiting conditions (Silva-Stenico et al. 2005; Kumar et al. 2008a).

Mesorhizobium loti MP6 (Chandra et al. 2007) and *S. fredii* KCC5 (Kumar et al. 2010) isolated from root nodules of *Mimosa pudica* and *Cajanus cajan*, respectively, secrete the hydroxamate type of siderophore and induce growth and yield of *Brassica campestris* and *Cajanus cajan*. Suppression of phytopathogens that is due to iron deficiency causes growth inhibition, decrease in nucleic acid synthesis, deformation of cell morphology, etc. (Mathiyazhagan et al. 2004).

Deryło et al. (1994) reported *Rhizobium* sp. as siderophore production in different host plants (Duhan et al. 1998; Deshwal et al. 2003a, b; Sridevi et al. 2008). Rhizobia producing siderophore resulting inhibition of other microorganisms further in plant growth promotion and higher crop yield. Some of the rhizobia producing siderophore are listed along with host plant (Table 6.4).

6.6 Present Status of Horse Gram and Common Bean

This chapter provides an overview studies to the potential of Rhizobia in productivity enhancement of *Macrotyloma uniflorum* L. and *Phaseolus vulgaris* L., two important legumes cultivated in the western Himalaya. Our aim is to provide a concise study on rhizobia as PGPR to increase crop production of these crops in high-altitude regions. PGPR applications in agriculture-based industries for economic development are in an eco-friendly manner. The overall strategy for increasing crop yields and sustaining them at high level by using indigenous rhizobia in two native legumes growing in the western Himalaya.

Table 6.4 Selected Rhizobia producing siderophore

S.No.	Rhizobia	Host plant	References
1.	*Bradyrhizobium japonicum*	*Glycine max* L.	Guerinot et al. (1990)
2.		*Melilotus alba* L.	Persmark et al. (1993)
3.		*Astragalus* spp.	Deryło et al. (1994)
4.	*S. meliloti*	*Ononis* spp.	Deryło et al. (1994
5.	*Rhizobium* spp.	*Genista* spp.	Deryło et al. (1994)
6.	*Rhizobia* spp.	*Cicer arietinum* L.	Roy et al. (1994)
7.	*B. japonicum*	*Glycine max* L.	Wittenberg et al. (1996)
8.	*R. cicero*	*Cicer arietinum* L.	Berraho et al. (1997)
9.	*Bradyrhizobium* spp.	*Cajanus cajan*	Duhan et al. (1998)
10.	*Rhizobium* spp.	*Cajanus cajan*	Duhan et al. (1998)
11.	*R. leguminosarum* bv. *viciae*	*Pisum sativum, Lens culinaris*	Dilworth et al. (1998)
12.	*R. leguminosarum* bv. *phaseoli*	–	Carrillo-Castañeda and Cano (2000)
13.	*R. leguminosarum* bv. *trifoli*	–	Lynch et al. (2001)
14.	*R. meliloti RMP3 and RMP5*	*Arachis hypogaea*	Arora et al. (2001)
15.	*Rhizobium* spp.	*Arachis hypogaea*	Deshwal et al. (2003a)
16.	*Bradyrhizobium* spp.	*Arachis hypogaea*	Deshwal et al. (2003a)
17.	*Sinorhizobium MPR3 and MPR4*	*Mucuna pruriens Linn.*	Kumar et al. (2006)
18.	*Rhizobia*	*Cajanus cajan*	Khan et al. (2006)
19.	*R. leguminosarum ICARDA 441*	*Vicia faba* L.	Mazen et al. (2008)
20.	*Mesorhizobium* spp.	–	Ahmad et al. (2008)
21.	*Mesorhizobium RC3*	*Cicer arietinum* L.	Wani et al. (2008)
22.	*Rhizobium* spp.	*Sesbania procumbens*	Sridevi et al. (2008)
23.	*Sinorhizobium KCC1, KCC5*	*Cajanus cajan*	Dubey et al. (2010)

6.6.1 Macrotyloma uniflorum *L. (Gahat)*

Macrotyloma uniflorum (Lam.) Verdc. (horse gram) is largely cultivated, especially in dry areas of India, Australia, Burma, and Sri Lanka. It is one of the important legume crops grown in tropics and subtropics regions in India. In India, it is commonly known as Gahat, kulat, horse grain, hurali, kalai, kallu, kerdekorn, kollu, etc. Horse gram is also grown in higher reaches of Uttarakhand state of Indian Himalaya. However, hilly area under cultivation varies and is cultivated more in areas in the Garhwal Himalayan region (60.3 %) than that in the Kumaon region (39.7 %) as stated by Shukla et al. (2006). At present, it ranks third among Indian pulses in area, covering about 1.7 million hectares with a total production of 0.74 million tons of the total cultivated area under pulses cultivation in India (Kumar 2006).

It is a major ingredient in the Pahadi cuisine of Himalayan areas of North India. It is cooked in a round iron saute-pan ("kadhai") to prepare Ras, a favorite of most

Kumaonis. Besides the Himalayan region, this grain legume is also grown in other Indian states, namely, Tamil Nadu, Karnataka, and Andhra Pradesh. The seeds are parched and then eaten after boiling or frying, either whole or as a meal (Pulseglove 1974) and proved important feed for cattle and horses. The stems, leaves, and husks are also known to be used as fodder; a good source of proteins, carbohydrates, and essential amino acids; and as an excellent source of iron and molybdenum (Katiyar 1984). It possesses many health beneficial components (Viswanatha et al. 2006) and is one of the major ingredients of Ayurvedic medicine "CYSTONE" prescribed commonly to the patients suffering from kidney (stone) ailments (Singhla and Kumar 1985; Mukherjee et al. 1984). The aqueous extract of this drug is known to inhibit the initial precipitation of calcium and phosphorus ions (Jethi et al. 1983) as evidenced by clinical studies. Earlier, this plant has been proved as one of the potential food sources by US.

Verdcourt (1982) studied various species of *Macrotyloma* and concluded that instead of *Dolichos*, it is grouped under a distinct genus as *Macrotyloma uniflorum*. Another species, *Macrotyloma axillare*, is identified as a wild relative and generally grown for fodder purpose. *Macrotyloma axillare*, *Macrotyloma africanum*, and *Macrotyloma daltonii* are three related species reported from Australia and are forage types. *Macrotyloma ciliatum* (Willd.) *Verdc.* is found in Tamil Nadu (Nair and Henry 1983; Matthew 1983) and Andhra Pradesh (Pullaiah and Chennaiah 1997). *Macrotyloma sar-garhwalensis* is wild relative of horse gram found in the Garhwal Himalayas (Gaur and Dangwal 1997). It is cultivated in the entire sub-Himalayan tracts up to 1,800 m in sunny and exposed places, and this crop is drought resistant but cannot withstand waterlogged condition and adapted to a wide range of soils from sands to gravels to clay loams and heavy clays. It is known to easily tolerate a pH range of 6.0–7.5 but is fairly tolerant of soil salinity.

The plant with a height of 100–110 cm is a slender, sub-erect annual herb with slightly twining, downy stems and branches. The leaves of this plant are trifoliate, while the flower is pale yellow in color. The linear and flattened pod is generally composed of about 5–7 seeds each. Flattened shiny seeds are small and 3–6 mm in length. The seed color varies which ranges from light red, brown, black, to mottled. Propagation of the plant is through both seed and vegetative methodologies. The sowing time is early rainy season from June to September and gets maturity within 125–130 days in field. The crop is also grown as a green manure meant to increase the soil fertility status.

6.6.2 Phaseolus vulgaris *L. (Rajma)*

Phaseolus vulgaris L. is one of the most ancient crops of world. It is being cultivated in Central America since 4000 BC. The word Phaseolus comes from the Greek phaselua, "which refers to a canoe-like boat reminiscent of a bean pod" (Albala 2007). It is dominant in the staple diets of lower income people in the Americas, Africa, and Asia, together with maize. Beans are extensively diverse

crops in terms of their uses for human nutrition and a major protein and calorie source in the world (Sharon 2003).

It is an important pulse crop of India cultivated in sub-Himalayan and higher Himalayan ranges at 1,200–1,800 m mainly in Maharashtra, Himachal Pradesh, Jammu and Kashmir, and Uttarakhand. Due to its nutritive components, it is one of the 10 most important crops of the world. In our country, the area under which common bean are cultivated is 9,700 million ha (only 36 % area) as compared to 27,086 million ha all over the world. While, its production in India is 4,330 million tons (only 23 %) as compared to 18,943 million tons in the world (FAO, Anonymous 2003). It is less preferred in comparison to other pulses like *Vigna radiata*, which consequently resulted in its limited cultivation restricted to certain parts of the country. Even then, this pulse crop has gained popularity among Indian farmers due to its high lucrative features like short growth cycle, good adaptability, and high market price, and the most important for poor farmers, particularly women, hence one of the names, is a woman's crop (Spence 2006; Kumar et al. 2008b).

It belongs to the subfamily Papilionaceae of Fabaceae. The common bean is a highly variable species with a long history. Bush varieties form erect bushes 20–60 cm tall, while pole or running varieties form vines 2–3 m long. All leaflets, are 6–15 cm long and 3–11 cm wide. The white, pink, or purple flowers are about 1 cm long and give way to pods 8–20 cm long; 1–1.5 cm wide; and green, yellow, black, or purple in color, each containing 4–6 beans. The beans are smooth, plump, kidney shaped, up to 1.5 cm long; range widely in color; and are often mottled in two or more colors.

Dry common bean is widely consumed throughout the world, and it is recognized as the major source of dietary protein in many African countries and India (Guzman-Maldonaldo and Paredes-Lopez 1998; Dursun 2007). A large variability exists in common bean seed; color and size are two important quality characteristics for the consumers. Seed size and weight depend on the genetic variation, cultivar, and environmental condition (Gonzalez de Mejia et al. 2005). The seed color of the beans is determined by the presence of concentration of flavonol glycosides, anthocyanins, and tannins (Beninger and Hosfield 2003; Aparicio-Fernandez et al. 2005).

Common bean seeds have a notable place in the folklore throughout the world and in the traditions of many cultures such a pharmacotherapeutic effects (Hangen and Bennick 2002; Mishra et al. 2010). The ripen dried pods and the beans are used for curing infections of urinary tracts, kidney, and bladder stones. It is also used as a diuretic and an antidiabetic. The juice of the fresh beans is applied over face to cure pimples. Leaf juice as liniment is used to alleviate pain due to sprain (Pullaiah 2006); because of the presence of various bioactive compound, common bean seeds can be associated with a decrease risk for a wide variety of chronic and degenerative diseases such as cancer, obesity, cardiovascular diseases, and diabetes. Its seeds are considered as a good dietary source (Ocho-anin Atchibri et al. 2010).

It is an interesting crop from the consumer, farmer, and processor's point of view. For the consumer, bean is important for its nutritive composition and its variable uses in different culinary forms. For farmers, crop contributes nitrogen to

the soil which often low, while dry seeds and fresh pods of specific land races attract high market prices (e.g., Harshil Rajma of Uttarakhand). The fresh pod crop, mainly field grown, can be produced during the coolest season in glasshouses. For the processor, it provides many possibilities such as canned and frozen seeds and pods (Escribano et al. 1997).

P. vulgaris is relatively important host for Rhizobia to nodulate in their root part. These rhizobia have also been recognized in other legumes including *Macrotyloma uniflorum*, *Vigna radiata*, *M. africanum*, and *Pisum sativum*. Jordan (1984) and Amarger et al. (1997) reported that some rhizobial species nodulating these legume crops are *R. giardini, R. gallicum, and R. leguminosarum* bv. *phaseoli*. Martinez-Romero et al. (1991) have also reported *R. tropici* as microsymbiont of *P. vulgaris*. *R. etli* is also reported as most dominating root-nodulating and nitrogen-fixing rhizobia of common bean (Segovia et al. 1993; Wang et al. 1999a; Beyene et al. 2004; Tamimi and Young 2004; Mouhsine et al. 2007; Grange and Hungria 2004; Stocco et al. 2008). Five additional rhizobia are reported by Mhamdi et al. (2002), i.e., *R. etli* bv. *phaseoli* (Silva et al. 2003; Shamseldin and Werner 2007), *R. gallicum* bv. *phaseoli*, *R. giardinii* bv. *giardinii*, *R. leguminosarum* bv. *phaseoli*, and *R. leguminosarum* bv. *viciae*, as asymbiotic microorganisms of common beans.

The distribution of bean rhizobial able to nodulate *P. vulgaris* varies between geographical locations, although *R. etli* and *R. tropici* appear to be widely distributed (Young et al. 2004; Amarger 2001). Mnasri et al. (2007a) characterized *Sinorhizobium meliloti* as salt-tolerating rhizobia and termed as *S. meliloti* bv. *mediterranense*. *R. yanglingense* is also reported to form nodules on *P. vulgaris* (Tan et al. 2001) (Table 6.5).

6.7 Productivity Enhancement

Productivity is a measure of the efficiency of crop production. It is measured in a ratio of production output (yield obtained from crop plant) to what is required to produce for inputs (consumption for mankind). In other words, it can be defined as a total output per one unit for the total input. Agriculturally important crop plant consumed by mankind for their food demand and hence the production of such crop are considered for high yield or increased productivity; the measure of the yield for their consumption from certain field area can be defined as productivity. The assumption of productivity enhancement is that the more the plant will grow and become healthy, the more yield will occur. Thus, intended to produce more yield, fertilization as well as biofertilization is practised. For biofertilization, microbial inoculants are exploited in the crop production. Rhizobial inoculants particularly in legume crops are capable to induce plant growth and further better yield *M. uniflorum*, and *P. vulgaris* with *Rhizobium leguminosarum* showed more pronounced increment in seedling growth (Ahmad et al. 2006; Bhatia et al. 2008; Husen et al. 2009; Singh et al. 2010; Minaxi and Saxena 2010; Kumar et al. 2011;

Table 6.5 Rhizobial species nodulating in *P. vulgaris* (common bean)

Rhizobial species	Reference
S. meliloti *R. leguminosarum* bv. *viciae* *R. tropici* *R. gallicum* bv. *gallicum*	Mouhsine et al. (2007)
R. leguminosarum *R. tropici*	Odee et al. (2002), Diouf et al. (2000), Anyango et al. (1995)
R. tropici *R. etli* *R. leguminosarum* *Mesorhizobium* spp. *Ensifer* spp.	Mostasso et al. (2002), Grange and Hungria (2004), Martinez-Romero et al. (1991), Stocco et al. (2008), Kaschuk et al. (2006)
R. giardinii *R. gallicum* *R. leguminosarum* bv. *phaseoli*	Amarger et al. (1997), Jordan (1984)
R. etli	Segovia et al. (1993), Beyene et al. (2004), Tamimi and Young (2004), Mouhsine et al. (2007)
R. gallicum bv. *gallicum* *R etli* bv. *phaseoli*	Silva et al. (2003)
Ensifer meliloti bv. *mediterranense*	Mnasri et al. (2007b)
R. etli bv. *phaseoli* *R. gallicum* bv. *gallicum*	Shamseldin and Werner (2007)

Kala et al. 2011) and shoot length (Hoque and Haq 1994; Patra and Bhattacharyya 1998; Shaharoona et al. 2006; Khalequzzaman and Hossain 2007; Ali et al. 2008; Minaxi and Saxena 2010; Kala et al. 2011) than that of seedling raised by non-bacterized seeds. Such increase in root length with greater surface area may be due to IAA secretion by both groups of bacteria that are involved in root initiation, cell division, and cell enlargement which enables the plant to access more nutrients from soil (Salisbury 1994; Mantelin and Touraine 2004) and act as an habitat/ecological niche to allow a greater number of introduced rhizobia during their symbiotic and root-colonizing activity which is an added advantage for supplementing the growth and development. Bashan et al. (2004) observed that seed treatment with a PGPR positively affect the root biomass and surface. An increase in root dimensions is directly proportional to aggressive colonization by desired beneficial rhizobia that may also be an influencing attribute for increase in plant productivity and phosphate solubilization process through which rhizobia enables the plant to uptake free P (soluble form) and is available in insoluble form in the soil. Rhizobia produced ACC deaminase, ACC of plant cell hydrolyzed due to bacterial-mediated ACCD into α-ketobutyrate and ammonia (Glick 1995), thereby restricting the overproduction of ethylene which leads to abnormal root growth and imparts a visible dent on growth and development of *M. uniflorum* and *Phaseolus vulgaris*. Thus, seeds bacterized with ACCD containing *Rhizobium* have

Table 6.6 Crop yield parameters of *M. uniflorum* (120 DAS) using MRG6 strain (unpublished data from corresponding authors lab)

Treatment	No. of pods/plant	Grain yield (kg/ha)	Biological yield (kg/ha)	Harvest index	Crop yield % rise over control
MRG6	19.333**	1,276	4,667	27.34	29.41
Control	15.000	986	3,863	25.48	
SEM'	0.197				
CD at 1 %	0.933				
CD at 5 %	0.642				

Values are mean of ten replicates; [a] = significant at 0.01 level of analysis of variance (ANOVA). ** = Significant at 0.01 level of LSD as compared to control; * = significant at 0.05 level of LSD as compared to control; [ns] = not significant at 0.05 level of LSD as compared to control

extensive root growth probably due to low level of ethylene and subsequent improvement in vegetative parameters. Such phenomenon is now well established in ACC containing PGPR role in growth promotion of crop plants as described by Saraf et al. (2010). *Rhizobium leguminosarum* are also effective at low phosphate level as reported by Xavier and Germida (2002). *R. leguminosarum* considerably increases dry biomass of shoot and seed, N and P content of plant as reported in green house trials (Mehdi et al. 2006). Further, the overall fresh and dry weight was enhanced significantly with *R. leguminosarum* MRG6 (AB569639) during our study (Table 6.6). Various other workers (Bhatia et al. 2008; Kumar et al. 2010, 2011; Singh et al. 2010; Minaxi and Saxena 2010; Agarwal and Ahmad 2010; Kala et al. 2011) also observed increase in early vegetative plant growth parameters due to application of different genera of PGPR including rhizobia.

More availability of nutrients uptake by plant made possible due to presence of desired strain in rhizosphere inoculated by seed bacterization helps in healthy plant growth. Rhizobia aggressively root colonize and hence showed enhancement in the average number of pods per plant which are similar to the finding observed in different host plants as observed by earlier workers (Bhatia et al. 2008; Kumar et al. 2010, 2011).

Thus, seed bacterization with rhizobial strains proved beneficial for raising healthy crops. Using biological growth-enhancing rhizobia thus increases the yield of *P. vulgaris* in given conditions. The enhancement of the yield parameters in *Phaseolus vulgaris* due to rhizobia over non-bacterized set of control plant is obvious due to multifarious activity of PGP attributes. As stated, an enhanced seed germination due to treatment with beneficial rhizobia leads to sturdier plants which give more yield in terms of grains produced per hectare. Further secretion of siderophores by these ACC deaminase-producing strains greatly enhances the ability of aggressive colonization target rhizosphere and warding off fungal pathogens by iron scavenging and competition for nutrients. Enhanced vegetative and reproductive growth achieved by *R. leguminosarum* MRG6 confer considerable benefits: an increase in crop yield by 32.2 % rise over control grain yield (1,189 kg/ha) and biological yield (4,603 kg/ha) (Table 6.7).

Table 6.7 Crop yield parameters of *P. vulgaris* (120DAS) using *Rhizobium leguminosarum* MRG6 strain (unpublished data from corresponding authors lab)

Treatment	No. of pods/plant	Grain yield (kg/ha)	Biological yield (kg/ha)	Harvest index	Crop yield % rise over control
MRG6	18.333**	1,189	4,603	25.83	32.2
Control	14.333	899	3,806	23.62	
SEM	0.316				
CD at 1 %	1.497				
CD at 5 %	1.030				

Values are mean of ten replicates; [a] = significant at 0.01 level of analysis of variance (ANOVA). ** = Significant at 0.01 level of LSD as compared to control; * = significant at 0.05 level of LSD as compared to control; [ns] = not significant at 0.05 level of LSD as compared to control

6.8 Conclusions

Rhizobia as a diverse group of microorganisms are potential PGPR, which are widely distributed in the agroecosystem and play a significant role in the enhancement of several agricultural crops. The importance of PGPR and its potential for plant growth promotion and enhancement in crop productivity of selected crops have been elaborated in this chapter. Although most research work conducted so far has largely focused on rhizobia in nitrogen fixation and other attributes such as IAA production, siderophore production and phosphate solubilization are added advantage to crop. Similar to other PGPR genera rhizobia exhibit both direct and indirect effects to the plants. An elaborate description is given on *M. uniflorum* and *P. vulgaris*. More studies are required to be carried out in those species which are neglected but cultivated by marginal farmers of the Himalayan states of India.

Acknowledgment Thanks are due to UCOST (Dehradun) and UGC (New Delhi) for providing financial support in the form of research project to DKM.

References

Abd-Alla MH (1994) Use of organic phosphorus by *Rhizobium leguminosarum* bv. *viciae* phosphatases. Boil Fert Soils 8:216–218

Abril A, Zurdo-Pineiro JL, Peix A, Rivas R, Velásquez E (2003) Solubilization of phosphate by a strain of *Rhizobium leguminosarum* bv *trifolii* isolated from *Phaseolus vulgaris* in El Chaco Arido soil (Argentina). In: Velázquez E (ed) First international meeting on microbial phosphate solubilization, Salamanca, Spain, 16–19 July 2002, pp 135–138

Aeron A, Kumar S, Piyush P, Maheshwari DK (2011) Emerging role of plant growth promoting rhizobacteria in agrobiology. In: Maheshwari DK (ed) Bacteria in agrobiology: crop ecosystem. Springer, Berlin, pp 1–36

Agarwal S, Ahmad Z (2010) Contribution of the *Rhizobium* inoculation on plant growth and productivity of two cultivars of berseem (*Trifolium alexandrinum* L.) in saline soil. Asian J Plant Sci 9:344–350

Ahmad ZI, Anjum MS, Rauf A (2006) Effect of rhizobia inoculation on growth and nodules formation of green gram. Int J Agri Biol 8(2):235–237

Ahmad F, Ahmad I, Khan MS (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res 163:173–181

Albala K (2007) Beans: a history. Berg, New York

Ali ME, Khanam D, Bhuiyan MAH, Khatun MR, Talukder MR (2008) Effect of *Rhizobium* inoculation to different varieties of gardenpea (*Pisum sativum* L.). J Soil Nature 2(1):30–33

Alikhani HA, Saleh-Rastin N, Antoun H (2006) Phosphate solubilization activity of rhizobia native to Iranian soils. Plant Soil 287:35–41

Amarger N (2001) Rhizobia in the field. Adv Agron 73:109–168

Amarger N, Macheret V, Laguerre G (1997) *Rhizobium gallicum* sp. nov. and *Rhizobium giardinii* sp. nov., from *Phaseolus vulgaris nodules*. Int J Syst Bacteriol 47:996–1006

Anonymous (2003) FAO production year book, vol 56, pp 109–110

Antoun H, Beauchamp CJ, Goussard N, Chabot R, Lalande R (1998) Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus sativus* L.). Plant Soil 204:57–67

Anyango B, Wilson KJ, Beynon JL, Giller KE (1995) Diversity of rhizobia nodulating *Phaseolus vulgaris* L. in two Kenyan soils with contrasting pHs. Appl Environ Microbiol 61:4016–4021

Aparicio-Fernandez X, Yousef GG, Loarca-Pina G, de Mejia E, Lila MA (2005) Characterization of polyphenolics in the seed coat of Black Jamapa bean (*Phaseolus vulgaris* L.). J Agric Food Chem 53(11):4615–4622

Arora NK, Kang SC, Maheshwari DK (2001) Isolation of siderophore producing strains of *Rhizobium meliloti* and their biocontrol potential against *Macrophomina phaseolina* that causes charcoal rot of groundnut. Curr Sci 81:673–677

Arshad M, Frankenberger WT Jr (1991) Microbial production of plant hormones. Plant Soil 133:1–8

Asadi RH, Afshari M, Khavazi K, Nourgholipour F, Otadi A (2005) Effects of common bean nodulating rhizobial native to Iranian soils on the yield and quality of bean. Iran J Soil Water Sci 19:215–222

Baca BE, Elmerich C (2007) Microbial production of plant hormones. In: Elmerich C, Newton WE (eds) Associative and endophytic nitrogen-fixing bacteria and cyanobacterial associations. Springer, Dordrecht, pp 113–143

Bajpai PD, Sundra Rao WVB (1971) Phosphate solubilising bacteria. Soil Sci Plant Nutr 17:46–53

Banik S, Dey BK (1982) Available phosphate content of an alluvial soil as influenced by inoculation of some isolated phosphate solubilizing microorganisms. Plant Soil 69:353–364

Banik S, Dey BK (1983) Phosphate solubilizing potentiality of the microorganisms capable of utilizing aluminium phosphate a sole phosphate source. Zentralbl Mikrobiol 138:17–23

Bardia MC, Gaur AC (1972) Rock phosphate dissolution by bacteria. Ind J Microbiol 12:269–271

Barea JM, Maria J, Rosario AP, Concepcion A (2005) Microbial co-operation in the rhizosphere. J Exp Bot 56(417):1761–1778

Bashan Y, Holguin G (1998) Proposal for the division of plant growth promoting rhizobacteria in two classifications: biocontrol-PGPB (plant growth promoting bacteria) and PGPB. Soil Biol Biochem 30(8/9):1225–1228

Bashan Y, Holguin G, de Bashan LE (2004) *Azospirillum*-plant relationships: physiological, molecular, agricultural, and environmental advances(1997–2003). Can J Microbiol 50:521–577

Beninger CW, Hosfield GL (2003) Antioxidant activity of extracts, condensed tannin fractions, and pure flavonoids from *Phaseolus vulgaris* L. seed coat color genotypes. J Agric Food Chem 51:7879–7883

Berg G, Zachow C (2011) PGPR interplay with rhizosphere communities and effect on plant and health. In: Maheshwari DK (ed) Bacteria in Agrobiology: Crop Ecosystems. Springer, Berlin, pp 97–109

Berge O, Lodhi A, Brandelet G, Santaella C, Marie-Anne R, Christen R, Heulin T, Achouak W (2009) *Rhizobium alamii* sp. nov., an exopolysaccharide-producing species isolated from legume and non-legume rhizospheres. Int J Syst Evol Microbiol 59:367–372

Berraho EL, Lesueur D, Diem HG, Sasson A (1997) Iron requirement and siderophore production in *Rhizobium ciceri* during growth on an iron-deficient medium. World J Microbiol Biotechnol 13:501–510

Beyene D, Kassa S, Ampy F, Assafa A, Gebremedhin T, van Berkum P (2004) Ethiopian soils harbor natural populations of rhizobia that form symbiosis with common bean (*Phaseolus vulgaris* L.). Arch Microbiol 181:129–136

Bhatia S, Maheshwari DK, Dubey RC, Arora DS, Bajpai VK, Kang SC (2008) Beneficial effects of fluorescent pseudomonads on seed germination, growth promotion, and suppression of charcoal rot in groundnut (*Arachis hypogea* L.). J Microbiol Biotechnol 18(9):1578–1583

Bloemberg GV, Lugtenberg BJJ (2001) Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Curr Opin Plant Biol 4:343–350

Briat JF (1992) Iron assimilation and storage in prokaryotes. J Gen Microbiol 138:2475–2483

Carrillo-Castañeda G, Cano MEA (2000) Characterization of siderophore-mediated iron transport from *Rhizobium leguminosarum* bv. *phaseoli*. J Plant Nutr 23(11–12):1669–1683

Chabot R, Antoun H, Cescas MP (1996) Growth promotion of maize and lettuce by phosphate solubilizing *Rhizobium leguminosarum* biovar *phaseoli*. Plant Soil 184:311–321

Chabot R, Beauchamp CE, Kloepper JW, Antoun H (1998) Effect of Phosphorus on root colonization and growth promotion of maize by bioluminescent mutants of phosphate solubilizing *Rhizobium leguminosarum* biovar *phaseoli*. Soil Biol Biochem 30:1615–1618

Chandra S, Choure K, Dubey RC, Maheshwari DK (2007) Rhizosphere competent *Mesorhizobium loti* MP6, induces root hair curling that enhances growth of Indian mustard (*Brassica campestris*) and inhibits *Sclerotinia sclerotiorum*. Braz J Microbiol 38:124–130

Chen WX, Yan GH, Li JL (1988) Numerical taxonomic study of fast-growing soybean rhizobia and a proposal that *rhizobium fredii* be assigned to *Sinorhizobium* gen. nov. Int J Syst Bacteriol 38(4):392–397

Chen WX, Tan ZY, Gao JL, Li Y, Wang ET (1997) *Rhizobium hainanense* sp. nov., isolated from tropical legumes. Int J Syst Bacteriol 47:870–873

Chen WM, Laevens S, Lee TM, Coenye T, De Vos P, Mergeay M, Vandamme P (2001) *Ralstonia taiwanensis* sp. nov., isolated from root nodules of *Mimosa* species and sputum of a cystic fibrosis patient. Int J Syst Evol Microbiol 51:1729–1735

Chen WM, Moulin L, Bontemps C, Vandamme P, Béna G, Boivin-Masson C (2003) Legume symbiotic nitrogen fixation by *β-Proteobacteria* is widespread in nature. J Bacteriol 185:7266–7272

Chen WM, James EK, Coenye T, Chou JH, Barrios E, de Faria SM, Elliott GN, Sheu SY, Sprent JI, Vandamme P (2006) *Burkholderia mimosarum* sp. nov., isolated from root nodules of *Mimosa* spp. from Taiwan and South America. Int J Syst Evol Microbiol 56(8):1847–1851

Chen WM, de Faria SM, James EK, Elliott GN, Lin KY, Chou JH, Sheu SY, Cnockaert M, Sprent J, Vandamme P (2007) *Burkholderia nodosa* sp. nov., isolated from root nodules of the woody Brazilian legumes *Mimosa bimucronata* and *Mimosa scabrella*. Int J Syst Evol Microbiol 57:1055–1059

Chen WM, de Faria SM, Chou JH, James EK, Elliott GN, Sprent JI, Bontemps C, Young JP, Vandamme P (2008) *Burkholderia sabiae* sp. nov., isolated from root nodules of *Mimosa caesalpiniifolia*. Int J Syst Evol Microbiol 58:2174–2179

Chen WM, Zhu W, Bontemps C, Young J, Peter W, Wei G (2009) *Mesorhizobium alhagi* sp. nov., isolated from root nodules of the wild legume *Alhagi sparsifolia*. Int J Syst Evol Microbiol 60:958–962

Chen WM, Zhu WF, Bontemps C, Young JP, Wei GH (2011) *Mesorhizobium camelthorni* sp. nov., isolated from *Alhagi sparsifolia*. Int J Syst Evol Microbiol 61:574–579

Choudhury B, Azad P (2005) Symbiotic properties in dual inoculation of Rhizobia and AM fungi for enhancement of pulse production in North-East Himalayan region. In: Podic GK, Varma A (eds) Biotechnological applications of microbes. IK International Pvt Ltd, Green Park Extension, New Delhi, pp 151–162

Cordell D, Drangert JO, White S (2009) The story of phosphorus: global food security and food for thought. Glob Environ Change 19:292–305

Costacurta A, Vanderleyden J (1995) Synthesis of phytohormones by plant-associated bacteria. Crit Rev Microbiol 21:1–18

Crosa JH, Walsh CT (2002) Genetics and assembly line enzymology of siderophore biosynthesis in bacteria. Microbiol Mol Biol Rev 66(2):223–249

Cummings SP, Humphry DR, Andrews M (2001) A review of the current taxonomy and diversity of symbiotic rhizosphere and bulk soil nitrogen-fixing bacteria, which are beneficial to plants. In: Aspects of applied biology 63 plant microbial interactions: positive interactions in relation to crop production and utilization, vol 63. Association of Applied Biologists, Wellesbourne, Warwick, pp 5–18

Cunningham JE, Kuiack C (1992) Production of citric and oxalic acids and solubilization of calcium phosphate by *Penicillium bilaii*. Appl Environ Microbiol 58:1451–1458

D'Haeze W, Leoff C, Freshour G, Noel KD, Carlson RW (2007) *Rhizobium etli* CE3 bacteroid lipopolysaccharides are structurally similar but not identical to those produced by cultured CE3 bacteria. J Biol Chem 282(23):17101–17113

Dakora FD (2003) Defining new roles for plant and rhizobial molecules in sole and mixed plant cultures involving symbiotic legumes. New Phytol 158:39–49

de Lajudie P, Willems A, Pot B, Dewettinck D, Maestrojuan G, Neyra M, Collins MD, Dreyfus B, Kersters K, Gillis M (1994) Polyphasic taxonomy of rhizobia: emendation of the genus *Sinorhizobium* and description of *Sinorhizobium meliloti* comb. nov., *Sinorhizobium saheli* sp. nov., and *Sinorhizobium teranga* sp. nov. Int J Syst Bacteriol 44:715–733

de Lajudie P, Laurent-Fulele E, Willems A, Torck U, Coopman R, Collins MD, Kersteres K, Dreyfus B, Gillis M (1998) *Allorhizobium undicola* gen. nov., sp. nov., nitrogen-fixing bacteria that efficiently nodulate *Neptunia natans* in Senegal. Int J Syst Bacteriol 4:1277–1290

de Werra P, Péchy-Tarr M, Keel C, Maurhofer M (2009) Role of gluconic acid production in the regulation of biocontrol traits of *Pseudomonas fluorescens* CHA0. Appl Environ Microbiol 75 (12):4162–4174

Dean DR, Jacobson MR (1992) Biochemical genetics of nitrogenase. In: Stacey G, Burris RH, Evans HJ (eds) Biological nitrogen fixation. Chapman and Hall, New York, pp 763–834

Deryło M, Choma A, Puchalski B, Suchanek W (1994) Siderophore activity in *Rhizobium* species isolated from different legumes. Acta Biochim Pol 41(1):7–11

Deshwal VK, Dubey RC, Maheshwari DK (2003a) Isolation of plant growth promoting *Bradyrhizobium* (*Arachis*) sp. with biocontrol potential against *Macrophomina phaseolina* causing charcoal root of peanut. Curr Sci 84:443–448

Deshwal VK, Pandey P, Kang SC, Maheshwari DK (2003b) Rhizobia as a biological control agent against soil borne pathogenic fungi. Indian J Exp Biol 41:1160–1164

Deshwal VK, Kumar T, Dubey RC, Maheshwari DK (2006) Long term effect of *Pseudomonas aeruginosa* GRC_1 on yield of subsequent crops of paddy after mustard seed bacterization. Curr Sci 91(4):423–424

Dey R, Pal KK, Bhatt DM, Chauhan SM (2004) Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. Microbiol Res 159:371–394

Dilworth MJ, Carson KC, Giles RGF, Byrne LT, Glenn AR (1998) *Rhizobium leguminosarum* bv. *viciae* produces a novel cyclic trihydroxamate siderophore, vicibactin. Microbiology 144:781–791

Diouf A, de Lajudie P, Neyra M, Kersters K, Gillis M, Martinez-Romero E, Gueye M (2000) Polyphasic characterization of rhizobia that nodulate *Phaseolus vulgaris* in West Africa (Senegal and Gambia). Int J Syst Evol Microbiol 50:159–170

Dobereiner J, Pedroza F (1987) Nitrogen-fixing bacteria in non-leguminous crop plants. Science Tech, Madison, WI, pp 1–15

Dreyfus B, Garcia JL, Gillis M (1988) Characterisation of *Azorhizobium caulinodans* gen. nov., a stem-nodulating nitrogen-fixing bacterium isolated from *Sesbania rostrata*. Int J Syst Evol Microbiol 38:89–98

Dubey RC, Maheshwari DK, Kumar H, Choure K (2010) Assessment of diversity and plant growth promoting attributes of rhizobia isolated from *Cajanus cajan* L. Afr J Biotechnol 9 (50):8619–8629

Duff RB, Webley DM (1959) 2-Ketogluconic acid as a natural chelator produced by soil bacteria. Chem Ind 1959:1376–1377

Duhan JS, Dudeja SS, Khurana AL (1998) Siderophore production in relation to N2 fixation and iron uptake in pigeon pea-*Rhizobium* symbiosis *Folia*. Microbiology 43:421–426

Duranti M, Guis C (1997) Legume seeds: protein content and nutritional value. Field Crops Res 53:31–45

Dursun A (2007) Variability, Heritability and correlation studies in bean (*Phaseolus vulgaris* L.) genotypes. World J Agric Sci 3(1):12–16

Egli DB (1998) Seed biology and the yield of grain crops. CAB International, Wallingford, UK

Epping B, Hansen A, Djlaji B, Martin P (1994) Symbiotic efficiency of four *Phaseolus vulgaris* genotypes after inoculation with different strains of *Rhizobium* under controlled conditions. Z Naturforsch 49:343–351

Escribano MR, Santalla M, de Ron AM (1997) Genetic diversity in pod and seed quality traits of common bean population from northwestern Spain. Euphytica 93:71–81

Esitken A (2011) Use of plant growth promoting rhizobacteria in horticultural crops. In: Maheshwari DK (ed) Bacteria in agrobiology: crop ecosystem. Springer, Berlin, pp 189–236

Estrella MJ, Muñoz S, Soto MJ, Ruiz O, Sanjuán J (2009) Genetic diversity and host range of rhizobia nodulating *Lotus tenuis* in typical soils of the Salado river basin (Argentina). Appl Environ Microbiol 75(4):1088–1098

Feng K, Lu HM, Sheng HJ, Wang XL, Mao J (2004) Effect of organic ligands on biological availability of inorganic phosphorus in soils. Pedosphere 14:85–92

Fernandez LA, Zalba P, Gomez M, Sagardoy MA (2007) Phosphate solubilization activity of bacterial strains in soil and their effect on soybean growth under greenhouse conditions. Biol Fertil Soils 43:805–809

Flaishman MA, Eyal Z, Zilberstein A, Voisarsd C, Haas D (1996) Suppression of *Septoria tritici* blotch and leaf rust of wheat by recombinant cyanide-producing strains of *Pseudomonas putida*. Mol Plant Microbe Interact 9:642–645

Frank B (1889) Uber die Pilzsymbiose der Leguminosen. Berichte der Deutschen Bereu Deut Bot Ges 7:332–346

Frankenberger WTJ, Arshad M (1990) Yield response of *Capsicum annuum* to the auxin precursor, L-tryptophan applied to soil. PGRSA Q 19:231–240

Fravel DR (2005) Commercialization and implementation of biocontrol. Annu Rev Phytopathol 43:337–359

Frommel ML, Nowak J, Lazarovits G (1991) Growth enhancement and developmental modifications of *in vitro* grown potato (*Solanum tuberosum* sp. t*uberosum*) as affected by a non-fluorescent *pseudomonas* sp. Plant Physiol 96:928–936

Fulchieir M, Lucangeli C, Bottini R (1993) Inoculation with *Azospirillum lipoferum* affects growth and gibberellin status on corn seedling roots. Plant Cell Physiol 34:1305–1309

Gamalero E, Glick BR (2011) Mechanisms used by plant growth-promoting bacteria. In: Maheshwari DK (ed) Bacteria in agrobiology: plant nutrient management. Springer, Berlin. doi:101007/978-3-642-21061-7_2

Garcia de Salamone IE, Hynes RK, Nelson LM (2001) Cytokinin production by plant growth promoting rhizobacteria and selected mutants. Can J Microbiol 47:404–411

Garcia-Fraile P, Rivas R, Willems A, Peix A, Martens M, Martinez-Molina E, Mateos PF, Velazquez E (2007) *Rhizobium cellulosilyticum* sp. nov., isolated from sawdust of *Populus alba*. Int J Syst Evol Microbiol 57:844–848

Garcia-Fraile P, Mulas-Garcia D, Peix A, Rivas R, Gonzalez-Andres F, Encarna V (2010) *Phaseolus vulgaris* is nodulated in northern Spain by *Rhizobium leguminosarum* strains harboring two *nod*C alleles present in American *Rhizobium etli* strains: biogeographical and evolutionary implications. Can J Microbiol 56(8):657–666

Gaur RD, Dangwal LR (1997) New species of *Macrotyloma* (Wight and Arn) Verdc (Fabaceae) from Garhwal Himalaya, UP, India. J Bombay Nat Hist Soc 94:381–383

Ghosh AC, Basu PS (2002) Growth behaviour and bioproduction of indole acetic acid by a *Rhizobium* sp. isolated from root nodules of a leguminous tree *Dalbergia lanceolaria*. Indian J Exp Biol 40(7):796–801

Ghosh S, Maiti TK, Basu PS (2008) Bioproduction of ascorbic acid in root nodule and root of the legume pulse *Phaseolus mungo*. Curr Microbiol 56(5):495–498

Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117

Glick BR, Karatuprovic DM, Newwll PC (1995) A novel procedure for rapid isolation of plant growth promoting pseudomonads. Can J Microbiol 41:533–536

Glick BR, Patten CL, Holguin G, Penrose DM (1999) Biochemical and genetic mechanism used by plant growth-promoting bacteria. Imperial College Press, London, UK

Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B (2007) Promotion of plant growth by ACC deaminase. Crit Rev Plant Sci 26:227–242

Goldstein AH (1986) Bacterial solubilization of microbial phosphates: a historical perspective and future prospects. Am J Altern Agric 1:51–57

Gonzalez de Mejia E, Valdez-Vega MC, Reynoso-Camacho R, Loarca-Pina G (2005) Tannins, trypsin inhibitors and lectin cytotoxicity in tepary (*Phaseolus acutifolius*) and common (*Phaseolus vulgaris*) beans. Plant Foods Hum Nutr 60:137–145

Grange L, Hungria M (2004) Genetic diversity of indigenous common bean (*Phaseolus vulgaris*) rhizobia in two Brazilian ecosystems. Soil Biol Biochem 36:1389–1398

Gressent F, Mantegazza N, Cullimore JV, Driguez H, Ranjeva R, Bono JJ (2002) High-affinity Nod factor binding site from *Phaseolus vulgaris* cell suspension cultures. Mol Plant Microbe Interact 15(8):834–839

Gu CT, Wang ET, Tian CF, Han TX, Chen WF, Sui XH, Chen WX (2008) *Rhizobium miluonense* sp. nov., a symbiotic bacterium isolated from *Lespedeza* root nodules. Int J Syst Evol Microbiol 58:1364–1368

Guan SH, Chen WF, Wang ET, Lu YL, Yan XR, Zhang XX, Chen WX (2008) *Mesorhizobium caraganae* sp. nov., a novel rhizobial species nodulated with *Caragana* spp. in China. Int J Syst Evol Microbiol 58(11):2646–2653

Guerinot ML, Meidl EJ, Plessener O (1990) Citrate as a siderophore in *Bradyrhizobium japonicum*. J Bacteriol 172:3298–3303

Gupta CP, Sharma A, Dubey RC, Maheshwari DK (1999) *Pseudomonas aeruginosa* as a strong antagonist of *Macrophomina phaseolina* and *Fusarium oxysporum*. Cytobios 99:183–189

Gupta CP, Sharma A, Dubey RC, Maheshwari DK (2001a) Antibiosis-mediated necrotrophic effect of *Pseudomonas* GRC_2 against two fungal plant pathogens. Curr Sci 81:90–94

Gupta CP, Sharma A, Dubey RC, Maheshwari DK (2001b) Effect of metal ions on the growth of *Pseudomonas aeruginosa* and siderophore and protein production. Indian J Exp Biol 39:1318–1321

Gupta CP, Dubey RC, Maheshwari DK (2002) Plant growth enhancement and suppression of *Macrophomina phaseolina* causing charcoal rot of peanut by fluorescent *Pseudomonas*. Biol Fert Soil 35:399–405

Gutierrez RM, Barraquio WL (2010) Acid-tolerant rhizobia of *Phaseolus vulgaris* L. from the intensively cropped soils of La Trinidad, Benguet, Philippines. Philip J Sci 139(1):79–90

Guzman-Maldonado SH, Paredes-Lopez O (1998) Functional products of plants indigenous of Latin America: amaranth, quinoa, common beans and botanicals. In: Mazz G, Thechnomic Lancaster PA (eds) Functional foods biochemical and processing aspects. CRC, Boca Raton, FL, pp 39–328

Haas D, Defago G (2005) Biological control soil born pathogen by fluorescent *Pseudomonas*. Nat Rev Microbiol 1038:1129–1141

Halder AK, Chakrobartty PK (1993) Solubilization of inorganic phosphate by *Rhizobium*. Folia Microbiol 38:325–330

Halder AK, Mishra AK, Bhattacharyya P, Chakrabartty PK (1990) Solubilization of rock phosphate by *Rhizobium* and *Bradyrhizobium*. J Gen Appl Microbiol 36:81–92

Han TX, Han LL, Wu LJ, Chen WF, Sui XH, Gu JG, Wang ET, Chen WX (2008a) *Mesorhizobium gobiense* sp. nov. and *Mesorhizobium tarimense* sp. nov., isolated from wild legumes growing in desert soils of Xinjiang, China. Int J Syst Evol Microbiol 58(11):2610–2618

Han TX, Wang ET, Han LL, Chen WF, Sui XH, Chen WX (2008b) *Rhizobium multihospitium* sp. nov., isolated from multiple legume species native of Xinjiang, China. Int J Syst Evol Microbiol 58:1693–1699

Hangen L, Bennick M (2002) Consumption of black beans and navy beans (*P. vulgaris*) to reduced azoxymethane-induced colon cancer in rats. Nutr Cancer 44:60–65

Hirsch AM, Fang Y, Asad S, Kapulnik Y (1997) The role of phytohormones in plant microbes symbiosis. Plant Soil 194:171–184

Höflich G, Wiehe W, Hecht-Buchholz C (1995) Rhizosphere colonization of different crops with growth promoting *Pseudomonas* and *Rhizobium* bacteria. Microbiol Res 150:139–147

Hoque MM, Haq MF (1994) Rhizobial inoculation and fertilization of lentil in Bangladesh. Lens Newsl 21:29–30

Huang HC, Erickson RS (2007) Effect of seed treatment with *Rhizobium leguminosarum* on *Pythium* damping-off, seedling height, root nodulation, root biomass, shoot biomass, and seed yield of pea and lentil. J Phytopathol 155:31–37

Husen E, Wahyudi AT, Suwanto A, Saraswati R (2009) Soybean seedling root growth promotion by 1-aminocyclopropane-1-carboxylate deaminase-producing pseudomonads. Indones J Agric Sci 10(1):19–25

Illmer P, Schinner F (1995) Solubilization of inorganic calcium phosphates-solubilization mechanisms. Soil Biol Biochem 27:257–263

Islam MS, Kawasaki H, Muramatsu Y, Nakagawa Y, Seki T (2008) *Bradyrhizobium iriomotense* sp. nov., isolated from a tumor-like root of the legume *Entada koshunensis* from Iriomote Island in Japan. Biosci Biotechnol Biochem 72:1416–1429

Jarvis BDW, Pankhurst CE, Patel JJ (1982) *Rhizobium loti*, a new species of legume root nodule bacteria. Int J Syst Bacteriol 32:378–380

Jarvis BDW, Van Berkum P, Chen WX, Nour SM, Fernandez MP, Cleyet-Marel JC, Gillis M (1997) Transfer of *Rhizobium loti*, *Rhizobium huakuii*, *Rhizobium ciceri*, *Rhizobium mediterraneum*, and *Rhizobium tianshanense* to *Mesorhizobium* gen. nov. Int J Syst Bacteriol 47:895–898

Jeffries S, Gianinazzi S, Perotto S, Turnau K, Barea JM (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol Fertil Soils 37:1–16

Jethi RK, Duggal B, Sahota RS, Gupta M, Sofat JB (1983) Important plants used in stone. Indian J Med Res 78:422–425

Jordan DC (1982) Transfer of *Rhizobium japonicum* Buchanan 1980 to *Bradyrhizobium* gen nov., a genus of slow-growing root nodule bacteria from leguminous plants. Int J Syst Bacteriol 32:136–139

Jordan DC (1984) Family III Rhizobeaceae. In: Krieg NR, Holt JG (eds) Bergey's manual of systemic bacteriology, vol 1. The Williams and Wilkins Co, Baltimore, pp 234–242

Jourand P, Giraud E, Bena G, Sy A, Willens A, Gillis M, Dreyfus B, Lajudie P (2004) *Methylobacterium nodulans* sp. nov., for a group of aerobic, facultatively methylotrophic, legume root-nodule-forming and nitrogen-fixing bacteria. Int J Syst Evol Microbiol 54:2269–2273

Kala TC, Christi RM, Bai NR (2011) Effect of *Rhizobium* inoculation on the growth and yield of horsegram (*Dolichos biflorus* L.). Plant Arch 11(1):97–99

Kang SC, Chul GH, Lee TG, Maheshwari DK (2002) Solubilization of insoluble inorganic phosphates by a soil inhabiting fungus, *Fomitopsis* spp. PS 102. Curr Sci 25:439–442

Kaschuk G, Hungria M, Anderde DS, Campo RJ (2006) Genetic diversity of rhizobia associated with common bean (*Phaseolus vulgaris* L.) grown under no tillage and conventional system in Southern Brazil. Appl soil Ecol 32:210–220

Katiyar RP (1984) 'Kulthi' a promising pulse crop for Himachal Hills. Indian Fmg 34(9):31–35

Katznelson H, Bose B (1959) Metabolic activity and phosphate dissolving capability of Bacterial isolates from wheat roots, rhizosphere and non-rhizosphere soil. Can J Microbiol 5:79–85

Khalequzzaman KM, Hossain I (2007) Effect of seed treatment with *Rhizobium* strains and biofertilizers on foot/root rot and yield of bushbean in *Fusarium solani* infested soil. J Agric Res 45(2):151–160

Khalequzzaman KM, Hossain I (2008) Effect of *Rhizobium* strains and biofertilizers on foot, root rot and yield of Bush bean in *Sclerotinia sclerotiorum* infested soil. J Bio Sci 16:73–78

Khan AG (2005) Role of soil microbes in the rhizosphere of plants growing on trace metal contaminated soils in phytoremediation. J Trace Elem Med Biol 18:355–364

Khan A, Geetha R, Akolkar A, Pandya A, Archana G, Desai AJ (2006) Differential cross-utilization of heterologous siderophores by nodule bacteria of *Cajanus cajan* and its possible role in growth under iron-limited conditions. Appl Soil Ecol 34(1):19–26

Kloepper JW, Schorth, MN (1978) Plant growth promoting rhizobacteria on radishes. In: Angers INRA (ed) Proceedings of the 4th international conference on plant pathogenic bacteria, vol 2, station de pathologie, Gibert-Clarey Tours, France, pp 879–882

Kloepper JW, Leong J, Teintze M, Schroth MN (1980) *Pseudomonas* siderophores: a mechanism explaining disease-suppressive soil. Curr Microbiol 4:317–320

Kloepper JW, Lifshitz R, Schorth MN (1988) *Pseudomonas* inoculants to benefit plant production. ISI Atlas of Science, Animal and Plant Science Institute for Public Information, Philadelphia, PA, pp 60–64

Kloepper JW, Lifshitz R, Zablotowich RK (1989) Free living bacterial inocula for enhancing crop productivity. Trends Biotechnol 7:39–43

Kloepper JW, Gutierrez-Estrada A, McInroy JA (2007) Photoperiod regulates elicitation of growth promotion but not induced resistance by plant growth-promoting rhizobacteria. Can J Microbiol 53:159–167

Kucey RMN, Janzen HH, Leggett ME (1989) Microbially mediated increases in plant-available phosphorus. Adv Agron 42:199–228

Kumar B (2006) Biocontrol potential of microbial consortium on *Macrophomina phaseolina* causing root-rot of *Sesamum indicum* L. PhD Thesis, Gurukul, Kangri University, Haridwar, Uttaranchal, India

Kumar B, Dubey RC, Maheshwari DK (2005) Biocontrol of *Macrophomina phaseolina*: Prospects and Constraints. In: Satyanarayana T, Johri BN (eds) Microbial diversity: current perspectives and potential applications. IK International Pvt Ltd, New Delhi, pp 471–492

Kumar B, Kumar MS, Annapurna K, Maheshwari DK (2006) Genetic diversity of plant growth promoting rhizobia isolated from a medicinal legume, *Mucuna pruriens* L. Curr Sci 91 (11):1524–1529

Kumar V, Dubey RC, Maheshwari DK (2008a) Coinoculation of PGPR for plant growth promotion and disease control. In: Maheshwari DK, Dubey RC (eds) Microorganism for sustainable agriculture: a techno-commercial perspective. IK International, New Delhi, pp 1–25

Kumar V, Sharma S, Sharma AK, Kumar M, Sharma S, Malik S, Singh KP, Sanger RS, Bhat KV (2008b) Genetic diversity in Indian common bean (*Phaseolus vulgaris* L.) using random amplified polymorphic DNA markers. Physiol Mol Plants 14(4):383–387

Kumar H, Bajpai VK, Dubey RC, Maheshwari DK, Kang SC (2010) Wilt disease management and enhancement of growth and yield of *Cajanus cajan* (L.) var Manak by bacterial combinations amended with chemical fertilizer. Crop Protect 29:591–598

Kumar H, Dubey RC, Maheshwari DK (2011) Effect of plant growth promoting rhizobia on seed germination, growth promotion and suppression of Fusarium wilt of fenugreek (*Trigonella foenum-graecum* L.). Crop Protect 30:1396–1403

Kuykendall LD, Saxena B, Devine TE, Udell SE (1992) Genetic diversity in *Bradyrhizobium japonicum* Jordan 1982 and a proposal for *Bradyrhizobium elkanii* sp. nov. Can J Microbiol 38:501–505

Laguerre G, Fernandez MP, Edel V, Normand P, Amarger N (1993) Genomic heterogeneity among French *Rhizobium* strains isolated from *Phaseolus Vulgaris* L. Int J Syst Bacteriol 43:761–767

Lee KD, Bai Y, Smith D, Han HS (2005) Supanjan isolation of plant-growth-promoting endophytic bacteria from bean nodules. Res J Agric Biol Sci 1(3):232–236

Leelahawonge C, Nuntagij A, Teaumroong N, Boonkerd N, Pongsilp N (2010) Characterization of root-nodule bacteria isolated from the medicinal legume *Indigofera tinctoria*. Ann Microbiol 60:65–74

Lhuissier FGP, de Ruijter NCA, Sieberer BJ, Esseling JJ, Emons AMC (2001) Time course of cell biological events evoked in legume root hairs by *Rhizobium* Nod factors: state of the art. Ann Bot 87:289–302

Li QQ, Wang ET, Chang YL, Zhang YZ, Zhang YM, Sui XH, Chen WF, Chen WX (2011) *Ensifer sojae* sp. nov., isolated from root nodules of *Glycine max* grown in saline-alkaline soils. Int J Syst Evol Microbiol 61(8):1981–1988

Libbert E, Silhengst P (1970) Interactions between plants and epiphytic bacteria regarding their auxin metabolism VIII Transfer of 14C-indoleacetic acid from epiphytic bacteria to corn coleoptiles. Physiol Plant 23:480–487

Lim HS, Kim YS, Kim SD (1991) *Pseudomonas stutzeri* YPL1 genetic transformation and antifungal mechanism against *Fusarium solani*, an agent of plant root rot. Appl Environ Microbiol 57:510–516

Lin DX, Wang ET, Tang H, Han TX, He YR, Guan SH, Chen WX (2008) *Shinella kummerowiae* sp. nov., a symbiotic bacterium isolated from root nodules of the herbal legume *Kummerowia stipulacea*. Int J Syst Evol Microbiol 58:1409–1413

Lin DX, Chen WF, Wang FQ, Hu D, Wang ET, Sui XH, Chen WX (2009) *Rhizobium mesosinicum* sp. nov., isolated from root nodules of three different legumes. Int J Syst Evol Microbiol 59:1919–1923

Lindstrom K (1988) *Rhizobium galegae*, a new species of legume root nodule. Int J Syst Evol Microbiol 39(3):365–367

Lindström K, Paulin L, Roos C, Suominen L (1995) Nodulation genes of *Rhizobium galegae*. In: Tikhonovitch IA, Provorov NA, Romanov VI, Nexton WE (eds) Nitrogen fixation: fundamentals and applications. Proceedings of the 10th international congress on nitrogen fixation. Kluwer, Dordrecht, pp 365–370

Lloret L, Ormeno-Orrillo E, Rincon R, Martinez-Romero J, Rogel-Hernandez MA, Martinez-Romero E (2007) *Ensifer mexicanus* sp. nov., a new species nodulating *Acacia angustissima* (Mill.) Kuntze in Mexico. Syst Appl Microbiol 30(4):280–290

Lopez-Lopez A, Rogel Marco A, Ormeno-Orrillo E, Martinez-Romero J, Martinez-Romero E (2010) *Phaseolus vulgaris* seed-borne endophytic community with novel bacterial species such as *Rhizobium endophyticum* sp. nov. Syst Appl Microbiol 33(6):322–327

Lu YL, Chen WF, Wang ET, Han LL, Zhang XX, Chen WX, Han SZ (2009a) *Mesorhizobium shangrilense* sp. nov., isolated from root nodules of *Caragana* species. Int J Syst Evol Microbiol 59(12):3012–3018

Lu YL, Chen WF, Han LL, Wang ET, Chen WX (2009b) *Rhizobium alkalisoli* sp. nov., isolated from *Caragana intermedia* growing in saline-alkaline soils in the north of China. Int J Syst Evol Microbiol 59(12):3006–3011

Lucy M, Reed E, Glick R (2004) Application of free living plant growth promoting rhizobacteria. Ant Van Leeuwen 86:1–25

Lynch JM, Bragg E (1985) Microorganisms and soil aggregate stability. Adv Soil Sci 2:133–171

Lynch D, O'Brien J, Welch T, Clarke P, ÓCuív P, Crosa JH, O'Connell M (2001) Genetic organization of the region encoding regulation, biosynthesis, and transport of rhizobactin 1021, a siderophore produced by *Sinorhizobium meliloti*. J Bacteriol 183(8):2576–2585

Ma W, Penrose DM, Glick BR (2002) Strategies used by rhizobia to lower plant ethylene levels and increase nodulation. Can J Microbiol 48:947–954

Mahmoud SAZ, Ramadan EM, Thabet FM, Khater T (1984) Production of plant growth promoting substances by Rhizosphere microorganisms. Zbl Mikrobiol 139:227–232

Malboobi MA, Behbahani M, Madani H, Owlia P, Deljou A, Yakhchali B, Moradi M, Hassanabadi H (2009) Performance evaluation of potent phosphate solubilizing bacteria in potato rhizosphere. World J Microbiol Biotechnol 25:1479–1484

Mantelin S, Touraine B (2004) Plant growth promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. J Exp Bot 55:27–34

Mantelin S, Saux MFL, Zakhia F, Bena G, Bonneau S, Jeder H, de Lajudie P, Cleyet-Marel JC (2006) Emended description of the genus *Phyllobacterium* and description of four novel species associated with plant roots: *Phyllobacterium bourgognense* sp. nov., *Phyllobacterium ifriqiyense* sp. nov., *Phyllobacterium leguminum* sp. nov. and *Phyllobacterium brassicacearum* sp. nov. Int J Syst Evol Microbiol 56(4):827–839

Martens M, Delaere M, Coopman R, De Vos P, Gillis M, Willems A (2007) Multilocus sequence analysis of *Ensifer* and related taxa. Int J Syst Evol Microbiol 57:489–503

Martinez-Romero E, Segovia L, Mercante FM, Franco AA, Graham P, Pardo MA (1991) *Rhizobium tropici*, a novel species nodulating *Phaseolus vulgaris* L. beans and *Leucaena* sp. trees. Int J Syst Bacteriol 41:417–426

Mathiyazhagan S, Kavitha K, Nakkeeran S, Chandrasekar G, Manian K, Renukadevi P, Krishnamoorthy AS, Fernando WGD (2004) PGPR mediated management of stem blight of *Phyllanthus amarus* (Schum and Thonn) caused by *Corynespora cassiicola* (Berk and Curt) Wei. Arch Phytopathol Plant Prot 37:183–199

Matiru VN, Dakora FD (2004) Potential use of rhizobial bacteria as promoters of plant growth for increased yield in landraces of African cereal crops. Afr J Biotechnol 3(1):1–7

Matthew KM (1983) The flora of the Tamil Nadu carnatic Part one polypetalae, The Rapinat Herbarium. St Joseph's College, Tiruchirapalli

Mazen MM, El-Batanony NH, Abd El-Monium MM, Massoud ON (2008) Cultural filtrate of *Rhizobium* spp. and arbuscular mycorrhiza are potential biological control agents against root rot fungal diseases of faba bean. Global J Biotechnol Biochem 3(1):32–41

Mehboob J, Zahir ZA, Arshad M, Tanveer A, Azam F (2011) Growth promoting activities of different *Rhizobium* spp. in wheat. Pak J Bot 43(3):1643–1650

Mehdi Z, Nahid S-R, Alikhani HA, Nasser A (2006) Response of lentil to co-inoculation with phosphate-solubilizing rhizobial strains and arbuscular mycorrhizal fungi. J Plant Nutr 29:1509–1522

Merabet C, Martens M, Mahdhi M, Zakhia F, Sy A, Le Roux C, Domergue O, Coopman R, Bekki A, Mars M, Willems A, de Lajudie P (2010) Multilocus sequence analysis of root nodule isolates from *Lotus arabicus* (Senegal), *Lotus creticus*, *Argyrolobium uniflorum* and *Medicago sativa* (Tunisia) and description of *Ensifer numidicus* sp. nov. and *Ensifer garamanticus* sp. nov. Int J Syst Evol Microbiol 60:664–674

Mhamdi R, Laguerre G, Aouani ME, Mars M, Amarger N (2002) Different species and symbiotic genotype of field rhizobia can nodulate *Phaseolus vulgaris* in Tunisian soils. FEMS Microbiol Ecol 41:77–84

Minaxi, Saxena J (2010) Disease suppression and crop improvement in moong beans (*Vigna radiata*) through *Pseudomonas* and *Burkholderia* strains isolated from semi-arid region of Rajasthan, India. BioControl 55:799–810

Mishra RK, Prakash O, Alam M, Dikshit A (2010) Influence of plant growth promoting rhizobacteria (PGPR) on the productivity of *Pelargonium graveolens* L. Herit. Rec Res Sci Tech 2(5):53–57

Mnasri B, Aouani ME, Mhamdi R (2007a) Nodulation and growth of common bean (*Phaseolus vulgaris*) under water deficiency. Soil Biol biochem 39:1744–1750

Mnasri B, Mrabet M, Laguerre G, Aouani ME, Mhamdi R (2007b) Salt-tolerant rhizobial isolated from a Tunisian oasis that are highly effective for symbiotic N2- fixation with *Phaseolus*

vulgaris constitute a novel biovar bv. *mediterranense* of *Sinorhizobium meliloti*. Arch Microbiol 187:79–85
Moghimi A, Tate ME, Oades JM (1978) Characterization of rhizosphere products especially 2-ketogluconic acid. Soil Biol Biochem 10(4):283–287
Mohammed Ahmed TH, Elsheikh EAE, Mahadi AA (2009) The *in vitro* compatibility of some *Rhizobium* and *Bradyrhizobium* strains with fungicides. Afr Crop Sci Conf Proc B:1171–1178
Mordukhova EA, Skvortsova NP, Kochaetko VV, Dubeikovskii AN, Boronin AM (1991) Synthesis of the phytohormone indole-3 acetic acid by rhizosphere bacteria by the genus *Pseudomonas*. Mikrobiologiya 60:494–500
Mostasso L, Mostasso FL, Dias BG, Vargas MAT, Hungria M (2002) Selection of bean (*Phaseolus vulgaris* L.) rhizobial strains for the Brazilian Cerrados. Field Crops Res 73:121–132
Mouhsine B, Prell J, Filali-Maltouf A, Priefer UB, Aurag J (2007) Diversity, phylogeny and distribution of bean rhizobia in salt affected soils of North-West Morocco. Symbiosis 43:83–96
Moulin L, Munive A, Dreyfus B, Boivin-Masson C (2001) Nodulation of legumes by members of the β subclass of Proteobacteria. Nature 411:948–950
Mukherjee T, Bhalla N, Singh G, Aulakh K, Jain HC (1984) Herbal drugs for urinary stones. Indian Drugs 21:224–228
Nair NC, Henry AN (1983) Flora of Tamil Nadu, India, vol I, Series I: analysis. Botanical Survey of India Southern circle, Coimbatore, pp 1–84
Nandasena KG, O'Hara GW, Tiwari RP, Willlems A, Howieson JG (2009) *Mesorhizobium australicum* sp. nov. and *Mesorhizobium opportunistum* sp. nov., isolated from *Biserrula pelecinus* L. in Australia. Int J Syst Evol Microbiol 59(9):2140–2197
Neilands JB, Leong SA (1986) Siderophores in relation to plant growth and disease. Annu Rev Plant Physiol 37:187–208
Neilands JB, Nakamura K (1991) Detection, determination, isolation, characterization and regulation of microbial iron chelates. In: Winkelmann G (ed) Handbook of microbial iron chelates. CRC, Boca Raton, FL, pp 1–14
Nick G, de Lajudie P, Eardly BD, Suomalainen S, Paulin L, Zhang X, Gillis M, Lindström K (1999) *Sinorhizobium arboris* sp. nov. and *Sinorhizobium kostiense* sp. nov., isolated from leguminous trees in Sudan and Kenya. Int J Syst Bacteriol 49:1359–1368
Noel TC, Sheng C, York CK, Pharis RP, Hynes MF (1996) *Rhizobium leguminosarum* as a plant growth-promoting rhizobacterium: direct growth promotion of canola and lettuce. Can J Microbiol 42:279–283
Nour SM, Cleyet-Marel JC, Beck D, Effosse A, Fernandez MP (1994) Genotypic and phenotypic diversity of *Rhizobium* isolated from chickpea (*Cicer arietinum* L.). Can J Microbiol 40:345–354
Nour SM, Cleyet-Marel JC, Normand P, Fernandez MP (1995) Genomic heterogeneity of strains nodulating chickpeas (*Cicer arietinum* L.) and description of *Rhizobium mediterraneum* sp. nov. Int J Syst Evol Microbiol 45(4):640–648
O'Sullivan DJ, O'Gara F (1992) Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. Microbiol Rev 56:662–676
Ocho-Anin Atchibri AL, Kouakou TH, Brou KD, Kouadio YJ, Gnakri D (2010) Evaluation of bioactive components in seeds of *Phaseolus vulgaris* L. (fabaceae) cultivated in Côte d'Ivoire. J Appl Biosci 31:1928–1934
Odee DW, Hukka K, McInroy SG, Sprent JI, Sutherland JM, Young JPW (2002) Genetic and symbiotic characterization of rhizobia isolated from tree and herbaceous legume grown in soils form ecologically diverse site in Kenya. Soil Biol Biochem 34:801–811
Ogasawara M, Suzuki T, Muthoh I, Annapurna K, Arora NK, Nishimura Y, Maheshwari DK (2003) *Sinorhizobium indiaense* sp. nov. and *Sinorhizobium abri* sp. nov. isolated from tropical legumes, *Sesbania rostrata* and *Abrus precatorius*, respectively. Symbiosis 34:53–68
Okon Y, Labandera-Gonzalez CA (1994) Agronomic applications of *azospirillum*: an evaluation of 20 years worldwide field inoculation. Soil Biol Biochem 26(12):1591–1601

Omar SA (1998) The role of rock-phosphate-solubilizing fungi and vesicular–arbuscular mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate. World J Microbiol Biotechnol 14:211–218

Osorio NW (2011) Effectiveness of phosphate solubilizing microorganism in increasing plant phosphate uptake and growth in tropical soils. In: Maheshwari DK (ed) Bacteria in agrobiology: plant nutrient management. Springer, Berlin, pp 65–80

Paio CG, Tang WH, Chen YX (1992) Study on the biological activity of yield-increasing bacteria. Chin J Microecol 4:55–62

Pandey P, Sahgal M, Maheswari DK, Johri BN (2004) Genetic diversity of rhizobia isolated from medicinal legumes growing in the sub-Himalayan region of Uttaranchal. Curr Sci 86:202–207

Park RY, Choi MH, Sun HY, Shin SH (2005) Production of catechol-siderophore and utilization of transferrin-bound iron in *Bacillus cereus*. Biol Pharm Bull 28(6):1132–1135

Patra DK, Bhattacharyya P (1998) Response of cowpea rhizobia on nodulation and yield of mungbean. J Mycopathol Res 36:17–23

Patten CL, Glick BR (2002) Role of *Pseudomonas putida* indole acetic acid in development of the host plan root system. Appl Environ Microbiol 68:3795–3801

Peng G, Yuan Q, Li H, Zhang W, Tan Z (2008) *Rhizobium oryzae* sp. nov., isolated from the wild rice *Oryza alta*. Int J Syst Evol Microbiol 58:2158–2163

Persmark M, Pittman P, Buyer JS, Schwyn B, Gill PRJ, Neilands JB (1993) Isolation and structure of rhizobactin 1021, a siderophore from the alfalfa symbiont *Rhizobium meliloti* 1021. J Am Chem Soc 115:3950–3956

Pongsilp N, Nuntagij A (2009) Genetic diversity and metabolite production of root-nodule bacteria isolated from medicinal legumes *Indigofera tinctoria*, *Pueraria mirifica* and *Derris elliptica* Benth grown in different geographic origins across Thailand. Am Eur J Agric Biol Sci 6:26–34

Prabhavati E, Mallaiah KV (2008) Production of indole acetic acid by *Rhizobium* sp. nodulating *Macrotyloma uniflorum* (Lam.) Verdc. Asian J Soil Sci 3(1):146–148

Puente ME, Bashan Y, Li CY, Lebsky VK (2004) Microbial populations and activities in the rhizoplane of rock-weathering desert plants I Root colonization and weathering of igneous rocks. Plant Biol 6:629–642

Pullaiah T (2006) Encyclopedia of world medicinal plants, vol 2. Regency Publications, New Delhi, pp 599–600

Pullaiah T, Chennaiah E (1997) Flora of Andhra Pradesh (India), vol 1, Ranaculace-Alangiaceae. Scientific Publishers, Jodhpur

Pulseglove JW (1974) Dolichos uniflorus. In: Tropical crops: dicotyledons. Longman, London, pp 263–264

Quan ZX, Bae HS, Baek JH, Chen WF, Im WT, Lee ST (2005) *Rhizobium daejeonense* sp. nov., isolated from a cyanide treatment bioreactor. Int J Syst Evol Microbiol 55(6):2543–2549

Ramirez-Bahena MH, Garcia-Fraile P, Peix A, Valverde A, Rivas R, Igual JM, Mateos PF, Martinez-Molina E, Velazquez E (2008) Revision of the taxonomic status of the species *Rhizobium leguminosarum* (Frank 1879) Frank 1889AL, *Rhizobium phaseoli* Dangeard 1926AL and *Rhizobium trifolii* Dangeard 1926AL *R trifolii* is a later synonym of *R leguminosarum* Reclassification of the strain *R leguminosarum* DSM 30132 (=NCIMB 11478) as *Rhizobium pisi* sp. nov. Int J Syst Evol Microbiol 58:2484–2490

Ramírez-Bahena MH, Peix A, Rivas R, Camacho M, Rodríguez-Navarro DN, Mateos PF, Martínez-Molina E, Willems A, Velázquez E (2009) *Bradyrhizobium pachyrhizi* sp. nov. and *Bradyrhizobium jicamae* sp. nov., isolated from effective nodules of *Pachyrhizus erosus*. Int J Syst Evol Microbiol 59:1929–1934

Reed MLE, Glick BR (2005) Growth of canola (*Brassica napus*) in the presence of plant growth-promoting bacteria and either copper or polycyclic aromatic hydrocarbons. Can J Microbiol 51:1061–1069

Ren DW, Chen WF, Sui XH, Wang ET, Chen WX (2011a) *Rhizobium herbae* sp. nov. and *Rhizobium giardinii*-related bacteria, minor microsymbionts of various wild legumes in China. Int J Syst Evol Microbiol 61:1912–1920

Ren DW, Wang ET, Chen WF, Sui XH, Zhang XX, Liu HC, Chen WX (2011b) *Rhizobium vignae* sp. nov., a symbiotic bacterium isolated from multiple legume species nov. Int J Syst Evol Microbiol 61:580–586

Renwick A, Campbell R, Coe S (1991) Assessment of *in-vivo* screening systems for potential biocontrol agents of *Gaeumannomyces graminis*. Plant Pathol 40:524–532

Rivas R, Willems A, Subba-Rao NS, Mateos PF, Dazzo FB, Kroppenstedt RM, Martı'nez-Molina E, Gillis M, Vela'zquez E (2003) Description of *Devosia neptuniae* sp. nov. that nodulates and fixes nitrogen in symbiosis with *Neptunia natans*, an aquatic legume from India. Syst Appl Microbiol 26:47–53

Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Rodriguez H, Fraga R, Gonzalez T, Bashan Y (2006) Genetics of phosphate solubilization and its potential applications for improving plant growth promoting bacteria. Plant Soil 287:15–21

Rome S, Fernandez MP, Brunel B, Normand P, Cleyet-Marel JC (1996) *Sinorhizobium medicae* sp. nov., isolated from annual *Medicago* spp. Int J Syst Bacteriol 46:972–980

Roy M, Basu PS (1989) Production of 3-indoleacetic acid by a *Rhizobium* sp. from *Mimosa pudica*. Folia Microbiol 34(2):120–126

Roy N, Bhattacharyya P, Chakrabartty PK (1994) Iron acquisition during growth in an iron deficient medium by *Rhizobium* sp. isolated from *Cicer arietinum*. Microbiology 140:2811–2820

Sahasrabudhe MM (2011) Screening of rhizobia for indole acetic acid production. Ann Biol Res 2 (4):460–468

Salisbury EB (1994) The role of plant hormones. In: Wilkinson RE, Dekker M (eds) Plant environment interactions. Dekker, New York, pp 39–81

Saraf M, Jha CK, Patel D (2010) The role of ACC deaminase producing PGPR in sustainable agriculture. In: Maheshwari DK (ed) Plant growth and health promoting bacteria. Springer, Berlin, pp 365–385

Saraf M, Rajkumar S, Saha T (2011) Perspectives of PGPR in Agri-Ecosystems. In: Maheshwari DK (ed) Bacteria in agrobiology: crop ecosystems. Springer, Berlin, pp 361–385

Sarita S, Sharma PK, Priefer UB, Prell J (2005) Direct amplification of rhizobial nodC gene sequence from soil total DNA and comparison of nodC diversity of root nodule bacteria. FEMS Microbiol Ecol 54:1–11

Segovia L, Young JPW, Martinez-Romero E (1993) Reclassification of American *rhizobium leguminosarum* biovar *phaseoli* type I strains as *Rhizobium etli* sp. nov. Int J Syst Bacteriol 43:374–377

Shaharoona B, Arshad M, Zahir ZA, Khalid A (2006) Performance of *Pseudomonas* spp. containing ACC-deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of nitrogenous fertilizer. Soil Biol Biochem 38:2971–2975

Shamseldin A, Werner D (2007) Presence of *Rhizobium etli* biovar *phaseoli* and *Rhizobium gallicum* biovar *gallicum* in Egyptian soils. World J Microbiol Biotechnol 23:285–289

Shamseldin AAY, Vinuesa P, Thierfelder H, Werner D (2005) *Rhizobium etli* and *Rhizobium gallicum* nodulate *Phaseolus vulgaris* in Egyptian soils and display cultivar- dependent symbiotic efficiency. Symbiosis 38:145–161

Shanahan P, O'Sullivan DJ, Simpson P, Glennon JD, O'Gara G (1992) Isolation of 2,4-diacetylphoroglucinol from a fluorescent pseudomonad and investigation of physiological parameters influencing its production. Appl Environ Microbiol 58:353–358

Sharon DA (2003) Dry bean production guide, Ith edn. North Dakota State University, Fargo North Dakota

Shiferaw B, Bantilan MCS, Serraj R (2004) Harnessing the potential of BNF for poor farmers: technological policy and institutional constraints and research need. In: Serraj R (ed) Symbiotic nitrogen fixation; prospects for enhanced application in tropical agriculture. Oxford and IBH, New Delhi, p 3

Shukla SK, Mahajan V, Gupta HS (2006) Horsegram in Uttaranchal-status, constraints and prospects. In: Kumar D (ed) Horsegram in India. Scientific Publishers, Jodhpur, India

Siddiqui ZA (2006) PGPR: prospective biocontrol agents of plant pathogens. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht, pp 111–142

Silva C, Vinuesa P, Eguiarte LE, Martinez-Romero E, Souza V (2003) *Rhizobium etli* and *Rhizobium gallicum* nodulate common bean (*Phaseolus vulgaris*) in a traditionally managed milpa plot in Mexico: population genetics and biogeographic implications. Appl Environ Microbiol 69:884–893

Silva-Stenico ME, Pacheco FTH, Rodrigues JLM, Carrilho E, Tsai SM (2005) Growth and siderophore production of *Xylella fastidiosa* under iron-limited conditions. Microbiol Res 160:429–436

Singh N, Kumar S, Bajpai VK, Dubey RC, Maheshwari DK, Kang SC (2010) Biocontrol of *Macrophomina phaseolina* by chemotactic fluorescent *Pseudomonas aeruginosa* PN1 and its plant growth promotory activity in chir pine. Crop Protect 29:1142–1147

Singhla SK, Kumar K (1985) Inhibitors of calcification from *Dolichos biflorus*. In: Proceedings of the third national conference of urolithiasis, vol 51. Society of India, Udaipur, pp 27–28

Spence N (2006) Bean root rot disease management in Uganda (DFID project ref, R8478, NR Int Code ZA0689). Final technical report, International Development Team, Central Science Laboratory (DEFRA)

Squartini A, Struffi P, Doring H, Selenska-Pobell S, Tola E, Giacomini A, Vendramin E, Velazquez E, Mateos PF, Martinez-Molina E, Dazzo FB, Casella S, Nuti MP (2002) *Rhizobium sullae* sp. nov. (formerly '*Rhizobium hedysari*'), the root-nodule microsymbiont of *Hedysarum coronarium* L. Int J Syst Evol Microbiol 52:1267–1276

Sridevi M, Mallaiah KV (2007a) Bioproduction of indole acetic acid by *Rhizobium* strains isolated from root nodules of green manure crop *Sesbania sesban* (L.) Merr. Iran J Biotechnol 5 (3):178–182

Sridevi M, Mallaiah KV (2007b) Production of indole-3-acetic acid by *Rhizobium* isolates from *Sesbania* species. Afr J Microbiol Res 1(7):125–128

Sridevi M, Kumar KG, Mallaiah KV (2008) Production of catechol-type of siderophores by *Rhizobium* sp. isolated from stem nodules of *Sesbania procumbens* (Roxb.) W and A. Res J Microbiol 3:282–287

Stevenson FJ, Cole MA (1999) Cycles of soils: carbon, nitrogen, phosphorus, sulfur, micronutrients, 2nd edn. Wiley, New York

Stocco P, Santos JCP, Vargas VP, Hungria M (2008) Avaliação da biodiversidade de rizóbios simbiontes do fejoeiro (*Phaseolus vulgaris* L.) em Santa Catarina. R Bras Ci Solo 32:1107–1120

Subba Rao NS (1982) Phosphate solubilization by soil microorganisms. In: SubbaRao NS (ed) Advances in agricultural microbiology. Butterworth Scientific, London, pp 295–303

Subba Rao NS (1993) Biofertilizers in agriculture and forestry. Oxford and IBH publishing Co. Pvt Ltd, Janpath, New Delhi

Takahashi S, Anwar MR (2007) Wheat grain yield, phosphorus uptake and soil phosphorus fraction after 23 years of annual fertilizer application to an andosol. Field Crops Res 101:160–171

Tamimi SM, Young JPW (2004) *Rhizobium etli* is the dominant common bean nodulating rhizobia in cultivated soils from different locations in Jordan. Appl Soil Ecol 26:193–200

Tan ZY, Kan FL, Peng GX, Wang ET, Reinhold-Hurek B, Chen WX (2001) *Rhizobium yanglingense* sp. nov., isolated from arid and semi-arid regions in China. Int J Syst Evol Microbiol 51:909–914

Tang WH (1994) Yield-increasing bacteria (YIB) and biocontrol of sheath blight of rice. In: Ryder MH, Stephens PM, Bowen GD (eds) Improving plant productivity with rhizosphere bacteria. Common wealth Scientific and Industrial Research Organisation, Adelaide, Australia, pp 267–278

Taylor JD, Day JM, Dudley CL (1983) The effect of *Rhizobium* inoculation and nitrogen fertilizer on nitrogen fixation and seed yield of dry beans (*Phaseolus vulgaris*). Ann Appl Biol 103:419–429

Teale WD, Paponov IA, Palme K (2006) Auxin in action: signalling, transport and the control of plant growth and development. Nature Rev Mol Cell Biol 7:847–859

Thakuria D, Talukdar NC, Goswami C, Hazarika S, Baro RC, Khan MR (2004) Characterisation and screening of bacteria from rhizosphere of rice grown in acidic soils of Assam. Curr Sci 86:978–985

Tian CF, Wang ET, Wu LJ, Han TX, Chen WF, Gu CT, Gu JG, Chen WX (2008) *Rhizobium fabae* sp. nov., a bacterium that nodulates *Vicia faba*. Int J Syst Evol Microbiol 58:2871–2875

Tien TM, Gaskin MH, Hubbel DH (1979) Plant growth substances produced by *A. brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). Appl Environ Microbiol 37:1016–1024

Tilak KVSR (1992) VAM-nitrogen fixing organisms interaction: an all India review. In: Mycorrhizae: an Asian overview. TERI, New Delhi, pp 31–37

Tilman D, Fargione J, Wolff B, D' Antonio C, Dobson A, Howarth R, Schindler D, Schlesinger WH, Daniel S, Swackhamer D (2001) Forecasting agriculturally driven global environmental change. Science 292(5515):281–284

Toledo I, Lloret L, Martinez-Romero E (2004) *Sinorhizobium americanus* sp. nov., a new *Sinorhizobium* species nodulating native *Acacia* spp. in Mexico. Syst Appl Microbiol 26:54–64

Trujillo ME, Willems A, Abril A, Planchuelo AM, Rivas R, Ludeña D, Mateos PF, Martínez-Molina E, Velázquez E (2005) Nodulation of *Lupinus* by strains of the new species *Ochrobactrum lupini* sp. nov. Appl Environ Microbiol 71:1318–1327

Valverde A, Velazquez E, Gutierrez C, Cervantes E, Ventosa A, Igual JM (2003) *Herbaspirillum lusitanum* sp. nov., a novel nitrogen fixing bacterium associated with root nodules of *Phaseolus vulgaris*. Int J Syst Evol Microbiol 53:1979–1983

Valverde A, Igual JM, Peix A, Cervantes E, Velázquez E (2006) *Rhizobium lusitanum* sp. nov., a bacterium that nodulates *Phaseolus vulgaris*. Int J Syst Evol Microbiol 56:2631–2637

Van Berkum P, Beyene D, Bao G, Campbell TA, Eardly BD (1998) *Rhizobium mongolense* sp. nov., is one of three rhizobial genotypes identified which nodulate and form nitrogen-fixing symbioses with *Medicago ruthenica* [(L.) Ledebour]. Int J Syst Bacteriol 48:13–22

Vandamme P, Mahenthiralingam E, Holmes B, Coenye T, Hoste B, De Vos P, Henry D, Speert DP (2000) Identification and population structure of *Burkholderia stabilis* sp. nov. (formerly *Burkholderia cepacia* genomovar IV). J Clin Microbiol 38:1042–1047

Vandamme P, Henry D, Coenye T, Nzula S, Vancanneyt M, LiPuma JJ, Speert DP, Govan JR, Mahenthiralingam E (2002) *Burkholderia anthina* sp. nov., and *Burkholderia pyrrocinia*, two additional *Burkholderia cepacia* complex bacteria, may confound results of new molecular diagnostic tools. FEMS Immunol Med Microbiol 33(2):143–149

Vandamme P, Govan JRW, LiPuma JJ (2007) Diversity and role of *Burkholderia* spp. In: Coenye T, Vandamme PAR (eds) *Burkholderia* genomics. Horizon Scientific Press, Norwich, pp 1–28

Vargas LK, Lisboa BB, Schlindwein G, Granada CE, Giongo A, Beneduzi A, Passaglia LMP (2009) Occurrence of plant growth-promoting traits in clover-nodulating rhizobia strains isolated from different soils in Rio Grande Do Sul State. R Bras Ci Solo 33:1227–1235

Velazquez E, Igual JM, Willems A et al (2001) *Mesorhizobium chacoense* sp. nov., a novel species that nodulates *Prosopis alba* in the Chaco Arido region (Argentina). Int J Syst Evol Microbiol 51:1011–1021

Verdcourt B (1982) A revision of *Macrotyloma* (*Leguminosae*). Hooker's Icones Plant 38:1–138

Verma JP, Yadav J, Tiwari KN (2010) Application of *Rhizobium* sp. BHURC01 and plant growth promoting rhizobacteria on nodulation, plant biomass and yields of chickpea (*Cicer arietinum* L.). Int J Agric Res 5(3):148–156

Vidal C, Chantreuil C, Berge O, Maure L, Escarre J, Bena G, Brunel B, Cleyet-Marel JC (2009) *Mesorhizobium metallidurans* sp. nov., a metal-resistant symbiont of *Anthyllis vulneraria* growing on metallicolous soil in Languedoc, France. Int J Syst Evol Microbiol 59:850–855

Viswanatha KP, Henry A, Tikka SBS, Kumar D (2006) Quality, uses and nutrition of horsegram. In: Kumar D (ed) Horsegram in India. Scientific Publishers, Jodhpur, pp 87–98

Wang ET, Van Berkum P, Beyene D, Sui XH, Dorado O, Chen WX, Martınez-Romero E (1998) *Rhizobium huautlense* sp. nov., a symbiont of *Sesbania herbacea* that has a close phylogenetic relationship with *Rhizobium galegae*. Int J Syst Bacteriol 48:687–699

Wang ET, Rogel MA, Garcia-de los Santos A, Martinez-Romero J, Rogel MA, Martinez-Romero E (1999a) *Rhizobium etli* bv mimosa, a novel biovar isolated from *Mimosa affinis*. Int J Syst Bacteriol 49:1479–1491

Wang ET, Van Berkum P, Sui XH, Beyene D, Chen WX, Martınez-Romero E (1999b) Diversity of rhizobia associated with *Amorpha fruticosa* isolated from Chinese soils and description of *Mesorhizobium amorphae* sp. nov. Int J Syst Evol Microbiol 49:51–65

Wang FQ, Wang ET, Liu J, Chen Q, Sui XH, Chen WF, Chen WX (2007) *Mesorhizobium albiziae* sp. nov., a novel bacterium that nodulates *Albizia kalkora* in a subtropical region of China. Int J Syst Evol Microbiol 57:1192–1199

Wang Y, Li P, Cao X, Wang X, Zhang A, Li X (2009) Identification and expression analysis of miRNAs from nitrogen-fixing soybean nodules. Biochem Biophys Res Commun 378:799–803

Wani PA, Khan MS, Zaidi A (2008) Chromium-reducing and plant growth-promoting *Mesorhizobium* improves chickpea growth in chromium-amended soil. Biotech Letter 30:159–163

Wei GH, Chen WX, Wang ET, Tan ZX, Zhu ME (2002) *Rhizobium indigoferae* sp. nov., and *Sinorhizobium kummerowiae* sp. nov., respectively isolated from *Indigofera* spp. and *Kummerowia stipulacea*. Int J Syst Evol Microbiol 52:2231–2239

Wei GH, Tan ZY, Zhu ME, Wang ET, Han SZ, Chen WX (2003) Characterization of rhizobia isolated from legume species within the genera *Astragalus* and *Lespedeza* grown in the Loess Plateau of China and description of *Rhizobium loessense* sp. nov. Int J Syst Evol Microbiol 53 (5):1575–1583

Weller DM (1988) Biological control of soil borne pathogens in the rhizosphere with bacteria. Annu Rev Phytopathol 26:279–407

White JGH (ed) (1989) Grain legumes in sustainable cropping systems: a review. Agronomy Society of New Zealand, Lincoln, Special publication no 7, pp 109–115

Whitelaw MA, Harden TJ, Helyar KR (1999) Phosphate solubilisation in solution culture by the soil fungus *Penicillium radicum*. Soil Biol Biochem 31:655–665

Wittenberg JB, Wittenberg BA, Day DA, Udvardi MK, Appleby CA (1996) Siderophore-bound iron in the peribacteroid space of soybean root nodules. Plant Soil 178:161–169

Xavier LJC, Germida JJ (2002) Response of lentil under controlled conditions to co-inoculation with arbuscular mycorrhizal fungi and rhizobia varying in efficacy. Soil Biol Biochem 34 (2):181–188

Yadav KS, Dadarwal KR (1997) Biotechnological approaches in soil microorganisms for sustainable crop production. Scientific Publishers, Jodhpur, India, pp 293–308

Yanni YG, Rizk RY, Corich V, Squartini A, Ninke K, Philip-Hollingsworth S, Orgambide G, de Bruijn F, Stoltzfus J, Buckley D, Schmidt TM, Mateos PF, Ladha JK, Dazzo FB (1997) Natural endophytic association between *Rhizobium leguminosarum* bv. *trifolii* and rice roots and assessment of its potential to promote rice growth. Plant Soil 194:99–114

Yao ZY, Kan FL, Wang ET, Chen WX (2002) Characterization of rhizobia that nodulate legume species of the genus *Lespedeza* and description of *Bradyrhizobium yuanmingense* sp. nov. Int J Syst Evol Microbiol 52:2219–2230

Young JPW, Haukka KE (1996) Diversity and phylogeny of rhizobia. New Phytol 133:87–94

Young JM, Park D, Weir D (2004) Diversity of 16S rDNA sequences of *Rhizobium* spp. implications for species determinations. FEMS Microbiol Lett 238:125–131

Zahir AZ, Yasin HM, Naveed M, Anjum MA, Khalid M (2010) L-Tryptophan application enhances the effectiveness of *Rhizobium* inoculation for improving growth and yield of mungbean (*Vigna Radiata* L. Wilczek). Pak J Bot 42(3):1771–1780

Zhang XX, Turner SL, Guo XW, Yang HJ, Debelle F, Yang GP, Denarie J, Young PW, Li FD (2000) The common nodulation genes of Astragalus sinicus rhizobia are conserved despite chromosomal diversity. Appl Environ Microbiol 66:2988–2995

Zhang RJ, Hou BC, Wang ET, Li YJ, Zhang XX, Chen WX (2011) *Rhizobium tubonense* sp. nov., isolated from root nodules of *Oxytropis glabra*. Int J Syst Evol Microbiol 61:512–517

Zhou PF, Chen WM, Wei GH (2010) *Mesorhizobium robiniae* sp. nov., isolated from root nodules of *Robinia pseudoacacia*. Int J Syst Evol Microbiol 60:2552–2556

Zurdo-Piñeiro JL, Rivas R, Trujillo ME, Vizcaíno N, Carrasco JA, Chamber M, Palomares A, Mateos PF, Martínez-Molina E, Velázquez E (2007) *Ochrobactrum cytisi* sp. nov., isolated from nodules of *Cytisus scoparius* in Spain. Int J Syst Evol Microbiol 57:784–788

Zurdo-Piñeiro JL, Garcia-Fraile P, Rivas R, Peix A, León-Barrios M, Willems A, Francisco Mateos P, Martinez-Molina E, Velazquez E, van Berkum P (2009) *Rhizobia* from Lanzarote, the Canary islands, that nodulate *Phaseolus vulgaris have* characteristics in common with *Sinorhizobium meliloti* isolates from mainland Spain. Appl Environ Microbiol 75 (8):2354–2359

Chapter 7
Root Nodule and Rhizosphere Bacteria for Forage Legume Growth Promotion and Disease Management

Nora Altier, Elena Beyhaut, and Carlos Pérez

7.1 Introduction

The rhizosphere—the volume of soil surrounding and under the influence of plant roots—is a unique environment that supports a large and metabolically active microbial population, where very important and intensive interactions take place among the plant, soil, and microorganisms (Kennedy 2005). Rhizosphere-competent bacteria that exert a beneficial effect on plant growth are termed collectively plant growth-promoting rhizobacteria (PGPR) (Kloepper 1993). The original definition of rhizobacteria was restricted to free-living bacteria, although some authors have used a broader description as any root-colonizing bacteria (Antoun and Prévost 2005). With the original definition, the root nodule bacteria with the ability of fixing nitrogen, here collectively called rhizobia, would not be considered as PGPR (Vessey 2003).

Rhizobia are well known as the microbial symbiotic partners of legumes, forming N_2-fixing root nodules. Since symbiotic biological nitrogen fixation (BNF) in legumes is mediated by rhizobia, these root nodule bacteria account for at least half of all biologically fixed nitrogen in agriculture (Lindström et al. 2010). The role of root nodule bacteria in plant growth promotion through BNF and their concomitant environmental benefits had already been reported in ancient times. Besides, rhizobia also share some characteristics with other PGPR (Sessitsch et al. 2002) by producing phytohormones, siderophores, HCN; solubilize phosphates; and can also colonize the roots of many non-legume plants (Antoun and Prévost 2005).

N. Altier (✉) • E. Beyhaut
Instituto Nacional de Investigación Agropecuaria (INIA), Ruta 48, km 10, Canelones, Uruguay
e-mail: naltier@inia.org.uy; ebeyhaut@inia.org.uy

C. Pérez
Departamento de Protección Vegetal, EEMAC, Universidad de la República, Ruta 3, km 363, Paysandú, Uruguay
e-mail: caperez@fagro.edu.uy

D.K. Maheshwari et al. (eds.), *Bacteria in Agrobiology: Crop Productivity*,
DOI 10.1007/978-3-642-37241-4_7,

PGPR may induce plant growth promotion by direct or indirect modes of action. Direct mechanisms include the production of volatile compounds and phytohormones and improvement of the plant nutrient status. Indirect effects originate when PGPR act like biocontrol agents (BCA) reducing diseases by changing microbial balance in the rhizosphere in favor of beneficial microorganisms (Siddiqui 2005). Based on their activities, Somers et al. (2004) classified PGPR as biofertilizers (increasing the availability of nutrients to plant) and biocontrol agents or biopesticides (controlling diseases mainly by the production of antibiotics and antifungal compounds and inducing systemic acquired resistance) (Siddiqui 2005; Weller and Thomashow 2010). Among diverse bacterial taxa of PGPR, *Pseudomonas* spp. are particularly suited as BCA because they are abundantly present in natural soils, especially in the rhizosphere. They can use plant exudates as nutrient source and improve plant performance directly promoting plant growth or controlling diseases by a variety of mechanisms (Höfte and Altier 2010).

Current research and farm production have shown that seed inoculation with rhizobia and PGPR, or direct application of PGPR bacteria to the soil, improves plant productivity, quality, and health and/or reduce the need for pesticides and chemical fertilization (Adesemoye et al. 2009; Berg 2009; Berg and Zachow 2011). The study of the indigenous microbial population diversity, isolate searching and characterization, and the stepwise screening are critical to develop commercial biofertilizers and BCA (Fravel 2005; Köhl et al. 2011).

Traditionally, the approach to develop inoculants was based on isolation, testing, and selection of single strains with desired biological properties, such as high nitrogen fixation efficiency in symbiosis with selected host plants, antagonistic potential, competitiveness, and tolerance to environmental conditions (Lindström et al. 2010). Although agronomic efficacy is the most important feature, other criteria for selection include aspects of microbial ecology, mass production, safety, environmental risks, protection of intellectual property rights, and marketing (Köhl et al. 2011). Culture collections that represent a broad biodiversity play a very important role in research, as sources of authenticated biological material (Dijkshoorn et al. 2010; Janssens et al. 2010).

Legumes offer a unique plant model to exploit the use of root nodule and rhizosphere bacteria for growth promotion and disease suppression. The understanding of the ecology of legume microbes is recognized as a key tool for developing sustainable agricultural systems. In this chapter we will discuss concepts and prospects of seed inoculation of forage legumes with rhizobia and pseudomonads. We will also discuss current experiences of co-inoculation with PGPR and rhizobia, focusing on the benefits for increasing yield.

7.2 Importance of Forage Legumes

Cow's milk and cattle meat rank first and third, respectively, among food and agricultural commodities throughout the world. Forage legumes are essential for an efficient animal-based agriculture worldwide. They have been the foundation of dairy and meat production for centuries as rich sources of protein, fiber, and energy (Graham and Vance 2003). Besides providing high-quality feed for livestock, they are a key component for the sustainability of crop-pasture rotations. Legumes offer the potential for enhancing productivity as well as sustainability of mixed, intercropped, and rotational cropping systems (Hardarson and Atkins 2003; Howieson et al. 2008). The value of forage legumes lies mainly in their ability to fix nitrogen (N_2) from the atmosphere in symbiosis with root nodule bacteria of the genera *Rhizobium*, *Sinorhizobium* (*Ensifer*), *Mesorhizobium*, and *Bradyrhizobium*, among others, collectively called rhizobia (Graham 2008; Graham and Vance 2003; Gualtieri and Bisseling 2000; Vance 1998). Symbiotic legumes are critical to the sustainable nitrogen economy, structure, and fertility of soils (Peoples et al. 2009).

7.3 Root Nodule Bacteria for Growth Promotion of Forage Legumes

Biological nitrogen fixation is second only to photosynthesis as the most important biochemical process on earth. It can provide substantial amounts of N_2 to plants and soil, reducing the need for industrial fertilizers (Carlsson and Huss-Danell 2003; Peoples et al. 2009). Use of legumes in pastures for soil improvement dates back to the Romans, with Varro (37 BC; cited by Fred et al. 1932) noting "*Legumes should be planted in light soils, not so much for their own crops as for the good they do to subsequent crops*" (Graham and Vance 2003).

The most recent estimates of annual nitrogen fixation inputs by crop legumes, as given in recent reports (Herridge et al. 2008; Peoples et al. 2009), were 21.45 million tons (Tg), and the inputs of pasture and fodder legumes, 12–25 Tg (Lindström et al. 2010). Perennial forage legumes are usually more effective and derive higher percentages of their N_2 from the atmosphere than most grain legume species (Hardarson and Atkins 2003). When grown in mixtures with grasses, the latter species can take a large fraction of their N_2 from the atmosphere, with average field measurements over 80 % (Carlsson and Huss-Danell 2003). Reported rates of N_2 fixation in aboveground plant tissues are in the range of up to 373 kg N ha^{-1} $year^{-1}$ in red clover (*Trifolium pratense* L.), 545 kg N ha^{-1} $year^{-1}$ in white clover (*Trifolium repens* L.), 350 kg N ha^{-1} $year^{-1}$ in alfalfa (*Medicago sativa* L.), and 138 kg N ha^{-1} $year^{-1}$ in birdsfoot trefoil (*Lotus corniculatus* L.) (Carlsson and Huss-Danell 2003; Gregerson et al. 1999; Vance et al. 1988).

Improved and cultivated pastures integrated by forage legumes form the primary bases of agriculture, dairy, and livestock production. Worldwide perennial

cultivated pastures cover extensive areas, while natural grasslands oversown with legumes sustain extensive cattle production. The most remarkable feature of utilization of forage legumes has been the impact on the effective management of N in the environment through N supply to natural and agroecosystems (Peoples et al. 2009). Their use has largely reduced N fertilization requirements while improving farmer profitability. Consequently, forage legume–rhizobia symbiosis does have a significant effect on world economy. On an average, one ton of urea (the most utilized N fertilizer) costs approximately $420 and supplies 460 kg N. With average estimates of N_2 fixation in forage legumes of about 230 kg N ha^{-1} $year^{-1}$, $210 is saved per hectare (Montañez et al. 2003). In Uruguay, dairy farms occupy an area of 750,000 ha, sown with legume-based pastures in mixture with grasses, mainly alfalfa, birdsfoot trefoil, and clovers (DIEA/MGAP 2009; Rebuffo et al. 2006). Extensive beef cattle production is sustained on natural grasslands improved with oversown exotic Mediterranean legumes, mainly *Lotus* spp., with *Lotus subbiflorus* representing 87.6 % (DIEA/MGAP 2002; Rebuffo et al. 2006). The total area sown to forage legumes covers over two million hectares, which represents 15 % of agricultural land. Considering these numbers, the country accomplishes savings ca. 420 million dollars per year through reducing imports of N fertilizer (Lindström et al. 2010).

7.3.1 *Rhizobial Inoculants for Forage Legumes: The Uruguayan System*

The perennial strategy of most temperate forage legumes like alfalfa (*Medicago sativa* L.), trefoils (*Lotus* spp.), and clover (*Trifolium* spp.) relies on the success of stand establishment and early development of healthy root systems to achieve high dry matter yields and optimal productivity. Microbial-based strategies that improve forage legume productivity, optimize N_2 fixation, conserve soil N, and augment the pool of soil N for the benefit of rotational nonleguminous crop have been exploited worldwide through rhizobial inoculant technology (Brockwell and Bottomley 1995; Carlsson and Huss-Danell 2003; Catroux et al. 2001; Herridge 2008).

Although the production and use of commercial rhizobial inoculants have now worldwide extent, Catroux et al. (2001) concluded that their quality remains poor despite available technologies. They further stated that legal requirements and official surveillance can improve the quality of inoculants and thus their efficacy. As observed in countries with standards and government control, the trend is to increase quality using sterile carriers or liquid inoculants in order to avoid contaminants and to support high numbers of rhizobia in the packages for at least 1 year of storage. Uruguay, together with Brazil, Canada, and France, has been recognized as one of the countries with regulatory authorities responsible for quality control services, supported by appropriate legislation (Brockwell and Bottomley 1995; Lupwayi et al. 2000).

The Uruguayan system for biological N_2 fixation technology has been recently described (Lindström et al. 2010; Montañez et al. 2003). Created in 1960, the key to its success has been the implementation of a national government-supported strategy, based on a strong functional relationship between public research, private industry, and farmers. Regulatory authorities (Ministry of Livestock, Agriculture and Fisheries, MGAP) are supported by appropriate legislation, setting requirements for inoculant registration, mandatory strain recommendation, and enforcement of inoculant quality control. Under a recent agreement between the MGAP and the National Institute for Agricultural Research (INIA), actions are performed in the Laboratory of Soil Microbiology and Inoculant Quality Control, located at INIA Las Brujas. The main activities of this laboratory consist of (1) characterization and selection of rhizobial strains, (2) preservation of the culture collection as a high-quality source of rhizobial germplasm for research, (3) supply of strains to industry, and (4) quality surveillance of commercial inoculants. High-quality standards are achieved using sterile peat carrier as well as liquid formulations, with high numbers of viable rhizobia in the packages being mandatory (2×10^9 rhizobia g^{-1} peat), as pointed out by Herridge (2008) and Lupwayi et al. (2000). Currently, four local manufacturers share the inner market with four imported brands. Moreover, high-quality rhizobial inoculants are exported to other South American and African countries. As a result of research and extension policies, inoculation technology has been broadly adopted by farmers.

As mentioned before, Uruguay bases its improvement of forage supply on the temperate legumes alfalfa, clover, and trefoils. The main rhizobial partner of alfalfa is the fast-growing species *Sinorhizobium* (*Ensifer*) *meliloti* (Vance et al. 1988); the fast-growing species *Rhizobium leguminosarum* biovar *trifolii* nodulates *Trifolium* spp. (Gualtieri and Bisseling 2000), while the moderately fast-growing *Mesorhizobium* spp. and slow-growing *Bradyrhizobium* spp. nodulate *Lotus* species (Díaz et al. 2005; Gregerson et al. 1999; Sotelo et al. 2011). Selected strains with superior symbiotic capacities need to be isolated and developed as inoculants (Hardarson and Atkins 2003; Lupwayi et al. 2000). Periodic assessment of commercial strains under field conditions is essential (Hardarson and Atkins 2003) and the need to monitor culture variability to maintain the quality of legume inoculants has been also emphasized (Bloem et al. 2002). In Uruguay, *S. meliloti* strain U45 (isolated from alfalfa, Uruguay) was formerly used for alfalfa commercial inoculant. However, variant cultures of this strain exhibited variability on the N_2-fixing effectiveness and competitiveness when inoculated to two alfalfa cultivars (Bloem et al. 2002). Therefore, it has been currently substituted by strain U143 (Synonym: MCH3, isolated from alfalfa, Uruguay) that has shown more stable results over time.

Within the genus *Lotus*, specific inoculants are marketed (Table 7.1). The commercial strain for *L. corniculatus*, used since 1972, is U510 (Synonyms: U226, B816, introduced from Australia); this strain, which has always been regarded as *Mesorhizobium loti*, was recently classified as *M. huakuii* biovar *loti* (Sotelo et al. 2011). The commercial strain for *L. subbiflorus* is U531 (Synonym: NC3, native to Uruguay), while for *L. uliginosus* the strain U1401 (Synonyms: U526, U416, NZP2309, introduced from New Zealand) is used. Both U531 and U1401 are slow-growing *Bradyrhizobium* sp.

Table 7.1 Summary of symbiotic compatibility of rhizobial strains on *Lotus* spp.

Strain[a]	Geographic origin	Microorganism[b]	Plant species			References
			L. corniculatus	*L. subbiflorus*	*L. uliginosus*	
U510 (Synonyms: U226, B816)	Australia	*Mesorhizobium huakuii*	**E**[c]	I	I	Baraibar et al. (1999), Monza et al. (1992, 1997, 2006), Sotelo et al. (2011)
U261 (Synonym: NZP2037)	New Zealand	*Mesorhizobium loti*	E	E	E	Baraibar et al. (1999), Díaz et al. (2005), Irisarri et al. (1996), Monza et al. (1992), Sotelo et al. (2011)
U531 (Synonym: NC3)	Uruguay	*Bradyrhizobium* sp.	E	**E**	E	Beyhaut com pers data not published
U1401 (Synonyms: U526, U416,NZP2309)	New Zealand	*Bradyrhizobium* sp.	I	E	**E**	Irisarri et al. (1996), Monza et al. (2006)

[a]Strain ID
[b]Rhizobial species
[c]Symbiotic compatibility: *E* effective, *I* ineffective. The strain mandatorily recommended for commercial inoculant is shown in underlined bold

7.3.2 *Diversity of Indigenous Rhizobia Nodulating Alfalfa and Lotus spp.*

The need for rhizobial strains with enhanced N_2 fixation and tolerance to edaphic constraints (i.e., soil pH) has been repeatedly emphasized (Catroux et al. 2001; Graham and Vance 2003; Langer et al. 2008). The development of inoculants on diverse continents has demonstrated the importance of diversity of indigenous rhizobial populations for both symbiotic nitrogen fixation and the success of inoculation (Lindström et al. 2010). A great diversity at species and strain levels is found in most soils but enhanced population size occurs where compatible legumes are grown. Several authors studied the occurrence, diversity, and symbiotic properties of alfalfa-nodulating strains isolated from acid soils of Uruguay and Argentina (Castro-Sowinski et al. 2002a; Del Papa et al. 1999; Segundo et al. 1999). Mid-acid-tolerant strains able to grow at pH 5.5 but not at pH 5.0 and acid-tolerant strains able to grow at pH 5.0 were characterized. Ten percent of the indigenous *S. meliloti* population in Uruguayan soils was tolerant to acidic conditions, and PCR analysis of the strains suggested that considerable diversity is present. Symbiotic analysis of the strains confirmed that they have the potential to improve alfalfa growth in acidic soils (Castro-Sowinski et al. 2002a) and may be considered for inoculant production (Segundo et al. 1999). Mid-acid-tolerant strains have also been characterized for laccase activity and melanin production. Interestingly, a plant growth-promoting effect in rice by a laccase-producing *S. meliloti* strain when co-inoculated with *Azospirillum brasilense* was observed (Castro-Sowinski et al. 2002b).

Symbiotic effectiveness and ecological characterization of indigenous rhizobia nodulating *Lotus* spp. has been extensively studied in Uruguay. Immunological, biochemical, and genetic properties were described for a large collection of strains (Baraibar et al. 1999; Díaz et al. 1995; Irisarri et al. 1996; Monza et al. 1992, 1997, 2006; Sotelo et al. 2011). Based on colony type and growth rates, isolates from nodules of *Lotus* spp. were separated into two groups corresponding to slow- and fast-growing strains, nodulating *L. subbiflorus* (Irisarri et al. 1996) and *L. corniculatus* (Monza et al. 1992, 1997), respectively. Partial 16S rDNA gene sequencing revealed that fast-growing strains could be identified as *Mesorhizobium loti* species and the slow-growing as *Bradyrhizobium* sp. (Monza et al. 2006). Sotelo et al. (2011) recently indicate that rhizobia nodulating *L. corniculatus* in Uruguay are genetically and phenotypically diverse. Phylogenetic analyses using 16S rRNA and atpD genes, and ITS sequences clustered all the isolates within genus *Mesorhizobium*. A great majority of the isolates likely belong to the species *M. huakuii*, as does the commercial strain U510 (Sotelo et al. 2011).

Although specificity is not yet completely defined in the genus *Lotus*, local studies have demonstrated its occurrence within indigenous population, showing different levels of efficiency when tested on various hosts (Table 7.1). Irisarri et al. (1996) found that the slow-growing isolates effectively nodulating *L. subbiflorus* were unable to form effective nodules on *L. corniculatus*. Similarly, Baraibar et al.

(1999) and Monza et al. (1992) found that all fast-growing isolates effectively nodulating *L. corniculatus* induced small and ineffective nodules in *L. subbiflorus*. An exception was the fast-growing strain U261 (Synonym: NZP2037), which formed effective root nodules on *L. corniculatus*, *L. subbiflorus*, and *L. uliginosus*, although suboptimal levels of nitrogenase activity were reported (Díaz et al. 2005). Inability of the inoculant strains to successfully compete with established rhizobia populations in soil has been frequently reported, and agronomical implications need to be considered. This underlines the need of rhizobial inoculation with specific inoculant-quality strains, particularly in land where a symbiotically incompatible *Lotus* species has been previously cultivated.

The importance of strains adapted to edaphic constraints is also relevant for *Lotus* spp. performance. Results reported by Baraibar et al. (1999) proved that 83 % of the indigenous rhizobia nodulating *Lotus* spp. was acid-tolerant in culture medium (pH 4.5); they lend support to the importance of selecting, among the latter, the most efficient and resistant strains to be included in the inoculants. As an example, the overwhelming increase in the area of natural grasslands oversown with *L. subbiflorus*, especially adapted to acidic soils, has been largely sustained by the selection of the indigenous strain U531 for commercial inoculant.

7.4 Rhizospheric Bacteria for Disease Management of Forage Legumes

Seedling diseases caused by soilborne pathogens, primarily *Pythium* species, are one of the main constraints for forage legume establishment (Altier and Thies 1995). Favorable environmental conditions for disease development are low soil temperatures and high soil moisture, which slow down germination rate and reduce seedling emergence (Altier and Thies 1995; Martin and Loper 1999; Pérez et al. 2000). Effective management of soilborne plant pathogens requires integrated strategies and the use of rhizospheric antagonistic microorganisms is a promising approach (Martin and Loper 1999; Weller et al. 2002, 2007).

Microbial-based strategies to improve forage legume establishment and optimize N_2 fixation have been deployed worldwide through rhizobial inoculant technology (Catroux et al. 2001). However, the study of rhizospheric bacteria for plant growth promotion and disease control of forage legumes has received less attention, and their agronomical use remains as a challenge (Handelsman et al. 1990; Jones and Samac 1996; Villacieros et al. 2003; Xiao et al. 2002). Fluorescent *Pseudomonas* spp. have been extensively reported as effective biocontrol agents (McSpadden Gardener 2007; Weller et al. 2007), including the control of *Pythium* seedling diseases in other crops (Loper 1988; Martin and Loper 1999). In addition, *Bacillus* spp. (Handelsman et al. 1990) and *Streptomyces* spp. (Jones and Samac 1996; Xiao et al. 2002) have been explored to control alfalfa seedling damping-off.

7.4.1 *Fluorescent Pseudomonads as Biocontrol Agents*

The highly diverse genus *Pseudomonas* contains very effective biocontrol agents that can increase plant growth and improve plant health. However, there is a lack of association of phylogenetic variation with biocontrol characteristics, which appear to be strain dependent. The most commonly reported mechanisms of biocontrol by fluorescent *Pseudomonas* spp. include production of antibiotics, hydrogen cyanide, cyclic lipopeptides (Raaijmakers et al. 2006), competition for nutrients and niches, competition for iron mediated by siderophores, and induced systemic resistance (De Vleesschauwer and Höfte 2009). The antibiotics 2,4-diacetylphloroglucinol (DAPG), pyoluteorin, pyrrolnitrin, and different phenazine derivatives have been described as the main weapons of these microorganisms with antagonistic activity (De La Fuente et al. 2004; Weller et al. 2007). Isolate screening remains essential to find strains that can effectively be used under local conditions. By testing large local collections of fluorescent *Pseudomonas*, strains can be selected with enhanced disease-suppressing and plant growth-promoting abilities to develop bacterial inoculants (Höfte and Altier 2010).

Research has been done to explore the biocontrol of *Pythium* seedling diseases using native fluorescent *Pseudomonas* isolated from Uruguayan soils (Bagnasco et al. 1998; Bajsa et al. 2005; De La Fuente et al. 2002, 2004; Pérez et al. 2000; Quagliotto et al. 2009; Yanes et al. 2004, 2012). Several strains with enhanced disease suppressing capability and plant growth-promoting abilities have been selected to develop bacterial inoculants (De La Fuente et al. 2002, 2004; Quagliotto et al. 2009; Yanes et al. 2004, 2012) (Table 7.2). Commercial registration of selected strains is currently under way.

7.4.2 *Phenotypic Characterization in the Laboratory and Under Controlled Conditions*

Initially, a collection of *P. fluorescens* with 700 bacterial strains was established. They were isolated from the rhizosphere of field-grown birdsfoot trefoil plants, collected from different agroecological regions in Uruguay. Laboratory studies such as in vitro assessment of antagonism against primary plant pathogens and production of antifungal compounds were performed. The presence of genes for antibiotic biosynthesis was also investigated (Bagnasco et al. 1998; De La Fuente et al. 2004). Three selected *P. fluorescens* strains, UP61, UP143, and UP148, demonstrated in vitro antagonism and were able to protect birdsfoot trefoil from the infection caused by *Pythium ultimum* and *Rhizoctonia solani* in vivo, under controlled conditions (Bagnasco et al. 1998) (Table 7.2). Hydrogen cyanide (HCN) and fluorescent siderophore production was detected among the factors possibly involved in their biocontrol activity (Bagnasco et al. 1998). In addition, *P. fluorescens* UP61 produced the antibiotics 2,4-diacetylphloroglucinol,

Table 7.2 Summary of *Pseudomonas fluorescens* strains native to Uruguay with biocontrol and plant growth-promoting effects

Strain	Plant species rhizosphere[a]	In vitro activity[b]					Agronomic effect[c]		References
		PA	AB	HCN P	SP	PO_4	IE	DWB	
UP61	BT	+	+	+	+	+	$+^{(A, BT, GC, F)}$	$+^{(A, BT, GC, F)}$	Bagnasco et al. (1998), Bajsa et al. 2005, De La Fuente et al. (2004), Quagliotto et al. (2009), Pérez et al. (2000)
UP143	BT	+	+	+	+	–	$+^{(A, BT, GC, F)}$	$+^{(A, BT, GC, F)}$	
UP148	BT	+	+	+	+	–	$+^{(A, BT, GC, F)}$	$+^{(A, BT, GC, F)}$	
αC119	A	+	–	+	+	–	$+^{(A, GC)}$	$-^{(A, GC)}$	Yanes et al. (2004, 2012)
αP271	A	+	–	+	+	+	$+^{(A, GC)}$	$+^{(A, GC)}$	
αP388	A	+	+	+	+	–	$+^{(A, GC)}$	$-^{(A, GC)}$	
αT633	A	+	–	–	+	–	$+^{(A, GC)}$	$+^{(A, GC)}$	
αT688	A	+	–	–	+	–	$+^{(A, GC)}$	$+^{(A, GC)}$	

[a]Plant rhizosphere from which strain was isolated *BT* birdsfoot trefoil, *A* alfalfa

[b]*PA* pathogen antagonism, *AB* antibiotic biosynthesis, *HCN P* hydrogen cyanide production, *SP* siderophore production, *PO_4* phosphate solubilization

[c]*IE* increased emergency, *DWB* dry weight biomass; in superscript parentheses: *A* alfalfa, *BT* birdsfoot trefoil, *GC* growth chamber data, *F* field data

pyoluteorin, and pyrrolnitrin (De La Fuente et al. 2004), whereas *P. fluorescens* UP148 produced a phenazine-derivative antifungal compound not previously described (Bajsa et al. 2005) (Table 7.2). The interaction of *P. fluorescens* UP61, UP143, or UP148 with rhizobial strains used locally as commercial inoculants was also assessed. In growth chamber conditions, birdsfoot trefoil and alfalfa seed inoculation with *Pseudomonas* strains did not affect different parameters of host-rhizobium symbiosis as observed in plant dry weight, nodulation rate, biological N_2 fixation efficiency, and rhizospheric colonization (Bagnasco et al. 1998; De La Fuente et al. 2002).

A second collection of 702 native *P. fluorescens* strains, isolated from the rhizosphere of field-grown alfalfa plants, was later established. A growth chamber in vivo assay was developed to screen the fluorescent *Pseudomonas* isolates for their ability to suppress disease and promote plant growth in the alfalfa-*Pythium* pathosystem, under controlled conditions (Yanes et al. 2004). When challenged against *Pythium debaryanum*, a wide response on disease suppression ability was found among *Pseudomonas* isolates. Twelve percent of the screened isolates protected alfalfa plants, increasing 81 % the emergence related to the non-inoculated control treatment (Yanes et al. 2004). A similar procedure, in the absence of the pathogen, was used to evaluate alfalfa growth-promoting ability of selected *Pseudomonas* strains as shown by biomass weight. Five *P. fluorescens* strains, αC119, αP271, αP388, αT633, and αT688, which showed ability to suppress disease and promote plant growth, were selected for further investigation under field conditions (Yanes et al. 2012) (Table 7.2).

7.4.3 Evaluation of Control Efficiency in Field Trials

During several years, experiments were conducted under field conditions to evaluate the ability of *P. fluorescens* UP61, UP143, and UP148 to suppress seedling diseases on alfalfa and birdsfoot trefoil (Bajsa et al. 2005; Pérez et al. 2000; Quagliotto et al. 2009). Combinations of different years, locations, and sowing dates resulted in twenty environments for each crop. The *P. fluorescens* strains successfully colonized alfalfa and birdsfoot trefoil roots at adequate densities for biocontrol activity (Table 7.2). Results demonstrated that bacterial seed inoculation provided a 10–13 % increase in the number of alfalfa plants established relative to the control, while in birdsfoot trefoil the increase ranged 6–10 % (Quagliotto et al. 2009). In the presence of biocontrol strains, the aboveground biomass was increased by 15–18 % and 6–10 % in alfalfa and birdsfoot trefoil, respectively (Table 7.2). Our results confirmed that an adequate stand of plants is initially required to forward the productive potential of the pasture (Quagliotto et al. 2009).

7.4.4 *Development of Bacterial Inoculants*

Laboratory assays were performed to identify a culture media for adequate biomass production of *P. fluorescens* at an industrial scale, using commercially available carbon and nitrogen sources. Sterile peat was assessed as the carrier for formulating the bacterial inoculant, following the rhizobial inoculant technology. Thus, *P. fluorescens* and rhizobia strains survived at 10^9 and 10^{10} CFU g^{-1}, respectively, in sterile peat inoculated with each bacterial species, when stored at 4 °C over 1 year (Bagnasco et al. 1998; De La Fuente et al. 2002).

Based on the strengths of already developed rhizobial inoculant technology, our research has been focused on the commercial development and agronomical performance of biological control agents. The key for the success of the Uruguayan biological N_2 fixation system has been the implementation of a national government-supported strategy, where regulatory authorities are sustained by appropriate legislation on inoculant registration, quality control, and usage (Brockwell and Bottomley 1995; Lupwayi et al. 2000). Therefore, the objective has been to develop an inoculant having both the rhizobia and the BCA; commercial registration of selected strains is currently under way.

7.5 Co-inoculation with Rhizobia and Pseudomonads

The exploitation of PGPR in combination with rhizobia constitutes an interesting alternative to improve nitrogen fixation of legume crops (Bai et al. 2002, 2003; Chebotar et al. 2001; De Leij et al. 2002; Fox et al. 2011; Villacieros et al. 2003). There have been an increasing number of reports of "helper" PGPR enhancing a variety of legume–rhizobia symbioses (Vessey 2003). The presence of PGPR can influence the activity of rhizobia to compete with indigenous populations for nodulation (Gupta et al. 2003). The effects of PGPR co-inoculated on legume symbioses include increases in nodule number and/or nodule weight and in some cases an enhancement of nitrogen fixation or N accumulation (Fox et al. 2011).

A variety of mechanisms have been proposed for the observed responses of symbiotic legumes to PGPR co-inoculation, including phytohormonal stimulation of root growth (Vessey and Buss 2002), increased production of nod gene products inducing flavonoids by the legume host (Andrade et al. 1998), stimulation of root hair development (Lucas García et al. 2004), and secretion of B vitamins by the PGPR enhancing rhizobial growth in the rhizosphere of red clover (Marek-Kozaczuk and Skorupska 2001). The production of the indole acetic acid (IAA) is of particular interest, since this phytohormone stimulates root elongation and increased density of both root hairs and lateral roots (Gray and Smith 2005). As roots are the initial point for nodule formation, increased growth could result in more colonizing sites for rhizobia.

Fox et al. (2011) demonstrated that co-inoculation of *Medicago truncatula* with *Sinorhizobium (Ensifer) meliloti* WSM419 and *Pseudomonas fluorescens* WSM3457 enhanced the rate of nodule initiation and development. It also resulted in increased number of nodules, total nitrogen accumulation, and dry shoot mass of barrel medic plants. Helper PGPR, such as *P. fluorescens* WSM3457, may offer a significant advantage enhancing early nodule initiation in legumes to increase the success of rhizobial inoculants under environmental conditions that result in rapid death of the inoculum. There is an incipient emergence of commercial inoculant products that contain plant growth-promoting microorganisms as well as rhizobia, though these are predominantly focused on phosphate solubilization (Leggett et al. 2007). Further research on development of co-inoculants for forage legumes is required to prove usefulness of this innovative technology in agriculture.

7.6 Concluding Remarks and Future Perspectives

Forage legumes form the primary bases of agriculture, dairy, and livestock production. Legume–rhizobium symbiosis is the most important route for sustainable nitrogen input into agroecosystems. BNF can be efficiently exploited by inoculating forage legumes with suitable rhizobia. With the global interest in microbial diversity, rhizobia have also become of interest for taxonomists, molecular biologists, and agronomists. However, many good inoculant strains are still poorly described, and molecular and taxonomic characteristics are missing. Research and extension policies which encourage rhizobial germplasm study and preservation, farmer training for proper inoculant use, and legal control of commercial inoculant quality have proven to be a successful approach to promoting the use of inoculants in forage legumes, while enhancing biological N_2 fixation at a national scale. It is our hope that information about the well-functioning Uruguayan system, in combination with the knowledge of rhizobial diversity and current taxonomy, could help to join together the diverse fields of rhizobia research.

Highly effective biocontrol agents can be found within the group of fluorescent pseudomonads. However, biocontrol capability is strain dependent. Thus, isolate screening remains important to identify effective strains adapted to local conditions. This can be done by testing large collections of local isolates for disease suppression or plant growth promotion. The attainment of well-structured scientific knowledge for developing biocontrol strategies has been demonstrated worldwide. However, scaling up, formulation, commercial production, quality control issues, and agronomical use remain a challenge.

The exploitation of PGPR inoculated along with rhizobia constitutes an interesting alternative to improve nitrogen fixation and reduce diseases of forage legumes. However, further research is needed for co-inoculants to prove usefulness to commercial agriculture.

Some actions must be strengthened on a global scale to recognize the ecology of forage legume microbes as a key tool for developing sustainable agricultural

systems: (1) establishment of regulatory legislation for registration and use of biocontrol agents, (2) risk assessment for human health and environment, (3) investment on research facilities, (4) recruitment and training of human resources, (5) support of technological ventures between public and private sector, (6) strengthening of international cooperation for collaborative research, and (7) education and extension policies for farmer adoption.

Acknowledgments The authors are thankful to INIA and Universidad de la República for providing support for research work.

References

Adesemoye AO, Torbert HA, Kloepper JW (2009) Plant growth promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58:921–929

Altier N, Thies JA (1995) Identification of resistance to *Pythium* seedling diseases in alfalfa using a culture plate method. Plant Dis 79:341–346

Andrade G, De Leij FAAM, Lynch JM (1998) Plant mediated interactions between *Pseudomonas fluorescens*, *Rhizobium leguminosarum* and arbuscular mycorrhizae on pea. Lett Appl Microbiol 26:311–316

Antoun H, Prévost D (2005) Ecology of plant growth promoting rhizobacteria. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, The Netherlands, pp 1–38

Bagnasco P, De La Fuente L, Gualtieri G, Noya F, Arias A (1998) Fluorescent *Pseudomonas* spp. as biocontrol agents against forage legume root pathogenic fungi. Soil Biol Biochem 30:1317–1322

Bai YM, Pan B, Charles TC, Smith DL (2002) Co-inoculation dose and root zone temperature for plant growth promoting rhizobacteria on soybean [*Glycine max* (L.) Merr] grown in soilless media. Soil Biol Biochem 34:1953–1957

Bai YM, Zhou X, Smith DL (2003) Enhanced soybean plant growth resulting from coinoculation of *Bacillus* strains with *Bradyrhizobium japonicum*. Crop Sci 43:1774–1781

Bajsa N, Quagliotto L, Yanes ML, Vaz P, Azziz G, De La Fuente L, Bagnasco P, Davyt D, Pérez C, Ducamp F, Altier N, Arias A (2005) Selección de *Pseudomonas* fluorescentes nativas para controlar enfermedades de implantación en praderas. Agrociencia 9:321–325

Baraibar A, Frioni L, Guedes ME, Ljunggren H (1999) Symbiotic effectiveness and ecological characterization of indigenous *Rhizobium loti* populations in Uruguay. Pesquisa Agropecuária Brasileira 34:1011–1017

Berg G (2009) Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84:11–18

Berg G, Zachow C (2011) PGPR interplay with rhizosphere communities and effect on plant growth and health. In: Maheshwari D (ed) Bacteria in agrobiology: crop ecosystems. Springer, The Netherlands, pp 97–109

Bloem JF, Botha WJ, Law IJ, Steyn PL (2002) Colony variation in *Sinorhizobium meliloti* inoculant strain U 45. Microbiol Res 157:283–292

Brockwell J, Bottomley PJ (1995) Recent advances in inoculant technology and prospects for the future. Soil Biol Biochem 27:683–697

Carlsson G, Huss-Danell K (2003) Nitrogen fixation in perennial forage legumes in the field. Plant Soil 253:353–372

Castro-Sowinski S, Carrera I, Catalán AI, Coll J, Martinez-Drets G (2002a) Occurrence, diversity and effectiveness of mid-acid tolerant alfalfa nodulating rhizobia in Uruguay. Symbiosis 32:105–118

Castro-Sowinski S, Martinez-Drets G, Okon Y (2002b) Laccase activity in melanin-producing strains of *Sinorhizobium meliloti*. FEMS Microbiol Lett 209:119–125

Catroux G, Hartmann A, Revellin C (2001) Trends in rhizobial inoculant production and use. Plant Soil 230:21–30

Chebotar VK, Ais CA, Akao S (2001) Production of growth-promoting substances and high colonization ability of rhizobacteria enhance nitrogen fixation in soybean when inoculated with *Bradyrhizobium japonicum*. Biol Fertil Soils 34:427–432

De La Fuente L, Quagliotto L, Bajsa N, Fabiano E, Altier N, Arias A (2002) Inoculation with *Pseudomonas fluorescens* biocontrol strains does not affect the symbiosis between rhizobia and forage legumes. Soil Biol Biochem 34:545–548

De La Fuente L, Thomashow L, Weller D, Bajsa N, Quagliotto L, Chernin L, Arias A (2004) *Pseudomonas fluorescens* UP61 isolated from birdsfoot trefoil rhizosphere produces multiple antibiotics and exerts a broad spectrum of biocontrol activity. Eur J Plant Pathol 110:671–681

De Leij FAAM, Dixon-Hardy JE, Lynch JM (2002) Effect of 2,4-diacetylphloroglucinol-producing and non-producing strains of *Pseudomonas fluorescens* on root development of pea seedlings in three different soil types and its effects on nodulation of *Rhizobium*. Biol Fertil Soils 35:114–121

De Vleesschauwer D, Höfte M (2009) Rhizobacteria-induced systemic resistance. Adv Bot Res 51:223–281

Del Papa MF, Balague LJ, Castro Sowinski S, Weneger C, Segundo E, Martinez Abarca E, Toro N, Niehaus K, Puhler A, Aguilar OM, Martinez Drets G, Lagares A (1999) Isolation and characterization of alfalfa-nodulating rhizobia present in acidic soils of central Argentina and Uruguay. Appl Environ Microbiol 65:1420–1427

Díaz P, Borsani O, Monza J (1995) Effect of inoculation and nitrate on nitrate reductase activity and acetylene reduction activity in *Lotus* sp.-*Rhizobium loti* symbiosis. Symbiosis 19:53–63

Díaz P, Borsani O, Monza J (2005) Lotus-related species and their agronomic importance. In: Márquez AJ (ed) *Lotus japonicus* handbook. Springer, The Netherlands, pp 25–37

DIEA/MGAP (2002) Encuesta Ganadera Año 2001. Serie Encuestas No.211. http://www.mgap.gub.uy/Diea/Encuestas/default.htm. Accessed 30 Apr 2012

DIEA/MGAP (2009) Producción lechera en el Uruguay Año 2007. Serie Encuestas No.278 http://www.mgap.gub.uy/Diea/Encuestas/Se278/Se278_ProduccionLecheraenelUruguay.pd. Accessed 30 Apr 2012

Dijkshoorn L, De Vos P, Dedeurwaerdere T (2010) Understanding patterns of use and scientific opportunities in the emerging global microbial commons. Res Microbiol 161:407–413

Fox SL, O'hara GW, Bräu L (2011) Enhanced nodulation and symbiotic effectiveness of *Medicago truncatula* when co-inoculated with *Pseudomonas fluorescens* WSM3457 and *Ensifer* (*Sinorhizobium*) *medicae* WSM419. Plant Soil 348:245–254. doi:10.1007/s11104-011-0959-8

Fravel DR (2005) Commercialization and implementation of biocontrol. Annu Rev Phytopathol 43:337–359

Fred EB, Baldwin IL, McCoy E (1932) Root nodule bacteria and leguminous plants. Madison, University of Wisconsin Press. http://digital.library.wisc.edu/1711.dl/HistSciTech.RootNodule. Accessed 12 Mar 2012

Graham PH (2008) Ecology of the root-nodule bacteria of legumes. In: Dilworth MJ, James EK, Sprent JI, Newton WE (eds) Leguminous nitrogen-fixing symbioses. Springer, The Netherlands, pp 23–58

Graham PH, Vance C (2003) Legumes: importance and constraints to greater use. Plant Physiol 131:872–877

Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signalling processes. Soil Biol Biochem 27:395–412

Gregerson RG, Lowell Robinson D, Vance CP (1999) Carbon and nitrogen metabolism in *Lotus*. In: Beuselinck PR (ed) Trefoil: the science and technology of *Lotus*, ASA-CSSA, CSSA special publication 28, pp 167–185

Gualtieri G, Bisseling T (2000) The evolution of nodulation. Plant Mol Biol 42:181–194
Gupta A, Saxena AK, Gopal M, Tilak KVBR (2003) Effects of co-inoculation of plant growth promoting rhizobacteria and *Bradyrhizobium* sp. (*Vigna*) on growth and yield of green gram [*Vigna radiata* (L.) Wilczek]. Trop Agr 80:28–35
Handelsman J, Raffel S, Mester EH, Wunderlich L, Grau CR (1990) Biological control of damping-off of alfalfa seedlings with *Bacillus cereus* UW85. Appl Environ Microbiol 56:713–718
Hardarson G, Atkins C (2003) Optimising biological N_2 fixation by legumes in farming systems. Plant Soil 252:41–54
Herridge DF (2008) Inoculation technology for legumes. In: Dilworth MJ, James EK, Sprent JI, Newton WE (eds) Leguminous nitrogen-fixing symbioses. Springer, The Netherlands, pp 77–115
Herridge DF, Peoples MB, Boddey RM (2008) Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil 311:1–18
Höfte M, Altier N (2010) Fluorescent pseudomonads as biocontrol agents for sustainable agricultural systems. Res Microbiol 161:464–471
Howieson JG, Yates RJ, Foster KJ, Real D, Besier RB (2008) Prospects for the future use of legumes. In: Dilworth MJ, James EK, Sprent JI, Newton WE (eds) Leguminous nitrogen-fixing symbioses. Springer, The Netherlands, pp 363–394
Irisarri P, Milnitsky F, Monza J, Bedmar EJ (1996) Characterization of rhizobia nodulating *Lotus subbiflorus* from Uruguayan soils. Plant Soil 180:39–47
Janssens D, Arahal DR, Bizet C, Garay E (2010) The role of public biological resource centers in providing a basic infrastructure for microbial research. Res Microbiol 161:422–429
Jones CR, Samac DA (1996) Biological control of fungi causing alfalfa seedling damping-off with a disease-suppressive strain of *Streptomyces*. Biol Cont 7:196–204
Kennedy AC (2005) Rhizosphere. In: Sylvia DM, Fuhrmann JJ, Harter PG, Zuberer DA (eds) Principles and applications of soil microbiology, 2nd edn. Upper Saddle River, NJ, Pearson, Prentice Hall, pp 242–262
Kloepper JW (1993) Plant-growth-promoting rhizobacteria as biological control agents. In: Metting FB Jr (ed) Soil microbial ecology. Dekker, New York, pp 255–273
Köhl J, Postma J, Nicot P, Ruocco M, Blum B (2011) Stepwise screening of microorganisms for commercial use in biological control of plant-pathogenic fungi and bacteria. Biol Cont 57:1–12
Langer H, Nandasena KG, Howieson JG, Jorquera M, Borie F (2008) Genetic diversity of *Sinorhizobium meliloti* associated with alfalfa in Chilean volcanic soils and their symbiotic effectiveness under acidic conditions. World J Microbiol Biotechnol 24:301–308
Leggett M, Cross J, Hnatowich G, Holloway G (2007) Challenges in commercializing a phosphate-solubilizing microorganism: *Penicillium bilaiae*, a case history. In: Velázquez E, Rodríguez-Barrueco C (eds) First international meeting on microbial phosphate solubilization. Springer, The Netherlands, pp 215–222
Lindström K, Murwira M, Willems A, Altier N (2010) The biodiversity of beneficial microbe-host mutualism: the case of rhizobia. Res Microbiol 161:453–463
Loper JE (1988) Role of fluorescent siderophore production in biological control of *Pythium ultimum* by a *Pseudomonas fluorescens* strain. Phytopathology 78:166–172
Lucas García JA, Probanza A, Ramos B, Barriuso J, Gutiérrez Manero FJ (2004) Effects of inoculation with plant growth promoting rhizobacteria (PGPRs) and *Sinorhizobium fredii* on biological nitrogen fixation, nodulation and growth of *Glycine max* cv. Osumi. Plant Soil 267:143–153
Lupwayi NZ, Olsen PE, Sande ES, Keyser HH, Collins MM, Singleton PW, Rice WA (2000) Inoculant quality and its evaluation. Field Crops Res 65:259–270
Marek-Kozaczuk M, Skorupska A (2001) Production of B-group vitamins by plant growth-promoting *Pseudomonas fluorescens* strain 267 and the importance of vitamins in the colonization and nodulation of red clover. Biol Fertil Soils 33:146–151

Martin FN, Loper JE (1999) Soilborne plant diseases caused by *Pythium* spp.: ecology, epidemiology and prospects for biological control. Crit Rev Plant Sci 18:111–181

McSpadden Gardener BB (2007) Diversity and ecology of biocontrol *Pseudomonas* spp. in agricultural systems. Phytopathology 97:221–226

Montañez A, Labandera C, Solari L (2003) Nitrogen fixation on a national scale. Leisa Magazine 19:9

Monza J, Fabiano E, Arias A (1992) Characterization of an indigenous population of rhizobia nodulating *Lotus corniculatus*. Soil Biol Biochem 24:241–247

Monza J, Diaz P, Borsani O, Ruiz-Argüeso T, Palacios JM (1997) Evaluation and improvement of the energy efficiency of nitrogen fixation in *Lotus corniculatus* nodules induced by *Rhizobium loti* strains indigenous to Uruguay. World J Microbiol Biotechnol 13:565–571

Monza J, Irisarri P, Díaz P, Delgado MJ, Mesa S, Bedmar EJ (2006) Denitrification ability of rhizobial strains isolated from *Lotus* sp. Antonie Van Leeuwenhoek 89:479–484

Peoples MB, Brockwell J, Herridge DF, Rochester IJ, Alves BJR, Urquiaga S, Boddey RM, Dakora FD, Bhattarai S, Maskey SL, Sampet C, Rerkasem B, Khan DF, Hauggaard-Nielsen H, Jensen ES (2009) The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. Symbiosis 48:1–17

Pérez C, De La Fuente L, Arias A, Altier N (2000) Uso de *Pseudomonas fluorescentes* nativas para el control de enfermedades de implantación en *Lotus corniculatus* L. Agrociencia 4:41–47

Quagliotto L, Azziz G, Bajsa N, Vaz P, Pérez C, Ducamp F, Cadenazzi M, Altier N, Arias A (2009) Three native *Pseudomonas fluorescens* strains tested under growth chamber and field conditions as biocontrol agents against damping-off in alfalfa. Biol Cont 51:42–50

Raaijmakers JM, de Bruijn I, de Kock MJD (2006) Cyclic lipopeptide production by plant-associated *Pseudomonas* spp.: diversity, activity, biosynthesis, and regulation. Mol Plant Microbe Interact 19:699–710

Rebuffo M, Bemhaja M, Risso DF (2006) Utilization of forage legumes in pastoral systems: state of art in Uruguay. Lotus Newsletter 36:22–33

Segundo E, Martínez-Abarca F, Van Dillewijn P, Fernández-López M, Lagares A, Martínez-Drets G, Niehaus K, Pühler A, Toro N (1999) Characterisation of symbiotically efficient alfalfa-nodulating rhizobia isolated from acid soils of Argentina and Uruguay. FEMS Microbiol Ecol 28:169–176

Sessitsch A, Howieson JG, Perret X, Antoun H, Martínez-Romero E (2002) Advances in *Rhizobium* research. Crit Rev Plant Sci 21:323–378

Siddiqui ZA (2005) PGPR: prospective biocontrol agents of plant pathogens. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, The Netherlands, pp 111–142

Somers E, Vanderleyden J, Srinivasan M (2004) Rhizosphere bacterial signalling: a love parade beneath our feet. Crit Rev Microbiol 30:205–240

Sotelo M, Irisarri P, Lorite MJ, Casaretto E, Rebuffo M, Sanjuán J, Monza J (2011) Diversity of rhizobia nodulating *Lotus corniculatus* grown in northern and southern regions of Uruguay. Appl Soil Ecol 49:197–207

Vance CP (1998) Legume symbiotic nitrogen fixation: agronomic aspects. In: Spaink HP, Kondorosi A, Hooykaas PJJ (eds) The Rhizobiaceae. Kluwer Academic Publishers, The Netherlands, pp 509–530

Vance CP, Heichel GH, Phillips DA (1988) Nodulation and symbiotic nitrogen fixation. In: Hanson AA, Barnes DK, Hill RR (eds) Alfalfa and alfalfa improvement. Agronomy 29. ASA-CSSA-SSSA, pp 229–257

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586

Vessey JK, Buss TJ (2002) *Bacillus cereus* UW85 inoculation effects on growth, nodulation, and N accumulation in grain legumes – controlled-environment studies. Can J Plant Sci 82:282–290

Villacieros M, Power B, Sánchez-Contreras M, Lloret J, Oruezabal RI, Martín M, Fernández-Piñas F, Bonilla I, Whelan C, Dowling DN, Rivilla R (2003) Colonization behaviour of

Pseudomonas fluorescens and *Sinorhizobium meliloti* in the alfalfa (*Medicago sativa*) rhizosphere. Plant Soil 251:47–54

Weller DM, Thomashow LS (2010) Biological control of soilborne pathogens: looking back and forward. The Phytopathological Society of Japan (PSJ) Soilborne Disease Workshop Report, vol 25, pp 48–66

Weller DM, McSpadden Gardener BB, Raaijmakers JM, Thomashow LS (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu Rev Phytopathol 40:309–348

Weller DM, Landa BB, Mavrodi OV, Schroeder KL, De La Fuente L, Blouin Bankhead S, Allende Molar R, Bonsall RF, Mavrodi DV, Thomashow LS (2007) Role of 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. in the defense of plant roots. Plant Biol 9:4–20

Xiao K, Kinkel LL, Samac DA (2002) Biological control of *Phytophthora* root rots on alfalfa and soybean with *Streptomyces*. Biol Cont 23:285–295

Yanes ML, Fernández A, Arias A, Altier N (2004) Método para evaluar protección contra *Pythium debaryanum* y promoción del crecimiento de alfalfa por *Pseudomonas* fluorescentes. Agrociencia 8:23–32

Yanes ML, De La Fuente L, Altier N, Arias A (2012) Characterization of native fluorescent *Pseudomonas* isolates associated with alfalfa roots in Uruguayan agroecosystems. Biol Cont 63:287–295. doi:10.1016/j.biocontrol.2012.08.006

Chapter 8
Bioinoculants: Understanding Chickpea *Rhizobia* in Providing Sustainable Agriculture

Hammad Khan and Nagina Parmar

8.1 Introduction

Mass production or high-input agriculture, once believed to be the jewel of economic sustainability, now has become a target of many environmental catastrophes. Pulse crop farming, orchards, rice fields, and cotton farming are the essence of economic stability for many developing countries (Ramarethinam et al. 2005). The agriculture push coupled with increased populations and consumer demands has left many farmers and agriculturalists seeking new methods of providing sustainable growth. To cope with the ever-increasing demand, farmers and horticulturists have turned to biotechnology as a means for creating more applicable fertilizers and creating genetically modified seedlings that can fix to variant environments by introducing selective microorganisms that interact to combat pesticide and rhizobial dwelling pathogens (Nautiyal 2000). These methods brought forth the wake known as the "green revolution" (Khanna-Chopra and Sinha 1998).

Environmental concerns over water availability, alkalinity, and soil health, through traditional methods of chemical applications, had begun to overshadow quality of production. Agrochemicals and pesticide usage became less desirable, opting farmers to push towards a more sustainable and self-sufficient organic control method, encouraging quality with increased production rather than quantity for mass production (Kakde et al. 2005). This has eliminated such traditional methods of chemically taxing harvest areas and therefore opened the gateway for subsidizing biological control agents, one of which is proving to make great strides in providing sustainable agriculture, known as bioinoculants (Kakde et al. 2005; Narain 1998).

H. Khan • N. Parmar (✉)
Department of Chemistry and Biology, Ryerson University, Toronto, ON, Canada M5B 2K3
e-mail: naginap@ryerson.ca

D.K. Maheshwari et al. (eds.), *Bacteria in Agrobiology: Crop Productivity*,
DOI 10.1007/978-3-642-37241-4_8,

Bioinoculants are defined as the concoctions of microbial entities that are supplemented as biocontrol agents to induce or suppress both biotic and abiotic factors in promoting sustained growth (Gupta et al. 2007). *Pseudomonas* and *Bacillus* spp. are common genera among bioinoculants that interact with diverse rhizobial communities. These bioinoculants undertake interactions between host and surrounding rhizosphere microorganisms by secreting and uptaking nutrients, known as root exudates (Hayat et al. 2010). Through associated, synergistic, and neutralistic interactions, plant growth and nodulation are promoted; however, antagonistic interactions may occur where competition for desired nutrients and production of antibiotic compounds may result in suppressing host characteristics (Nautiyal 2000).

Bioinoculant interactions are very important in low-lying nutrient-deprived soils. They are used in promoting the uptake of nutrients such as nitrogen and phosphorus and are used for their interactive capabilities to promote desired and suppress less desirable rhizospheric microorganisms (Borde et al. 2009). Plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhiza (AM) fungi improve root nodulation and other plant growth parameters, respectively, by mechanisms that increase surface area, improve root and shoot length, encourage sporulation, and eliminate the need for harsh chemical applications such as chemical fertilizers and insecticidal sprays (Wang et al. 2005).

Understanding the complexity of rhizobial interactions is crucial in determining sustainability; however, abiotic factors such as temperature, acidity, and soil composition may be delimiting factors that determine growth. Variant temperatures coupled with limited aeration and pH gradients promote the survival of microbes adapted to conditions suited for such environments (Singh et al. 2011). Regions where water is limited and shade is minimal would have high levels of evapotranspiration, thus requiring deep root and shoot integration. Rhizobial flora, in such an environment, would exist deep within the soils, requiring the bacterium to be tolerant of conditions where oxygen and available atmospheric nitrogen concentrations are limited (Upadhyay et al. 2000).

Applications must carefully be assessed before administering any foreign bacterium to a population of native bacteria. If rejection is encountered, survival of the native rhizobacteria as well as the supplemented microorganisms will be unlikely. Native dwelling rhizobacteria can initiate defense mechanisms to combat potential invading microbes by inducing the production of antibiotics or by releasing flavonoids and acting as phytoalexins, which may tax the plant and hinder the plant growth (Parmar and Dufresne 2011). Selecting microorganisms as potential applicants must be carefully tested through rigorous field studies to fully understand interactive traits. Bioinoculants essentially provide the potential of combating both biotic and abiotic factors as well as eliminating the need for harsh insecticides and chemicals, all the while promoting sustainable growth of the microflora within the rhizosphere.

8.2 Factors Affecting Sustainable Growth

Overcropping and mass production have become nationwide concerns for many agriculturists for reasons beyond systemic productivity. Traditional methods of chemical and insecticidal applications not only have reduced annual harvests; rather, their use has resulted in altered soil chemistry, disrupting the balance between plant–microbe interactions and chemical/ion/nutrient exchange. More so, using these strategies to increase rate of production has resulted in microbial sterility and decreased diversity of many beneficial microorganisms including rhizobial inhabitants (Wang et al. 2005). For such factors, examining and understanding how old practices, once believed to be the dawn of the agrochemical boom, have become the essence of failed intervention and hindered successive generational growth. Here, we examine some key factors that have hampered soil sustainability, thereby truncating successive growth yields.

8.2.1 Overgrazing, Excessive Cropping, and Improper Agricultural Practices

Maximizing crop yield and fertility has been at the forefront for agribusinesses across the globe, pushing towards high production, quicker harvests, and lower costs. Excessive cropping and the expenditure of resources towards erratic practices such as overgrazing have left soils in dire shape (Kakde et al. 2005). Soil erosion, through less mitigating factors such as water or wind, is one direct consequence of such unsanctioned practices. Soil infertility and degradation in India alone has resulted in uncultivable top soil, reaching approximately 18.5 %, which is a statistical reading recorded as the maximum global loss (Sharma 2005). Also, 175 million ha of land from a total of 329 million ha geographical area is considered to be partially degraded in one form or another caused by excessive cropping and improper agricultural practices (Bhadauria et al. 2010). As such, natural vegetation, essential nutrients, stored organic matter, and microbial entities cannot perform/supply effective quantities to sustain sufficient growth nor provide fertility within the soil flora. Through continuous tilling and agitation, the soil becomes taxed and arid; as a result, organic/biological control methods become less responsive, as essential precursor elements become less available (Krebs 2000). Subsidizing organic controls, such as bioinoculants, to regions devastated by overcropping and overgrazing is a tedious and time-consuming process. Selecting appropriate microorganisms such as PGPR, understanding interactive characteristics of the indigenous microflora, and then mediating symbiosis may provide soil fertility in the long run. Unfortunately, organic turnover is slow and needs time to adapt to environmental conditions before even attempting to correct the damage left by such practices (Upadhyay et al. 2000). As is often the case, farmers and agriculturists turn towards aggressive chemical treatments to essentially save a harvest season,

potentially risking further tillage and mediating negative biological response. Reality is, without aggressive intervention in remediating such soils, natural replenishment of supplement nutrients and organic matter cannot provide an environment needed for supplying sustainable growth conditions.

8.2.2 Soil Salinity

Sustained practices and soil quality are two key characteristics widely recognized as mitigating factors that determine successive growth. Through some abiotic factors and unconventional practices such as overgrazing and application of chemical fertilizers, insecticides, and pesticides, soils have become vulnerable to withering and anoxic conditions. Regions marked by such practices often turn saline as a result (Qadir et al. 2008). Without the necessary remediation tools, salinity can extend over fertility dynamics such as determining viability of microbial entities and consequently altering the rhizoplane structure itself. Salinity and nutrient stresses account for over 100 million ha of damaged farmland across the globe (Ashraf et al. 2009). In India, where pulse crop and leguminous harvests are major economic contributors to a high-population market, 8.5 million ha is considered degraded and highly saline, with 1.3 million ha reflecting in state of Uttar Pradesh (Bhadauria et al. 2010). Characteristics of saline soils are categorized by two parameters: one being soluble salts available in soil and the other, soil reaction. Soluble salts accumulate in soil through waterlogging and secondary salinization mechanisms, whereby these immobilized salts seep into the rhizoplane and adversely prompt changes in physicochemical properties. Such salts can also accumulate through the application of fertilizers, from atmospheric salt depositions, as seen near coastlines and weathering of soil minerals (Wang et al. 2009).

The significance behind "salting of the soils" describes the chemical shift or deviation in soil, which consequently effects rhizospheric competency. As more salt-tolerant bacteria capable of adapting in such environments proliferate, nutrients and minerals sequestered from soil become limited and specific. As a result, the affected region encounters what is described as a shift in microbial hierarchy or dominancy to counteract the chemical shift (FAO and IAEA 2010). This may further be accompanied by a shift in soil composition and plant demographics to reflect such environmental parameters. As the salt concentrations rise, osmotic stresses, which may also be initiated by arid or semiarid conditions, activate the plants to initiate defense mechanisms (Cordovilla et al. 1995). In this process, bacteria may go through a physiological change to try to adapt to the saline condition. Intracellular accumulation of low molecular weight organic solutes, called osmolytes, tries to counteract dehydration parameters in the plant by increases in potassium (K^+) concentrations. Increasing K^+ level acts to control magnesium (Mg^{2+}) flux's during osmotic shock as magnesium ions combat inhibitory response (Zahran 1999). Such a response eliminates any possibility of interacting with the newly colonizing microbes. Microbial activity can be

suppressed by transformation, through physiological changes, accompanied by the release of phytohormones and flavonoids, which may suppress microbial activity. More concerning, however, is the plant itself which may become chemically deprived by not receiving adequate amounts of essential nutrients needed to replenish itself and gear for survival (Singh et al. 2011).

8.2.3 Abiotic Factors

Abiotic stresses have been at the forefront of many failed agribusinesses. Limiting factors, such as soil pH, aridity, aeration, and irrigation, occur naturally due to land topography, limited resources (e.g., water scarcity), climate, and landscape.

8.2.3.1 pH Factors Affecting Sustainable Growth

pH gradients in an ecosystem vary region by region depending on many factors. Of those, salinity, soil composition, and localized plantation are considered influencing factors to pH profiles. Acidic soils require microorganisms that are capable of adapting in pH gradients less than 7. Acidic conditions promote growth factors that stimulate physical dismemberment of soil parameters and plant protective mechanisms, such as cysts and spores, in response to environmental stresses (Sethi et al. 1994). Acidic regions marked by coniferous plantations and aridity often contain soils with limited productivity and marginal diversity. Limited nitrate concentrations are characteristics of acidic soils which reflect truncated nitrogen fixation levels and often are counteracted by high pH, which inhibit growth regulators (Sharma 2005; Torimitsu et al. 1985). In addition, acidic soils are associated with high levels of manganese (Mn), iron (Fe), and aluminum ions (Al^{3+}) (Busse and Bottomley 1989). These toxic elements act as inhibitors by disrupting cell differentiation and morphology, suppress nutrient uptake, and undermine plant growth. Aluminum specifically inhibits root growth and phosphorous uptake, while Mn initiates physiological changes, such as black necrotic spots on leaves and chlorosis on leaf margins and cuppings (Busse and Bottomley 1989; Government of Alberta 2002). Essential nutrients such as phosphorus are needed to regulate metabolism and be utilized as energy. Phosphate is returned to soil in organic forms as organic phosphate is readily used by *Rhizobia*. In acidic conditions, phosphorus becomes difficult to attain as the organic element transforms into inorganic phosphate by anionic bonding to cations, such as Al or Fe, becomes fixed to the soil, and essentially is no longer available for nitrogen fixation, thus hindering growth (Wiederholt and Johnson 2005).

To limit toxicological effects of Mn, Al^{3+}, and Mn, remediation is selected towards application of buffering solutions, primarily carbonates and bicarbonates, to counteract acidic stresses (Powell 1994). Alkaline soils between pH 6.5 and 8.5 are preferred conditions where many plants are capable of adapting growth

parameters due to buffering capacities coordinated by host plant and rhizobia. In a study conducted by Bhadauria et al. (2010), using biological intervention for remediating alkaline wastelands, it was observed that appropriate selection of microbes and stimulation of ecological parameters could be maintained. Within a 3-year time frame, soil in such regions marked by alkaline pHs could be reclaimed at pH 8.5, accounting for a growth of 681 diverse tree species and 21 different tree types (Bhadauria et al. 2010). Microbes present in these alkaline soils promote the breakdown of calcium carbonates and calcium hydroxides which act as buffering compounds reducing hydrogen ions from suppressing growth. In such an environment, rhizobial colonies flourish by reducing acidic ions, leading to more available phosphorus, thereby leaving the surrounding rhizosphere as a neutral environment for interactions to occur without inhibitory stresses (Jakasaniya and Trivedi 2004). Selecting biological methods to naturally shift acidic soils towards more alkaline soils is encouraged. This process is quite slow and much work is needed before implementation; however, engineered bioinoculant controls, such as acid- or alkaline-tolerant bacterium which can promote growth in the rhizosphere, are gaining recognition and may be at the frontier of engineered soil inoculants in the near future.

8.2.3.2 The Arid Soil Effect on the Rhizosphere: Limited Aeration and Irrigation

The significance and intensity of the arid soil effect on the rhizosphere and plant growth parameters are largely influenced by land topography, climate, and drainage. The moisture content of the soil without adequate irrigation through evapotranspiration may be lost at higher rates before soil can be replenished (Bhadauria et al. 2010). As a response to such environmental stresses, soil properties have manifested mechanisms in which water and nutrients can be retained, all in lower quantities, permitting potential correspondence with host plants (Vevrek and Campbell 2002). Arid and semiarid soils are fairly porous and aerated, allowing water and nutrients to penetrate into deep layers of soil, serving as a reservoir to existing soil microbes. To access this "reservoir" of nutrients and water, plants must go through physiological changes to accommodate their need for survival (Radwan 2009). In arid and semiarid regions, plant roots must stretch beyond surface layers into deep layers, often ranging between 7 and 10 f. (Rao 2002; FAO and IAEA 2010). This physiological change can be regarded as an adaptive response to aid in survival and, however, often may take several cultivation years before naturally adapting. Plants of tropical regions may not be able to survive in such environmental conditions. These plants are catered towards lateral surface and subsurface elongation in means of attaining water and nutrients. Without intervention in the form of bioinoculant controls, PGPR/AM fungi or a genetically engineered organism, these plants will struggle to survive, as evapotranspiration rates will eventually dehydrate the plant, resulting in shutdown and formation of cysts (Singh et al. 2009).

It is estimated that currently over 70 million ha of farming land is affected by drought/arid-like conditions, with numbers projected to rise. By 2025, the Food and Agriculture Organization (FAO) estimates 1.8 billion people will be affected by water scarcity with length of cultivable growing seasons ranging between 120 days in drylands and 74, or less, days in arid regions (FAO and IAEA 2010; Kassas 2008). Intensive irrigation, tilling, and soil composition are directly affected by precipitation and drainage efficiency in arid regions; however, often such soils are subject to higher saline concentrations, erosion, and soil degradation. As a result, a common mechanism associated with such factors is an increase in the water table due to waterlogging (Kassas 2008). Waterlogging exists through irony as water is the contributing factor leading to aridity. Contrary to how arid soils were described earlier, Sharma (2005) positions waterlogged regions by describing how the actual porous capability of soil in such a region has been truncated due to excessive tilling. The air pockets associated with naturally developed arid and semiarid lands due to environmental parameters are subsequently reduced. As a result, soil is less aerated and more compact with limited capabilities for water penetration deep into soil (FAO 1989). Anoxia is often the mitigating factor which mediates plant and rhizobial death in such practices (Jackson 2004). Sustained respiration and synthesis of metabolites and the rapid exchange of O_2 and CO_2 become limited and difficult to attain with the influx of water (Setter and Belford 1990). The plant essentially fixates from its own redox reactions as facultative anaerobes eliminate nitrate utilization by converting nitrate into nitrogen gas through a process of denitrification. More concerning is chemical oxides such as Mn and Fe reduced into highly soluble forms Mn^{2+} and Fe^{2+}, leading to chemical toxicity that enters roots and disrupts cell morphology and differentiation (Arshad and Frankenberger 1990; Laanbroek 1990).

Further, microbial activity and viability become suppressed as soluble salts accumulate in subsurface layers, resulting in altered chemistry around the roots. With inadequate irrigation or drainage, the waterlogged regions remain stagnated with limited permeability, and as a result evaporation rates increase, leaving behind a highly concentrated saline layer (Sharma 2005). Waterlogging has been estimated to occur approximately in 10 % of all irrigated farmland, resulting in a 20 % decrease in crop productivity (Jackson 2004). Without supplementing biotechnology or corrective irrigational means, soil in such regions will continue to become more saline. As harvest seasons prolong, limited yields, fertility, and allocated growing periods will decline as soil chemistry, microflora, and vegetation will all shift to cope with arid pressures (Sharma 2005).

8.3 Effects of PGPR Bioinoculants

The microbial flora within the rhizosphere exists as a continuous complex of interactions and sustained mechanisms. Diverse microbial communities interact among one another in the aim of attaining sustainability, be it through synergism,

neutralism, associated or antagonistic interactions. This is known as developing rhizospheric competency (Nautiyal 2000). These interactions determine soil health and plant viability as a means of characterizing which species will dominate within the rhizosphere (de Selincourt 1996). Through chemical breakdown and uptake of essential nutrients, a dominant species, such as PGPR, will encourage growth and proliferation of plant parameters as well as reduce invasion from competitors by inducing mechanisms that readily fix nitrogen, secrete siderophores for iron utilization, and promote the synthesis of phytohormones (Glick 1995). Either of these mechanisms used by PGPR bioinoculants can provide conditions that stimulate secretion of root exudates from host plants, thereby encouraging colonial growth of the novel species complimentary to the PGPR bioinoculant (Lynch 1990).

Interaction and uptake are of essence the mitigating factors for successive colonization and proliferation of PGPR with an existing indigenous population. The concentration of bacteria surrounding the rhizosphere as per gram of soil compared to that of the bacteria found existing in aggregates dispersed throughout the soil is generally found at much higher folds (Lynch 1990). This accounts for the high levels of metabolic activity occurring within root regions. Nutrients such as atmospheric nitrogen, phosphorus, and carbon are readily available in agro-rich regions. Rokhzadi et al. (2008) demonstrated nutrient acquisition capabilities by studying the interactions of symbiotic bacterium *Mesorhizobium ciceri* and nonsymbiotic rhizobacteria from the *Azospirillum*, *Azotobacter*, and *Pseudomonas* genra on growth and yield of *Cicer arietinum* (Rokhzadi et al. 2008). Combined inoculation with mutually inclusive traits promote symbiotic activities that often result in increased nutrient acquisition by activating host characteristics that allow recognition and release of root exudates into soil (Sindhu et al. 2002). Rhizobacteria mutually respond by uptaking soil nutrients and fixating them so they can be used for plant synthesis. Stimulation inevitably results from the sustained nutrient supply and exchange within the root, promoting cellular respiration and differentiation in plant tissues (Rokhzadi et al. 2008; Zhang et al. 2011). This mechanism is similar in both bacteria and fungi, displaying characteristics of growth fixation, uptake, and release of nutrients by host plant and surrounding microbes (Zhang et al. 2011).

8.3.1 PGPR's Mechanism of Biocontrol Within the Rhizosphere

Under environmental norms, mechanisms implemented to support PGPR's inhabitation, growth, and proliferation in the rhizosphere lie towards secondary methods of protection to complement the symbiotic invasion: the production of phytohormones and the release of flavonoids and phytoalexins (Parmar and Dufresne 2011). These are secreted as a by-product of an endosymbiotic interaction to provide the plant with support and protection against invasive or damaging stresses (Glick 1995). We will focus on the role of phytohormones on plant growth

promotion and then try to correlate these findings with mechanisms employed by PGPR treated in *Cicer* dwelling rhizospheres.

8.3.1.1 Phytohormones Supplemented with PGPR Inoculants

Phytohormones are described as plant growth-promoting hormones active in regulating response to biotic and abiotic stresses through synergistic or antagonistic actions. This is referred to as signaling cross talk (Schmelz et al. 2003). These hormones induce cell elongation, aid in cell division and differentiation, and promote lateral root development to allow nutrients and minerals to be sequestered from distant and localized regions, essentially aiding in plant versatility under conditions of limited surface nutrient availability (Hong et al. 1991). We will focus primarily on auxins as hormones responsible for plant growth promotion due to the interactive traits displayed against PGPR. Auxins are defined for their characteristic as a plant hormone containing indole-3-acetic acid (IAA), which, through its synthesis, stimulates rapid cell growth and differentiation (Cleland 1990). PGPR accelerate cell differentiation through its capacity to synthesize auxin, a role typically reserved to plants which now is able to transmit dual synthesis, accelerating cell growth and proliferation (Gaudin et al. 1994). However, Gaudin et al. (1994) suggested, to positively benefit from maximum auxin synthesis, the primary objective is to distinguish between the degree of auxin synthesis in plants, void of PGPR, and compare that to the level of auxin synthesis when a bioinoculant, such as PGPR, is supplemented in the rhizosphere (Gaudin et al. 1994). To understand such characteristics, a wheat plant was supplemented with a mutant of *Azospirillum brasilense* strain, and trace quantities of IAA synthesis were found (Glick 1995). Compared to the wild-type strain, production of IAA was much limited, and as a result, limited IAA cannot effectively promote formation of laterals roots, thereby eliminating such physiological characteristics associated with PGPR stimulation (Glick 1995).

A study done by Khalid et al. (2004) demonstrated how effective PGPR is as an auxin synthesizer and to which degree PGPR's characteristics can promote survival in foreign rhizospheres. The team used tryptophan (L-TRP) as their method of control. The significance behind L-TRP is that it is an amino acid readily secreted in root exudates, which holds as a precursor for the biosynthesis of auxins in plants and microbes (Frankenberger and Arshad 1995). PGPR supplemented in L-TRP-deficient soils were found to synthesize plant auxins in varied amounts; however, when comparing this number to TRP-positive PGPR-deficient inoculums, PGPR prompted synthesis at much higher levels even in the absence of TRP. In the presence of TRP, auxin synthesis was heightened several folds, with Glick (1995) suggesting that PGPR symbiotically coordinates uptake and chemical breakdown with the plant's vascular system in an effort to enhance auxin synthesis (Glick 1995). Khalid et al. (2004) also tested the versatility of PGPR strains inoculated in sterile and non-sterile soils, a test to understand compatibility of PGPR with the indigenous microflora. Monitored through auxin synthesis analysis, it was found

that PGPR effectively prompted auxin synthesis at higher levels in both sterilized and non-sterilized soils as compared to the preexisting uninoculated strains. However, uninoculated strains in non-sterilized soil provided higher degrees of synthesis compared to single strain PGPR inoculants in sterilized soils. Furthermore, PGPR inoculated in non-sterilized soils, in tandem with the preexisting microflora, substantially accelerated auxin synthesis, which was achieved through the secretion of plant growth-promoting substances which encourage other PGPR bioinoculants to take forth in the mutually inclusive interaction (Okon and Vanderleyden 1997). Once established, the symbiotic partners fix nitrogen, promote metabolic activities, and indirectly stimulate the plant to release much needed exudates to enrich the soil (Parmar and Dadarwal 1997). As a result, PGPR-inoculated plants showed early germination, early development and flowering, and increases in dry weight of root and shoot parameters, all of which correlated to higher yields and increased biomass (Khalid et al. 2004).

8.3.2 *Synergistic Relations of PGPR with Native* Cicer Rhizobia

Cicer arietinum, also known as chickpea, is among the high-demand pulse crop selection geared towards serving Middle Eastern and south Asian diets. With such demands, productivity and marketing of *Cicer* and other pulse crops is essential in establishing successive harvests and generational fertility to serve a largely vegetarian population (Reddy et al. 2000). Traditionally, production was often heightened through the use of chemical fertilizers and insecticidal sprays to combat invasive species and promote soil fertility; however, after subsequent application, physiological side effects and growth yield began to shrink. Rajasthan's state environmental policy 2010 reported truncated growing periods and necrosis of cellular components in plant, and indigenous populations began to propagate, suppressing biological activity and shifting the plant towards defense/survival mechanisms. Through such a response, production inevitability falters as host symbiosis no longer can be sustained, bringing about a microbial shift and consequently altering the interactions within the rhizosphere (Department of Rajasthan 2010).

Coupled with the interactions of applied bioinoculants, PGPR can exist in any form that promotes growth and fixation between host and the native microflora population (Glick 1995). PGPR regulates successive growth and coordination through two mechanisms in *Cicer* sp. and other leguminous crops: endosymbiosis interactions with host *Rhizobia* and biocontrol stimulatory response mechanisms and differentiation in cellular components (Zhang et al. 2011; Glick 1995). PGPR and *Rhizobium* interactions are marked by selective integration and release of intermediary metabolites that induce uptake and growth. Such metabolites include flavonoids; phytohormones, such as auxins as mentioned earlier; iron-chelating

siderophores; and antibiotics (Glick and Pasternak 2003). It is well known that *Rhizobia* are equipped with specialized *Nod* genes; these *Nod* genes are inactive in the absence of host legumes, such as *Cicer*. Together with *Nod* factors, signal transduction between symbionts is expressed through an affinity for receptor and signaling molecules adjacent to *Nod* (Tilak et al. 2010). *Nod* genes induce response in the epidermis near the distal part of the nodule infection zone where infection threads and bacteria are released (Mirabella et al. 2005). These infection sites harvest regions that provide additional occupancy for *Rhizobia* to colonize, enhance the solubilization of inorganic phosphates, and provide protection for the plant from phytopathogens (Hayat et al. 2010). This ultimately promotes growth through enhanced nodule and root hair formation along root structures and phosphorus utilization, heightening physiological function of plant respiration and indirectly prompting soil fertility (Tilak et al. 2010).

8.3.2.1 Growth Promotion by *Pseudomonas* spp.

Pseudomonas spp. have been identified as novel forms of PGPR that act synergistically with indigenous populations to promote growth and proliferation of plant parameters (Antoun and Prévost 2005). In vivo and in vitro studies have shown that supplementing *Pseudomonas* spp. as a PGPR-directed bioinoculant causes significant increases in nodule yield, weight, and root and shoot biomass of various legumes and marked increases in soil fertility (Parmar and Dufresne 2011). The secondary function observed with *Pseudomonas* spp. while promoting nitrogen fixation and symbiosis with the native bacteria population is to reduce infection from phytopathogens by acting as an antagonist towards soilborne plant pathogens (Khare et al. 2011).

Production of indoleacetic acid (IAA) stimulates cell elongation and cell division by activating aminocyclopropane-1-carboxylic acid (ACC) deaminase activity (Jacobson et al. 1994). The use of *Pseudomonas fluorescens* as a biological control method for chickpea wilt was demonstrated by supplementing *P. fluorescens* within the rhizosphere. This not only promoted growth and interactions of the native rhizobacteria but acted as an antagonist towards *Fusarium oxysporum* f. sp. *ciceri* (Vidhyasekaran and Muthamilan 1995). *Fusarium oxysporum* f. sp. *ciceri* is one of the most devastating known soilborne fungal pathogens keen on disrupting cellular processes and translocation of water and nutrients through development of spores, causing vascular wilt, chlorosis, flaccidity, and discoloration in chickpea plants (Cho and Muehlbauer 2004; Buddenhagen and Workneh 1988). The stages of infection caused by *Fusarium oxysporum* f. sp. *ciceri* in chickpea xylem vessels were captured by Gupta et al. using scanning electron microscopy (Gupta et al. 2010). Symptoms of the pathogenic infection were analyzed over a subset of 4 days postinoculation (DPI) in a susceptible breed of chickpea plant, JG62, to measure degree of wilt and internal vascular disintegration. At 4 DPI, the onset of beginning stages of tissue damage in the xylem vessels was seen with microspores beginning to propagate within the xylem tissue interior. At 8 DPI, larger numbers of spores

were found causing pronounced vascular tissue damage in the xylem. At 12 DPI, the appearance of microconidia was apparent, translating into complete demolishment of the original structure of the xylem tissue. In comparison, using the wilt-resistant WR315 chickpea plant, it was found that, at 15 DPI, no damage or sporadic activity within the xylem was noticed. Only after 22–24 DPI, fungal spores were detected and after 28 DPI colonization and slight tissue damage were seen; however, no fungal division was present (Gupta et al. 2010).

Using synergistic biocontrol methods, Nautiyal supplemented *P. fluorescens* NBRI1303 as an active antagonist to pathogenic *Fusarium oxysporum* f. sp. *ciceri*, *Rhizoctonia bataticola*, and *Pythium* sp., three of the most devastating pathogens affecting chickpea (Nautiyal 1997b). When inoculated among chickpea seed cultivars with *P. fluorescens* NBRI1303, seed germination increased by 25 %, the number of diseased plants reduced by 45 %, and seedling dry weight and shoot as well as root length increased between 16 and 18 %. These results suggested that the PGPR bacterium (*P. fluorescens*) actively and aggressively interacted with chickpea *Rhizobia* to colonize and mediate mechanisms to readily promote, sequester, and regulate nutrient–soil homeostasis, all the while maintaining suppressive behavior of phytopathogenic species (Nautiyal 1997a, b). Recently, Maheshwari et al. (2011) reported co-inoculation of urea- and DAP-tolerant *Sinorhizobium meliloti* and *Pseudomonas aeruginosa* as an integrated approach of growth enhancement of *Brassica juncea*. Gupta et al. (2010) further elaborated on this notion of wilt resistance using *Pseudomonas* spp. in chickpea seedlings. Phenotypic changes in chickpea plants over a subset of three 4 DPI intervals with infected JG62 and resistant WR315 were studied. Despite slight yellowing of the roots, WR315 plants after 12 DPI remained unaffected as compared to JG62, which by 8 DPI began showing major signs of wilt, chlorosis, browning of roots, and retardation of branching and growth. By 12 DPI, the plant suffered major loss with chlorosis, accompanied by root blackening due to increased phenolic deposition (Gupta et al. 2010). Similar antagonistic interactions were also observed by Parmar and Dadarwal (1997), studying sustainability and effectiveness through co-inoculation of rhizosphere bacteria such as *Bacillus* spp. (*Pseudomonas* sp.) with chickpea *Rhizobium*. Results indicated significant increases in nodule weight, nitrogen uptake, and root and shoot biomass. *Pseudomonas* spp. "CRP55b" strain acted symbiotically to induce increased production of flavonoids like compounds in roots on seed bacterization accounting for enhancement in growth and percent yield (Parmar and Dadarwal 1997). More recently Rokhzadi et al. (2008) used a combination of bioinoculant strains *Azospirillum* spp., *A. chroococcum* 5, *Mesorhizobium ciceri* SWR17, and *P. fluorescens* P21 to mimic similar responses against chickpea cultivars. The combined effects of the bioinoculants and PGPR strain enhanced nitrogen and phosphorus consumption and availability, increased supply of nutrients, and enriched production of growth-promoting substances. Secondary effects markedly reduced phytopathogen populations and competitively inhibited antagonistic populations. These factors accounted for improved nodulation, increased dry matter content in roots and shoots, and promotion of grain, biomass, and protein yields (Rokhzadi et al. 2008). Inoculation with PGPR increased growth

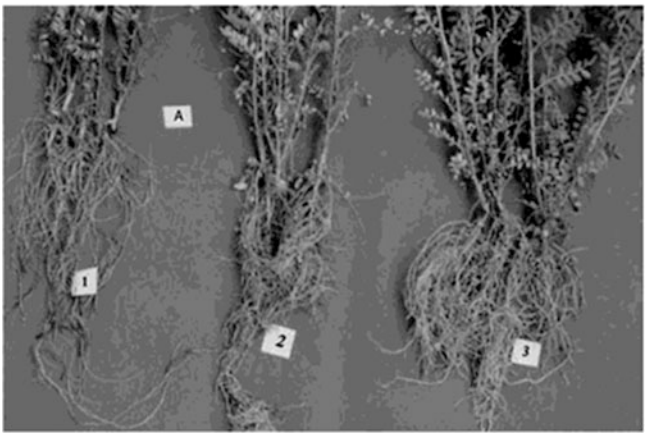


Fig. 8.1 Comparing *Cicer arietinum* (chickpea) growth inoculated with modified rhizobial symbionts (Parmar and Dadarwal 1999)

parameters in chickpea plants with increased biomass and lateral root formation in our studies (Fig. 8.1).

8.3.2.2 Growth Promotion by *Bacillus* spp.

Bacillus spp. is another type of soil-dwelling rhizobacteria often used for its growth-promoting capabilities. *Bacillus* spp. mode of action orients itself towards providing biological control methods through suppression of plant pathogenic organisms, production of iron-chelating siderophores, and release of antibiotics (Timmusk et al. 1999; Pal et al. 2000; Chakraborty et al. 2006). The capacity of *Bacillus* spp. to sequester iron and other heavy metal compounds from soil prevents redox reactions from converting heavy metal compounds into radical forms, which are known toxicological elements geared towards suppressing plant metabolism (Miethke and Marahiel 2007; Tian et al. 2009). Furthermore, the siderophore activity prevents pathogenic organisms from uptaking iron; starving the pathogens thus creates an environment unsuitable for growth and infection (Saharan and Nehra 2011).

Bacillus spp. mode of interaction and growth compared to *Pseudomonas* spp. is much similar. Both PGPR are capable of effectively solubilizing insoluble phosphate, hence commonly described as phosphate-solubilizing microorganisms (PSM). Both genera have potential to increase yield and biomass content, and both mediate symbiosis with plant and validate expression on biocontrol mechanisms (Saharan and Nehra 2011). In a study oriented towards understanding inoculated characteristics of PGPR and *Rhizobium* in chickpeas, Verma et al. (2010) found significant increases in nodule formation, nodule dry weight and nutrient concentration, root and shoot biomass production, and grain and straw

yield. Tests were conducted in field for a period of 2 years using inoculated seeds and control (no bacteria), *Rhizobium* spp., *Rhizobium* spp. + *A. chroococcum*, *Rhizobium* spp. + *P. fluorescens*, and *Rhizobium* spp. + *B. megaterium*. The only considerable differences captured between coinoculated *Rhizobia* + *Pseudomonas* and *rhizobia* + *Bacillus* were the following: earlier combinations produced higher levels of indoleacetic acid (IAA), siderophore production, HCN utilization, and the inhibition of *Fusarium oxysporum* as compared to rhizobia +*Bacillus megaterium*. This difference in IAA levels account for *Pseudomonas* spp. ability to synthesize tryptophan, which, as described earlier, is one of the most recognized auxins involved in promoting cell and stem elongation (Verma et al. 2010). *Bacillus* spp. marked advantage as a bioinoculant lies towards its capacity to survive within extremes and irregular environments. *Bacillus* spp. has been recognized as moderate halophiles or halotolerant bacteria. In the rhizosphere, *Bacillus cereus* 80 is capable of adapting to various concentrations of salt, ranging in soil from 0 to 5 % NaCl (Welsh 2000). In cultivating chickpea, Indian farmlands are often characterized by soil salinity or alkalinity, requiring corrective remediation and inoculation with successive strains to counteract such adverse conditions (Bhadauria et al. 2010). Through seed inoculation of *B. subtilis*, growth factors in chickpea were maintained at various conditions and often showed enhanced biomass and nodule formation (Siddiqui and Mahmood 1995). Furthermore, *B. subtilis*, a well-recognized antagonistic bacterium against phytopathogens, can survive high soil and arid conditions ranging from 30 to 60 °C (Brock 1978; Edwards 1990). These physiological traits marked *Bacillus* spp. as one of the most recognized and versatile PGPR species. In association, as a single bioinoculant or selected part of a co-inoculant, *Bacillus* spp. and *Pseudomonas* spp. exhibit traits that induced proliferation geared towards suppressing soilborne pathogens and promoting plant growth parameters.

8.3.3 The Role of AM Fungi in Soil and as a Potential Bioinoculant

When considering fungi as a source of soil inoculums, often negative connotations propelled by the intensive degradation by fungal species (e.g., *Fusarium oxysporum*) are contributing factors to agricultural condemnation. However, recent advances towards biotechnology have identified fungal species capable of promoting successive growth and increasing soil fertility (Sharif and Moawad 2006). The major groups of fungi that establish mutualistic symbiosis are categorized for their ability to interact with the roots of various plant species, referred to as mycorrhizal symbionts (Ahmad et al. 2008a). Arbuscular mycorrhizal fungi (AMF) have been identified as existing entities in most agroecosystems, colonizing the root cortex biotrophically and establishing a mycelium bridge (hyphal network), connecting root to surrounding microhabitats (Egamberdiyeva et al. 2004). AM fungi are

considered as obligate microbial symbionts, dependent on colonization of host plants to maintain viability in the system. This mutually exclusive relationship benefits the host through correspondence with the mycorrhizal hyphal network, providing a larger surface area for absorption of essential immobile ions such as phosphate, copper, and zinc needed by the plant for sustaining growth (Paraskevopoulou Paroussi et al. 1997; Masoumeh et al. 2009). Mycorrhizal symbiosis also provides the plant with versatility against various biotic and abiotic stresses through formation of stable soil aggregates, selective proliferation of synergistic microbial colonies, and formation of macropore structures in soil to facilitate aeration and water penetration to deep surface layers (Piotrowski et al. 2004). These compositional structure modifications and branching complexes allow nutrients to be sequestered from various deep soil reserves, mandating a push towards plant fitness and tolerance, increasing the probability of survival when subsurface nutrient concentrations are limited or faced with harsh environmental conditions (Ahmad et al. 2008b).

Macrophomina phaseolina (tassi) is a common root rot fungus, infecting about 500 plant species, one of which being *Cicer arietinum* (Srivastva et al. 2001). *Rhizobia* provide an initial barrier to fungal pathogens; however, through the use of AM fungi species, potential for remediating pathogenesis while promoting growth is possible (Siddiqui and Akhtar 2009; Ozgonen and Erkilic 2007; Akkopru and Demir 2005). Akhtar and Siddiqui (2010) studied the influence of four AM fungi species, *Glomus intraradices*, *G. aggregatum*, *G. claroideum*, *and Glomus* sp., for biocontrol of *M. phaseolina* on *Cicer arietinum* pod growth, nodulation, chlorophyll, nitrogen, phosphorus, potassium concentrations, and effectiveness of controlling root rot. The experimental design consisted of five randomized blocks, each with different treatments: (1) *G. intraradices*, (2) *G. aggregatum*, (3) *G. claroideum*, (4) *Glomus* sp., and (5) control in the presence and absence of *M. phaseolina* (Akhtar and Siddiqui 2010). The plants were harvested 90 days after inoculation and grown in sandy loam soil mixed with washed river sand and farm yard manure at 3:2:1. The inoculation of all four AM fungi species without treatment of *M. phaseolina* exercised all growth parameters as compared to the uninoculated control. Increases in shoot dry weight, number of pods per plant, the number of nodules per root system, nitrogen, potassium, phosphorus, chlorophyll, and degree of root colonization by AM fungi were all exhibited after the 90-day harvest period, with *G. intraradices* optimizing greatest yields. Under the influence of *M. phaseolina*, interestingly enough shoot dry weight also increased, recording higher percentages, and then control and non-pathogen treatment. This gain corresponded to the increased shoot dry weight of pathogenic fungus manifested through AM fungi colonization, however; this also resulted in considerable decreases to the number of pods per plant as compared to non-*M. phaseolina* treatment (Akhtar and Siddiqui 2010). The number of nodules per root system stayed relatively the same, while root colonization of AM fungi was found to be considerably lower, suggesting formulation of spores and/or the activation of plant defense mechanisms, inhibiting growth and colonization (Demir and Akkopru 2005). Through the influence of AM fungi on *M. phaseolina*-treated plants, a

reduction in root rot index was seen, suggesting that the uninoculated control (index of 4) was less effective in secreting enzymes and biocontrol compounds necessary to maintain viability after infection (Pozo et al. 1999).

8.3.3.1 PGPR Interactions with AM Fungi as a Potential Bioinoculant

Diversity in the rhizosphere and surrounding microhabitats is marked by various interactive microfloras, stimulating mechanisms to promote or suppress microbial activity. AM fungi establish host specificity by infecting the host cortical cells, forming arbuscules along the plant root architecture. In this, the soil-dwelling *Rhizobium* and PGPR bacteria interact through endosymbiosis, forming an AM fungal endosymbiotic bacteria capable of promoting rhizobial interactions with mycorrhizae and plant (Bianciotto and Bonfante 2002). The typical rhizobacteria–AM fungi interaction describes PGPR as the "mycorrhizae-helper microorganism/bacteria," active in stimulating mycelial growth and/or enhancing mycorrhizal formation (Garbaye 1994). PGPR or soil-dwelling *Rhizobia* interact with the mycorrhizal fungi by adhering to fungal spores and hyphal structures, initiating exposure and spread to other microorganisms capable of symbiosis within the rhizosphere (Bianciotto and Bonfante 2002). As PGPR or *Rhizobia* interact with the host plant, the rate of exudate expulsion increases. When aided by the presence of AM fungi, the secretion of root exudates stimulates mycelial growth in the rhizosphere and initiates root penetration by the fungus (Azcon-Aguilar and Barea 1992).

Furthermore, as Azcon-Aguilar and Barea (1992, 1995) observed, the rhizobial interaction influences presymbiotic stages of AM fungal development such as spore germination and mycelia growth, when coupled by the release of plant hormones, instigate AM establishment within the rhizosphere and root cortex. Such morphological transformations induce physiological changes within the plant and surrounding environment to complement the interaction. Symbiosis alters the chemical composition of root exudates through changes in host's physiology, establishing shifts in mineral nutrient disposition of plant tissues, carbon allocation and utilization, and hormonal balances. However, physical development of AM mycelium in the rhizosphere/rhizoplane induces the synthesis and metabolism of essential plant and microbial parameters by acting as an abundant source of carbon (Barea et al. 2005). Secretion, uptake, and availability of root exudates, phytoalexins and, phenolic compounds become more abundant, prompting soil composition to become systemically modified to accommodate elevated interactions (Duponnois et al. 2005), thereby inducing physiological changes in the rhizobial community, marketing both quantitative and qualitative production of viable active symbionts, such as PGPR (Barea et al. 2005). This well-nourished and rich region of interaction and growth of mycorrhizae and mycelia is referred to as the mycorrhizosphere (Linderman 1988; Gryndler 2000). In the mycorrhizosphere, the principle of interaction is oriented towards promoting phosphorus uptake. Through the extensive branching between AM fungal mycelium and host root

structures, access to phosphate ions in soil can be elevated, extending beyond the phosphate depleting zone and into deeper regions in soil (Smith and Read 1997). Aside from providing the vessel for transport and available carbon, AM fungi contributed to phosphorous capture by linking the biotic and geochemical portions of the soil ecosystem, thereby affecting both phosphorous cycling rates and patterns (Jeffries and Barea 2001).

Supplementing artificial phosphate feeds in aims of enriching soil content and interactions has shown mediocre gains. It has been suggested that through ecological soil exploration, the naturally occurring uptake of phosphate from bulk soils produce greater levels of activation and response between indigenous microflora and host plant parameters (Gupta et al. 2007). Due to the fact that the availability of appropriate enzymes and secretion of stimulated growth factors promote rhizobial and soil competency, physiological and adaptive traits catered towards synchronizing symbiosis are induced (Barea et al. 2005). However, large doses of phosphorous fertilizer may potentially inhibit or hinder mycorrhizal growth and efficiency. As surface area is more prevalent, host and PGPR may absorb more phosphorous at higher rates; however, biological response to meet the surplus may be overwhelmed and hinder escalation to appropriate metabolite requirements without taxing the plant of other essential compounds (Gupta et al. 2007).

8.3.3.2 Promotion by AM Fungi–PGPR Symbiosis

The mode of interaction between AM fungi and PGPR is a universally recognized interaction, marketing each symbiont as an individual entity capable of inducing growth. PGPR interact with host plants and indigenous *Rhizobia* through endosymbiosis and release stimulatory control compounds, while AM fungi interact by forming infection sites (spores) on host plant roots, increasing susceptibility for *Rhizobia* and PGPR induction, all the while increasing surface area through hyphal extensions (Bianciotto and Bonfante 2002). On co-inoculation, AM fungi and PGPR initiate morphological, physiological, and biological changes in the rhizosphere and mycorrhizosphere in aims of attaining prolonged growth and fertility in various types of soil conditions. Such parameters are generated through interactions which promote nutrient acquisition, nitrogen fixation, phosphorus capture, exudates secretion, and release of antipathogenic compounds (Barea et al. 2005). It was observed that AM fungi, in association with nitrogen-fixing bacteria, *Azospirillum brasilense*, increase plant productivity by stimulating AM fungi root colonization, thereby increasing the number of internal vesicles relaying nutrient capture and flow (Linderman and Paulitz 1990). Furthermore, inoculation of *Rhizobium* sp. with phosphate-solubilizing microorganism (PSM) *Pseudomonas striata* and AM fungi species *Glomus fasciculatum* enhanced plant yield and nutrient and phosphorus uptake for chickpea plants in phosphorus-deficient sandy clay loam soils (Zaidi et al. 2001).

In fact, the postinoculation period between 45 and 90 days was marked by significant levels of growth through collective combinations of PSM on root

infection and spore density (Zaidi et al. 2001). This persistent symbiotic behavior between AM fungi, PGPR, and rhizobia suggested similar results can be obtained in environmentally stressed soils where viable growth is hindered due to source availability. AM fungi species *Glomus intraradices* as a co-inoculant with *P. fluorescens* exhibited varying deficit intensities. Individually, in water-deprived soil, *P. fluorescens* (Pf) had limited grain and biomass production, while co-inoculation with AM fungi increased assimilation of phosphorus and nitrogen concentrations, equivalent to that of chemical phosphorus treatment. However, when inoculated in water-deficient soil, dual inoculation with phosphorus fertilizer and AM + Pf inoculation significantly increased grain phosphorous and nitrogen concentrations as compared to uninoculated well-watered treatments (control). Root colonization was significantly higher in applications with dual inoculants, against control (uninoculated) and phosphorus fertilizer treatment in well-watered soils (Ehteshami et al. 2007). Such increased levels of colonization coincide with increased ACC-deaminase and chitinase activity (Shaharoona et al. 2006). Further, Ehteshami et al. (2007) suggest these gains market proliferation through the aid of plant hormones (phytohormones) and release of regulatory metabolites to counteract and maintain vitality during erratic intensities of water deficit (Ehteshami et al. 2007). Earlier, Subramanian et al. (2006) suggested that the increased absorptive surface area and densely proliferated root growth in the mycorrhizosphere complement increased root colonization and infection. These characteristics support the use of bioinoculants as potential remediation tools to combat water-deficit stresses. However, water uptake through a plant vascular system can be hindered if severe stresses disrupt root architecture and distribution, thereby affecting the rate of water absorption per unit root (Auge 2001). In such case, naturally occurring bioinoculants may not be as effective to counteract such stresses; however, a tool is out there to market biocontrol with higher degree of success and adaptability: the development of anti-pathogens and genetically engineering bioinoculative strains.

8.4 Engineering Bioinoculants as Remediation Tools in Agriculture

With an increase of biotic and abiotic stresses plaguing agricultural sustainability, scientists are aggressively pushing towards biological controls as a means of primary remediation. Through the study of soil ecology and interaction, scientists harvest the knowledge of symbiosis existing as a biological phenomenon involving dynamic changes in the genome, metabolism, and signaling network (Kawaguchi and Minamisawa 2010). Dynamic changes in genome are of particular interest, as genetic engineering provides the capacity to manipulate biological growth-promoting strains to correspond with indigenous microflora in order to maximize productivity in harsh soils (Upadhyay et al. 2000). By mimicking indigenous traits, engineered bioinoculants are capable of adapting to various stresses through

production of antimetabolites to inhibit nodule occupancy of native *rhizobia*, enhance regulation of plant–microbe signaling, adapt to environmental stresses, and enhance nutrition and Exudates sequestration and usage (Archana 2010). Others have linked engineering bioinoculants through beneficial relation of the plants to resist soilborne pathogens, become better hosts to symbiotic microbes, remediate toxic waste, and even attract communities of soil microbes to enhance plant growth (O'Connell et al. 1996). These methods have been provided to increase growth, fertility, and viability throughout harvesting seasons so farmers are capable of competing and succeeding against the demanding agriculture market without sacrificing quality and yield.

8.4.1 Engineering Bioinoculants as Anti-pathogens

Scientists are seeking innovative ways to engineer the rhizosphere in the aim to create a biased rhizosphere which essentially engineers the plants to secrete nutrients that specifically enhance the growth of mutualistic microbes (O'Connell et al. 1996). In such an attempt, to maximize efficiency, selecting to control root rot and pathogen invasion was of primary concern. Without adequate pathogen control, invasive species will try to persist as the engineered rhizosphere is now the epicenter of nutrient and chemical exchange. A lag phase between plant–microbe symbioses may hinder development and seed fertilization due to the initial competition in the rhizosphere, depriving both the plant and microbes of essential energy and compounds needed for sustained cellular and respiratory functions (Glick 1995). As mentioned earlier, to control chickpea root rot, *P. fluorescence* NBRI1303 was supplemented in soil to act as a pathogen antagonist towards *R. bataticola*, *F. oxysporum* f. sp. *ciceri*, and *Pythium* sp. (Nautiyal 1997a). Engineering, without genetic manipulation, a chickpea rhizosphere-competent strain involved greenhouse assays to evaluate the root-colonizing capacity of native chickpea rhizosphere. By selecting out and inoculating the spontaneous chromosomal Rifr strains to seeds, without checking for mutation, the isogenic form of the Rifr strain could be compared against survival and competition with that of the isogenic parent and one another to exhibit specific traits. These strains could then be added to a mixture of isolates and observed for stable growth and treatment against soilborne pathogens or pests (Nautiyal 1997a). The NBRI1303 was identified as the first reported single biocontrol bacterium active against the three most devastating pathogenic fungi of chickpea (Nautiyal 1997b). Rifampicin-resistant mutant *P. fluorescens* strain NBRI1303R confirmed NBRI1303 capacity to control pathogen infection by observing the rapid and aggressive root colonization. In particular, strain NBRI1303 reduced the number of diseased plants by 45 %, significantly promoted seed germination, and increased yield, length, and overall biomass of chickpea (Nautiyal 1997b).

Treatment using PGPR has also provided an alternative against combating viral diseases without the use of abrasive chemical pesticides and sprays through induced

systemic resistance (ISR), which characterizes increased synthesis of defense enzymes (M'piga et al. 1997; Zehnder et al. 2000). ISR was best described by Kirankumar et al. (2008) expressing resistance against the tomato mosaic virus, reporting a reduction of weight up to 59.0 % with a mean disease incidence recording at 55.98 % (Kirankumar et al. 2008; Cherian and Muniyapppa 1998). Several of the PGPR isolates were able to control early blight disease of tomato caused by *Alternaria solani* through induced system resistance (Earnapalli et al. 2005). ISR's mechanism behind establishing resistance lies through PGPR's ability to conform physiological and biochemical reactions of the host, resulting in the synthesis and secretion of defense chemicals against pathogenic organisms (Van Loon et al. 1998). As a result, phenol content, peroxidase and phenylalanine ammonia lyase, (PALase) enzymes witnessed a multiple fold of augmented activity. The major biological properties of phenolic compounds are reflected towards establishing antimicrobial activity, while peroxidase is a key enzyme in the biosynthesis of lignin and oxidation of hydroxyl-cinnamyl alcohols into free radical intermediates, which has been correlated with viral disease resistance (Saini et al. 1988; Bruce and West 1989). PALase is responsible for biosynthesis of various defense chemicals in phenylpropanoid metabolism and promotes plant functions that elicit strength and repair of the cell wall, antimicrobial activity, and signaling (Daayf et al. 1997). In addition, ISR-expressing plants have the capacity to convert, aminocyclopropane-1-carboxylate (ACC), an essential precursor molecule to ethylene biosynthesis, which acts as a suppressant against phytopathogens during initial stages of pathogen attack (Niranjan Raj et al. 2005).

Understanding the signaling pathways and supplementing advantageous microbes to the rhizosphere mediate selective remediation where biological recognition and response are tightly monitored. *P. fluorescens* supplemented in soil has shown remarketed beneficence in growth of various legumes, with secondary characteristics geared towards reflecting sustained pathogen control. *P. fluorescens* produce salicylic acid, which acts as local and systemic signaling molecule, inducing resistance in plants through activation and adherence to secondary plant hormones, jasmonic acid, and ethylene (De Meyer and Hofte 1997). Signaling compounds such as salicylic acid (SA) and ethylene (ET) play roles in regulating and inducing basal resistance. SA is a key regulator of pathogen-induced systemic acquired resistance (SAR), while ET is initiated through rhizobacteria-mediated induced systemic resistance (ISR) (Niranjan Raj et al. 2005). Root colonization of *A. thaliana* by *P. fluorescens* WCS417r has shown to elicit ISR against *P. syringae* pv. tomato (PST) (Knoester et al. 1999). Mutant ethylene-response *P. fluorescens* WCS417r strains revealed ISR function suppression, while SAR function remained unaffected (Knoester et al. 1999). SAR differs with regard to its capacity to be effective against pathogens that non-induced plants are resisted through SA-dependent defenses, while ISR are effective against pathogens in non-induced plants and dependent on ET-producing compounds (Ton et al. 2002). Knoester et al. (1999) found diminished ethylene production in roots/leaves and limited expression of the ethylene biosynthetic enzymes, ACC synthase and ACC oxidase, and suggested that the expression of ISR requires complete submission of the signal

transduction pathway. Thus, the potential to mediate signal transduction on *P. fluorescens* WCS417r strains with nonmutant ET-dependent pathways is possible and can be implemented to similar biochemically inducing ISR pathways in plants.

8.4.1.1 Engineering Resistance Through Rhizospheric Competency

Rhizospheric competency and ecology is a complex correlation between abiotic and biotic factors. Supplementing bioinoculants or PGPR in the rhizosphere and proposing effective microbe and plant symbiosis are a process that in vitro is highly effective. However, in field conditions, factors such as soil chemistry, mineral availability, and diversity of phytopathogen species may be delimiting factors to sustained colonization and effective pathogen control (Glick 1995). The capacity of PGPR to initiate defense mechanisms against phytopathogens requires engineering to characterize specific traits complementing a particular pathogen genome. This process, all be it highly effective, requires tedious interaction monitoring and genetic manipulation to suppress or activate specific molecular markers or sequences that complement, methylate, and destroy pathogen DNA/RNA (Prins et al. 2008). As a result of this complexity, biotechnology ventured into understanding soil characteristics and whether it is possible to use soil chemistry as a novel characteristic to engineer PGPR and utilize rhizosphere components for diverse suppression of various phytopathogens. Understanding PGPR and such soil characteristics, earlier Castignetti and Smarrelli (1986) suggested supplementing the rhizosphere with PGPR that are capable of producing and secreting siderophore molecules with a very high affinity for iron (Fe^{3+}).

The theory behind selecting high-affinity iron-binding siderophore molecules lies parallel to the fact that Fe^{3+} is only sparingly available in nature at a sustainable soil pH of 7.4 (Neilands et al. 1987). By engineering the PGPR to secrete siderophore that binds at higher affinities, most of the available Fe^{3+} in the rhizosphere is quickly taken up, leaving the surrounding area barren and essentially starving pathogens through the lack of iron uptake (O'Sullivan and O'Gara 1992). Biotechnology can engineer the bacterium to contain a receptor on the outer cell membrane that specifically compliments the iron–siderophore complex, transports it back to the microbial cell, and encourages utilization for microbial growth and proliferation (O'Sullivan and O'Gara 1992; Neilands and Leong 1986).

In an attempt to justify this mechanism of pathogen resistance, Vandenbergh and Gonzalez (1984) tested pathogen resistance against *F. oxysporum* in tomatoes by using a mutant strain of *P. putida* that overproduced siderophore molecules. The study revealed that overproduction of siderophore molecules in the mutant *P. fluorescens* strain was better suited to provide protection against *F. oxysporum* as compared to the wild-type *P. fluorescens* strain (Vandenbergh and Gonzalez 1984). Similarly, a mutant *P. aeruginosa* strain incapable of producing siderophore molecules was tested for its efficiency to control pathogen, *Pythium* sp., in tomato plants. Results confirmed *Pythium sp*. infection in tomato, as parameters marketing iron consumption were solely induced by *Pythium* sp., rendering the PGPR

siderophore complex inactive (Buysens et al. 1994). *Pseudomonas* sp. WCS417r strain was previously identified for the bacteria's capacity to induce systemic resistance through an ethylene-dependent signaling pathway. This strain has also shown marketability in inducing systemic resistance to Fusarium wilt on carnation caused by *F. oxysporum* f. sp. *dianthi* (Fod). Duijff et al. (1993) demonstrated this by using mutant WCS417r, defective in its capacity for siderophore biosynthesis (sid-), and compared this to *Pseudomonas putida* strain WCS358r. The team inhibited conidial germination by purified pseudobactins, which are siderophore molecules of *Pseudomonas* species, and found that the ferrated pseudobactins inhibited germination significantly less than the unferrated pseudobactins. Furthermore, sid-mutant WCS358 was ineffective in inhibiting Fod, whereas sid-WCS417r was still able to inhibit Fod. Treatment with WCS358r strain on carnation was able to reduce fusarium wilt, suggesting inhibition of Fod was induced solely on siderophore-mediated competition for iron. WCS417r strain significantly reduced wilt incidence, while mutant sid-WCS417r strain showed intermediate effectiveness in reducing wilt, suggesting WCS417 strain mechanism of pathogen control extends beyond siderophore inhibition, involving multiple mechanisms of control (Duijff et al. 1993). Such binding capacities essentially mediate effective biocontrol of disease through competitive bacterium–pathogen interactions where sustainability is dependent on soil parameters. This mechanism can be sustained by plants even at low Fe^{3+} concentrations as plants are independent of the physical uptake process and, however, dependent on PGPR siderophore uptake and release into plant cellular components (Crowley et al. 1988; Wang et al. 1993). Thus, engineering rhizobacteria to compliment soil characteristics and actively suppress pathogens through competitive antagonisms is one method of active pathogen inhibition through rhizosphere competency.

8.4.1.2 Engineering PGPR-Mediated Antibiotic Resistance

PGPR-mediated antibiotic resistance has provided scientists another avenue of integrated phytopathogenic suppression through direct involvement of antibiotic genes displaying antiviral, antimicrobial, antifeedant, phytotoxic, antioxidant, cytotoxic, and plant growth-promoting activities (Glick 1995; Fernando et al. 2005). Maurhofer et al. (1992) engineered a wild-type *Pseudomonas fluorescens* CHA0 strain to overproduce antibiotics pyoluteorin and 2, 4-diacetylphloroglucinol (DAPG). The strain was tested for its ability to protect cucumber plants against disease caused by *Pythium ultimum* and compare it to levels of wild-type *P. fluorescens* CHA0 inhibition. Together with similar findings of Schnider et al. (1994), Maurhofer and team elucidated strong correlation of increased synthesis of antibiotics by mutant *P. fluorescens* CHA0 strain results in better protection and suppression of *P. ultimum* in cucumber as compared to wild-type *P. fluorescens* CHA0 (Maurhofer et al. 1992; Schnider et al. 1994). DAPG and pyoluteorin are antibiotics classified as nonvolatile polyketides produced by *P. fluorescens* capable of a broad spectrum of actions against pathogenic fungi, bacteria, and nematodes

(Haas and Keel 2003). To actively suppress invasive species, *P. fluorescens* relay signaling molecules such as N-acyl-homoserine lactones (AHL) to mediate communication between different rhizobial dwelling bacteria as a means of antibiotic gene expression (Pierson et al. 1998). DAPG induces its own biosynthesis and acts as a diffusible signal for increasing the synthesis of DAPG by increasing the expression of DAPG biosynthetic genes (Maurhofer et al. 2004). The regulation of secondary metabolite production involves a two-component regulatory system, consisting of cellular homeostasis and transcription of antibiotic biosynthetic genes (Elander et al. 1968; Haas et al. 2000). A complex known as the GacS/GacA system acts to facilitate active response to changes in gene expression and sensory signals once AHL in most *Pseudomonas* sp. is recognized, exerting a positive impact on cell density-dependent gene regulation. Upon activation, GacS/GacA modulates expression of exoenzymes, antibiotics, and HCN during cellular transition from exponential to stationary phase of growth to mandate cell-to-cell communication and establish competency when antimetabolites are released in soil medium (Fuqua et al. 1994; Sacherer et al. 1994; Heeb and Haas 2001). Several other genetic regulators and signaling genes are involved, but for the purpose of explaining systemic antibiotic regulation, AHL and the GacS/GacA system are sufficient. These regulatory genes, coupled with the symbiotic soil bacterium, diversify PGPR's capacity to initiate, selectively suppress, and regulate the rhizosphere from incidence of attack (Fernando et al. 2005).

Antibiotics produced by various PGPR have a broad spectrum of activity. With *P. fluorescens* synthesizing DAPG, Cronin et al. (1997) used purified DAPG against nematode *Globodera rostochiensis* to exemplify suppressive abilities of the PGPR. Cronin et al. (1997) observed a decrease in the emergence of nematode cysts and reduced juvenile mobility. Similarly, *B. cereus* and *B. thuringiensis* exhibited pathogen resistance by producing antibiotic, Zwittermicin A (Fernando et al. 2005). Bacillus strains that produce Zwittermicin A are found at a minimum of 10^4 cfu/g of soil worldwide and contain a gene responsible for self-resistance against the action of its own antibiotic (Raffel et al. 1996). *Helicoverpa armigera* (pod borer) and a homopteran group of sucking insects, *Aphis craccivora*, represent two of the most potent pests to chickpea growth (Das 2005). Insecticidal Cry proteins derived from *Bacillus thuringiensis* (Bt) are transcribed into *Cicer arietinum* genomes (Cowgill and Lateef 1996). Cry proteins are classified as δ-endotoxins that bind to the midgut epithelial cells, inducing osmotic lysis in the invading pest, causing reduced activity and eventual death (Herrera-Estrella et al. 2005). High expression of Bt lines carrying the Cry2Aa gene has shown to confer near-complete protection, reporting 98 % mortality of *H. armigera* larvae (Sarmah 2006). Such a characteristic enables *B. thuringiensis* to persist as a novel insecticidal strain in suppressing oomycete disease of plants and other pathogenic fungi (Emmert et al. 2004; Silo-Suh et al. 1998). Thus it is recognized that with the utility of PGPR, isolating genes that encode the biosynthesis of antibiotics engineered or naturally found in expressing resistance can provide optimal growth and sustained resistance against a wide range of phytopathogens (Glick 1995; Gill and Warren 1988). Furthermore, by secreting antibiotics in the rhizosphere, the proliferation of

unwanted soil microorganisms indirectly becomes limited, reducing occupancy and competition for nutrients, thus prompting ideal parameters for sustainability (Glick 1995).

8.5 Conclusions and Future Perspectives

Biotechnology has revolutionized modern-day agriculture. The use of bioinoculants encourages selective integration of compatible rhizobia and genetic traits which correspond to the host and surrounding environment. PGPR and AM fungi market trait specificity within the rhizosphere through active chemical fixation, nutrient cycling, and induced methods of pathogen resistance. *Cicer arietinum* (chickpea) biomass, yield, nodulation, dry weight, and root and shoot lengths all increased, while incidence of root rot and infection from pathogenic organisms decreased. As co-inoculants, PGPR and AM fungi elicited greater response as compared to chemical alternatives such as insecticidal sprays and fertilizers. By reducing chemical alternatives, soil chemistry is managed through biological fixation and reduction. In doing so, beneficial rhizobacteria adapt and proliferate at higher levels, establishing a colony where continuous feedback is generated and competition is controlled. Hormones and iron-chelating compounds such as phytoalexins and siderophores released by the chickpea plant mediated control around the rhizosphere, establishing an interactive zone favorable to rhizobia expressing particular lines of symbiosis. In harsh or heavily deprived environments, the use of antibiotics and development of transgenic plants and PGPR enable pathogen-derived and pathogen-induced systemic resistance towards combating abiotic and biotic stresses. With acquired/selected genetic traits, plants and microbes are able to perform and enhance growth parameters without sacrificing quality. A continuous effort to establish rhizosphere competency using mutually inclusive rhizobia and enhanced resistance against a broad range of pathogens and viruses is being made; however, many tests and trials must be conducted before marketing for public applications. The push for such developments will take time and patience from both farmers and biotechnologists; however, the possibility to sustain growth in a once infertile piece of land is worth the wait.

References

Ahmad F, Ahmad I, Aqil F, Khan MS, Hayat S (2008a) Diversity and potential of non-symbiotic diazotrophic bacteria in promoting plant growth. In: Ahmad I, Pichtel J, Hayat S (eds) Plant bacteria interactions: strategies and techniques to promote plant growth. Wiley-VCH, Weinheim, pp 81–102

Ahmad I, Pichtel J, Hayat S (2008b) Plant bacteria interactions: strategies and techniques to promote plant growth. Wiley-VCH, Weinheim

Akhtar MS, Siddiqui ZA (2010) Effects of AM fungi on the plant growth and root-rot disease of chickpea. Am Eurasian J Agric Environ Sci 8(5):544–549

Akkopru A, Demir S (2005) Biological control of *Fusarium wilt* in tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* by AMF *Glomus intraradices* and some rhizobacteria. J Phytopathol 153:544–550

Antoun H, Prévost D (2005) Ecology of plant growth promoting rhizobacteria. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht, pp 1–38

Archana G (2010) Engineering nodulation competitiveness of rhizobial bioinoculants in soils. In: Khan MS et al (eds) Microbes for legume improvement. Springer, Wien, pp 157–190

Arshad M, Frankenberger WTJ (1990) Production and stability of ethylene in soil. Biol Fertil Soil 10:29–34

Ashraf M, Ozturk M, Athar HR (2009) Salinity and water stress, improving crop efficiency, vol 44, Tasks for vegetation science. Springer, Dordrecht

Auge RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42

Azcon-Aguilar C, Barea JM (1992) Interactions between mycorrhizal fungi and other rhizosphere micro-organisms. In: Allen MF (ed) Mycorrhizal functioning: an integrative plant–fungal process. Chapman & Hall, New York, pp 163–198

Azcon-Aguilar C, Barea JM (1995) Saprophytic growth of arbuscular–mycorrhizal fungi. In: Hock B, Varma B (eds) Mycorrhiza structure, function, molecular biology and biotechnology. Springer, Heidelberg, pp 391–407

Barea JM, Pozo MJ, Azcon R, Azcon-Aguilar C (2005) Microbial cooperation in the rhizosphere. J Exp Bot 56(417):1761–1778

Bhadauria S, Sengar RMS, Mohan D, Singh C, Kushwah BS (2010) Sustainable land use planning through utilization of alkaline wasteland by biotechnological intervention. Am Eurasian J Agric Environ Sci 9(3):325–337

Bianciotto V, Bonfante P (2002) Arbuscular mycorrhizal fungi: a specialized niche for rhizospheric and endocellular bacteria. Ant Van Leeuwenhoek 81:365–371

Borde M, Dudhane M, Jite PK (2009) Role of bioinoculant (AM fungi) increasing in growth, flavor content and yield in *Allium sativum* L. under field condition. Not Bot Hort Agrobot Cluj 37(2):124–128

Brock TD (1978) Thermophilic microorganisms and life at high temperatures. Springer, New York, pp 465

Bruce RJ, West CA (1989) Elicitation of lignan biosynthesis and isoperoxidase activity by pectic fragments in suspension cultures of castor bean. Plant Physiol 91:889–897

Buddenhagen IW, Workneh F (1988) Fusarium wilt of chickpea in California. Phytopathology 78:1563

Busse MD, Bottomley PJ (1989) Growth and nodulation responses of *Rhizobium meliloti* to water stress induced by permeating and nonpermeating solutes. Appl Environ Microbiol 55:2431–2436

Buysens S, Poppe J, Hofte M (1994) Role of siderophores in plant growth stimulation and antagonism by *Pseudomonas aeruginosa* 7NSK2. In: Ryder MH, Stephens PM, Bowen GD (eds) Improving plant productivity with rhizosphere bacteria. Commonwealth Scientific and Industrial Research Organization, Adelaide, pp 139–141

Castignetti D, Smarrelli JJR (1986) Siderophores, the iron nutrition of plants, and nitrate reductase. FEBS Lett 209:147–151

Chakraborty U, Chakraborty B, Basnet M (2006) Plant growth promotion and induction of resistance in *Camellia sinensis* by *Bacillus megaterium*. J Basic Microbiol 46(Suppl 3):186–195

Cherian S, Muniyapppa V (1998) ELISA based survey and host range of tomato mosaic tobamovirus. Ind J Virol 14:65–69

Cho S, Muehlbauer FJ (2004) Genetic effect of differentially regulated fungal response genes on resistance to necrotrophic fungal pathogens in chickpea (*Cicer arietinum* L.). Physiol Mol Plant Pathol 64:57–66

Cleland RE (1990) Auxin and cell elongation. In: Davies PJ (ed) Plant hormones and their role in plant growth and development. Kluwer Academic, Dordrecht, pp 132–151

Cordovilla MP, Ocana A, Ligero LC (1995) Salinity effects on growth analysis and nutrient composition in four grain legumes-rhizobium symbiosis. J Plant Nutr 18:1595–1609

Cowgill SE, Lateef SS (1996) Identification of antibiotic and antixenotic resistance to *Helicoverpa armigera* (Lepidoptera: Noctuidae) in chickpea. J Econ Entomol 89:224–229

Cronin D, Moenne-Loccoz Y, Fenton A, Dunne C, Dowling DN, O'Gara F (1997) Role of 2,4-diacetylphloroglucinol in the interactions of the biocontrol pseudomonad strain F113 with the potato cyst nematode *Globodera rostochiensis*. Appl Environ Microbiol 63:1357–1361

Crowley DE, Reid CPP, Szaniszlo PJ (1988) Utilization of microbial siderophores in iron acquisition by oat. Plant Physiol 87:680–685

Daayf F, Bel-Rhild R, Belanger RR (1997) Methyl ester of P-coumaric acid: a phytoalexin like compound from long English cucumber leaves. J Chem Eco 23:1517–1526

Das S (2005) Expression of insecticidal proteins in chickpea (*Cicer arietinum* L.) in order to develop resistance to important pests, *Aphis craccivora* and *Helicoverpa armigera*. ISCB final programme report–second phase (2004–2007)

De Meyer G, Hofte M (1997) Salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 induces resistance to leaf infection by *Botrytis cinerea* on bean. Phytopathology 87:58–593

de Selincourt K (1996) Intensifying agriculture the organic way. Ecologist 26:271–272

Demir S, Akkopru A (2005) Use of arbuscular mycorrhizal fungi for biocontrol of soilborne fungal plant pathogens. In: Chincholkar SB, Mukerji KG (eds) Biological control of plant disease. Howarth, New York, pp 17–37

Department of Rajasthan (2010) Rajasthan state environmental policy 2010. http://india.gov.in/allimpfrms/alldocs/15379.pdf

Duijff BJ, Meijer JW, Bakker PAHM, Schippers B (1993) Siderophore-mediated competition for iron and induced resistance in the suppression of Fusarium wilt of carnation by fluorescent *Pseudomonas* spp. Neth J Plant Pathol 99:277–289

Duponnois R, Colombet A, Hien V, Thioulouse J (2005) The mycorrhizal fungus *Glomus intraradices* and rock phosphate amendment influence plant growth and microbial activity in the rhizosphere of *Acacia holosericea*. Soil Biol Biochem 37:1460–1468

Earnapalli VN, Jagadeesh KS, Krishnaraj PU, Moon SS (2005) Mechanisms in the biocontrol of early blight of tomato by native isolates of tomato. In: Proceedings of the Asian conference on emerging trends in plant-microbe interactions, University of Madras, Chennai, 8–10 Dec 2005

Edwards C (1990) Microbiology of extreme environments. McGraw-Hill, New York, pp 55–92

Egamberdiyeva D, Qarshieva D, Davranov K (2004) The use of *Bradyrhizobium japonicum* to enhance growth and yield of soybean varieties in Uzbekistan conditions. Plant Growth Regul 23:54–57

Ehteshami SMR, Aghaalikhani M, Khavazi K, Chaichi MR (2007) Effect of phosphate solubilizing microorganisms on quantitative and qualitative characteristics of maize (*Zea mays* L.) under water deficit stress. Pak J Biol Sci 10:3585–3591

Elander RP, Mabe JA, Hamill RH, Gorman M (1968) Metabolism of tryptophans by *Pseudomonas aureofaciens*. VI. Production of pyrrolnitrin by selected *Pseudomonas* spp. Appl Environ Microbiol 16:753–758

Emmert BAE, Klimowicz KA, Thomas GM, Handelsman J (2004) Genetics of Zwittermicin A production by *Bacillus cereus*. Appl Environ Microbiol 70:104–113

FAO (1989) The arid environments. In: Arid zone forestry: a guide for field technicians. Ver.20: Chapter I. http://www.fao.org/docrep/T0122E/t0122e03.htm

FAO & IAEA (2010) Agricultural biotechnologies in developing countries: options and opportunities in crops, forestry, livestock, fisheries and agro-industry to face the challenges of food insecurity and climate change (ABDC-10). http://www.fao.org/docrep/meeting/019/al258e.pdf

Fernando WGD, Nakkeeran S, Zhang Y (2005) Biosynthesis of antibiotics by PGPR and its relation in biocontrol of plant diseases. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht, pp 67–109
Frankenberger WTJ, Arshad M (1995) Phytohormones in soil: microbial production and function. Marcel Dekker, NY, p 503
Fuqua WC, Winans SC, Greenberg EP (1994) Quorum sensing in bacteria: the LuxR–LuxI family of cell density-responsive transcriptional regulators. J Bacteriol 176:269–275
Garbaye J (1994) Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol 128:197–210
Gaudin V, Vrain T, Jouanin L (1994) Bacterial genes modifying hormonal balances in plants. Plant Physiol Biochem 32:11–29
Gill PR, Warren GJ (1988) An iron-antagonized fungistatic agent that is not required for iron assimilation from a fluorescent rhizosphere pseudomonad. J Bacteriol 170:163–170
Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117
Glick BR, Pasternak JJ (2003) Plant growth promoting bacteria. In: Glick BR, Pasternak JJ (eds) Molecular biology – principals and application of recombinant DNA, 3rd edn. ASM, Washington, pp 436–454
Government of Alberta (2002) Agriculture and rural development: liming acid soils. http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex3684
Gryndler M (2000) Interactions of arbuscular mycorrhizal fungi with other soil organisms. In: Kapulnik Y, Douds DD Jr (eds) Arbuscular mycorrhizas: physiology and function. Kluwer Academic, Dordrecht, pp 239–262
Gupta RP, Kalia A, Kapoor S (2007) Bioinoculants: a step towards sustainable agriculture. New India Publishing Agency, New Delhi
Gupta S, Chakraborti D, Sengupta A, Basu D, Das S (2010) Primary metabolism of chickpea is the initial target of wound inducing early sensed *Fusarium oxysporum* f. sp. *ciceri* race I. PLoS One 5(2):e9030
Haas D, Keel C (2003) Regulation of antibiotic production in root colonizing *Pseudomonas* spp., and relevance for biological control of plant disease. Annu Rev Phytopathol 79:117–153
Haas D, Blumer C, Keel C (2000) Biocontrol ability of fluorescent pseudomonads genetically dissected: importance of positive feedback regulation. Curr Opin Biotechnol 11:209–297
Hayat R, Safdar Ali S, Amaru U, Khalid R, Ahmed I (2010) Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol 60:579–598
Heeb S, Haas D (2001) Regulatory roles of GacS–GacA two component system in plant associated and other gram-negative bacteria. Mol Plant Microbe Interact 14:1351–1363
Herrera-Estrella L, Simpson J, Martinez-Trujillo M (2005) Transgenic plants: an historical perspective. In: Peña L (ed) Transgenic plants: methods and protocols. Humana, New Jersey, pp 3–30
Hong Y, Glick BR, Pasternak JJ (1991) Plant-microbial interaction under gnotobiotic conditions: a scanning electron microscope study. Curr Microbiol 23:111–114
Jackson MB (2004) The impact of flooding stress on plants and crops. http://www.plantstress.com/Articles/waterlogging_i/waterlog_i.htm
Jacobson CB, Pasternak JJ, Glick BR (1994) Partial purification and characterization of ACC deaminase from the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12–2. Can J Microbiol 40:1019–1025
Jakasaniya MS, Trivedi BS (2004) Transformation of added phosphorus into inorganic P-fractions in soils of Gujarat. Madras Agric 91:163–167
Jeffries P, Barea JM (2001) Arbuscular mycorrhiza: a key component of sustainable plant–soil ecosystems. In: Hock B (ed) The mycota: fungal associations, vol 9. Springer, Berlin, pp 95–113
Kakde TD, Wadaskar RM, Siddhabhatti PM, Nemade P, Tajne VS (2005) Marketing of biological based products: potential and reality. Pestology XXIX(4):32–37

Kassas M (2008) Aridity, drought and desertification. In: Arab environment future challenges. Technical Publications and Environment & Development Magazine, Lebanon, pp 96–110

Kawaguchi M, Minamisawa K (2010) Plant–microbe communications for symbiosis. Plant Cell Physiol 51:1377–1380

Khalid A, Arshad M, Zahir Z (2004) Screening plant growth promoting rhizobacteria for improving growth and yield of wheat. J Appl Microbiol 96:473–480

Khanna-Chopra R, Sinha SK (1998) Prospects of success of biotechnological approaches for improving tolerance to drought stress in crop plants. Curr Sci 74:25–34

Khare E, Singh S, Maheshwari DK, Arora NK (2011) Suppression of charcoal rot of chickpea by fluorescent pseudomonas under saline stress condition. Curr Microbiol 62:1548–1553

Kirankumar R, Jagadeesh KS, Krishnaraj PU, Patil MS (2008) Enhanced growth promotion of tomato and nutrient uptake by plant growth promoting rhizobacterial isolates in presence of tomato mosaic virus pathogen. Karnataka J Agric Sci 21:309–311

Knoester M, Pieterse CMJ, Bol JF, Van Loon LC (1999) Systemic resistance in *Arabidopsis* induced by rhizobacteria requires ethylene-dependent signaling at the site of application. Mol Plant Microbe Interact 12:720–727

Krebs J (2000) Ecology: the experimental analysis of distribution and abundance. Harper and Row, New York

Laanbroek HJ (1990) Bacterial cycling of minerals that affect plant growth in waterlogged soils: a review. Aqua Bot 38:109–125

Linderman RG (1988) Mycorrhizal interactions with the rhizosphere microflora. The mycorrhizosphere effects. Phytopathology 78:366–371

Linderman RG, Paulitz TC (1990) Mycorrhizal-rhizobacterial interactions. In: Hornby D, Cook RJ (eds) Biological control of soil-borne plant pathogens. CAB International, Wallingford, pp 261–283

Lynch JM (1990) The rhizosphere. Wiley-Interscience, Chichester

Maheshwari DK, Kumar S, Kumar B, Pandey P (2011) Co-inoculation of urea and DAP tolerant *Sinorhizobium meliloti* and *Pseudomonas aeruginosa* as integrated approach for growth enhancement of Brassica juncea. Indian J Microbiol 50(4):425–431

M'piga P, Belanger RR, Paulitz TC, Benhamou N (1997) Increased resistance to *Fusarium oxysporum* f. sp. *Radicis Lycopersici* in tomato plants treated with enophytic bacterium *Pseudomonas fluorescens*, 63-68. Physiol Mol Plant Pathol 50:301–320

Masoumeh F, Wichmann S, Vierheilig H, Kaul HP (2009) The effects of arbuscular mycorrhiza and nitrogen nutrition on growth of chickpea and barley. Pflanzenbauwissenschaften 13(1):15–22

Maurhofer M, Keel C, Schnider U, Voisard C, Haas D, Defago G (1992) Influence of enhanced antibiotic production in *Pseudomonas fluorescens* strain CHAO on its disease suppressive capacity. Phytopathology 82:190–195

Maurhofer M, Baehler E, Notz R, Martinez V, Keel C (2004) Cross talk between 2,4-diacetylphloroglucinol – producing biocontrol pseudomonads on wheat roots. Appl Environ Microbiol 70:1990–1998

Miethke M, Marahiel M (2007) Siderophore-based iron acquisition and pathogen control. Microbiol Mol Biol Rev 71(Suppl 3):413–451

Mirabella R, Hartog M, Franken C, Geurts R, Bisseling T (2005) Expression patterns of DMI genes in *Medicago* nodules. In: Wang Y-P, Lin M, Tian Z-X, Elmerich C, Newton WE (eds) Biological nitrogen fixation, sustainable agriculture and the environment. Springer, Dordrecht, pp 153–155

Narain P (1998) A dialectical perspective of agricultural research for sustainable development. Curr Sci 74:663–665

Nautiyal CS (1997a) A method for selection and characterization of rhizosphere competent bacteria of chickpea. Curr Microbiol 34:12–17

Nautiyal CS (1997b) Selection of chickpea-rhizosphere competent *Pseudomonas fluorescens* NBR11303, antagonistic to *Fusarium oxysporum* f. sp. *ciceri*, *Rhizoctonia bataticola and Pythium* sp. Curr Microbiol 35:52–58

Nautiyal CS (2000) Biocontrol of plant disease for agricultural sustainability. In: Upadhyay RK, Mukerji KG, Chamola BP (eds) Biocontrol potential and its exploitation in sustainable agriculture, vol 1. Kluwer Academic/Plenum, New York, pp 9–23

Nehl DB, Allen SJ, Brown JF (1996) Deleterious rhizosphere bacteria: an integrating perspective. Appl Soil Ecol 5:1–20

Neilands JB, Leong SA (1986) Siderophores in relation to plant growth and disease. Annu Rev Plant Physiol 37:187–208

Neilands JB, Konopka K, Schwyn B, Coy M, Francis RT, Paw BH, Bagg A (1987) Comparative biochemistry of microbial iron assimilation. In: Winkelmann G, van der Helm D, Neilands JB (eds) Iron transport in microbes, plants and animals. VCH, Weinheim, pp 3–33

Niranjan Raj S, Shetty HS, Reddy MS (2005) Plant growth promoting rhizobacteria: potential green alternative for plant productivity. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht, pp 197–216

O'Connell KP, Goodman RM, Handelsman J (1996) Engineering the rhizosphere expressing a bias. Trends Biotechnol 14:83–88

O'Sullivan D, O'Gara F (1992) Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. Microbiol Rev 56:662–676

Okon Y, Vanderleyden J (1997) Root associated *Azospirillum* sp. can stimulate plants. Am Soc Microbiol News 63:366–370

Ozgonen H, Erkilic A (2007) Growth enhancement and phytophthora blight (*Phytophthora capsici* Leonian) control by arbuscular mycorrhizal fungal inoculation in pepper. Crop Protect 26:1682–1688

Pal KK, Tilak KVBR, Saxena AK, Dey R, Singh CS (2000) Antifungal characteristics of a *fluorescent Pseudomonas* strain involved in the biological control of *Rhizoctonia solani*. Res Microbiol 155(Suppl 3):233–242

Paraskevopoulou Paroussi G, Karagiannidis N, Paroussie E, Sponomitsios G (1997) The effect of mycorrhiza on nutrient uptake and plant development of three strawberry cultivars. In: Proceedings of the third international strawberry symposium, Acta Hort 439, vol 2.

Parmar N, Dadarwal KR (1997) Rhizobacteria from rhizosphere and rhizoplane of chickpea (*Cicer arietinum* L.). Ind J Microbiol 37:205–210

Parmar N, Dadarwal KR (1999) Stimulation of nitrogen fixation and induction of flavonoid like compounds by rhizobacteria. J Appl Microbiol 86:3–44

Parmar N, Dufresne J (2011) Beneficial interactions of plant growth promoting rhizosphere microorganisms. In: Singh A et al (eds) Bioaugmentation, biostimulation and biocontrol. Springer, Berlin, pp 27–43

Pierson LS, Wood DW, Pierson EA, Chancey ST (1998) *N*-acyl homoserine lactone-mediated gene regulation in biological control by fluorescent pseudomonads: current knowledge and future work. Eur J Plant Pathol 104:1–9

Piotrowski JS, Denich T, Klironomos JN, Graham JM, Rillig MC (2004) The effects of arbuscular mycorrhizas on soil aggregation depend on the interaction between plant and fungal species. New Phytol 164:365–373

Powell M (1994) Selecting and testing nitrogen fixing trees for acid soils. Agroforestry for the Pacific technologies. Forest, farm and community tree network. No. 10. http://www.winrock.org/fnrm/factnet/FACTPUB/AIS_web/AIS10.html

Pozo MJ, Azcon-Aguilar C, Dumas-Gaudot E, Barea JM (1999) Beta-1, 3 glucanase activities in tomato roots inoculated with arbuscular mycorrhizal fungi and/or *Phytophthora parasitica* and their possible involvement in bioprotection. Plant Sci 141:149–157

Prins M, Laimer M, Noris E, Schubert J, Wassenegger M, Tepfer M (2008) Strategies for antiviral resistance in transgenic plants. Mol Plant Pathol 9(1):73–83

Qadir M, Tubeileh A, Akhtar J, Larbi A, Minhas PS, Khan MA (2008) Productivity enhancement of salt-affected environments through crop diversification. Land Degr Dev 19:429–453

Radwan S (2009) Phytoremediation for oily desert soils. In: Singh A, Kuhad RC, Ward OP (eds) Soil biology: advances in applied bioremediation, vol 17. Springer, Heidelberg, pp 280–293

Raffel SJ, Stabb EV, Milner JL, Handelsman J (1996) Genotypic and phenotypic analysis of Zwittermicin A-producing strains of *Bacillus cereus*. Microbiology 142:3425–3436

Ramarethinam S, Murugesan NV, Rajalakshmi N (2005) Effect of liquid formulation of biofertilizer on the root association of AM fungi and chili roots. Pest XXIX(4):12–16

Rao AV (2002) Conservation of soil productivity through adoption of soil bio-technological approaches in Indian arid zone. 12th ISCO conference.

Reddy MV, Srinivasulu B, Pramila Devi T (2000) Biocontrol of pulse diseases. In: Upadhyay RK, Mukerji KG, Chamola BP (eds) Biocontrol potential and its exploitation in sustainable agriculture, vol 1. Kluwer Academic/Plenum, New York, pp 239–242

Rokhzadi A, Asgazadeh A, Darvish F, Nour-Mohammed G, Majidi E (2008) Influence of plant growth promoting rhizobacteria on dry matter accumulation and yield of chickpea (*Cicer arietinum* L.) under filed conditions. Am Eurasian J Agric Environ Sci 3:253–257

Sacherer P, Défago G, Haas D (1994) Extracellular protease and phospholipase C are controlled by the global regulatory gene *gacA* in the biocontrol strain *Pseudomonas fluorescens* CHA0. FEMS Microbiol Lett 116:155–160

Saharan BS, Nehra V (2011) Plant growth promoting rhizobacteria: a critical review. Life Sci Med Res Volume 2011: LSMR-21

Saini RS, Arora YK, Chawla HKL, Wagle DS (1988) Total phenols and sugar content in wheat cultivars resistant and susceptible to *Ustilago nuda* (Jens) rostrup. Biochem Physiol Pglanzon 183:89–93

Sarmah BK (2006) Development of transgenic chickpeas resistant to insect pests. international workshop on legume genomics. In: ISCB final programme report – second phase (2004–2007)

Schmelz EA, Engelberth J, Alborn HT, O'Donnell P, Sammons M, Toshima H, Tumlinson JH III (2003) Simultaneous analysis of phytohormones, phytotoxins and volatile organic compounds in plants. Proc Natl Acad Sci USA 100(18):10552–10557

Schnider U, Blumer C, Troxler J, Defago G, Haas D (1994) Over production of the antibiotics 2,4-diacetylphloroglucinol and pyoluteorin in *Pseudomonas fluorescens* strain CHAO. In: Ryder MH, Stephens PM, Bowen GD (eds) Improving plant productivity with rhizosphere bacteria. Commonwealth Scientific and Industrial Research Organization, Adelaide, pp 120–121

Sethi RM, Nair GKM, Rao S, Singh RR (1994) Status of land degradation in India. J Soil Water Cons 38(3–4):163–172

Setter T, Belford R (1990) Waterlogging: how it reduces plant growth and how plants can overcome its effects. J Agric West Aust 31:51–55

Shaharoona B, Arshad M, Zahir ZA, Khalid A (2006) Performance of *Pseudomonas* spp. containing ACC-deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of nitrogenous fertilizer. Soil Biol Biochem 38:2971–2975

Sharif M, Moawad AM (2006) Arbuscular mycorrhizal incidence and infectivity of crops in northwest frontier province of Pakistan. World J Agric Sci 2:123–132

Sharma JP (2005) Comprehensive environmental studies: natural resources. Laxmi, New Delhi

Siddiqui ZA, Akhtar MS (2009) Effect of antagonistic fungi, plant growth promoting rhizobacteria, an arbuscular mycorrhizal fungi alone and in-combination on reproduction of *Meloidogyne incognita* and growth of tomato. J Gen Plant Pathol 75:144–153

Siddiqui ZA, Mahmood I (1995) Biological control of *Heterodera cajani* and *Fusarium udum* by *Bacillus subtilis*, *Bradyrhizobium japonicum* and *Glomus fasciculatum* on pigeonpea. Fund Appl Nematol 18:559–566

Silo-Suh LA, Stab VE, Raffel SR, Handelsman J (1998) Target range of Zwittermicin A, an aminopolyol antibiotic from *Bacillus cereus*. Curr Microbiol 37:6–11

Sindhu SS, Suneja S, Goel AK, Parmar N, Dadarwal KR (2002) Plant growth promoting effects of *Pseudomonas* sp. on coinoculation with *Mesorhizobium* sp. Cicer strain under sterile and "wilt sick" conditions. Appl Soil Ecol 19:57–64

Singh A, Kuhad RC, Ward OP (2009) Advances in applied bioremediation. Springer, Heidelberg

Singh A, Parmar N, Kuhad RC (2011) Bioaugmentation, biostimulation and biocontrol. Springer, Berlin

Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic, San Diego
Srivastva AK, Singh T, Jana TK, Arora DK (2001) Induced resistance and control of charcoal rot in *Cicer arietinum* (chickpea) by *Pseudomonas fluorescens*. Can J Bot 79:787–795
Subramanian KS, Santhanakrihnan P, Balasubramanian P (2006) Responses of field grown tomato plants to arbuscular mycorrhizal fungal colonization under varying intensities of drought stress. Sci Hortic 107:245–253
Tian F, Ding Y, Zhu H, Yao L, Du B (2009) Genetic diversity of siderophore-producing bacteria of tobacco rhizosphere. Braz J Microbiol 40(Suppl 2):276–284
Tilak KVBR, Pal KK, Dey R (2010) Microbes for sustainable agriculture. I.K. International, New Delhi
Timmusk S, Nicander B, Granhall U, Tillberg E (1999) Cytokinin production by *Paenibacillus polymyxa*. Soil Biol Biochem 31(Suppl 13):1847–1852
Ton J, Van Pelt JA, Van Loon LC, Pieterse CMJ (2002) Differential effectiveness of salicylate-dependent and jasmonate/ethylene-dependent induced resistance in arabidopsis. Mol Plant Microbe Interact 15:27–34
Torimitsu K, Hayashi M, Ohta E, Sakata M (1985) Effect of K^+ and H^+ stress and role of Ca^{2+} in the regulation of intracellular K^+ concentration in mung-bean roots. Physiol Plant 63:247–252
Upadhyay RK, Mukerji KG, Chamola BP (2000) Biocontrol potential and its exploitation in sustainable agriculture. Kluwer Academic/Plenum, New York
Van Loon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Ann Rev Phytopathol 36:453–483
Vandenbergh PA, Gonzalez CF (1984) Method for protecting the growth of plants employing mutant siderophore producing strains of *Pseudomonas putida*. US Patent No. 4 479 936
Verma JP, Yadav J, Tiwari KV (2010) Application of *Rhizobium* sp. BHURC01 and plant growth promoting rhizobacteria on nodulation, plant biomass and yields of chickpea (*Cicer arietinum* L.). Int J Agric Res 5:148–156
Vevrek MC, Campbell WJ (2002) Identification of plant traits that enhance biodegradation of oil. 9th Annual international petroleum conference
Vidhyasekaran P, Muthamilan M (1995) Development and formulations of *Pseudomonas fluorescens* for control of chickpea wilt. Plant Dis 79:782–786
Wang Y, Brown HN, Crowley DE, Szaniszlo PJ (1993) Evidence for direct utilization of a siderophore, ferrioxamine B, in axenically grown cucumber. Plant Cell Environ 16:579–585
Wang Y, Lin M, Tian ZX, Elmerich C, Newton W (2005) Biological nitrogen fixation, sustainable agriculture and the environment. Springer, Dordrecht
Wang L, Katsutoshi S, Miyazaki T, Ishihama Y (2009) The cause of soil alkalization in the songnen plain of northeast China. Paddy Water Environ 7(3):259–270
Welsh DT (2000) Ecological significance of compatible solute accumulation by microorganisms: from single cells to global climate. FEMS Microbiol Rev 24:263–290
Wiederholt R, Johnson B (2005) Phosphorus behavior in the environment. North Dakota State University. http://www.ag.ndsu.edu/pubs/h2oqual/watnut/nm1298w.htm
Zahran HH (1999) *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol Mol Biol 63(4):968–989
Zaidi A, MdS K, Md A (2001) Interactive effect of rhizotrophic microorganisms on yield and nutrient uptake of chickpea (*Cicer arietinum* L.). Eur J Agron 19(1):15–21
Zehnder GW, Yao C, Murphy JF, Slkora ER, Kloepper W (2000) Induction of resistance in tomato against *Cucumber Mosaic Cucumo virus* by plant growth promoting rhizobacteria. BioCont 45:127–137
Zhang H, Wu X, Li G, Qin P (2011) Interactions between arbuscular mycorrhizal fungi and phosphate-solubilizing fungus (*Mortierella* sp.) and their effects on *Kosteletzkya virginica* growth and enzyme activities of rhizosphere and bulk soils at different salinities. Biol Fertil Soils 47:543–554

Chapter 9
Plant Growth-Promoting Rhizobacteria as Zinc Mobilizers: A Promising Approach for Cereals Biofortification

Fauzia Yusuf Hafeez, Muhammad Abaid-Ullah, and Muhammad Nadeem Hassan

9.1 Introduction

Zinc (Zn) is an essential element necessary for plants, humans, and microorganisms (Broadley et al. 2007; Prasad 2008b; Cakmak 2008). Humans and other living things require Zn throughout life in little quantities to orchestrate a complete array of physiological functions (Canadian UNICEF Committee 2006). Zinc is a vital mineral of "exceptional biological and public health importance" (Hambidge and Krebs 2007). Furthermore 100 specific enzymes are found in which zinc serves as structural ions in transcription factors and is stored and transferred in metallothionein (Silvera and Ronan 2001; United States National Research Council 2000). It is typically "the second most abundant transition metal in organisms" after iron, and it is the only metal which appears in all enzyme classes (King 2006; Broadley et al. 2007).

Biofortification is a current approach aimed at increasing the bioavailability of micronutrients such as Zn and Fe in the staple crops of specific region (Stein 2010). In this regard beneficial free-living soil bacteria which have been shown to improve plant health or increase yield can also mobilize micronutrients. In this chapter, the collective results highlight the importance of Zn with comparison of various strategies to meet its required quantity in major food crops. The next technological revolution to eradicate Zn malnutrition would be the plant growth-promoting microorganisms enabling better availability of Zn and other micronutrients through their economical, beneficial, and eco-friendly nature.

F.Y. Hafeez (✉) • M. Abaid-Ullah • M.N. Hassan
Department of Biosciences, COMSATS Institute of Information Technology, Park road, 44000 Islamabad, Pakistan
e-mail: fauzia_y@yahoo.com

D.K. Maheshwari et al. (eds.), *Bacteria in Agrobiology: Crop Productivity*,
DOI 10.1007/978-3-642-37241-4_9,

9.1.1 Role of Zinc in Plants

Zinc is an important micronutrient for plants which plays numerous functions in life cycle of plants (Hirschi 2008). Crop growth, vigor, maturity, and yield are very much reliant upon essential micronutrient such as Zn. It is involved in many physiological functions in plants. It is responsible for synthesis of auxin and catalyzes the photochemical reaction of chlorophyll. Zn is also required for the stability of biological membranes and is important for the activity of various enzymes, e.g., Cu/Zn superoxide dismutase (SOD) and carbonic anhydrase which contain structurally bound Zn and plant growth regulator, i.e., indoleacetic acid (IAA). It influences the synthesis of nucleic acid, lipids, and proteins by which the grain quality becomes superior (Seilsepour 2006; Hershfinkel 2006; Kramer and Clemens 2006). Physiologically it activates metabolism of carbohydrates, auxin, RNA, and ribosome's functions. Zn has also been reported for increased growth, yield, and yield components as well as improved leaves and flowers nutrient content and plant chemical constituents, i.e., pigments, carbohydrates, and flowers oil concentration (Khalifa et al. 2011). It has been proved that Zn application to wheat increases its concentration in flag leaves and grains (Ranjbar and Bahmaniar 2007; Cakmak 2008; Waters et al. 2009). Higher absorption of Zn produced higher grain yield (Han et al. 2006).

9.1.2 Role of Zinc in Humans

Humans cannot attain normal vigorous growth without essential elements like Zn (Calder and Jackson 2000). In developing countries, supplementation with Zn was found to lower frequency and severity of infections like diarrhea and pneumonia and decrease mortality (Black et al. 2008). Biologically Zn plays catalytic, co-catalytic, or structural roles in more than 300 enzymes. The six enzyme classes, namely, oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases, depend on Zn for their activity. Although 86 % is in skeletal muscle, there are certain parts, prostate, hippocampus, pancreas, and kidney cortex, where zinc concentration is particularly high and may represent functional significance (Vallee and Falchuk 1981).

Furthermore, in the synthesis of proteins and metabolism of DNA, RNA, and metabolic homeostasis in the human body, zinc is critically involved. Strong evidence indicates the presence of a number of zinc-containing proteins, which directly influence gene expression (Welch 2001). Exposure to high doses has toxic effects, but intoxication by excessive exposure is rare.

9.1.3 Zinc Deficiency and Malnutrition

Zn deficiency is among the top micronutrient deficiencies reported in human beings which influences almost one third of the world's populations (Hotz and Brown 2004; Zhang et al. 2011; Stein 2010). Zn is an essential metal element for human health. Its deficiency caused by malnutrition is the 11th major risk factor of disease trouble in the global distribution linked with 1.8 million deaths yearly (WHO 2002). About 100 million people mainly living in rural areas undergo Zn deficiency in China (Zhang et al. 2011; Ma et al. 2008). Studies of inhibition of spermatogenesis and different abnormalities of sperm production in human have shown Zn deficiency (Prasad 2008a). It is estimated that globally two billion people are at threat of zinc deficiency (Gibson and Ferguson 1998). Additionally 37 % of children less than five years of age are at risk of zinc deficiency in Pakistan (Harvest Plus 2012).

In vitro trials have illustrated that zinc supplementation can decrease the brutality of morbidity from a numeral common babyhood infections (Harvest Plus 2012). WHO also recommended Zn supplementation during diarrheal infection and for treatment of severe malnutrition (WHO 2004). A study in China has proved that zinc-fortified flour could improve its deficiency in women of childbearing age (Brown et al. 2009). Zn absorption is influenced by various factors, i.e., binding to a ligand secreted by the pancreas increases absorption, luminal amino acids bind Zn and prevent its precipitation by substances such as phosphate and phytate, whereas pregnancy, corticosteroids, and endotoxin all enhance absorption while phytate, phosphate, iron, copper, lead, and calcium hinder absorption of Zn (Davies 1980). Zn deficiency is widespread and has a detrimental impact on growth, neuronal development, and immunity (Plum et al. 2010). The reason behind Zn deficiency is insufficient nutritional ingestion of Zn and Fe in majority of the cases (Welch and Graham 2004; Cakmak et al. 2010). Conversely the concentration of a number of minerals especially zinc, iron, iodine, and selenium is inherently poor in plants as compared to animal-derived foods. As a consequence more than three billion people globally suffer from micronutrient starvation (White and Broadley 2009: Cakmak 2008). As reported by the Alloway (2004), most of the wheat crop was harvested on the zinc deficient which resulted in lower zinc content of wheat grain. The development of high-yielding genotypes has aggravated this dilemma (Zhao and McGrath 2009; Cakmak et al. 2010; Stein 2010). Moreover, the processing of wheat significantly decreases the concentration of Zn as well as other minerals, which promote Zn deficiency (Zhang et al. 2010; Kutman et al. 2011). To overcome this problem, improvement of Zn bioavailability in cultivated soils may enhance Zn contents in the staple food grains which would possibly diminish the major health risks attributable to this micronutrient deficiency. Plant scientists are formulating different methodologies to tackle the zinc deficiencies in human populations through fertilizer applications and/or by means of plant breeding strategies to augment the absorption and/or bioavailability of zinc in grain crops.

9.2 Zinc Status of Soil

Most of the soils are either Zn deficient or contain Zn in fixed form, i.e., unavailable to the plant. According to FAO reports, 50 % of the soils are deficient in Zn (FAO 2002). Deficiency of Zn is frequent in calcareous and neutral soils, paddy soils, intensively harvested soils and inadequately drained soils, saline and sodic soils, peat soils, soils with elevated level of phosphorus and silicon, sandy soils, extremely weathered acid, and coarse-textured soils (Sillanpaa 1982; Alloway 2008). Zn deficiency may also be related with the nature of soil such as in calcareous soils; Zn^{2+} may exist as low as 10^{-11}–10^{-9} M and can reduce crop growth (Hacisalihoglu and Kochian 2003). Approximately half of the agricultural soils in China has been affected by zinc deficiency, while in India zinc-deficient soils has engaged almost 50 % of the agricultural part, and the same is the situation in Turkey (FAO 2002). In Pakistan, 70 % of agricultural land has been reported as Zn deficient (Hamid and Ahmad 2001; Kauser et al. 2001).

Occurrence of Zn in soil is found as ZnS (sphalerite); further less frequent Zn-containing mineral ores include smithsonite ($ZnCO_3$), zincite (ZnO), zinkosite ($ZnSO_4$), franklinite ($ZnFe_2O_4$), and hopeite [$Zn_3(PO_4)_2{\cdot}4H_2O$]; however, availability of Zn from these sources depends on various factors. The natural sources of zinc to soil include (a) chemical and physical weathering of parent rocks (Alloway 1995) and (b) atmospheric contribution of zinc to soils (e.g., volcanoes, forest fires, and surface dusts) (Friedland 1990; International Zinc Association 2011).

Micronutrient uptake from the rhizosphere is the primary step for its accumulation into the plant before translocation to seeds (Giehl et al. 2009). Plant roots uptake Zn as Zn^{2+} cation which is constituent of synthetic and organic compounds (Havlin et al. 2005; Oliveira and Nascimento 2006). Plants adsorb available zinc from the soil solution in a reactive form. Accessible amount of zinc to plants is controlled by the soil factors, e.g., the total zinc concentration, pH, organic matter, clay and calcium carbonate, redox conditions, microbial activity in the rhizosphere, soil moisture, concentrations of other trace elements, and concentrations of macronutrients, especially phosphorus and climate (Alloway 2008). Zn supply is mainly affected by the soil pH in soil pools; in view of the fact that this element is readily adsorbed in exchange cation sites at over-neutral pH and made accessible at low pH values (Broadley et al. 2007; Havlin et al. 2005). Cereals have very little Zn concentration in grains as compared to animal-based foods or pulses. Currently, Indian soils are Zn deficient particularly in wheat cropping system and it will further decrease grain Zn concentration in cereals (Prasad 2005; Gupta 2005). Generally cereal grain contains low concentration of Zn due to the existence of anti-nutrition factor such as phytic acid (PA) which reduces the mineral bioavailability (Pahlvan-Rad and Pressaraki 2009). The lesser bioavailability of soil zinc directly affects grain zinc concentration and human health.

9.3 Strategies to Overcome the Zinc Deficiency

To address the problem of Zn deficiency, micronutrients biofortification of grain crops is getting increased interest in developing countries (Cakmak 2008; Bouis and Welch 2010; Zhao and McGrath 2009). Several approaches have been projected and practiced for fortification of cereals (Bouis 2003; Pfeiffer and McClafferty 2007). Enhancing Zn concentration of cereal grains has been recognized as an approach of tackling humane Zn deficiency (Pahlvan-Rad and Pressaraki 2009). Plant scientists are formulating different methodologies to tackle the Zn deficiencies in crops through fertilizer applications and/or by means of plant breeding strategies to augment the absorption and/or bioavailability of Zn in grain crops (Cakmak 2008; White and Broadley 2009). Various dietary factors, e.g., organic acids (citrate), amino acids (histidine and methionine), and chelators such as EDTA, seem to support the bioavailability of zinc whereas fibers and some minerals such as copper, iron, and calcium may decrease it in some situations (Lonnerdal 2000). Recent studies have also demonstrated that enhanced Zn bioavailability reduces the phosphorus and the phytic acid concentration in grain (Cakmak et al. 2010). Absorption of Zn is improved by citric acid, malic acid, lactic acid, and ascorbic acid. EDTA can assist to solubilize Zn from more insoluble phytate-Zn compound, forming Zn-EDTA. Crop fortification is the best approach, and it is aimed to increase the average Zn contents of wheat from 25 ppm in Pakistan (HarvestPlus 2012). It involves two strategies: genetic biofortification and agronomic biofortification.

9.3.1 Genetic Biofortification

Genetic biofortification comprises the developing varieties with increase Zn content of grain through conventional breeding and genetic engineering.

9.3.1.1 Breeding Practices

Altering the genetics of plants with the intention to produce desired characteristics is known as plant breeding. Breeding and biotechnology are the most important tactics of the plant biofortification. Genetic strategies are powerful approaches for altering the nutrient balance in the food crop. In the earlier period, agronomists and policy makers focused on yields only without considering the nutritional worth of the crops, thus generating the mineral malnutrition in humans (Khoshgoftarmanesh et al. 2009). It has been reported that increase in yield of crops is found to associate for reduction in micronutrients. The zinc concentration is low in the edible tissues of higher yielding varieties as compared to the low-yielding varieties (White and Broadley 2009; Zhao and McGrath 2009; Monasterio and Graham 2000).

Therefore, it is fundamental to believe whether any enhancement in tissue zinc concentration is just the result of slower growth or low yields (White and Broadley 2011).

Despite the fact that the improvements achieved in development of novel genotypes consisting of high zinc are admirable (Pfeiffer and McClafferty 2007; Bouis and Welch 2010; Cakmak et al. 2010), there exist some issues with this strategy. Similar to Zn, toxic metals like Cd can be translocated in the same pathway (Intawongse and Dean 2006) and has a bioavailability that is much greater than that of other heavy metals (Reeves and Chaney 2008). Moreover many ZIP family proteins (metal transporter in plants) can transport Cd (Yang et al. 2009; Assunc et al. 2010), which makes it difficult to ignore the risk in breeding programs. The important issue associated with these breeding strategies is the instability of newly incorporated Zn trait in different genotypes and relatively limited genotypic variation for grain Zn concentration among wheat cultivars of cereals (Welch and Graham 2004). It has been proved that Zn translocation in the wheat grain was highly influenced by the genotype, climate, and their interactions (Gomez-Becerra et al. 2010; Zhang et al. 2010). Secondly, it is a time-consuming approach and takes several years to develop a biofortified variety (Cakmak 2008), and further breeding program is also constrained by high cost and complexity of laboratory analysis (Monasterio et al. 2007).

9.3.1.2 Transgenic Approach

Transgenic approach is also contributing in developing the biofortified crops. Numerous transgenic food crops have been produced with the better zinc concentrations in the edible parts than conventional cultivars. Studies have shown that constitutive expression of transcription factors *bZIP19* and *bZIP23* could be used to enhance the zinc accumulation in the edible parts of food crop plants (Assunc et al. 2010). Different transport proteins of plasma membrane are the targets for the manipulations of zinc concentrations in the different portions of plants. These transport proteins make possible the uptake and sequestration of zinc in the vacuole together with enzymes concerned in the synthesis of substances that bind Zn^{2+} in the rhizosphere. Overexpressing genes encoding a transport protein of root will specifically increase the uptake of Zn^{2+} in the root portion (Gustin et al. 2009). Wheat grain zinc concentration can be raised by the overexpression of NAC-transcription factor (NAM-B1) which is responsible to increase the remobilization of mineral elements from leaves to the developing grain and the senescence (Uauy et al. 2006).

9.3.2 Agronomic Practices

Different authors have evaluated agronomic strategies in the perspectives of both global health and sustainable economic progress to increase the concentrations of zinc in edible parts of major crops. Many studies have described profit for edible crop production and human health by agronomic zinc biofortification of grain crops (Cakmak 2008; White and Broadley 2009). Zinc fertilization of food crops signifies a short-term remedy of ensuring Zn translocation (Cakmak 2008). Different genotypes have different capability for the zinc accumulation. Zn is transportable, and different fertilizers such as zinc sulfate ($ZnSO_4$) can enhance the yield of grain crops in zinc-deficient soils and can raise zinc concentration in the grain (White and Broadley 2005). Combined application of zinc through soil as well as through foliar considerably enhanced the concentration of zinc in wheat grain (Cakmak 2008; Zhang et al. 2011; Zhao et al. 2011). Zn fertilization also diminishes the antinutrient concentration in grain and decreases PA to Zn molar ratio, which is generally expressed as an indicator of Zn bioavailability in diets (Cakmak et al. 2010). The water soluble Zn is low in soil solution and even in Zn-contaminated soils (Knight et al. 1997). Chelate-mediated bioavailability involves the utilization of synthetic chelators, e.g., ethylenediaminetetraacetate (EDTA) (Piechalak et al. 2003; Sahi et al. 2002). In nutrient-deficient ecosystems, the application of nitrogen–phosphorous–potassium (NPK) fertilizers is necessary for obtaining the high yield of field crops. These three macronutrients simultaneously augment root growth and result in an elevated transport of micronutrients from the soil to the plant.

Conversely availability of zinc applied to the soil also depends on pH of soil, e.g., NH_4^+ causes acidification of the rhizosphere which increases transfer of Zn from the soil to the plant, while NO_3^- causes more alkalinity to the soil, dropping this transfer rate. If the efficient absorption of minerals occurs, it can raise mineral levels in leaves but not essentially in fruits or seeds, for the reason that the relative efficiency of mineral transfer differs depending on the different parts of plant (Hartikainen 2005). Application of Zn-containing fertilizers seem to be a quick and easy solution to the Zn deficiency problem, but resource-poor farmers especially in developing countries cannot afford application of micronutrient fertilizers. On the other hand, it is reported that several synthetic chelators are costly and cause a hazard to the soil quality and groundwater (Kos and Lestan 2003). Plant growth can be restricted and also influence the other soil organisms if large amount of metal is applied to soils. Abandoned use of chemical inputs in cultivation has escalated the expenses of production and damaged the soil, water, and biological resources globally. There is also a dire need to improve Zn application methods in provisions of form, dose, and effective time for the application of Zn fertilizers. Table 9.1 shows the comparative prospective of diverse strategies to assuage the micronutrients deficiency in cereals.

Table 9.1 Comparative look of the strategies used for the improvement of Zn deficiency in plants

Strategies	Merits	Demerits	References
Chemical fertilizations	• Short-time solution • Foliar zinc application is also effective • Readily diminish the phytic acid conc. in the grains	• Expensive • Extremely reliant upon crop and cultivar • Not promising to target edible parts • Not eco-friendly	Cakmak (2008, 2009), Galloway et al. (2008), Smith et al. (2008)
Conventional breeding	• Exploits inherent properties of crops • Feasible to improve the zinc deficiency • Increase micronutrients density in the edible parts of plants	• Depends on existing trait diversity of gene pool • Long-term strategy • Traits might need to be introgressed from wild relatives • Possible intellectual property restraint	Raboy (2002), Bouis and Welch (2010), Cakmak et al. (2010), Pfeiffer and McClafferty (2007)
Transgenic techniques	• Rapid • Independent of gene pool • Targeted expression in edible parts • Applicable directly to elite cultivars	• Similar to zinc translocation, many ZIP family proteins can transport toxic metals (e.g., Cd) • Regulatory landscape • Socioeconomic and political issues concerning with transgenic plants • Possible intellectual property restraint	Perfus-Barbeoch (2002), Yang et al. (2009), Assunc et al. (2010), Johnson et al. (2011), Lee et al. (2011), Chowdhury et al. (2009)
Biofertilizer application	• Economical • Eco-friendly • Increase macro- and micronutrient uptake (P, Fe, Zn, Si, etc.) • Natural • Helpful against pathogenic microorganisms • Valuable for bioremediation • Significantly increases the yield of crops	• Limited shelf life • Slow action • Affected by environment	Hafeez et al. (2002, 2006), Ahmed et al. (2011), Bahrani et al. (2010), Badr et al. (2009), Metin et al. (2010), Kaewchai et al. (2005)

9.3.3 Plant Growth-Promoting Rhizobacteria as Zn Mobilizers

Plant growth-promoting rhizobacteria (PGPR) are one of the key factors that have important function in sustainable agriculture. PGPR are a diverse group of bacteria that can be found in the rhizosphere on root surfaces as well as in association with roots (Maheshwari et al. 2012; Ahmad et al. 2008). These bacteria move around from the bulk soil to the living plant rhizosphere and antagonistically colonize the rhizosphere and roots of plants (Hafeez et al. 2001; Yasmin et al. 2004; Kloepper and Schroth 1978). Soil bacteria which are important for plant growth are termed as PGPR (Hafeez et al. 2001; Hayat et al. 2010; Yasmin et al. 2004). PGPR can be alienated into two groups according to their relationship with the plants: symbiotic bacteria and free-living rhizobacteria (Khan 2005). These are comprised of naturally occurring beneficial microorganisms in soil that make available nutrients to plants through several mechanisms by fixing atmospheric nitrogen, solubilizing the nutrients fixed in soil, and by producing phytohormones (Hafeez et al. 2005; Jilani et al. 2007; Jacobs et al. 2008; Yao et al. 2008; Siddiqui et al. 2008).

In addition to phosphate mobilization, they are responsible to play key role in carrying out the bioavailability of soil phosphorous, potassium, iron, zinc, and silicate to plant roots (Tariq et al. 2007; Ahmad 2007; Saravanan et al. 2011; Abaid-Ullah et al. 2011). Many studies have revealed that inoculations of potent strains of Zn mobilizer rhizobacteria increased the yield of field crops such as rice, wheat, barley, and maize. Tariq et al. (2007) have described the effect of Zn-mobilizing PGPR which significantly alleviated the deficiency symptoms of Zn and regularly increased the total biomass (23 %), grain yield (65 %), and harvest index in addition to Zn concentration in the grain of rice. Furthermore inoculation of Zn-mobilizing PGPR had a notably positive impact on root weight (74 %), root length (54 %), root area (75 %), root volume (62 %), shoot weight (23 %), and panicle emergence index (96 %) and exhibited the maximum Zn mobilization efficiency as compared to the un-inoculated control. Besides it was also confirmed that PGPR strains can efficiently solubilize the Zn in liquid culture which was accessible for rice plant. Interestingly, the yield data has indicated that the PGPR contributed larger storage of assimilates in rice grains (Tariq et al. 2007). Ahmad (2007) screened the best Zn-mobilizing strain out of fifty strains on the basis of clear zone formation by plate assay isolated from maize rhizosphere. Similar work accomplished by Yasmin (2011) determined the Zn-solubilizing ability of *Pseudomonas* sp. Z5 isolated from rhizosphere of rice plants. In a study of doctoral dissertation, Abaid-Ullah et al. (2011) screened nine out of fifty Zn-solubilizer PGPR qualitatively and quantitatively on different insoluble Zn ores such as ZnO, ZnS, Zn $(CO_3)_2$, and Zn $(PO_4)_3$. A positive correlation of Zn solubilization was found between qualitative and quantitative screening of *Serratia* sp. Likewise higher Zn solubilization was noted with ZnO as compared to other insoluble ores. The beneficial effect of the efficient Zn mobilizer *Serratia* sp. was tested in vivo which significantly increased the yield and yield parameters of wheat crop at various locations with respect to climatic conditions of Pakistan (Abaid-Ullah

et al. 2010, 2011). Viable applications of PGPR are being tested and are repeatedly promising; however, a good understanding of the microbial interactions that result in plant growth increase will significantly raise the success rate of field applications (Burr et al. 1984: Saravanan et al. 2011; Saharan and Nehra 2011).

9.3.3.1 Mechanism of Zinc Solubilization by PGPR

About 90 % of the soil's Zn exists in insoluble form and is inaccessible for plant uptake (Barber 1995). Solubilization of metal salts is an imperative feature of PGPR as mobilized compound are accessible for the plants. Bacterial comparative and functional genomics research has opened new avenues for exploring these underlying mechanisms at biochemical and molecular level. Various studies have been conducted to explore the mechanisms of Zn-solubilizing PGPR. Generally PGPR solubilize the nutrients (essential trace elements) through acidification, chelation, exchange reactions, and release of organic acids (Chung et al. 2005; Hafeez et al. 2005). It is found that mobilization mechanism of Zn and iron possibly involves the siderophore production (Tariq et al. 2007; Burd et al. 2000; Wani et al. 2007; Saravanan et al. 2011), gluconate, or the derivatives of gluconic acids, e.g., 2-ketogluconic acid (Fasim et al. 2002), 5-ketogluconic acid (Saravanan et al. 2007a, b), and various other organic acids by PGPR (Wani et al. 2007; Di Simine et al. 1998; Tariq et al. 2007) as described in Table 9.2. Soil–plant–microbe interactions are complex, and there are lots of ways in which the outcome can influence the crop vigor and yield (Pieterse et al. 2003; Hafeez et al. 2002). The precise mechanism through which PGPR promote plant growth is not completely understood yet.

9.3.3.2 Screening of Zinc-Mobilizing PGPR

Zinc-mobilizing PGPR can be screened by plate assay, and their relative Zn-solubilizing capacity can be quantified through atomic absorption spectrophotometer (AAS) analysis. Screening of Zn-mobilizing PGPR can be made qualitatively by agar plate assay. The solubilizing potential can be visualized by the zone formation on the modified Bunt and Rovira (1955) media containing insoluble Zn ores (ZnO, $ZnPO_4$, ZnS, Zn $(CO_3)_2$, etc.). Quantification of Zn-mobilizing ability of PGPR is also obtainable by LB media amended with different insoluble Zn ores (ZnO, $ZnPO_4$, ZnS, $ZnCO_3$, etc.) through AAS (Hafeez and Hassan 2012; Abaid-Ullah et al. 2010).

9.3.3.3 Formulation and Delivery of Zn-Solubilizing PGPR

Biofertilizer formulation is an industrial art of converting a promising laboratory-proven bacterium into a commercial field product (Bashan 1998; Hafeez et al. 2006). Formulations of PGPR are composed of the active component, i.e.,

Table 9.2 Zn-mobilizing plant growth-promoting rhizobacteria (PGPR) producing various compounds helpful for plant growth

No.	Zinc-mobilizing PGPR	Plant/source	PGPR traits	References
1.	*Pseudomonas fluorescens*	Forest soil	Zn, P solubilizer, citric acid, and gluconic acid production	Di Simine et al. (1998)
2.	*Pseudomonas aeruginosa*	Air environment of a tannery	Zn solubilizer, low gluconic acid but higher amount of 2-ketogluconic acid production	Fasim et al. (2002)
3.	*Gluconacetobacter diazotrophicus*	*Saccharum officinarum*	Zn solubilizer, 5-ketogluconic acid production	Saravanan et al. (2007a, b)
4.	*Rhizobia* spp.	*Pisum sativum*	Zn solubilizer/tolerant, phytohormones production	Wani et al. (2008)
5.	*Pseudomonas* sp. PsM6, *P. jessenii* PjM15	*Ricinus communis*	Zn, Ni, and Cu mobilizers; ACC deaminase, siderophore, and IAA production; biosorption	Rajkumar and Freitas (2008)
6.	*Flavobacterium* sp.	*Orychophragmus violaceus*/ sewage sludge	Zn mobilization and accumulation	He et al. (2010)
7.	*Pseudomonas* sp. Z5	*Oryza sativa*	Zn solubilization	Yasmin (2011)
8.	*Serratia* sp.	*Triticum aestivum*	Zn and P solubilization	Abaid-Ullah et al. (2011)

rhizobacteria which are carried by an inert stuff used to deliver the active ingredients to the target (Hafeez and Hassan 2012). These microbial inoculums not only defeat loss of viability for the period of storage but also have longer shelf life and strength over a range of temperature −5 to 30 °C as in the marketing supply change (Hafeez 2009; Bashan 1998). The PGPR are formulated in solid carrier materials and commercialized with different trade names throughout the world. The biofertilizers with trade names Azotobakterin™ consisting of *Azotobacter chroococcum and* Phosphobacterin™ consisting of *Bacillus megaterium* var. *phosphaticum* are being used as seed treatment and soil drenching. The liquid-based formulations of biofertilizers such as Nitragin™ containing *Rhizobia* cells and Nitro™ containing *Azotobacter* cells are also being used as seed treatment in the USA since 1885. Solid formulation-based biofertilizer Gmax™ and Nitromax™ consisting of *Azotobacter* cells and *Azospirillum* cells, respectively, are being commercialized.

In Pakistan, mixture of potent PGPR has been formulated in sugarcane filter cake and marketed with the trade name of *Biopower* as nitrogen and phosphatic fertilizer for different field crops by National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan, in collaboration with public sector.

Certain other phosphate-solubilizing PGPR have been formulated in humic acid carrier by the COMSATS Institute of Information Technology, Islamabad, Pakistan, and being marketed with the trade name of *Humiphos* and *Biophos* by the private sector; AURIGA Chemical Enterprises, Lahore, Pakistan (Khan et al. 2010; Hafeez and Hassan 2011; Hafeez and Hassan 2012). Zn-mobilizing PGPR inoculants exploited as biofertilizers can accelerate rehabilitation of degraded land and improvement of soil fertility, enhance survival and growth of plants, increase grain yield, lower malnutrition rates, and reduce dependence on chemical fertilizers (Hafeez et al. 2001). Moreover biofertilizers are economical, eco-friendly, and its use can augment crop productivity (Hafeez et al. 2002). Hence biofertilizer formulation for Zn deficiency in cereal crops may represent a natural, environment-friendly, and inexpensive alternate to replace already existing chemical fertilizer hazard. Using Zn-solubilizing rhizobacteria together with all other beneficial traits will be a key advantage for the formulation of effective biofertilizers. It will be their binary beneficial nutritional effect resulting together from phosphate solubilization, N_2-fixation (Zaidi and Mohammad 2006; Gull et al. 2004), and their well-documented synergistic interactions with arbuscular mycorrhizal fungi (Ordookhani et al. 2010). The exploitation of PGPR inoculants as biofertilizers and/or antagonists of phytopathogens offers a promising alternative to chemical fertilizers and pesticides. It was practically determined that the application of PGPR (*Pseudomonas fluorescens* and *Paenibacillus polymyxa*) to rice plant enhanced the induced systematic resistance in a pot experiment (Hafeez and Naureen 2011; Naureen et al. 2009; Umashankari and Sekar 2011).

Current trends in agriculture are focused on the reduction of the pesticides and inorganic fertilizers utilization, forcing the study for alternative ways to progress a more sustainable agriculture (Kloepper et al. 1989; Mahdi et al. 2010a, b; Hafeez and Gull 2009). The outcome of the studies has shown that a *Bacillus* sp. (Zn-solubilizing bacteria) can be exploited as biofertilizer for zinc or in soils where native zinc is elevated or in conjunction with insoluble cheaper zinc compounds like zinc oxide (ZnO), zinc carbonate ($ZnCO_3$), and zinc sulfide (ZnS) as an alternative of expensive zinc sulfate (Mahdi et al. 2010a, b). Subsequent studies of PGPR have shown that several best strains are multifunctional, and secondly, PGPR traits are frequently disseminated among various different species and genera of microorganisms, many of which are native members of the soil microbial community. Generally individual strains differ significantly in performance. Native PGPR can affect the performance of introduced PGPR inoculants comparatively. Hence, lack of information about the background PGPR function; it is not easy to predict the response to soil inoculations. A lot of PGPR frequently solubilize nutrients (phosphorus, iron, zinc, silicate, etc.), produce auxins which stimulate root development, and produce siderophore and antibiotics that may help in inhibition of root infection. During environmental stress the plants produce ethylene or hydrogen cyanide and reactive oxygen species (ROS) that may be degraded by substances (enzymes) produced by these PGPR.

9.4 Conclusion

Zn deficiency is a serious constraint causing a numbers of health disorders in humans. The micronutrient deficiency particularly exists for the inhabitants of developing countries where cereal crops are consumed as staple food; in addition the prevalent high-yield cultivars in the underdeveloped regions are zinc deficient. Such mal-conditions are rigorous in the areas with calcareous and sandy soil due to their high vulnerability to micronutrient deficiency. More than 50 % soil is zinc deficient according to various survey reports for countries like India, Pakistan, and China. Monotonous food style of consumers in developing countries has focused the researchers to improve the micronutrient contents in the respective staple food crops. Existing strategies like chemical fertilization, agronomic practices, and transgenic plants development for the improvement of the Zn contents of food crops seem to have potential; however, these engaged practices have raised the environmental pollution, high-cost, socioeconomic, and political issues. Use of PGPR for the improvements of micronutrients deficiency is promising due to its ecological, economic, and eco-friendly nature. The net increase in nutrient contents and yield has been reported by the recent studies in the last decades for different grain yielding crops. The application of microbial technologies in agriculture is presently growing quite rapidly with the recognition of novel bacterial strains, which are additionally effective in promoting plant growth. Exploitation of the multifunction PGPR (P, Fe, Zn, Si solubilizer, production of phytohormones, bacteriocin, etc.) will be a good candidate for the formulation of effective biofertilizers. Thus, in the near future, promising use of biofortification is the best solution for preventing micronutrients deficiency and enhancing sustainable agriculture.

Acknowledgment I am thankful to Dr. Muhammad Imran, Assistant Professor, CIIT, for assisting me in writing and correcting the English.

References

Abaid-Ullah M, Hassan MN, Nawaz MK, Hafeez FY (2010) Screening of zinc mobilizing strains from wheat (*Triticum aestivum* L.) rhizosphere. Proceedings of international science conference on "utilization of modern agriculture techniques in challenging environmental perspectives". Azad Jammu and Kashmir University, Pakistan, p 390

Abaid-Ullah M, Hassan MN, Nawaz MK, Hafeez FY (2011) Biofortification of wheat (*Triticum aestivum* L.) through Zn mobilizing PGPR. Proceedings of international science conference "prospects and challenges to sustainable agriculture". Azad Jammu and Kashmir University, Pakistan, p 298

Ahmad M (2007) Biochemical and molecular basis of phosphate and zinc mobilization by PGPR in rice. M. Phil Dissertation, NIBGE, Faisalabad

Ahmad P, Wani AE, Saghir MD, Khan AE, Zaidi A (2008) Effect of metal-tolerant plant growth-promoting *Rhizobium* on the performance of pea grown in metal-amended soil. Arch Environ Contam Toxicol 55:33–42

Ahmed Amal MA, Ahmed G, Magda Mohamed H, TawfikIntegrated MM (2011) Effect of organic and biofertilizers on wheat productivity in new reclaimed sandy soil. Res J Agric Biol Sci 7(1):105–114

Alloway BJ (1995) Heavy metals in soils, 2nd edn. Blackie Academic & Professional, London

Alloway BJ (2004) Zinc in soils and crop nutrition. International Zinc Association (IZA) Publications, Brussels

Alloway BJ (2008) Zinc in soils and plant nutrition. International zinc association (IZA) and IFA Brussels, Belgium and Paris, p 139

Assunc AGL, Herrero E, Lin YF, Huettel B, Talukdar S, Smaczniak C, Immink RGH, Eldik MV, Fiers M, Schat H, Aarts MGM (2010) *Arabidopsis thaliana* transcription factors bZIP19 and bZIP23 regulate the adaptation to zinc deficiency. Proc Natl Acad Sci USA 107:10296–10301

Badr Elham A, Ibrahim OM, El-Kramany MF (2009) Interaction effect of biological and organic fertilizers on yield and yield components of two wheat cultivars. Egypt J Agron 31(1):17–27

Bahrani A, Pourreza J, Hagh Joo M (2010) Response of winter wheat to co-inoculation with Azotobacter and arbuscular mycorrhizal fungi (AMF) under different sources of nitrogen fertilizer. Am Eurasian J Sustain Agric 8(1):95–103

Barber SA (1995) Soil nutrient bioavailability, 2nd edn. Wiley, New York

Bashan Y (1998) *Azospirillum* plant growth-promoting strains are nonpathogenic on tomato, pepper, cotton, and wheat. Can J Microbiol 44:168–174

Black RE, Lindsay HA, Bhutta ZA, Caulfield LE, De Onnis M, Ezzati M, Mathers C, Rivera J (2008) Maternal and child undernutrition: global and regional exposures and health consequences. Lancet 371:243–260

Bouis HE (2003) Micronutrient fortification of plants through plant breeding: can it improve nutrition in man at low cost? Proc Nutr Soc 62:403–411

Bouis HE, Welch RM (2010) Biofortification: a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. Crop Sci 50:20–32

Broadley MR, White PJ, Zelko I, Lux A (2007) Zinc in plants. New Phytol 173(4):677–702

Brown KH, Hambidge KM, Ranum P, Tyler V, The Zinc Fortification Working Group (2009) Zinc fortification of cereal flours: current recommendations and research needs. Food Nutr Bull 31: S62–S74

Bunt JS, Rovira AD (1955) Microbiological studies of some subantarctic soils. J Soil Sci 6:119–128

Burd GI, Dixon DG, Glick BR (2000) Plant growth promoting bacteria that decrease heavy metal toxicity in plants. Can J Microbiol 46:237–245

Burr TJ, Caesar AM, Schrolh N (1984) Beneficial plant bacteria. Crit Rev Plant Sci 2(Suppl 1):1–20

Cakmak I (2008) Enrichment of cereal grains with zinc: agronomic or genetic bio-fortification? Plant Soil 302:1–17

Cakmak I (2009) Enrichment of fertilizers with zinc: an excellent investment for humanity and crop production in India. J Trace Elem Med Biol 23:281–289

Cakmak I, Pfeiffer WH, McClafferty B (2010) Biofortification of durum wheat with zinc and iron. Cereal Chem 87:10–20

Calder PC, Jackson AA (2000) Under nutrition, infection and immune function. Nutr Rev 8:129–133

Canadian UNICEF Committee (2006) Global child survival and health, p 67

Chowdhury S, Meenakshi JV, Tomlins K, Owori C (2009) Are consumers willing to pay more for biofortified foods? Evidence from a field experiment in Uganda. HarvestPlus working paper 3. International Food Policy Research Institute, Washington

Chung H, Park M, Madhaiyan M, Seshadri S, Song J, Cho H (2005) Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil Biol Biochem 37:1970–1974

Davies NT (1980) Studies on the absorption of zinc by rat intestine. Br J Nutr 43:189–203
Di Simine CD, Sayer JA, Gadd GM (1998) Solubilization of zinc phosphate by strain of *Pseudomonas fluorescens* isolated from forest soil. Biol Fertil Soils 28:87–94
FAO WHO (2002) Human vitamin and mineral requirements. Food and Agriculture Organization of the United Nations, Bangkok, Thailand. ISBN 1014–9228
Fasim F, Ahmed N, Parsons R, Gadd GM (2002) Solubilization of zinc salts by bacterium isolated by the air environment of tannery. FEMS Microbiol Lett 213:1–6
Friedland AJ (1990) The movement of metals through soils and ecosystems. In: Shaw AJ (ed) Heavy metal tolerance in plants: evolutionary aspects. CRC, Boca Raton, FL, pp 7–19
Galloway JN, Townsend AR, Erisman JW (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320:889–892
Gibson RS, Ferguson EL (1998) Nutrition intervention strategies to combat zinc deficiency in developing countries. Nutr Res Rev 11:115–131
Giehl RFH, Meda AR, Wiren N (2009) Moving up, down, and everywhere: signaling of micronutrients in plants. Curr Opin Plant Biol 12:320–327
Gomez-Becerra HF, Yazici A, Ozturk L, Budak H, Peleg Z, Morgounov A, Fahima T, Saranga Y, Cakmak I (2010) Genetic variation and environmental stability of grain mineral nutrient concentrations in *Triticum dicoccoides* under five environments. Euphytica 171:39–52
Gull M, Hafeez FY, Saleem M, Malik KA (2004) Phosphate-uptake and growth promotion of chickpea (*Cicer arietinum* L.) by co-inoculation of mineral phosphate solubilizing bacteria and a mixed rhizobial culture. Aust J Exp Agric 44(6):623–628
Gupta AP (2005) Micronutrient status and fertilizer use scenario in India. J Trace Elem Med Biol 18:325–331
Gustin JL, Loureiro ME, Kim D, Na G, Tikhonova M, Salt DE (2009) MTP1-dependent Zn sequestration into shoot vacuoles suggests dual roles in Zn tolerance and accumulation in Zn hyper-accumulating plants. Plant J 57:1116–1127
Hacisalihoglu G, Kochian LV (2003) How do some plants tolerate low levels of soil zinc? Mechanisms of zinc efficiency in crop plants. New Phytol 159:341–350
Hafeez FY, Gull M (2009) Role of siderophores in plant disease suppression. In: Saghir MD, Zaidi A, Musarrat J (eds) Microbes in sustainable agriculture. Nova, Hauppauge, NY, pp 167–182
Hafeez FY, Hassan MN (2011) Use of biotechnological approaches to explore PGPR. Curr Opin Biotechnol 22
Hafeez FY, Hassan MN (2012) Utilization of plant associated rhizobacteria to improve plant nutrition and protection: a cost effective and ecofriendly strategy. In: Brebia CA (ed) Sustainability today. WIT press, Southampton, pp 231–238
Hafeez FY, Naureen Z (2011) Biological control of sheath blight disease of rice by siderophore producing rhizobacterial strains and their role in efficient mobilization of micronutrients from soil. Curr Opin Biotechnol 22
Hafeez FY, Hameed S, Ahmad T, Malik KA (2001) Competition between effective and less effective strains of *Bradyrhizobium* spp. for nodulation on *Vigna radiata*. Biol Fertil Soils 33:382–386
Hafeez FY, Hameed S, Zaidi AH, Malik KA (2002) Biofertilizer for sustainable agriculture. In: Azam F, Iqbal MM, Inayatullah C, Malik KA (eds) Technologies for sustainable agriculture. NIAB, Faisalabad, pp 67–74
Hafeez FY, Naeem F, Naeem R, Zaidi AH, Malik KA (2005) Symbiotic effectiveness and bacteriocin production by *Rhizobium leguminosarum* bv. *viciae* isolated from agriculture soils in Faisalabad. Environ Exp Bot 54:142–147
Hafeez FY, Yasmin S, Ariani D, Naranchimeg D, Delgermaa B, Rahman M, Malik KA, Zafar Y (2006) Plant growth-promoting bacteria as biofertilizer. Agron Sustain Dev 26:143–150
Hambidge KM, Krebs NF (2007) Zinc deficiency: a special challenge. J Nutr 137(4):1101
Hamid A, Ahmad N (2001) Paper at regional workshop on integrated plant nutrition system (IPNS): development and rural poverty alleviation, Bangkok, 18–21 Sept

Han JL, Li YM, MaC Y (2006) Effect of zinc fertilization on accumulation and transportation of N, P, K and Zn after anthesis of wheat. Plant Nutr Fert Sci 12:313–320

Hartikainen H (2005) Biogeochemistry of selenium and its impact on food chain quality and human health. J Trace Elem Med Biol 18:309–318

HarvestPlus (2012) Breeding crops for better nutrition. Web page of HarvestPlus. International Food Policy Research Institute, Washington, DC. Available via http://www.harvestplus.org/content/zinc-wheat

Havlin JL, Beaton JD, Tisdale SL, Nelson WL (2005) Soil fertility and fertilizers. Pearson, Upper Saddle River, NJ, p 515

Hayat R, Ali S, Amara U, Khalid R, Ahmed I (2010) Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol 60:579–598

He CQ, Tan GE, Liang X, Du W, Chen YL, Zhi GY (2010) Effect of Zn-tolerant bacterial strains on growth and Zn accumulation in *Orychophragmus violaceus*. Appl Soil Ecol 44:1–5

Hershfinkel M (2006) Zn^{2+}, a dynamic signaling molecule. In: Tamás MJ, Martinoia E (eds) Molecular biology of metal homeostasis and detoxification: from microbes to man. Springer, Heidelberg, pp 131–152

Hirschi K (2008) Nutritional improvements in plants: time to bite on biofortified foods. Trends Plant Sci 13:459–462

Hotz C, Brown KH (2004) Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull 25:S91–S204

Intawongse M, Dean JR (2006) Uptake of heavy metals by vegetable plants grown on contaminated soil and their bioavailability in the human gastrointestinal tract. Food Addit Contam 23:36–48

International Zinc Association IZA (2011) http://www.zinc.org/sustainability

Jacobs JL, Fasi AC, Ramette A, Smith JJ, Hammerschmidt R, Sundin GW (2008) Identification and onion pathogenicity of *Burkholderia cepacia* complex isolates from the onion rhizosphere and onion field soil. Appl Environ Microbiol 74:3121–3129

Jilani G, Akram A, Ali RM, Hafeez FY, Shamsi IH, Chaudhry AN, Chaudhry AG (2007) Enhancing crop growth, nutrients availability, economics and beneficial rhizosphere microflora through organic and biofertilizers. Ann Microbiol 57(2):177–183

Johnson AAT, Kyriacou B, Calla-han DL, Carruthers L, Stangoulis J, Lombi E, Tester M (2011) Constitutive over expression of the OsNAS gene family reveals single-gene strategies for effective iron- and zinc-biofortification of rice endosperm. PLoS One 6:e24476. doi:10.1371/journal.pone.0024476

Kaewchai S, Soytong K, Hyde KD (2005) Mycofungicides and fungal biofertilizers. Fungal Divers 38:25–50

Kauser MA, Hussain F, Ali S, Iqbal MM (2001) Zinc and Cu nutrition of two wheat varieties on a calcareous soil. Pak J Soil Sci 20:21–26

Khalifa RKHM, Shaaban SHA, RAWIA A (2011) Effect of foliar application of zinc sulfate and boric acid on growth, yield and chemical constituents of iris plants. Ozean J Appl Sci 4(2):130–144

Khan AG (2005) Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. J Trace Elem Med Biol 18:355–364

Khan MA, Haq I, Hassan MN, Hafeez FY (2010) Effect of phosphate solubilizing strains PCMC-1 and PCMC-2 on maize crop. Oral presentation. In: 13th National soil science congress held at Serena, 24–26 Mar 2010

Khoshgoftarmanesh AH, Schulin R, Chaney RL, Daneshbakhsh B, Afyuni M (2009) Micronutrient-efficient genotypes for crop yield and nutritional quality in sustainable agriculture. A review. Agron Sustain Dev 30:83–110

King JC (2006) Zinc. In: Shils ME, Shike M (eds) Modern nutrition in health and disease, 10th edn. Lippincott Williams & Wilkins, Philadelphia, PA, pp 271–285

Kloepper JW, Schroth MN (1978) Plant growth-promoting rhizobacteria on radishes. In: Proceedings of the 4th international conference on plant pathogen bacteria, vol 2, pp 879–882

Kloepper JW, Lifshitz R, Zablotowicz RM (1989) Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol 7(2):39–43

Knight B, Zhao FJ, McGrath SP, Shen ZG (1997) Zinc and cadmium uptake by the hyperaccumulator *Thlaspi caerulescens* in contaminated soils and its effects on the concentration and chemical speciation of metals in soil solution. Plant Soil 197:71–78

Kos B, Lestan D (2003) Induced phytoextraction/soil washing of lead using biodegradable chelate and permeable barriers. Environ Sci Technol 37:624–629

Kramer U, Clemens S (2006) Functions and homeostasis of zinc, copper, and nickel in plants, molecule. In: Tamas MJ, Martinoia E (eds) Molecular biology of metal homeostasis and detoxification: from microbes to man. Springer, Heidelberg, pp 216–271

Kutman UB, Yildiz B, Cakmak I (2011) Improved nitrogen status enhances zinc and iron concentrations both in the whole grain and the endosperm fraction of wheat. J Cereal Sci 53:118–125

Lee S, Persson DP, Hansen TH, Husted S, Schjoerring JK, Kim YS, Jeon US, Kim YK, Kakei Y, Masuda H, Nishizawa NK, An G (2011) Bio-available zinc in rice seeds is increased by activation tagging of nicotianamine synthase. Plant Biotechnol J 9:865–873

Lonnerdal IB (2000) Dietary factors influencing zinc absorption. J Nutr 130:1378–1387

Ma G, Jin Y, Li Y, Zhai F, Kok FJ, Jacobsen E, Yang X (2008) Iron and zinc deficiencies in China: what is a feasible and cost-effective strategy? Public Health Nutr 11:632–638

Mahdi SS, Hassan GI, Samoon SA, Rather HA, Dar SA, Zehra B (2010a) Bio-fertilizers in organic agriculture. J Phytopathol 2(10):42–54

Mahdi SS, Dar SA, Ahmad S, Hassan GI (2010b) Zinc availability – a major issue in agriculture. Res J Agric Sci 3(3):78–79

Maheshwari DK, Kumar S, Maheshwari NK, Patel D, Saraf M (2012) Nutrient availability and management in the rhizosphere by microorganisms. In: Maheshwari DK (ed) Bacteria in agrobiology: stress management. Springer, Berlin, pp 301–326

Metin TA, Medine GB, Ramazan CC, Taskin OF, Sahin D (2010) The effect of PGPR strain on wheat yield and quality parameters. Proceeding of world congress of soil science, soil solutions for a changing world, Brisbane, Australia, 1–6 Aug

Monasterio I, Graham RD (2000) Breeding for trace minerals in wheat. Food Nutr Bull 21:392–396

Monasterio JIO, Rojas NP, MengE PK, Trethowan R, Pena RJ (2007) Enhancing the mineral and vitamin content of wheat and maize through plant breeding. J Cereal Sci 46:293–307

Naureen Z, Hafeez FY, Roberts MR (2009) Induction of systemic resistance against rice blast disease by PGPR isolated from the rhizosphere of rice. In: Hafeez FY, Malik KA, Zafar Y (eds) Microbial technologies for sustainable agriculture. Crystal press, Islamabad, p 269. ISBN 978-969-8189-14-3

Oliveira AB, Nascimento CWA (2006) Formas de manganês e ferro em solos de referência de Pernambuco. Rev Bras Ciênc Solo 30(1):99–110

Ordookhani K, Khavazi K, Moezzi A, Rejali F (2010) Influence of PGPR and AMFon antioxidant activity, lycopene and potassium contents in tomato. African J Agric Res 5(10):1108–1116

Pahlvan-Rad MR, Pressaraki M (2009) Response of wheat plant to zinc, iron and manganese applications and uptake and concentration of zinc, iron and manganese in wheat grains. Commun Soil Sci Plant Anal 40:1322–1332

Perfus-Barbeoch L (2002) Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. Plant J 32:539–548

Pfeiffer WH, McClafferty B (2007) Harvest plus: breeding crops for better nutrition. Crop Sci 47:88–105

Piechalak A, Tomaszewska B, Baralkiewics D (2003) Enhancing phytoremediative ability of *Pisum sativum* by EDTA application. Phytochemistry 64:1239–1251

Pieterse CMJ, Pelt JA, Verhagen BWM, Jurriaan T, Wees SCM, Léon-Kloosterziel KM, Loon LC (2003) Induced systemic resistance by plant growth-promoting rhizobacteria. Symbiosis 35 (Suppl 1–3):39–54

Plum LM, Rink L, Haase H (2010) The essential toxin: impact of zinc on human health. Int J Environ Res Public Health 7:1342–1365

Prasad R (2005) Rice wheat cropping system. Adv Agron 86:255–339

Prasad AS (2008a) Zinc in human health: effect of zinc on immune cells. Mol Med 14(5–6):353

Prasad AS (2008b) Zinc deficiency. Brit Med J 326:409–410

Raboy V (2002) Progress in breeding low phytate crops. J Nutr 132:503S–505S

Rajkumar M, Freitas H (2008) Influence of metal resistant-plant growth-promoting bacteria on the growth of *Ricinus communis* in soil contaminated with heavy metals. Chemosphere 71:834–842

Ranjbar GA, Bahmaniar MA (2007) Effect of soil and foliar application of zinc fertilizer on yield and growth characteristic of bread wheat (*Triticum aestivum* L.) cultivars. Asian J Sci 6:1000–1005

Reeves PG, Chaney RL (2008) Bioavailability as an issue in risk assessment and management of food cadmium: a review. Sci Total Environ 398:13–19

Saharan BS, Nehra V (2011) Plant growth promoting rhizobacteria: a critical review. Life Sci Med Res 1–7

Sahi SZ, Bryant NL, Sharma NC, Singh SR (2002) Characterization of a lead hyperaccumulator shrub, *Sesbania drummondii*. Environ Sci Technol 36:4676–4680

Saravanan VS, Madhaiyan M, Thangaraju M (2007a) Solubilization of zinc compounds by the diazotrophic, plant growth promoting bacterium *Gluconacetobacter diazotrophicus*. Chemosphere 66:1794–1798

Saravanan VS, Osborne J, Madhaiyan M, Mathew L, Chung J, Ahn K, Sa T (2007b) Zinc metal solubilisation by *Gluconacetobacter diazotrophicus* and induction of pleomorphic cells. J Microbiol Biotechnol 17(9):1477–1482

Saravanan VS, Kumar MR, Sa TM (2011) Microbial zinc solubilisation and their role on plants. In: Maheshwari DK (ed) Bacteria in agrobiology: plant nutrient management. Springer, Berlin, pp 47–63

Seilsepour M (2006) Study of zinc effects on quantitative and qualitative traits of winter wheat in saline soil condition. Biaban/Desert 11:17–23

Siddiqui ZA, Akhtar MS, Futai K (2008) Mycorrhizae: sustainable agriculture and forestry. Springer, Dordrecht

Sillanpaa M (1982) Micronutrients and the nutrient status of soils. A global study. FAO Soil Bulletin No. 48. FAO, Rome, Italy

Silvera S, Ronan T (2001) Trace element and cancer risk. A review of the epidemiological evidences. Cancer Cause Control 18(1):7–27

Smith P, Martino D, Cai Z (2008) Greenhouse gas mitigation in agriculture. Philos Trans R Soc B 363:789–813

Stein AJ (2010) Global impacts of human mineral malnutrition. Plant Soil 335:133–154

Tariq M, Hameed S, Malik KA, Hafeez FY (2007) Plant root associated bacteria for zinc mobilization in rice. Pak J Bot 39(1):245–253

Trethowan RM (2007) Breeding wheat for high iron and zinc at CIMMYT: state of the art, challenges and future prospects. In: Proceedings of the seventh international wheat conference, Mar del Plata, Argentina

Uauy C, Brevis JC, Dubcovsky J (2006) The high grain protein content gene Gpc-B1 accelerates senescence and has pleiotropic effects on protein content in wheat. J Exp Bot 57:2785–2794

Umashankari J, Sekar C (2011) Comparative evaluation of different bioformulations of PGPR cells on the enhancement of induced systemic resistance (ISR) in rice *P. oryzae* pathosystem under upland condition. Curr Bot 2(3):12–17

United States National Research Council, Institute of Medicine (2000) Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academies Press, Washington, DC, pp 442–455

Vallee BL, Falchuk KH (1981) Zinc and gene expression. Philos Trans R Soc Lond B Biol Sci 294:185–197

Wani PA, Khan MS, Zaidi A (2007) Impact of zinc tolerant plant growth promoting rhizobacteria on lentil grown in zinc amended soil. Agron Sustain Dev 28:449–455. doi:10.1051/agro-2007048

Wani PA, Khan MS, Zaidi A (2008) Effect of metal tolerant plant growth promoting *Rhizobium* on the performance of pea grown in metal amended soil. Arch Environ Contam Toxicol 55:33–42

Waters BM, Uauy C, Dubcovsky J, Grusak MA (2009) Wheat (*Triticum aestivum*) NAM proteins regulate the translocation of iron, zinc and nitrogen compounds from vegetative tissues to grain. J Exp Bot 60:4263–4274

Welch RM (2001) Impact of mineral nutrients in plants on human nutrition on a worldwide scale. In: Horst WJ et al (eds) Plant nutrition–food security and sustainability of agro-ecosystems. Kluwer, Dordrecht, pp 284–285

Welch RM, Graham RD (2004) Breeding for micronutrients in staple food crops from a humane nutrition perspective. J Exp Bot 55:353–364

White PJ, Broadley MR (2005) Biofortifying crops with essential mineral elements. Trends Plant Sci 10:586–593

White PJ, Broadley MR (2009) Biofortification of crops with seven mineral elements often lacking in humane diets: iron zinc copper calcium magnesium selenium and iodine. New Phytol 182:49–84

White PJ, Broadley MR (2011) Physiological limits to zinc biofortification of edible crops. Front Plant Sci 2:1–11

WHO (2002) The world health report: reducing risks, promoting healthy life. World Health Organization Geneva, Switzerland

WHO (2004) Clinical management of acute diarrhea. World Health Organization Geneva, Switzerland

Yang X, Huang J, Jiang Y, Zhang HS (2009) Cloning and functional identification of two members of the ZIP (Zrt, Irt-like protein) gene family in rice (*Oryza sativa* L.). Mol Biol Rep 36:281–287

Yao T, Yasmin S, Malik KA, Hafeez FY (2008) Potential role of Rhizobacteria isolated from North-western China for enhancing wheat and oat yield. J Agric Sci 146:49–56

Yasmin S (2011) Characterization of growth promoting and bioantagonistic bacteria associated with rhizosphere of cotton and rice. NIBGE, Faisalabad

Yasmin S, Bakar MAR, Malik KA, Hafeez FY (2004) Isolation, characterization and beneficial effects of rice associated PGPRs from Zanzibar soils. J Basic Microbiol 44:241–252

Zaidi A, Mohammad S (2006) Co-inoculation effects of phosphate solubilizing microorganisms and glomus fasciculatum on green gram-Bradyrhizobium symbiosis. Agric Sci 30:223–230

Zhang Y, Song Q, Yan J, Tang J, Zhao R, Zhang Y, He Z, Zou C, Ortiz-Monasterio I (2010) Mineral element concentrations in grains of Chinese wheat cultivars. Euphytica 174:303–313

Zhang YQ, Sun YX, Ye YL, Karim MR, Xue YF, Yan P, Meng QF, Cui ZL, Cakmak I, Zhang FS, Zou CQ (2011) Zinc biofortification of wheat through fertilizer applications in different locations of China. Field Crops Res 125:1–7

Zhao FJ, McGrath SP (2009) Biofortification and phytoremediation. Curr Opin Plant Biol 12:373–380

Zhao A, Lu X, Chen Z, Tian X, Yang X (2011) Zinc fertilization methods on zinc absorption and translocation in wheat. J Agric Sci 3:28–35

Chapter 10
Functional Aspect of Phosphate-Solubilizing Bacteria: Importance in Crop Production

Mohammad Saghir Khan, Ees Ahmad, Almas Zaidi, and Mohammad Oves

10.1 Introduction

Phosphorus (P) is one of the major macronutrients essentially required by plants and plays a critical role in photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis, and respiration (Fernandez et al. 2007; Ahemad et al. 2009). After uptake by plants, P also stimulates root development and facilitates flower formation and quality and quantity of fruits and seed formation (Ahemad et al. 2009). Additionally, sufficient P concentration may increase the resistance ability of plants to diseases and adverse conditions. On the other hand, majority of the soils around the world are deficient in P, and hence, only 1–5 % of total soil P is available to plants (Molla and Chaudhury 1984). As a result of the acute deficiency, P is, therefore, applied in agronomic operations from external sources in order to fulfill the phosphatic demands of plants. The use of consistent and sometimes excessively higher rates of chemicals including phosphatic fertilizers in current high-input agricultural practices has, however, resulted in the damaging effects on composition and functions of rhizosphere microbes. Subsequently, the fertility of soil is disturbed. These factors together lead to losses in crop production. The reduction in overall growth of plants following excessive application of P occurs primarily due to poor P uptake ability of plants and rapid fixation/sorption ability of P with soil constituents as calcium, aluminum, and iron phosphate (Lindsay et al. 1989; Vassilev and Vassileva 2003; Tao et al. 2008). In order to reduce chemical addition to soils and spiraling cost, and undeniable deleterious environmental impacts of P fertilizers, there is an urgent need to find a suitable/feasible alternative to chemical fertilizers. In this regard, microbial communities capable of transforming insoluble/bound P into soluble and available forms, collectively called

M.S. Khan (✉) • E. Ahmad • A. Zaidi • M. Oves
Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh 202002, Uttar Pradesh, India
e-mail: khanms17@rediffmail.com

D.K. Maheshwari et al. (eds.), *Bacteria in Agrobiology: Crop Productivity*,
DOI 10.1007/978-3-642-37241-4_10,

as phosphate-solubilizing microorganisms (PSM), may be applied to overcome such barriers. Considering these, many researchers around the world have isolated PS bacteria from different soils (Perveen et al. 2002; Pérez et al. 2007; Chen et al. 2008; Khan et al. 2009a; Ahemad and Khan 2010; Hui et al. 2011; Xiang et al. 2011) and tested their ability as inoculants to see whether they have any impact on plant growth or not (Zaidi 1999; Zaidi et al. 2003, 2009a; Chen et al. 2006; Kumari et al. 2009; Khan et al. 2010). Interestingly, among microbiological option, many of PS bacteria belonging largely to the genera pseudomonads (Behbahani 2010; Ahemad and Khan 2011a), bacilli (Wani et al. 2007a; Behbahani 2010; Sanjotha et al. 2011; Yadav et al. 2011), rhizobia (Abd-Alla 1994; Alikhani et al. 2006; Abril et al. 2007; Chandra et al. 2007; Marra et al. 2011), and *Azotobacter* (Ivanova et al. 2006; Yi et al. 2008) when used as phosphatic inoculants have been found effective and more practical in sustainable agricultural practices for enhancing crop production by providing available forms of P to different plants (Bojinova et al. 2008; Adesemoye and Kloepper 2009; Oliveira et al. 2009; Yu et al. 2011) in different agro-ecological niches (Zaidi et al. 2003; Khan et al. 2007). In addition to P, the PSM including bacteria (Zaidi et al. 2009b; Zhu et al. 2011) and fungi (El-Azouni 2008; Khan et al. 2010) increase the growth of plants by other mechanisms like N_2 fixation, by providing various growth-regulating substances to plants (Wani et al. 2007a; Mittal et al. 2008; Ahemad and Khan 2011b), such as siderophores (Oves et al. 2009; Ahemad and Khan 2012) and antibiotics (Lipping et al. 2008; Khan et al. 2010), and by protecting plants from pathogen damage (Hamadali et al. 2008). Documented results have shown that microphos (microbial cultures with PS activity) having such vast and varied activities when used either alone (Chen et al. 2008; Poonguzhali et al. 2008) or as mixture with other plant growth-promoting rhizobacteria (PGPR), a modifier of soil fertility and facilitator of plant establishment (Zaidi and Khan 2006; Wani et al. 2007b; Vikram and Hamzehzarghani 2008; Khan et al. 2009a, b) increased the biological and chemical characteristics of plants grown in various agro-ecosystems (Rodríguez et al. 2006; Khan et al. 2009b; Ahemad and Khan 2011b).

10.2 Mechanism of P-Solubilization and Development of Inoculant: A Brief Account

Naturally abundant yet unavailable insoluble forms of P such as tricalcium phosphate $(Ca_3PO_4)_2$, aluminum phosphate (Al_3PO_4), and iron phosphate (Fe_3PO_4) may be converted to soluble P by P-solubilizing bacteria inhabiting different soil ecosystems (Song et al. 2008; Khan et al. 2010; Ahemad and Khan 2011a). Soil microorganisms in this regard have generally been found more effective in making P available to plants from both inorganic and organic sources by solubilizing (Toro 2007; Wani et al. 2007b) and mineralizing difficultly available P (Bishop et al. 1994; Ponmurugan and Gopi 2006), respectively. Several workers have

documented their findings in order to better understand as to how the microbial populations including bacteria cause the solubilization of insoluble P (Cunningham and Kuiack 1992; Illmer and Schinner 1995; Buch et al. 2008; Song et al. 2008). Of the various strategies adopted by microbes, the involvement of low molecular mass organic acids (OA) secreted by microorganisms has been well recognized and a widely accepted theory as a principal means of P solubilization (Maliha et al. 2004). The OA produced by bacterial cultures (Table 10.1) in the natural environment or under in vitro conditions chelate mineral ions or decrease the pH to bring P into solution (Maliha et al. 2004; Pradhan and Shukla 2005). Consequently, the acidification of microbial cells and their surrounding leads to the release of P ions from the P mineral by H^+ substitution for Ca^{2+} (Goldstein 1994). However, there are also reports which suggest that insoluble P could be transformed into soluble forms of P without OA production by microbes (Asea et al. 1988; Illmer and Schineer 1992, 1995; Chen et al. 2006). For example, Altomare et al. (1999) while investigating the P-solubilizing ability of plant growth-promoting and biocontrol fungus *Trichoderma harzianum* T-22 did not record OA production (rock P was used as insoluble P source) under in vitro condition. It was concluded from this study that the insoluble P could be solubilized by mechanisms other than acidification process. Also, the fungal-solubilizing activity was credited both to chelation and to reduction processes, which may be useful in the management of phytopathogens. Apart from the OA theory, some of the inorganic acids (Reyes et al. 2001; Richardson 2001) such as HCl (Kim et al. 1997), nitric acid, and sulfuric acids (Dugan and Lundgren 1965) produced by chemoautrophs and the H^+ pump, for example, in *Penicillium rugulosum*, have also been reported to solubilize the insoluble P (Reyes et al. 1999). The inorganic acids convert tri-calcium phosphate to di- and monobasic phosphates with the net result of an enhanced availability of the element to plants.

The advent of P-solubilizing potentials among renewable resources like the bacterial populations has been one of the most important biological traits that have resulted in reducing the dependence on synthetic P fertilizers and consequently preserving soil fertility and environmental safety from chemical toxicity. And therefore, the use of PS bacteria as an alternative to chemical fertilizer has attracted greater attention of agronomists than microbiologists in recent times. In order to develop microphos, organisms with P-solubilizing activity may be isolated from either conventional or derelict environment using standard methods. The isolated bacterial cultures showing greatest P-solubilizing activity (Fig. 10.1) on any media designed especially to select P-solubilizing bacteria, for example, Pikovskaya medium (Pikovskaya 1948) are selected and used to develop as microbial inoculants following standard procedure (Fig. 10.2). Subsequently, the microphos are tested both under pot house and field environment using seed treatment, seedling dipping, or soil application methods for their ultimate transfer to practitioner/farmers for application in agricultural practices as a cheap and viable phosphatic option.

Table 10.1 Organic acid production and P solubilisation by PS bacteria

PS bacteria	Organic acid produced	Initial pH	Final pH	Amount of P solubilised (μg/ml)	Time (h)	Reference
Pseudomonas trivialis (BIHB 769)	GA, 2-KGA, LA, SA, FA, MA	7 ± 0.2	3.70	806.4 ± 2.3	120	Vyas and Gulati (2009)
P. poae (BIHB 808)	GA, 2-KGA, SA, CA, MA	7 ± 0.2	3.58	821.4 ± 1.7	120	Vyas and Gulati (2009)
P. fluorescens (BIHB 740)	GA, 2-KGA, SA, FA, CA, MA	7 ± 0.2	3.97	768.3 ± 2.6	120	Vyas and Gulati (2009)
Pseudomonas spp. (BIHB 751)	OA, GA, 2-KGA, FA, MA	7 ± 0.2	4.20	318.7 ± 2.0	120	Vyas and Gulati (2009)
Enterobacter Hy-401	OA, GA, MA, LA, CA, SA, FuA	7–7.5	4.32±0.02	623.6 ± 23.0	120	Yi et al. (2008)
Arthrobacter Hy-505	OA, GA, LA, CA	7–7.5	5.50 ± 0.04	428.9 ± 15.3	120	Yi et al. (2008)
Azotobacter Hy-510	OA, GA, TA, LA, SA, FuA	7–7.5	4.69 ± 0.05	229.03 ± 15.2	120	Yi et al. (2008)
Enterobacter Hy-402	OA, GA, TA, CA, SA, FuA	7–7.5	4.51 ± 0.02	111.73 ± 8.07	120	Yi et al. (2008)
Rhodococcus erythropolis(CC-BC11)	GA	7–6.8	5.3	186.9	72	Chen et al. (2006)
Bacillus megaterium (CC-BC10)	CA, LA, PA	7–6.8	5.1	270.2	72	Chen et al. (2006)
Arthrobacter sp. (CC-BC03)	CA, LA	7–6.8	4.9	519.7	72	Chen et al. (2006)
A. ureafaciens (CC-BC02)	CA	7–6.8	5.0	316.1	72	Chen et al. (2006)
Serratia marcescens (CC-BC14)	CA, GA, SA, LA	7–6.8	4.9	421.8	72	Chen et al. (2006)
Delftia (CC-BC21)	SA,	7–6.8	4.9	346.1	72	Chen et al. (2006)
Chryseobacterium (CC-BC05)	CA	7–6.8	6.0	298.9.	72	Chen et al. (2006)
Phyllobacterium myrsinacearum (CC-BC19)	GA	7–6.8	5.2	201.2	72	Chen et al. (2006)
Gordonia (CC-BC07)		7–6.8	6.0	31.5	72	Chen et al. (2006)
Enterobacter intermedium	2-KGA	8	2.8	65×10^3	240	Hwangbo et al. (2003)
Bacillus amyloliquefaciens	AA, IBA, IVA, LA, SA	ND	ND	60 (approx)	24	Vazquez et al. (2000)

B. atrophaeus	PA, IBA, IVA, VA, ISA, SA	ND	ND	110 (approx)	24	Vazquez et al. (2000)
B. licheniformis	IBA, VA, LA, FuA, SA	ND	ND	105 (approx)	24	Vazquez et al. (2000)
V. proteolyticus	AA, LA	ND	ND	475 (approx)	24	Vazquez et al. (2000)
P. macerans	IBA, IVA, LA, SA	ND	ND	85 (approx)	24	Vazquez et al. (2000)
X. agilis	IBA, IVA	ND	ND	190 (approx)	24	Vazquez et al. (2000)

GA Gluconic acid, *2-KGA-2α* ketogluconic acid, *LA* Lactic acid, *SA* Succinic acid, *FA* Formic acid, *MA* Malic acid, *CA* Citric acid, *OA* Oxalic acid, *FuA* Fumaric acid, *TA* Tartaric acid, *PA* Propionic acid, *AA* Acetic acid, *IBA* Isobutyric acid, *IVA* Isovaleric acid, *VA* Valeric acid, *ISA* Isocaproic acid, *ND* not determined

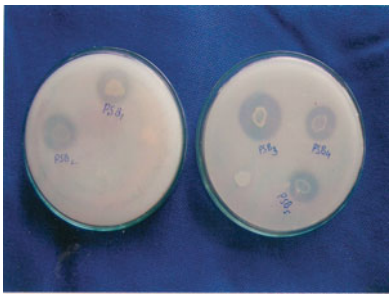


Fig. 10.1 Halo formation by phosphate-solubilizing bacteria on Pikovskaya agar plate

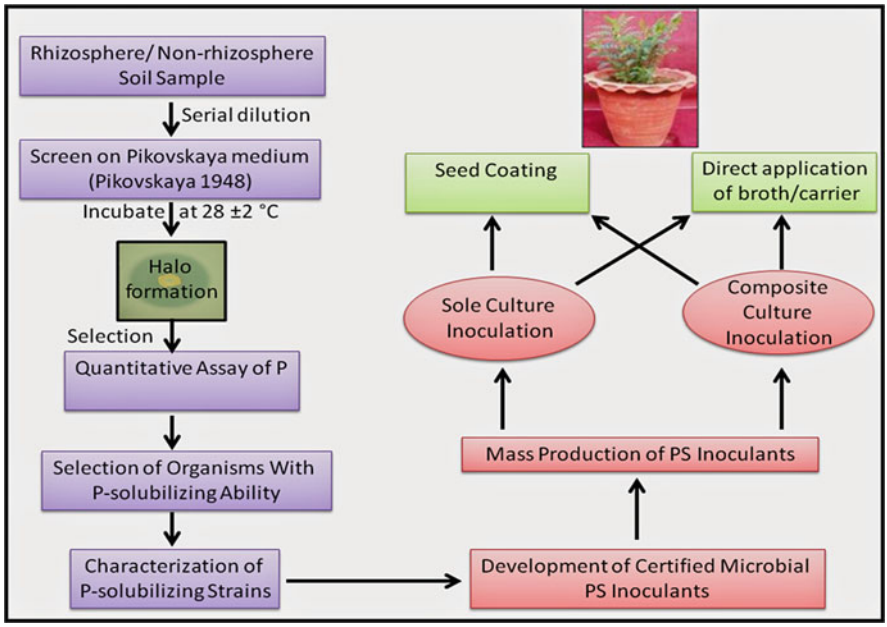


Fig. 10.2 Isolation, selection and formulation of PS bacteria (Modified from Zaidi et al. 2009a, b)

10.3 Functional Diversity Among Phosphate-Solubilizing Bacteria

Principally, P-solubilizing microorganisms in general are widely known to increase the overall performance by providing soluble P to plants in different production systems. However, they also benefit plants by other mechanisms (Fig. 10.3). They exhibit multifunctional properties (Vikram et al. 2007a; Singh et al. 2010; Vassileva et al. 2010; Yadav et al. 2011), for example, they are known to synthesize siderophores (Matthijs et al. 2007; Hamadali et al. 2008; Viruel et al. 2011),

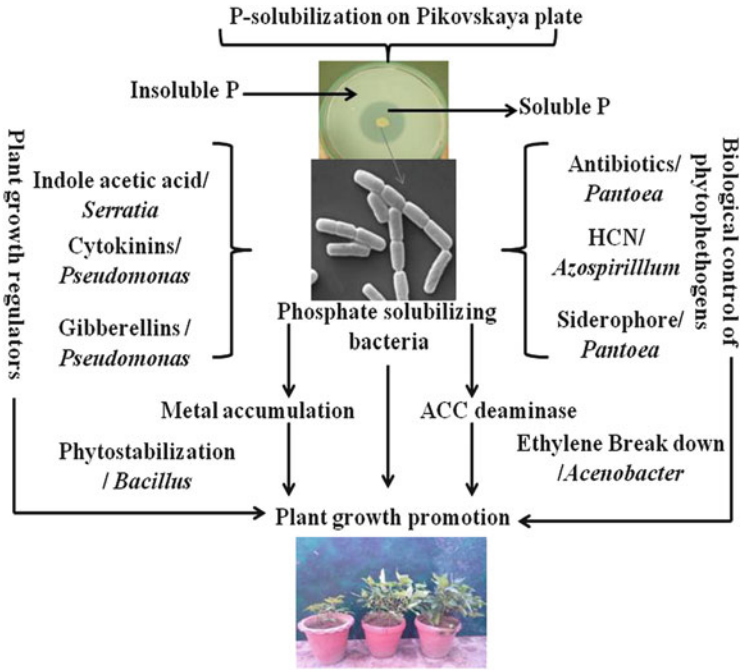


Fig. 10.3 An illustration depicting functional diversity among PS bacteria (Modified from Oves et al. 2009; photograph of PSB, courtesy M. Oves)

indoleacetic acid (IAA), and gibberellic acid (Sattar and Gaur 1987; Souchie et al. 2007; Viruel et al. 2011). Phosphate-solubilizing bacteria such as Gram-negative *P. fluorescens*, *P. aeruginosa*, and *Chromobacterium violaceum* also secretes cyanide, a secondary metabolite which is ecologically important (Siddiqui et al. 2006; Wani et al. 2007a), and gives a selective advantage to the producing strains (Rudrappa et al. 2008). Besides strict P solubilizers, a few genera of rhizobia, for example, *Bradyrhizobium* and *Rhizobium*, have also been found to solubilize P and secrete IAA (Pandey and Maheshwari 2007; Badawi et al. 2011). Interestingly, the ability of PSB, for example, *Serratia marcescens*, to secrete siderophores and cyanide is critical in managing various diseases inflicted by the plant pathogens (Vassilev et al. 2006) and indirectly promoting the plant growth (Badawi et al. 2011). Some of the compounds synthesized by P-solubilizing bacteria with possible effect on plant growth promotion are listed in Table 10.2.

Table 10.2 Examples of plant growth-promoting substances released by phosphate-solubilizing bacteria

Phosphate-solubilizing bacteria	Plant growth-promoting substances	Reference
Pseudomonas fluorescens, *P. putida*	IAA, siderophore, ACC deaminase	Zabihi et al. (2011)
Serratia nematodiphila	IAA, siderophore, HCN	Dastager et al. (2011a)
Pontibacter niistensis	IAA, HCN, ACC deaminase and siderophore	Dastager et al. (2011b)
Klebsiella spp.	IAA, siderophore, HCN, ammonia, EPS	Ahemad and Khan (2011b)
Pantoea agglomerans	IAA	Mishra et al. (2011)
Arthrobacter, *Bacillus*	IAA, antifungal activity, HCN, NH_3	Banerjee et al. (2010)
Paenibacillus alvei, *Bacillus cereus*	IAA, siderophore	
Pantoea	IAA, siderophore, antifungal activity	Taurian et al. (2010)
Pseudomonas aeruginosa	IAA, siderophore, antifungal activity, HCN, EPS	Ahemad and Khan (2010)
P. mendocina, *P. stutzeri* and *P. putida*	IAA, gibberellic acid, trans-zeatin riboside and abscisic acid	Naz and Bano (2010)
Enterobacter aerogenes, *E. cloacae*, *E. asburiae*	IAA, siderophore, HCN	Deepa et al. (2010)
P. alvei	IAA	Hassen and Labuschagne (2010)
Pseudomonas sp.	ACC deaminase, IAA, siderophore	Poonguzhali et al. (2008)
Serratia marcescens	IAA, siderophore, HCN	Selvakumar et al. (2008)
Acinetobacter sp., *Pseudomonas* sp.	ACC deaminase, IAA, antifungal activity, N_2-fixation	Indiragandhi et al. (2008)
Enterobacter sp.	ACC deaminase, IAA, siderophore	Kumar et al. (2008)
Burkholderia	ACC deaminase, IAA, siderophore	Jiang et al. (2008)
Pseudomonas jessenii	ACC deaminase, IAA, siderophore	Rajkumar and Freitas (2008)
P. aeruginosa	ACC deaminase, IAA, siderophore	Ganesan (2008)
Azotobacter sp., *Pseudomonas* sp., *Bacillus* sp.	IAA, siderophore, antifungal activity, ammonia production, HCN	Ahmad et al. (2008)
Fluorescent pseudomonas	IAA, siderophores, HCN, antifungal activity	Shweta et al. (2008)
Pseudomonas vancouverensis	IAA, HCN, siderophore, antifungal activity	Mishra et al. (2008)
Bacillus sp.	IAA, siderophores, ammonia production, HCN	Wani et al. (2007a, 2007b)
Klebsiella oxytoca	IAA, nitrogenase activity	Jha and Kumar (2007)

10.4 Importance of Phosphate-Solubilizing Bacteria in Crop Improvement

Phosphate-solubilizing bacteria among biological materials are one of the most important soil constituents which play a central role in maintaining soil fertility. Consequently, they support plants to grow in a well-directed manner because starting from seed germination until the seed production or maturation stages, plants remain in close proximity with PSB. Considering the vast and varied activities, researchers around the world have either attempted or included the use of this novel group of economically feasible biological materials in agronomic operation for sustainable crop production with variable results (Tables 10.3 and 10.4). The role of PSB in maintaining soil fertility vis-a-vis increasing crop productivity is briefly discussed in the following section.

10.4.1 Phosphate Solubilizers–Legume Interactions: Current Perspective

The sole or composite application of PSB for raising legume production has received considerable attention worldwide (Zaidi et al. 2004; Vikram et al. 2007b; Shaharoona et al. 2008; Collavino et al. 2010). Considering the success of PSB application achieved so far in agronomic practices, we have attempted in the following section to focus on the role of PSB exclusively in the improvement of legumes grown in different agro-ecosystems.

10.4.1.1 Impact of Monoculture of PSB on Legume Improvement

Phosphate-solubilizing fluorescent pseudomonads isolated from the groundnut (*Arachis hypogaea*) rhizosphere, when used as phosphatic biofertilizer against groundnut plants, enhanced germination by 30 % while it increased grain yield by 77 %. To test the biocontrol potential of this PSB strain, a plant pathogen *Macrophomina phaseolina* alone was also included, which, however, decreased the grain yield substantially by 57 %. The increase in the yield of ground following PSB application, however, suggested that *Pseudomonas* strains used in this study had two basic traits (1) pseudomonads acted as biocontrol agent against *M. phaseolina* and (2) that they provided available form of P and consequently enhanced the yield of groundnut (Shweta et al. 2008). Dey et al. (2004) in yet another study observed a significantly higher pod yields, haulm yield, and nodule dry weight in PSB (*P. fluorescens*)-inoculated peanut plants compared to those recorded for un-inoculated plants grown in pots and field trials. The seed bacterization also resulted in higher N and P contents in soil. In addition, the pod yields were increased by 23–26 %; other plant characteristics such as root length, pod number,

Table 10.3 Examples of phosphate solubilizing bacteria used for raising crop production

Phosphate-solubilizing bacteria	Crop tested	Botanical name	Reference
Pantoea agglomerans	Maize	*Zea mays*	Mishra et al. (2011)
Pseudomonas fluorescens, *B. cepacia*, *Aeromonas vaga*	Mung bean	*Vigna radiata*	Jha et al. (2012)
Pseudomonas fluorescens, *P. putida*	Wheat	*Triticum aestivum* L.	Zabihi et al. (2011)
Bacillus	Rice	*Oryza sativa*	Panhwar et al. (2011)
Serratia nematodiphila	black pepper	*Piper nigrum* L	Dastager et al. (2011a)
Pseudomonas chlororaphis, *Bacillus cereus* and *P. fluorescens*	Walnut	*Juglans siggillata* L	Yu et al. (2011)
Enterobacter aerogenes	Kidney bean	*Phaseolus vulgaris*	Collavino et al. (2010)
Pseudomonas, *Bacillus*	Alfalfa	*Medicago sativa* L	Guiñazú et al. (2010)
Pantoea	Peanut	*Arachis hypogaea*	Taurian et al. (2010)
P. aeruginosa	Green gram	*Vigna radiata* (L.) Wilczek	Ahemad and Khan (2010)
E. aerogenes, *E. cloacae*, *E. asburiae*	Cowpea	*Vigna unguiculata* (L.)	Deepa et al. (2010)
Pseudomonas synxantha, *Burkholderia gladioli*, *Enterobacter hormaechei* and *Serratia marcescens*	Chinese aloe	*Aloe barbadensis*	Mamta et al. (2010)
Bacillus megaterium var. *phosphaticum*	Flax	*Linum usitatissimum* L	El-Nagdy et al. (2010)
Bacillus simplex, *B. megaterium*, *B. cereus*, *Paenibacillus alvei*	Tomato, wheat	*Lycopersicon esculentum* Mill.	Hassen and Labuschagne (2010)
B. amyloliquefaciens and *B. pumilus*	Tomato	*Solanum lycopersicum*	Adesemoye et al. (2009)
B. megaterium, *B. subtilis*, *Pseudomonas corrugate*	Rice	*Oryza sativa*	Trivedi et al. (2007)

100-kernel mass, shelling out-turns, and nodule numbers were also increased following bacterial inoculation. Seed treatment with *P. fluorescens* also depressed incidence of soil-borne fungal diseases, like collar rot and charcoal rot of peanut (Bhatia et al. 2008), caused by *A. niger*. While considering the overall improvement in inoculated peanut, it was inferred that the increase was due to (1) the synthesis of IAA, ACC-deaminase and siderophore, and (2) antifungal activity against various fungal pathogens. Similar increase in the biological and chemical characteristics and quality of pea (*Pisum sativum*) and chickpea (*Cicer arietinum*) under both controlled conditions and field environment following P-solubilizing, auxin, ACC deaminase, ammonia, and siderophore-producing strains of *Acinetobacter*

Table 10.4 Examples of sole and composite inoculation effects of phosphate-solubilizing bacteria on biological and chemical characteristics of different plants

Organisms applied				
Sole	Composite	Crop	Plant attributes	Reference
P. agglomerans NBRISRM		Maize, chickpea	Shoot length, leaves, seed, N, P and K uptake	Mishra et al. (2011)
P. chlororaphis, *P. fluorescens*, *B. cereus*		Walnut	Plant height, root and shoot dry weight, P, N and K uptake	Yu et al. (2011)
P. fluorescens, *P. putida*		Wheat	Plant height, tillers, number of grains/spike, 1,000-grain weight, grain and straw yield, N, P and K uptake	Zabihi et al. (2011)
Enterobacter sp		Cowpea	Root and shoot length, dry biomass, seedling length	Deepa et al. (2010)
P. fluorescens, *Pantoea*		Peanut	Plant length, Dry weight, N and P content	Taurian et al. (2010)
P. aeruginosa		Green gram	Plant height, plant dry weight, nodulation, chlorophyll, leghaemoglobin, N and P content, seed yield	Ahemad and Khan (2010)
Citrobacter, *Pantoea*, *Klebsiella and Enterobacter*		Pigeon pea	Shoot P content, dry shoot/root ratio, dry weight	Patel et al. (2010)
Bacillus sp.		Chickpea	Root and shoot length, nodulation, dry weight	Wani and Khan (2010)
Burkholderia gladioli, *Enterobacter aerogenes* and *Serratia marcescens*		*Stevia rebaudiana*	Shoot and root length, leaf and stem dry weight, shoot biomass and glycoside contents	Mamta et al. (2010)
A. calcoaceticus SE370		Cucumber, Chinese cabbage and Crown daisy	Shoot length, plant height, dry weight	Kang et al (2009)
Pseudomonas aeruginosa	*Sinorhizobium meliloti*	Mustard	Root and shoot fresh weight and dry weight, yield	Maheshwari et al. (2011)
Pontibacter niistensis		Cowpea	Root and shoot weight, dry weight, seedling growth	Dastager et al. (2011b)

(continued)

Table 10.4 (continued)

Organisms applied		Crop	Plant attributes	Reference
Sole	Composite			
P. fluorescens	*Burkholderia cepcia*, *Aeromonas vaga*	Mung bean	Root and shoot length, dry weight, leaf area, photosynthetic yield, P content in leaf	Jha et al. (2012)
Pseudomonas	*Bacillus*	Strawberry	Fruit yield and weight, vit. C, reducing sugar	Esitken et al (2010)
Bacillus, *Pseudomonas*	*Sinorhizobium meliloti*	Alfalfa	Root and shoot dry weight, root length, N content in shoot	Guiñazú et al (2010)
Paenibacillus alvei	*Bacillus simplex*, *Bacillus cereus*	Wheat	Shoot and root biomass and total root length	Hassen and Labuschagne (2010)
Bacillus megaterium	*Bacillus simplex*, *Bacillus cereus*	Tomato	Shoot and root biomass and total root length	Hassen and Labuschagne (2010)
P. putida	*B. japonicum*	Soybean	Root and shoot dry weight, nodulation	Rosas et al. (2006)
P. putida	*S. meliloti*	Alfalfa	Root and shoot dry weight, nodulation	Rosas et al. (2006)

rhizosphaerae and *Mesorhizobium mediterraneum* (PECA21) has been reported (Peix et al. 2001; Gull et al. 2004; Gulati et al. 2009). Likewise, inoculation of green gram [*Vigna radiata* (L.) Wilczek] seeds with PSB demonstrated an extensive nodulation and increased shoot dry matter and total dry matter, P-content, and P uptake in green gram plants 45 days after sowing relative to either rock phosphate (RP) or single super phosphate (SSP) application (Vikram and Hamzehzarghani 2008).

10.4.1.2 Synergistic Effect of Phosphate-Solubilizing Bacteria with Other PGPR/AM-Fungi

Even though P is available in plenty in many soils, application of phosphatic fertilizers is essentially required to cover up losses caused due to P fixation by soil constituents and phosphate runoff in P-loaded soil (Goldstein 1986; Del Campillo et al. 1999). On the contrary, the use of phosphate solubilizers to provide exclusively P to plants and also along with other compatible PGPR for increasing quality of crops have been studied intensively (Zaidi and Khan 2006; Afzal et al. 2010; Zaidi et al. 2010). The beneficial microbes involved in P solubilization in addition to P can also enhance plant growth by improving the efficiency of BNF, by

accelerating the availability of other trace elements, and by production of phytohormones (Wani et al. 2007a). Accordingly, increase in yield of various legumes have been observed following seed or soil inoculation with N_2-fixing organisms, PSB, or PSB when used with nodule bacteria (Maheshwari et al. 2011) and AM fungus (Zaidi and Khan 2006; Khan and Zaidi 2007).

Like other PGPR, PSB within soil forms a close relationship with microbes and play important role in improving crop yields additively or synergistically. For example, the composite application of N_2-fixing *Sinorhizobium meliloti* and P-solubilizing bacterium *Bacillus* sp. M7c and *Pseudomonas* sp. FM7d significantly enhanced the N-fixing efficiency of alfalfa plants. Of these, *Pseudomonas* sp. FM7d resulted in enhanced dry matters production in plant organs such as root and shoot, length and surface area of roots, number and symbiotic properties of alfalfa (*Medicago sativa* L.) plants (Guiñazú et al. 2010). It was concluded from this study that *S. meliloti* B399 and *Bacillus* sp. M7c proved effective for developing mixed phosphatic inoculants. In a similar experiment, Bansal (2009) observed a dramatic increase in nodulation and grain yield of mung bean treated simultaneously with *Rhizobium*, PGPR, and PSB. The tripartite treatments were followed by dual inoculation of *Rhizobium* with PGPR and *Rhizobium* alone in terms of nodulation and grain yield increases in *kharif* seasons. The pooled analysis also gave significantly highest number of nodules/plant (21/plant), dry weight of nodules/plant (87.7 mg), and grain yield (12.9 q/ha) following combined inoculation of *Rhizobium*, PGPR, and PSB. The increase in yield (12 q/ha) was at par with *Rhizobium* used with PGPR. In a follow-up study, Dutta and Bandyopadhyay (2009), while conducting a field experiment during the winter seasons, observed that P and biofertilizers, phosphobacterin (*Pseudomonas striata*) and co-inoculation of *Rhizobium* with phosphobacterin, when applied together, enhanced the early vegetative growth, symbiotic properties like nodule production and excessive synthesis of leghaemoglobin in nodules, nitrogenase activity (NA), and yield components such as seed yields, harvest index (HI), and P uptake by chickpea cultivar Mahamaya-2 plants grown in entisol (laterite soil) under rainfed conditions. Of the various combination treatments, seed inoculation of phosphobacterin with *Rhizobium* was significantly better than that of rest of the treatments.

When P (26.2 kg/ha) was also added to the mixture of *Rhizobium* and phosphobacterin, the biological and chemical properties of chickpeas were further improved relative to other levels of P used with biofertilizer. In other parts of the world like Erzurum (29°55′N and 41°16′E with an altitude of 1,950 m), Turkey, a similar investigation was carried out by Elkoca et al. (2008) where they used *Rhizobium*, N_2-fixing *Bacillus subtilis* (OSU-142), and P-solubilizing *B. megaterium* (M-3) to inoculate chickpea plants. Under the field trials, single, dual, and triple inoculations with *Rhizobium*, OSU-142, and M-3 significantly increased plant height, shoot, root, and nodule dry weight, N%, chlorophyll content, pod numbers, seed yield, total biomass yield, and seed protein content compared with the control treatment, equal to or higher than N, P, and NP treatments. Interestingly, the mixture containing *Rhizobium* was comparatively better in

terms of nodulation than the sole application of *Rhizobium*. Increase in the seed yield under different inoculation treatments ranged between 18 % (*Rhizobium*) and 31 % (*Rhizobium* with OSU-142 and M-3) over the control whereas N, P, and NP applications corresponds to an increase of 27 %, 11 %, and 33 %, respectively. Dual and triple inoculations in general were more effective than other treatments which could probably be due to P activity of *Enterobacter*.

Coinoculation with rhizosphere PSM and AMF of soils with high phosphate-fixation capacity may overcome the limitation mentioned on the effectiveness of PSM in enhancing plant P uptake. First, mycorrhizal plants can release higher amounts of carbonaceous substance in to rhizosphere (Linderman 1988; Rambelli 1973) than non-mycorrhizal plants. Rhizosphere PSM can use these carbon substrates for their metabolic process, which are responsible for organic acid production in the rhizosphere and/or protein excretion (Azcon and Barea 1996). Second, the extensive mycorrhizal network formed around roots can efficiently take up P released by PSM thus minimizing its re-fixation. Barea et al. (2002) reported that the combined inoculation with PSB, mycorrhizal fungi, and *Rhizobium* increased the P uptake by several legumes fertilized with rock phosphate. Mycorrhizal interaction with PSM has been found beneficial and has shown dramatic improvement in plant P uptake in highly weathered soil in contrast to the results obtained for less-weathered soils. Osorio (2011) in his experiments while using PSM alone and in combination with mycorrhizal fungi in order to assess their impact on growth of *Leucaena leucocephala* found that the overall growth of test plant was highly dependent on the nature of P sorption capacity of soil. The sole application of PSM significantly increased plant growth of *Leucaena* in low P sorption soil, while in high P sorption soil mixture of PSM and AMF was significantly greater than single application of PSM. This finding suggested that the effectiveness of PSM in increasing plant P uptake and growth is controlled by the P sorption capacity. In soils with low P sorption ($P_{0.3} < 100$) capacity, though PSM inoculation alone can increase plant growth but in soils with medium and high P sorption ($100 < P_{0.2} < 500 < P_{0.2}$), PSM alone is less effective or even ineffective, their effectiveness depends on the presence of mycorrhizal association.

In other study, Osorio (2008) observed that PSM could desorb P from mineral and soil samples, but this was controlled by the P desorption (higher P desorption at low $P_{0.2}$ value). For minerals, the magnitude on which P desorbed was in the order montmorillonite > kaolinite > goethite > allophone (null description) and consequently for soils the order was mollisol > oxisol > ultisol > andisol. The amount of P desorbed by the PSM was higher when the minerals or soils had higher levels of sorbed P; this is when saturation of sorption sites was higher.

In addition to the PGPR, PSB has been found to form symbiotic relationship with AM fungi (Wang et al. 2011). For example, Toro et al. (2008) conducted an experiment to test the efficacy of composite microbial inoculations such as a wild-type (WT) *R. meliloti* strain, its genetically modified (GM) derivative, the AM fungus *G. mosseae* (Nicol. and Gerd) Gerd and Trappe, and a PSB *Enterobacter* sp. and rock phosphate (RP) on N and P acquisition by alfalfa plants. Interestingly, all the microbial cultures were established well within root tissues

and/or in the alfalfa rhizosphere and had no antagonistic effect towards each other. Also, the population of PSB was stimulated following both AM colonization and RP application and GM *Rhizobium* application. Subsequently, there was tremendous improvement in N and P accumulation in alfalfa plants following composite microbial inoculations. Even though the *Enterobacter* application had no noticeable effect on N or P accumulation in soil treated with RP, it showed an obvious effect in the non-RP-amended controls. In addition, ^{15}N:^{14}N ratio in plant shoots indicated enhanced N_2 fixation rates in *Rhizobium*-inoculated AM plants, compared to those obtained by the same *Rhizobium* strain in non-mycorrhizal plants. Regardless of the *Rhizobium* strain and of whether or not RP was added, AM-inoculated plants showed a lower specific activity (^{32}P:^{31}P) than did their comparable non-mycorrhizal controls suggesting that the plant was using otherwise unavailable P sources. The P-solubilizing, AM-associated, microbiota could in fact release P ions, either from the added RP or from the indigenous "less-available" P. Additionally, the proportion of plant P derived either from the labeled soil P (labile P pool) or from RP was similar for AM-inoculated and non-mycorrhizal controls (without *Enterobacter* inoculation) for each *Rhizobium* strain, but the total P uptake, regardless of the P source, was far higher in AM plants which could probably be due to P mobilization by AM fungi.

10.4.2 Inoculation Effects of Phosphate Solubilizers on Cereal Crops

The use of PSB in agricultural practices dates back to 1950s when some Russian and European scientists applied *Megaterium viphosphateum*, which later on was identified as *Bacillus megaterium* var. *phosphaticum*. The preparation of this bacterium was subsequently called as phosphobacterin (Cooper 1959; Menkina 1963), and when this was used, increased crop yields from 0 % to 70 % in Soviet soils. However, similar experiments conducted in USA failed to produce any significant effect (Smith et al. 1961). Despite conflicting reports on the performance of PSB in variable agro-ecosystem against a multitude of crops (Yarzábal 2010), they have since been applied and have shown promising results in some parts of the world (Chesti and Ali 2007; Baig et al. 2011). For example, in a trial conducted under both pot and field environments, the biomass and total P of winter wheat (*Triticum aestivum*) were significantly increased following sole application of *Phosphobacterium* strain 9320-SD. However, there was no significant difference in height of the test plants (Chen et al. 2006). Similarly, PSB isolated from stressed environment such as cold temperature region contained *Serratia marcescens* with inherent PGP traits such as IAA, HCN, and siderophore production profoundly enhanced the plant biomass and nutrient uptake of wheat seedlings when grown in cold environment (Selvakumar et al. 2008). In a follow-up study, wheat plants inoculated with ACC deaminase-secreting PSB, *P. fluorescens* and *P. fluorescens*

biotype F, had higher growth, yield, and nutrient use efficiency, when grown in soil treated simultaneously with varying levels of three major nutrients like N, P, and K (at 0 %, 25 %, 50 %, 75 %, and 100 % of recommended doses). However, the overall growth of inoculated wheat plants decreased both under pot and field trials with increasing concentration of synthetic fertilizers.

Hence, in most of the cases, significant negative linear correlations were recorded between percentage increases in growth and yield parameters of even inoculated wheat plants. The decline in growth and yield of bacterized wheat plants when grown with increasing chemical fertilizers, however, raised certain questions. For example, do the rates of fertilizers greater than recommended ones have any direct impact on composition and functional activities of bacteria or excessive rates have any inhibitory effect on plants metabolism? In this context, it is speculated that low fertilizer application causes reduction in the ACC deaminase activity of PS strains and thereby leads to reduction in the synthesis of stress (nutrient)-induced inhibitory levels of ethylene in the roots through ACC hydrolysis into NH_3 and α-ketobutyrate. Based on this finding, the study suggested that *Pseudomonads* could be used in combination with appropriate doses of fertilizers for better plant growth and savings of fertilizers (Shaharoona et al. 2008) as also observed by Kumar et al. (2009) and Maheshwari et al. (2011). Such increase in cereal production following PSB such as *P. fluorescens* 153, *P. fluorescens* 169, *P. putida* 4, and *P. putida* 108 application has been attributed to both PSA of PSB and their ability to synthesize growth-promoting substances (such as ACC deaminase and IAA-like products) in natural soil ecosystem (Zabihi et al. 2011). Interestingly, *P. putida* 108 among the bacterial cultures displayed enhanced P uptake (96 % and 80 %) and grain yield (58 % and 37 %) in wheat under greenhouse and field conditions, respectively. Even though this finding suggested that *Pseudomonas* sp. could serve as an alternative to expensive P application in wheat production system, the better results can be achieved when a compatible bioinoculant is added as mixture with 50 % (25 kg/ha P_2O_5) P fertilization. In a recent follow-up study, Abbasi et al. (2011) isolated eight PGPR strains and assessed their morphological and cultural characteristics, PSA and their ability to secrete IAA. Invariably all strains produced IAA (ranging from 5.5 to 31.0 mg/ml) while only four of them showed P-solubilizing traits. Subsequently, strains WPR-32, WPR-42, and WPR-51 grouped under PGPR category were used both as single and coculture along with two levels (50 and 100 kg N/ha) of N to evaluate their effect against wheat under greenhouse conditions. As expected, application of PGPR resulted in significant increase in plant height (25 %), shoot fresh weight (45 %), and shoot dry weight (86 %), while it was 27 %, 102 %, and 76 % increase in root length, root fresh and dry weight, respectively, over uninoculated plants. In addition, the number of tillers per plant, 1,000-grain weight, and grain yield were enhanced by 23 %, 48 %, and 59 %, respectively, over control. The nutrient (N and P) uptake by plant organs like shoot was increased threefolds, while K uptake was increased by 58 % following PGPR application.

However, the growth, yields, and nutrient uptake were increased even further when bacterial cultures were used together with varying levels of N. Apart from the

direct effect of PGPR on wheat plants, the concentration of NO^{-3}, N, and available P in soil also increased with PGPR application. Moreover, of the varying treatments, mixed bacterial cultures showed better efficiency than the individual ones suggesting that there is no reason to doubt why application of PGPR with N fertilizer cannot increase N contents and N uptake by plants. Also, application of PGPR even with low fertilizer rates could be a more viable option for achieving optimum benefits while reducing the dependence on chemical inputs (Kumar et al. 2009). An interactive and positive effect of PSB, N_2 fixer, and AM fungi on plant vigor, nutrient uptake, and yield in wheat plants was observed following composite application of *Pseudomonas striata* + *Azotobacter chroococcum* + *Glomus fasciculatum*. The available P contents in soil enhanced significantly due to triple inoculation of *A. chroococcum*, *P. striata*, and *G. fasciculatum*. The residual N content of soil, however, did not change appreciably even among the treatments. The density of *A. chroococcum*, PSB, percentage root infection, and spore density of the AM fungus in inoculated treatments increased at 80 days of wheat growth (Zaidi and Khan 2005).

Inoculation of *Burkholderia vietnamiensis* to rice (*Oryza sativa*) cultivars in two pot and four field trials at different locations of Vietnam showed an enhancement of 33 %, 57 %, 30 %, and 13 % in shoot weight, root weight, leaf area, and number of tillers/hill, respectively, compared to non-inoculated plants. In other study, strain of *Rhodobacter capsulatus* significantly increased the plant dry weight, number of productive tillers, grain and straw yields of rice var. Giza 176, grown in pot treated with different levels of N fertilizer compared to non-inoculated plants (Elbadry et al. 1999). The results of this study concluded that N fertilizer could be saved up to 50 % while applying bacterial fertilizers. Similarly, an increase of 41 %, 12 %, 11.2–20 %, and 18.7 % in root weight, straw yield, grain yield, and total biomass, respectively, due to PGPR inoculation over non-inoculated rice is reported (Sherchand 2000; Mehnaz et al. 1998). The liquid culture (for pot experiments) or carrier-based preparation (for field trials) of three bacterial species, such as *Bacillus megaterium*, *B. subtilis*, and *Pseudomonas corrugata*, isolated from temperate locations in the Indian Himalayan region and exhibiting phosphate-solubilizing activity (PSA) in the order *P. corrugata* > *B. megaterium* > *B. subtilis*, when tested caused a dramatic increase in overall performance of rice. While comparing the effect of three cultures, *B. subtilis* had the most promising effect and increased the grain yield by 1.7- and 1.6-fold in pot and field trials, respectively (Trivedi et al. 2007).

Similar variable effects of PSB on other cereals used either alone or in combination with other chemical fertilizers have been reported (Panhwar et al. 2011; Yazdani et al. 2011). For example, like wheat and cereals, there has also been a substantial increase in the biomass of maize (*Zea mays*) plants inoculated with *S. marcescens* (EB 67) and *Pseudomonas* sp. (CDB 35) (Hameeda et al. 2008). In this experiment, strain EB 67 enhanced the dry matter accumulation by 99 % while it was 94 % by strain CDB 35. Grain yield of inoculated maize increased by 85 % and 64 %, following EB 67 and CDB 35 application, respectively. When applied as mixture with arbuscular mycorrhizal (AM) fungi *Glomus intraradices*, the PSB

Pseudomonas fluorescens (Pf) had a positive impact on plant growth, nutrient uptake, grain yield, and yield components in maize plants. Composite inoculation of the two cultures significantly increased grain yield, yield components, harvest index, grain N and P, soil available P, and root colonization percentage under water stress conditions. However, some of the assayed characteristics under well-watered conditions were nonsignificantly higher in chemical fertilizer treatment compared to those observed for dual inoculation treatments. However, the effect of sole application of *P. fluorescens* (Pf) was poor relative to the composite application of AM fungus with PSB or single application of AM fungi. The measured parameters of inoculated plants were in general higher than un-inoculated plants under water deficit stress conditions. In addition, the characteristics determined for coinoculated plants grown under severe water-stressed conditions were significantly lower than coinoculated plants grown under well-watered and moderate-stressed conditions. This finding suggested that PSB can interact positively with other organism like AM fungi as observed in this study and can be used to facilitate plant growth and P uptake by maize plants, leading to plant tolerance improving under water deficit stress conditions (Ehteshami et al. 2007). In a recent study, Rajapaksha et al. (2011) conducted experiments under both pot and field environment to assess the substitutability of triple superphosphate (TSP) by a P fertilizer mixture (PFM) involving TSP, RP, and PSB inoculants for wetland rice. For these studies, six single and two dual inoculants were formulated with *Enterobacter gergoviae* and five *Bacillus* species. In pot trials, the mixture of *E. gergoviae* and *B. mycoides* and the sole application of *B. subtilis* enhanced yields by 32 % and 25 %, respectively, relative to single application of TSP. The results observed in pot trials were validated under field environment where dual culture of *E. gergoviae* with *B. subtilis* and *E. gergoviae* with *B. pumilus* augmented grain yield by 22–27 % compared to TSP application alone (574 gm^{-2}). Overall, it was suggested that about 50 % of TSP could be saved when RP is applied with *E. gergoviae*, *B. pumilus*, and *B. subtilis*, as seed inoculant for raising the productivity of rice both under pot and field conditions.

10.5 Conclusion and Future Prospects

Considering the documented data and literature presented in this chapter, it seems feasible that the soil nutrient pool especially P using renewable resources like microbes can be increased by (1) careful management of existing microbial populations to optimize their competence to solubilize/mobilize P and (2) applying microbial inoculants especially designed/developed to provide P to plants. Despite repeated claims of making P available to plants or enhancing soil P by PSB, limited success in terms of their wide and regular application in agronomic practices has, however, been achieved so far. The reason for this low popularity of microphos could be both unawareness about the performance of PSB among practitioners or their varying activity under natural but fluctuating environments. Therefore, to

make PSM more attractive and cost-effective measures for increasing crop productivity in different agro-ecological regions, we need to have a detailed and meaningful understanding of microbial interactions occurring in soil environment.

Moreover, how soil and farm management practices influence the processes mediated by PSM needs to be elucidated. In this context, molecular tools and metagenomic approaches have provided some insight to uncover the structure and functions of PSM. Genetic manipulation of some PSB and plants for important features such as P mobilization or growth promotion besides generating specific mutants with traits such as organic anion release in *Pseudomonas* spp. could play pivotal roles in deciphering mechanistic basis and evaluating their contribution to increased P availability in soil. Even some success has been achieved here and there by using molecular tools; there is greater need to develop area-specific microphos which may be suitable for application in any specific region. If developed with suitable multiple traits, such microphos can be applied back into the same environment from where they originated. This approach is, therefore, likely to reduce the impact of fluctuating environment on the performance of PSM when used for raising the production of different crops grown in many variable regions across the world.

References

Abbasi MK, Sharif S, Kazmi M, Sultan T, Aslam M (2011) Isolation of plant growth promoting rhizobacteria from wheat rhizosphere and their effect on improving growth, yield and nutrient uptake of plants. Plant Biosyst 145:159–168

Abd-Alla MH (1994) Phosphatases and the utilization of organic phosphorus by *Rhizobium leguminosarum* biovar viceae. Lett Appl Microbiol 18:294–296

Abril A, Zurdo-Pineiro JL, Peix A, Rivas R, Velazquez E (2007) Solubilization of phosphate by a strain of *Rhizobium leguminosarum* bv trifolii isolated from *Phaseolus vulgaris* in El Chaco Arido soil (Argentina). In: Velazquez E, Rodrıguez-Barrueco C (eds) First international meeting on microbial phosphate solubilization. Springer, Berlin, pp 135–138

Adesemoye AO, Kloepper JW (2009) Plant-microbes interactions in enhanced fertilizer-use efficiency. Appl Microbiol Biotechnol 85:1–12

Adesemoye AO, Torbert HA, Kloepper JW (2009) Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58:921–929

Afzal A, Bano A, Fatima M (2010) Higher soybean yield by inoculation with N-fixing and P-solubilizing bacteria. Agron Sustain Dev 30:487–495

Ahmad F, Ahmad I, Khan MS (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res 163:173–181

Ahemad M, Khan MS (2010) Phosphate-solubilizing and plant-growth-promoting *Pseudomonas aeruginosa* PS1 improves green gram performance in quizalafop-p-ethyl and clodinafop amended soil. Arch Environ Contam Toxicol 58:361–372

Ahemad M, Khan MS (2011a) *Pseudomonas aeruginosa* strain PS1 enhances growth parameters of green gram [*Vigna radiata* (L.) Wilczek] in insecticide-stressed soils. J Pestic Sci 84:123–131

Ahemad M, Khan MS (2011b) Toxicological effects of selective herbicides on plant growth promoting activities of phosphate solubilizing *Klebsiella* sp. strain PS19. Curr Microbiol 62:532–538

Ahemad M, Khan MS (2012) Evaluation of plant-growth-promoting activities of rhizobacterium *Pseudomonas putida* under herbicide stress. Ann Microbiol. doi:10.1007/s13213-011-0407-2

Ahemad M, Zaidi A, Khan MS, Oves M (2009) Biological importance of phosphorus and phosphate solubilizing microbes. In: Khan MS, Zaidi A (eds) Phosphate solubilizing microbes for crop improvement. Nova Science, New York, pp 1–14

Alikhani HA, Saleh-Rastin N, Antoun H (2006) Phosphate solubilization activity of rhizobia native to Iranian soils. Plant Soil 287:35–41

Altomare C, Norvell AW, Bjorkman T, Harman GE (1999) Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295–22. Appl Environ Microbiol 65:2926–2933

Asea PEA, Kucey RMN, Stewart JWB (1988) Inorganic phosphate solubilization by two *Penicillium* species in solution culture and soil. Soil Biol Biochem 20:459–464

Azcon C, Barea JM (1996) Interactions of arbuscular mycorrhizal with rhizosphere microorganisms. In: Guerrero E (ed) Mycorrhiza. Biological soil resource. FEN, Bogota, pp 47–68

Badawi FSF, Biomy AMM, Desoky AH (2011) Peanut plant growth and yield as influenced by co-inoculation with *Bradyrhizobium* and some rhizo-microorganisms under sandy loam soil conditions. Ann Agric Sci 56:17–25

Baig KS, Arshad M, Shaharoona B, Khalid A, Ahmed I (2011) Comparative effectiveness of *Bacillus* spp. possessing either dual or single growth-promoting traits for improving phosphorus uptake, growth and yield of wheat (*Triticum aestivum* L.). Ann Microbiol. doi:10.1007/s13213-011-0352-0

Banerjee S, Palit R, Sengupta C, Standing D (2010) Stress induced phosphate solubilization by *Arthrobacter* sp. and *Bacillus* sp. isolated from tomato rhizosphere. AJCS 4:378–383

Bansal RK (2009) Synergistic effect of *Rhizobium*, PSB and PGPR on nodulation and grain yield of mung bean. J Food Legumes 22:37–39

Barea JM, Toro M, Orozco M, Campos E, Azcon R (2002) The application of isotopic (^{32}P and ^{15}N) dilution technique to evaluate the interactive effect of phosphate solubilizing rhizobacteria, mycorrhizal fungi and *Rhizobium* to improve the agronomic efficiency of rock phosphate for legume crops. Nutr Cycling Agroecosyst 63:35–42

Behbahani M (2010) Investigation of biological behavior and colonization ability of Iranian indigenous phosphate solubilizing bacteria. Sci Hortic 124:93–399

Bhatia S, Maheshwari DK, Dubey RC, Arora DS, Bajpai VK, Kang SC (2008) Beneficial effect of fluorescent Pseudomonads on seed germination, growth promotion and suppression of charcoal rot in ground nut (*Arachis hypogea* L.). J Microbiol Biotechnol 18:1578–1583

Bishop ML, Chang AC, Lee RWK (1994) Enzymatic mineralization of organic phosphorus in a volcanic soil in Chile. Soil Sci 157:238–243

Bojinova D, Velkova R, Ivanova R (2008) Solubilization of Morocco phosphorite by *Aspergillus niger*. Bioresour Technol 99:7348–7353

Buch A, Archana G, Kumar GN (2008) Metabolic channelling of glucose towards gluconate in phosphate-solubilizing *Pseudomonas aeruginosa* P4 under phosphorus deficiency. Res Microbiol 159:635–642

Chandra S, Choure K, Dubey RC, Maheshwari DK (2007) Rhizosphere competent *Mesorhizobium loti* MP6 induces root hair curling, inhibits *Sclerotinia sclerotiorum* and enhances growth of Indian mustard (*Brassica campestris*). Braz J Microbiol 38:124–130

Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl Soil Ecol 34:33–41

Chen Z, Ma S, Liu L (2008) Studies on phosphorus solubilizing activity of a strain of phosphobacteria isolated from chestnut type soil in China. Bioresour Technol 99:6702–6707

Chesti MH, Ali T (2007) Effect of integrated phosphorus management on yield, nutrient availability and phosphorus transformation in green gram. J Res 6:243–248

Collavino MM, Sansberro PA, Mroginski LA, Aguilar OM (2010) Comparison of *in vitro* solubilization activity of diverse phosphate-solubilizing bacteria native to acid soil and their ability to promote *Phaseolus vulgaris* growth. Biol Fertil Soils 46:727–738

Cooper R (1959) Bacterial fertilizers in Soviet Union. Soil Fertil 22:327–333

Cunningham J, Kuiack C (1992) Production of citric and oxalic acids and solubilization of calcium phosphate by *Penicillium bilaii*. Appl Environ Microbiol 58:1451–1458

Dastager SG, Deepa CK, Pandey A (2011a) Potential plant growth-promoting activity of *Serratia nematodiphila* NII-0928 on black pepper (*Piper nigrum* L.). World J Microbiol Biotechnol 27:259–265

Dastager SG, Deepa CK, Pandey A (2011b) Plant growth promoting potential of *Pontibacter niistensis* in cowpea (*Vigna unguiculata* (L.) Walp.). Appl Soil Ecol 49:250–255

Deepa CK, Dastager SG, Pandey A (2010) Isolation and characterization of plant growth promoting bacteria from non-rhizospheric soil and their effect on cowpea (*Vigna unguiculata* (L.) Walp.) seedling growth. World J Microbiol Biotechnol 26:1233–1240

Del Campillo SE, Van der Zee SE, Torrent J (1999) Modelling long-term phosphorus leaching and changes in phosphorus fertility in excessively fertilized acid sandy soils. Eur J Soil Sci 50:391–399

Dey R, Pal KK, Bhatt DM, Chauhan SM (2004) Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth promoting rhizobacteria. Microbiol Res 159:371–394

Dugan PR, Lundgren DG (1965) Energy supply for chemoautotroph *Ferrobacillus ferrooxidans*. Can J Biochem 45:1547–1556

Dutta D, Bandyopadhyay P (2009) Performance of chickpea (*Cicer arietinum* L.) to application of phosphorus and bio-fertilizer in laterite soil. Arch Agron Soil Sci 55:147–155

Ehteshami SM, Aghaalikhani M, Khavazi K, Chaichi MR (2007) Effect of phosphate solubilizing microorganisms on quantitative and qualitative characteristics of maize (*Zea mays* L.) under water deficit stress. Pak J Biol Sci 10:3585–3591

El-Azouni IM (2008) Effect of phosphate solubilizing fungi on growth and nutrient uptake of soybean (*Glycine max* L.) plants. J Appl Sci Res 4:592–598

El-Nagdy GA, Nassar DMA, El-Kady EA, El-Yamanee GSA (2010) Response of flax plant (*Linum usitatissimum* L.) to treatments with mineral and bio-fertilizers from nitrogen and phosphorus. J Am Sci 6:207–217

Elbadry M, Gamal-Eldin H, Elbana K (1999) Effects of *Rhodobacter capsulatus* inoculation in combination with graded levels of nitrogen fertilizer on growth and yield of rice in pots and lysimeter experiments. World J Microbiol Biotechnol 15:393–395

Elkoca E, Kantar F, Sahin F (2008) Influence of nitrogen fixing and phosphate solubilizing bacteria on nodulation, plant growth and yield of chickpea. J Plant Nutr 33:157–171

Esitken A, Yildiz HE, Ercisli S, Donmez MF, Turan M, Gunes A (2010) Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry. Sci Hortic 124:62–66

Fernandez LA, Zalba P, Gómez MA, Sagardoy MA (2007) Phosphate-solubilization activity of bacterial strains in soil and their effect on soybean growth under greenhouse conditions. Biol Fertil Soils 43:805–809

Ganesan V (2008) Rhizoremediation of cadmium soil using a cadmium-resistant plant growth promoting rhizopseudomonad. Curr Microbiol 56:403–407

Goldstein AH (1986) Bacterial solubilization of mineral phosphates: historical perspective and future prospects. Am J Altern Agric 1:51–57

Goldstein AH (1994) Involvement of the quinoprotein glucose dehydrogenase in the solubilization of exogenous mineral phosphates by Gram negative bacteria. In: Torriani-Gorni A, Yagil E, Silver S (eds) Phosphate in microorganisms: cellular and molecular biology. ASM Press, Washington, DC, pp 197–203

Guiñazú LB, Andrés JA, Florencia MDP, Pistorio M, Rosas SB (2010) Response of alfalfa (*Medicago sativa* L.) to single and mixed inoculation with phosphate-solubilizing bacteria and *Sinorhizobium meliloti*. Biol Fertil Soils 46:185–190

Gulati A, Vyas P, Rahi P, Kasana RC (2009) Plant growth-promoting and rhizosphere-competent *Acinetobacter rhizosphaerae* strain BIHB 723 from the cold deserts of the Himalayas. Curr Microbiol 58:371–377

Gull FY, Hafeez I, Saleem M, Malik KA (2004) Phosphorus uptake and growth promotion of chickpea by co-inoculation of mineral phosphate solubilizing bacteria and a mixed rhizobial culture. Aust J Exp Agric 44:623–628

Hamadali H, Hafidi M, Virolle MJ, Ouhdouch Y (2008) Rock phosphate solubilizing actinomycetes: screening for plant growth promoting activities. World J Microbiol Biotechnol 24:2565–2575

Hameeda B, Harini G, Rupela OP, Wani SP, Reddy G (2008) Growth promotion of maize by phosphate solubilizing bacteria isolated from composts and macrofauna. Microbiol Res 163:234–242

Hassen AI, Labuschagne N (2010) Root colonization and growth enhancement in wheat and tomato by rhizobacteria isolated from the rhizoplane of grasses. World J Microbiol Biotechnol 26:1837–1846

Hui L, Xiao-Qin W, Jia-Hong R, Jian-Ren Y (2011) Isolation and identification of phosphobacteria in poplar rhizosphere from different regions of china. Pedosphere 21:90–97

Hwangbo H, Park RD, Kim YW, Rim YS, Park KH, Kim TH, Suh JS, Kim KY (2003) 2-Ketogluconic acid production and phosphate solubilization by *Enterobacter intermedium*. Curr Microbiol 47:87–92

Illmer P, Schineer F (1992) Solubilization of insoluble phosphates by microorganisms isolated from forest soils. Soil Biol Biochem 24:389–395

Illmer P, Schinner F (1995) Solubilization of inorganic calcium phosphates-solubilization mechanisms soil. Soil Biol Biochem 27:257–263

Indiragandhi P, Anandham R, Madhaiyan M, Sa TM (2008) Characterization of plant growth promoting traits of bacteria isolated from larval guts of diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae). Curr Microbiol 56:327–333

Ivanova R, Bojinova D, Nedialkova K (2006) Rock phosphate solubilization by soil bacteria. J Univ Chem Technol Metallurgy 41:297–302

Jha PN, Kumar A (2007) Endophytic colonization of *Typha australis* by a plant growth-promoting bacterium *Klebsiella oxytoca* strain GR-3. J Appl Microbiol 103:1311–1320

Jha A, Sharma D, Saxena J (2012) Effect of single and dual phosphate-solubilizing bacterial strain inoculations on overall growth of mung bean plants. Arch Agro Soil Sci 58:967–981

Jiang C, Sheng X, Qian M, Wang Q (2008) Isolation and characterization of a heavy metal resistant *Burkholderia* sp. from heavy metal-contaminated paddy field soil and its potential in promoting plant growth and heavy metal accumulation in metal-polluted soil. Chemosphere 72:157–164

Kang S, Joo G, Hamayun M, Na C, Shin D, Kim HY, Hong J, Lee I (2009) Gibberellin production and phosphate solubilization by newly isolated strain of *Acinetobacter calcoaceticus* and its effect on plant growth. Biotechnol Lett 31:277–281

Khan MS, Zaidi A (2007) Synergistic effects of the inoculation with plant growth-promoting rhizobacteria and an arbuscular mycorrhizal fungus on the performance of wheat. Turk J Agric For 31:355–362

Khan MS, Zaidi A, Wani PA (2007) Role of phosphate solubilizing microorganisms in sustainable agriculture-a review. Agron Sustain Dev 27:29–43

Khan MS, Zaidi A, Wani PA, Ahemad M, Oves M (2009a) Functional diversity among plant growth-promoting rhizobacteria. In: Khan MS, Zaidi A, Musarrat J (eds) Microbial strategies for crop improvement. Springer, Berlin, pp 105–132

Khan AA, Jilani G, Akhtar MS, Naqvi SMS, Rasheed M (2009b) Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. J Agric Biol Sci 1:48–58

Khan MS, Zaidi A, Ahemad M, Oves M, Wani PA (2010) Plant growth promotion by phosphate solubilizing fungi–current perspective. Arch Agron Soil Sci 56:73–98

Kim KY, Jordan D, Krishnan HB (1997) *Rahnella aquatilis*, bacterium isolated from soybean rhizosphere, can solubilize hydroxyapatite. FEMS Microbiol Lett 153:273–277

Kumar KV, Singh N, Behl HM, Srivastava S (2008) Influence of plant growth promoting bacteria and its mutant on heavy metal toxicity in *Brassica juncea* grown in fly ash amended soil. Chemosphere 72:678–683

Kumar S, Pandey P, Maheshwari DK (2009) Reduction in dose of chemical fertilizers and growth enhancement of Sesame (*Sesamum indicum* L.) with application of rhizospheric competent *Pseudomonas aeruginosa* LES4. Eur J Soil Biol 45:334–340

Kumari M, Vasu D, Ul-Hasan Z, Dhurwe UK (2009) Effects of PSB (Phosphate Solubilizing Bacteria) on morphological characters of *Lens culinaris Medic*. Biol Forum 1:5–7

Linderman RG (1988) Mycorrhizal interaction with the rhizosphere microflora: the mycorhizosphere effect. Phytopathology 78:366–371

Lindsay WL, Vlek PLG, Chien SH (1989) Phosphate minerals. In: Dixon JB, Weed SB (eds) Minerals in soil environment, 2nd edn. Soil Science Society of America, Madison, WI, pp 1089–1130

Lipping Y, Jiatao X, Daohong J, Yanping F, Guoqing L, Fangcan L (2008) Antifungal substances produced by *Penicillium oxalicum* strain PY-1 potential antibiotics against plant pathogenic fungi. World J Microbiol Biotechnol 24:909–915

Maheshwari DK, Kumar S, Kumar B, Pandey P (2011) Co-inoculation of urea and DAP tolerant *Sinorhizobium meliloti* and *Pseudomonas aeruginosa* as integrated approach for growth enhancement of *Brassica Juncea*. Indian J Microbiol 50:425–431

Maliha R, Samina K, Najma A, Sadia A, Farooq L (2004) Organic acid production and phosphate solubilization by phosphate solubilizing microorganisms under *in vitro* conditions. Pak J Biol Sci 7:187–196

Mamta RP, Pathania V, Gulati A, Singh B, Bhanwra RK, Tewari R (2010) Stimulatory effect of phosphate-solubilizing bacteria on plant growth, stevioside and rebaudioside-A contents of *Stevia rebaudiana* Bertoni. Appl Soil Ecol 46:222–229

Marra LM, de Oliveira SM, Soares CRFS, deSouza Moreira FM (2011) Solubilisation of inorganic phosphates by inoculants strains from tropical legumes. Sci Agric 68:603–609

Matthijs S, Tehrani KA, Laus G, Jackson RW, Cooper RM, Cornelis P (2007) Thioquinolobactin, a *Pseudomonas* siderophore with antifungal and anti-*Pythium* activity. Environ Microbiol 9:425–434

Mehnaz S, Mirza MS, Hassan U, Malik KA (1998) Detection of inoculated plant growth promoting rhizobacteria in rhizosphere of rice. In: Malik KA, Mirza MS, Ladha JK (eds) Nitrogen fixation with non-legumes. Kluwer Academic, London, pp 75–83

Menkina RA (1963) Bacterial fertilizers and their importance for agricultural plants. Microbiology 33:352–358

Mishra A, Chauhan PS, Chaudhry V, Tripathi M, Nautiyal CS (2011) Rhizosphere competent *Pantoea agglomerans* enhances maize (*Zea mays*) and chickpea (*Cicer arietinum* L.) growth, without altering the rhizosphere functional diversity. Antonie van Leeuwenhoek 100:405–413

Mishra PK, Mishra S, Selvakumar G, Bisht SC, Bisht JK, Kundu S, Gupta HS (2008) Characterisation of a psychrotolerant plant growth promoting *Pseudomonas* sp. strain PGERs17 (MTCC 9000) isolated from North Western Indian Himalayas. Ann Microbiol 58:561–568

Mittal V, Singh O, Nayyar H, Kaur J, Tewari R (2008) Stimulatory effect of phosphate-solubilizing fungal strains (*Aspergillus awamori* and *Penicillium citrinum*) on the yield of chickpea (*Cicer arietinum* L. cv. GPF2). Soil Biol Biochem 40:718–727

Molla M, Chaudhury AA (1984) Microbial mineralization of organic phosphate in soil. Plant Soil 78:393–399

Naz I, Bano A (2010) Biochemical, molecular characterization and growth promoting effects of phosphate solubilizing *Pseudomonas* sp. isolated from weeds grown in salt range of Pakistan. Plant Soil 334:199–207

Oliveira CA, Alves VMC, Marriel IE, Gomes EA, Scotti MR, Carneiro NP, Guimara CT, Schaffert RE, Sá NMH (2009) Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome. Soil Biol Biochem 41:1782–1787

Osorio NW (2008) Effectiveness of microbial solubilization of plant phosphate in enhancing plant phosphate uptake in tropical soils and assessment of the mechanism of solubilization. Ph.D. dissertation, University of Hawai, Honululu

Osorio NW (2011) Effectiveness of phosphate solubilizing microorganism in increasing plant phosphate uptake and growth in tropical soils. In: Maheshwari DK (ed) Bacteria in agrobiology: plant nutrient management. Springer, Berlin, pp 65–80

Oves M, Zaidi A, Khan MS, Ahemad M (2009) Variation in plant growth promoting activities of phosphate-solubilizing microbes and factors affecting their colonization and solubilizing efficiency in different agro-ecosystems. In: Khan MS, Zaidi A (eds) Phosphate solubilizing microbes for crop improvement. Nova Science, New York, pp 247–263

Pandey P, Maheshwari DK (2007) Two-species microbial consortium for growth promotion of *Cajanus cajan*. Curr Sci 92:1137–1142

Panhwar QA, Radziah O, Rahman AZ, Sariah M, Razi MI, Naher UA (2011) Contribution of phosphate-solubilizing bacteria in phosphorus bioavailability and growth enhancement of aerobic rice. Span J Agric Res 9:810–820

Patel KJ, Singh AK, Nareshkumar G, Archana G (2010) Organic-acid-producing, phytate-mineralizing rhizobacteria and their effect on growth of pigeon pea (*Cajanus cajan*). Appl Soil Ecol 44:252–261

Peix A, Mateos PF, Rodriguez-Barrueco C, Martínez-Molina E, Velazquez E (2001) Growth promotion of common bean [*Phaseolus vulgaris* L.] by a strain of *Burkholderia cepacia* under growth chamber. Soil Biol Biochem 33:1927–1935

Pérez E, Sulbarán M, Ball MM, Yarzábal LA (2007) Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. Soil Biol Biochem 39:2905–2914

Perveen S, Khan MS, Zaidi A (2002) Effect of rhizospheric microorganisms on growth and yield of green gram (*Phaseolus radiatus* L.). Indian J Agric Sci 72:421–423

Pikovskaya RI (1948) Mobilization of phosphorus in soil connection with the vital activity of some microbial species. Microbiologiya 17:362–370

Ponmurugan P, Gopi C (2006) *In vitro* production of growth regulators and phosphatase activity by phosphate solubilizing bacteria. Afr J Biotechnol 5:348–350

Poonguzhali S, Madhaiyan M, Sa T (2008) Isolation and identification of phosphate solubilizing bacteria from chinese cabbage and their effect on growth and phosphorus utilization of plants. J Microbiol Biotechnol 18:773–777

Pradhan N, Shukla LB (2005) Solubilization of inorganic phosphates by fungi isolated from agriculture soil. Afr J Biotechnol 5:850–854

Rajapaksha RMCP, Herath D, Senanayake AP, Senevirathne MGTL (2011) Mobilization of rock phosphate phosphorus through bacterial inoculants to enhance growth and yield of wetland rice. Commun Soil Sci Plant Anal 42:301–314

Rajkumar M, Freitas H (2008) Influence of metal resistant-plant growth-promoting bacteria on the growth of *Ricinus communis* in soil contaminated with heavy metals. Chemosphere 71:834–842

Rambelli A (1973) The rhizosphere of mycorrrhizae. In: Marks GC, Kozlovasky TT (eds) Ectomycorrrhizae, their ecology and philosophy. Academic, London, pp 299–343

Reyes I, Bernier L, Simard R, Antoun H (1999) Effect of nitrogen source on solubilization of different inorganic phosphates by bacterial strain of *Penicillium rugulosum* and two UV induced mutants. FEMS Microbiol Ecol 28:281–290

Reyes I, Baziramakenga R, Bernier L, Antoun H (2001) Solubilization of phosphate rocks and minerals by a wild-type strain and two UV induced mutants of *Penicillium rugulosum*. Soil Biol Biochem 33:1741–1747

Richardson AE (2001) Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Aust J Plant Physiol 28:897–906

Rodríguez H, Fraga R, Gonzalez T, Bashan Y (2006) Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil 287:15–21

Rosas SB, Andre's JA, Rovera M, Correa NS (2006) Phosphate-solubilizing *Pseudomonas putida* can influence the rhizobia–legume symbiosis. Soil Biol Biochem 38:3502–3505

Rudrappa T, Splaine RE, Biedrzycki ML, Bais HP (2008) Cyanogenic pseudomonads influence multitrophic interactions in the rhizosphere. Publ Libr Sci 3:e2073

Sanjotha P, Mahantesh P, Patil CS (2011) Isolation and screening of efficiency of phosphate solubilizing microbes. Int J Microbiol Res 3:56–58

Sattar MA, Gaur AC (1987) Production of auxins and gibberellins by phosphate dissolving microorganisms. Zentral Microbiol 142:393–395

Selvakumar G, Mohan M, Kundu S, Gupta AD, Joshi P, Nazim S, Gupta HS (2008) Cold tolerance and plant growth promotion potential of *Serratia marcescens* strain SRM (MTCC 8708) isolated from flowers of summer squash (*Cucurbita pepo*). Lett Appl Microbiol 46:171–175

Shaharoona B, Naveed M, Arshad M, Zahir ZA (2008) Fertilizer-dependent efficiency of Pseudomonads for improving growth, yield, and nutrient use efficiency of wheat (*Triticum aestivum* L.). Appl Microbiol Biotechnol 79:147–155

Sherchand K (2000) Responses of effective microorganisms and other nutrients to rice and wheat under field conditions at Khumaltar. Nepal EM World J 1:40–44

Shweta B, Maheshwari DK, Dubey RC, Arora DS, Bajpai VK, Kang SC (2008) Beneficial effects of fluorescent pseudomonads on seed germination, growth promotion, and suppression of charcoal rot in groundnut (*Arachis hypogea* L.). J Microbiol Biotechnol 18:1578–1583

Siddiqui IA, Shaukat SS, Sheikh IH, Khan A (2006) Role of cyanide production by *Pseudomonas fluorescens* CHA0 in the suppression of root-knot nematode, *Meloidogyne javanica* in tomato. World J Microbiol Biotechnol 22:641–650

Singh N, Kumar S, Bajpai VK, Dubey RC, Maheshwari DK, Kang SC (2010) Biological control of *Macrophomina phaseolina* by chemotactic fluorescent *Pseudomonas aeruginosa* PN1 and its plant growth promotory activity in chir-pine. Crop Prot 29:1142–1147

Smith JH, Allison FE, Soulides DA (1961) Evaluation of phosphobacterin as a soil inoculants. Soil Sci Soc Am Proc 25:109–111

Song OR, Lee SJ, Lee YS, Lee SC, Kim KK, Choi YL (2008) Solubilization of insoluble inorganic phosphate by *Burkholderia cepacia* DA23 isolated from cultivated soil. Braz J Microbiol 39:151–156

Souchie EL, Azcon R, Barea JM, Saggin-Júnior OJ, da Silva EMR (2007) Indoleacetic acid production by P-solubilizing microorganisms and interaction with arbuscular mycorrhizal fungi. Acta Sci Biol Sci 29:315–320

Tao GC, Tian SJ, Cai MY, Xie GH (2008) Phosphate-solubilizing and mineralizing abilities of bacteria isolated from soils. Pedosphere 18:515–523

Taurian T, Anzuay MS, Angelini JG, Tonelli ML, Ludueña L, Pena D, Ibáñez F, Fabra A (2010) Phosphate-solubilizing peanut associated bacteria: screening for plant growth-promoting activities. Plant Soil 329:421–431

Toro M (2007) Phosphate solubilizing microorganisms in the rhizosphere of native plants from tropical savannas: an adaptive strategy to acid soils? In: Velazquez C, Rodriguez-Barrueco E (eds) Developments in plant and soil sciences. Springer, Amsterdam, pp 249–252

Toro M, Azcon R, Barea JM (2008) The use of isotopic dilution techniques to evaluate the interactive effects of *Rhizobium* genotype, mycorrhizal fungi, phosphate-solubilizing rhizobacteria and rock phosphate on nitrogen and phosphorus acquisition by *Medicago sativa*. New Phytol 138:265–273

Trivedi P, Kumar B, Pandey A, Palni LMS (2007) Growth promotion of rice by phosphate solubilizing bioinoculants in a Himalayan location. In: First international meeting on microbial phosphate solubilization, pp 291–299

Vassilev N, Vassileva M (2003) Biotechnological solubilization of rock phosphate on media containing agroindustrial wastes. Appl Microbiol Biotechnol 61:435–440

Vassilev N, Vassileva M, Nikolaeva I (2006) Simultaneous P-solubilizing and biocontrol activity of microorganisms: potentials and future trends. Appl Microbiol Biotechnol 71:137–144

Vassileva M, Serrano M, Bravo V, Jurado E, Nikolaeva I, Martos V, Vassilev N (2010) Multifunctional properties of phosphate-solubilizing microorganisms grown on agro-industrial wastes in fermentation and soil conditions. Appl Microbiol Biotechnol 85:1287–1299

Vazquez P, Holguin G, Puente ME, Lopez-Cortes A, Bashan Y (2000) Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. Biol Fertil Soils 30:460–468

Vikram A, Hamzehzarghani H (2008) Effect of phosphate solubilizing bacteria on nodulation and growth parameters of green gram (*Vigna radiata* L. Wilczek). Res J Microbiol 3:62–72

Vikram A, Hamzehzargani H, Al-Mighrabi KI, Krishnaraj PU, Jagadesh KS (2007a) Interaction between *Pseudomonas fluorescens* FPD-15 and *Bradyrhizobium* spp. in peanut. Biotechnology 6:292–298

Vikram A, Hamzehzarghani H, Alagawadi AR, Krishnaraj PU, Chandrashekar BS (2007b) Production of plant growth promoting substances by phosphate solubilizing bacteria isolated from vertisols. J Plant Sci 2:326–333

Viruel E, Lucca ME, Siñeriz F (2011) Plant growth promotion traits of phosphobacteria isolated from Puna, Argentina. Arch Microbiol 193:489–496

Vyas P, Gulati A (2009) Organic acid production *in vitro* and plant growth promotion in maize under controlled environment by phosphate-solubilizing fluorescent *Pseudomonas*. BMC Microbiol 9:1–15

Wang X, Pan Q, Chen F, Yan X, Liao H (2011) Effects of co-inoculation with arbuscular mycorrhizal fungi and rhizobia on soybean growth as related to root architecture and availability of N and P. Mycorrhiza 21:173–181

Wani PA, Khan MS (2010) *Bacillus* species enhance growth parameters of chickpea (*Cicer arietinum* L.) in chromium stressed soils. Food Chem Toxicol 48:3262–3267

Wani PA, Khan MS, Zaidi A (2007a) Synergistic effects of the inoculation with nitrogen fixing and phosphate solubilizing rhizobacteria on the performance of field grown chickpea. J Plant Nutr Soil Sci 170:283–287

Wani PA, Khan MS, Zaidi A (2007b) Effect of metal tolerant plant growth promoting *Bradyrhizobium* sp. (vigna) on growth, symbiosis, seed yield and metal uptake by green gram plants. Chemosphere 70:36–45

Xiang WL, Liang HZ, Liu S, Luo F, Tang J, Li MY, Che ZM (2011) Isolation and performance evaluation of halotolerant phosphate solubilizing bacteria from the rhizospheric soils of historic Dagong Brine Well in China. World J Microbiol Biotechnol 27:629–2637

Yadav S, Kaushik R, Saxena AK, Arora DK (2011) Diversity and phylogeny of plant growth-promoting bacilli from moderately acidic soil. J Basic Microbiol 51:98–106

Yarzábal LA (2010) Agricultural development in tropical acidic soils: potential and limits of phosphate-solubilizing bacteria. Soil Biol Agric Trop 21:209–233

Yazdani M, Bagheri H, Ghanbari-Malidarreh A (2011) Investigation on the effect of biofertilizers, phosphate solubilization microorganisms (PSM) and plant growth promoting rhizobacteria (PGPR) on improvement of quality and quantity in corn (Zea *mays* L.). Adv Environ Biol 5:2182–2185

Yi Y, Huang W, Ge Y (2008) Exo-polysaccharide: a novel important factor in the microbial dissolution of tricalcium phosphate. World J Microbiol Biotechnol 24:1059–1065

Yu X, Liu X, Hui TZ, Liu GH, Mao C (2011) Isolation and characterization of phosphate-solubilizing bacteria from walnut and their effect on growth and phosphorus mobilization. Biol Fertil Soils 47:437–446

Zabihi HR, Savaghebi GR, Khavazi K, Ganjali A, Miransari M (2011) Pseudomonas bacteria and phosphorous fertilization, affecting wheat (*Triticum aestivum* L.) yield and P uptake under greenhouse and field conditions. Acta Physiol Plant 33:145–152

Zaidi A (1999) Synergistic interactions of nitrogen fixing microorganisms with phosphate mobilizing microorganisms. Ph.D. Thesis, Aligarh Muslim University, Aligarh

Zaidi A, Khan MS (2005) Interactive effect of rhizotrophic microorganisms on growth, yield, and nutrient uptake of wheat. J Plant Nutr 28:2079–2092

Zaidi A, Khan MS (2006) Co-inoculation effects of phosphate solubilizing microorganisms and G*lomus fasciculatum* on green gram-*Bradyrhizobium* symbiosis. Turk J Agric 30:223–230

Zaidi A, Khan MS, Amil M (2003) Interactive effect of rhizotrophic microorganisms on yield and nutrient uptake of chickpea (*Cicer arietinum* L.). Eur J Agron 19:15–21

Zaidi A, Khan MS, Aamil M (2004) Bioassociative effect of rhizospheric microorganisms on growth, yield, and nutrient uptake of green gram. J Plant Nutr 27:599–610

Zaidi A, Khan MS, Ahemad M, Oves M (2009a) Plant growth promotion by phosphate solubilizing bacteria. Acta Microbiol Immunol Hung 56:263–284

Zaidi A, Khan MS, Oves M, Ahemad M (2009b) Strategies for development of microphos and mechanisms of phosphate-solubilization. In: Khan MS, Zaidi A (eds) Phosphate solubilizing microbes for crop improvement. Nova Science, New York

Zaidi A, Ahemad M, Oves M, Ahmad E, Khan MS (2010) Role of phosphate-solubilizing bacteria in legume improvement. In: Khan MS, Zaidi A, Musarrat J (eds) Microbes for legume improvement. Springer, Wien, pp 273–292

Zhu F, Qu L, Hong X, Sun X (2011) Isolation and characterization of a phosphate-solubilizing halophilic bacterium *Kushneria* sp. YCWA18 from Daqiao Saltern on the coast of yellow sea of China. Evid Based Complement Alternat Med. doi:10.1155/2011/615032

Chapter 11
The Role of Siderophores in Plant Growth-Promoting Bacteria

Ana Fernández Scavino and Raúl O. Pedraza

11.1 Introduction

Iron is the fourth most abundant element in the earth's crust, and living organisms require iron for growth. Although abundant in the environment, iron is not readily available. Under aerobic conditions, free ferrous iron, Fe(II), is oxidized to ferric iron, Fe(III), forming oxy-hydroxide polymers, which are not very easily soluble (Neilands 1995).

Iron is physiologically indispensable since a great number of proteins require iron for their activities, particularly the enzymes involved in redox reactions. Organisms have developed different mechanisms to scavenge iron from the abundant but biologically unusable sources in the environment. Examples of them are (1) reduction of extremely insoluble forms of ferric ion to soluble forms of ferrous ion that can be used easily, (2) use of iron present in hemoglobin by the destruction of erythrocytes and hydrolysis of hemoglobin, (3) direct use of the iron stored in ferritin (complexes that store iron in a form that is soluble, bioavailable, and nontoxic), and (4) enzymatic degradation of compounds that bind ironlike transferrin (Vasil and Ochsner 1999). But among the various mechanisms employed, the production of iron-binding compounds called siderophores is the best studied.

The term siderophore stands for "iron carriers" or "iron bearers" in Greek. They are water-soluble, low-molecular-weight, organic ligands with high affinity and specific for iron binding (Kraemer 2004). This constitutes a high-affinity system for the uptake of iron from the external medium, present in many microorganisms. This

A. Fernández Scavino (✉)
Facultad de Química, Universidad de la República, Gral. Flores 2124, Casilla de Correo 1157 Montevideo, Uruguay
e-mail: afernand@fq.edu.uy

R.O. Pedraza
Facultad de Agronomía y Zootecnia, Universidad Nacional de Tucumán, Av. Kirchner 1900, 4000 Tucumán, Argentina

D.K. Maheshwari et al. (eds.), *Bacteria in Agrobiology: Crop Productivity*,
DOI 10.1007/978-3-642-37241-4_11,

system has three components: a siderophore that acts as a high-affinity ferric-ion-specific ligand that is usually released to the extracellular environment by microbes, a membrane receptor for iron-bound siderophore (ferri-siderophore) complex that transports the chelated iron across the microbial membrane, and an enzymatic system that is present within the cell that can release ferric ion bound to the siderophore. The siderophores form soluble complexes with ferric ion which, in natural environments, is extracted from insoluble iron hydroxides, protein-bound iron from cellular detritus, or from other iron chelates.

This system of high-affinity acquisition and receptor-dependent transport of ferric ion is associated with growth or germination factors and with virulence factors (Crichton and Charloteaux-Wauters 1987). Due to which the siderophores production is a common trait of invasive pathogenic microorganisms, synthetic analogs of bacterial siderophores attract increasing interest as potential drugs for the treatment of infections (Bergeron et al. 1999).

Recently, siderophores production proved in different plant growth-promoting bacteria (PGPB) as an important attribute in the plant growth and phytosanitary protection (Compant et al. 2005, Maheshwari 2011). Considering the important role that siderophores production can play in agronomic ecosystems, the iron content as a limiting nutrient for living organisms, the bacterial siderophores production particularly in PGPB, and the biotechnological applications of siderophores in agriculture are presented in this chapter.

11.2 Iron as a Limited Nutrient

Iron is an essential trace nutrient for most known organisms. The abundance of iron in soils is 1–6 % by weight, and its solubility is dependent on pH. In most environments iron deficiency is not caused by low total iron concentrations but by low iron bioavailability (Kraemer 2004). In aerobic environment iron is found as Fe(III), which is insoluble under physiological conditions (Powell et al. 1980; Matzanke et al. 1989).

More than 100 enzymes involved in primary and secondary metabolism possess iron-containing cofactors such as iron–sulfur cluster or heme groups. The reversible Fe(II)/Fe(III) redox pair is best suited to catalyze a broad spectrum of redox reactions and to mediate electron chain transfer (Miethke and Marahiel 2007). These enzymes and cofactors participate in various processes such as respiration, activation of oxygen, degradation of hydrogen peroxide and hydroxyl radicals, amino acid and pyrimidine biosynthesis, the citric acid cycle, DNA synthesis, nitrogen fixation, carbon fixation metabolism, photosynthesis, and oxygen binding (Andrews 1998). In addition, several transcriptional and posttranscriptional regulators interact with iron to sense its intracellular level or the current status of oxidative stress in order to efficiently control the expression of a broad array of genes involved mainly in iron acquisition or in the reactive oxygen species protection (Hantke 2001).

The cellular uptake of iron is restricted to its physiologically most relevant species, ferrous, i.e., Fe(II), and ferric, i.e., Fe(III). Ferrous form is more soluble in aqueous solutions at neutral pH and then sufficiently available for living cells if remains in the reductive status. Generally, Fe(II) form can be taken up by ubiquitous divalent metal transporters, although specific ferrous uptake systems are known in bacteria and yeasts (Miethke and Marahiel 2007).

Though iron is required by a majority of microorganisms, there are some exceptions like the lactic acid bacteria, as they do not contain heme enzymes and the iron-containing ribonucleotide reductase (Neilands 1995). On the other hand, iron can be toxic for certain organisms. High intracellular concentration of ferrous ion may produce hydroxyl radicals (Crichton and Charloteaux-Wauters 1987). This problem is alleviated with enzymes such as superoxide dismutase, catalase, and peroxidase that can degrade reactive oxygen species. Iron toxicity is also alleviated by the presence of antioxidants such as glutathione and endonucleases that repair damages caused to DNA during redox stress (Andrews 1998). It is also well known that the iron imports toxicity towards rice plants in lowland environments. After inundation, reduction of iron oxides and hydroxides results in the accumulation of large amounts of ferrous ion that disrupt or overexpress metabolic processes that result in damage of the rice plant (Becker and Asch 2005).

11.2.1 Iron Bioavailability

The iron pools in soils and aquatic environments contain iron complexes (ferric complexes with other ligands different from siderophores), iron-bound enzymes from detritus plant and microbial cells, iron bound to humic and fulvic substances, and iron-bearing minerals. A major iron pool in terrestrial and aquatic systems is constituted by iron oxides (Kraemer 2004). Some pathogens can mobilize ferric iron directly from iron-containing eukaryotic host proteins, like transferrin, lactoferrin, and ferritin, or from heme using a heme oxygenase (Winkelmann 2007). The siderophores production is a particularly efficient and specialized iron-acquisition system that confers competitive advantage to many organisms in biotic and abiotic ecosystems. Most of the information in biological iron acquisition is focused on aerobic systems since reducing conditions lead to a strong increase of iron solubility and is unlikely to encounter iron-limiting conditions in reduced systems (Kraemer 2004). The iron availability is limited by the solubility, and the slow dissolution kinetics of iron-bearing mineral phases particularly occurs in neutral or alkaline environments. The solubility of iron oxides in aerobic systems depends on the properties of the solids, on the particle size, and on the pH, ionic strength, and concentration of organic ligands in solution (Kraemer 2004). At neutral pH and oxic conditions, Fe(II) quickly oxidizes to Fe(III) (Stumm and Morgan 1995). In the absence of a strong organic ligand, Fe(III) precipitates rapidly as a hydrous ferric oxide, and citrate is too weak to bind iron and prevent Fe(III) precipitation in the culture medium (Konigsberger et al. 2000).

In the soil environment, at around neutral pH, the free Fe(III) concentration in equilibrium with ferric oxide hydrates is around 10^{-17} M (Budzikiewicz 2010). But living microorganisms require higher concentrations (10^{-6} M), and when cells detect concentrations below this threshold, the siderophores production begins (Miethke and Marahiel 2007). Siderophores have a pronounced effect on the solubility of iron oxides over a wide range of pH due to the extraordinary thermodynamic stability of soluble siderophore–iron complexes. Very small concentrations of free siderophores in solution have a large effect on the saturation state of iron oxides. This siderophore-induced disequilibrium can drive dissolution mechanism such as proton-promoted or ligand-promoted iron oxide dissolution. The adsorption of siderophores to oxide surfaces also induces a direct siderophore-promoted surface-controlled dissolution mechanism (Kraemer 2004). In addition, iron can also be mobilized by exudation of non-siderophore ligands that are ubiquitous in soil. Organic acids such as lactate, succinate, fumarate, malate, acetate, and amino acids exuded by roots of iron-stressed plants can also contribute to the Fe(III) solubilization and influence microbial iron acquisition (Fan et al. 1997).

11.2.2 Siderophores from Different Organisms

Various plants belong to family *Poaceae* (graminaceous grasses); fungi and several bacterial genera are known to sequester iron using siderophores (Neilands 1957; Takagi 1976; Winkelmann 1992).

A specialized mechanism for iron uptake is observed in *Poaceae* plants which, via roots, release iron-chelating nonproteinogenic amino acids called phytosiderophores. According to Römheld and Marschner (1986), there are two strategies for the acquisition of iron by plants under iron deficiency. Strategy I (in most non-*Poaceae* species) is characterized by an inducible plasma membrane-bound reductase and an enhancement of H^+ release. Strategy II (in grasses) is characterized by an enhanced release of phytosiderophores and by a highly specific uptake system for Fe(III) phytosiderophores. This strategy seems to have several ecological advantages over strategy I such as solubilization of sparingly soluble inorganic Fe(III) compounds in the rhizosphere and less inhibition by high pH. Thus, mugineic acid is produced by barley, distichonic acid by barley, avenic acid A by oats, deoxymugineic acid by wheat, hydroxymugineic acid by rye, and nicotinamide by tobacco. Some plant like barley is able to take up ferriphytosiderophores 100–1,000 times faster than other ferri-chelators (Castignetti and Smarrelli 1986). It has been observed that the lower affinities of phytosiderophores by iron, compared to microbial siderophores, are partly compensated by high exudation rates by *Poaceae* plant roots resulting in local ligand concentrations in the millimolar range in the rhizosphere, whereas the bacterial hydroxamate siderophore concentration is four orders lower (Römheld 1991).

Most fungi produce a variety of different types of siderophores, and individual organism may produce a set of siderophores covering a wide range of physicochemical properties. This diversity allows fungi to overcome the adverse local conditions of iron solubility and the outcompetition by motile bacteria that can migrate towards increasing nutrient concentrations (Winkelmann 2007). More than 100 structurally different fungal siderophores are known, though all of them have a peptidic ring in common. One of the four major classes, the ferrichromes, comprises diverse structures that are recognized by their resistance to degradation in the environment, particularly when they are complexed with iron (Winkelmann 2007). Virtually all aerobic bacteria and fungi produce siderophores (Neilands and Leong 1986). Though, this property is a clear advantage for microorganisms inhabiting aerobic environments. Most of the facultative bacteria isolated of rice paddy soils reported as siderophore producers (Loaces et al. 2011). It remains to be elucidated if these bacteria are effectively producing siderophores in such anoxic soils where iron probably is present as Fe(II).

There are microorganisms which are unable to produce siderophores. S*accharomyces cerevisiae* lacks the ability to synthesize siderophores, although it can utilize siderophores produced by other species via reductive and nonreductive iron assimilation (Eissendle et al. 2003). In addition, Pandey et al. (1994) studied 23 strains of lactic acid bacteria for their ability to produce siderophores. The growth of several strains tested was unaffected by an iron deficiency, and no direct effect due to iron chelation by a synthetic iron chelator was observed. Hence, the authors confirmed that these strains of lactic acid bacteria do not require iron.

11.2.3 Siderophores in Soil

In most environmental systems, siderophores mainly exist in complexed form (Kraemer 2004). Their concentrations in soil depend on the soil horizons, but the rhizosphere shows higher concentrations than bulk soil (Bossier et al. 1988). Powell et al. (1980) have estimated hydroxamate siderophore concentrations in soil solutions between 10^{-7} and 10^{-8} M. Römheld (1991) has estimated that phytosiderophore concentrations can reach local concentrations of up to 10^{-3} M since plants are able to exude phytosiderophore at high rates into the rhizosphere. The concentration of microbial siderophores depends on the environmental conditions. Ferrioxamine B-type siderophores, produced by most actinomycetes (Neilands and Leong 1986), were the most abundant siderophore producer in a tiller-amended soil system, whereas the ferrichrome type produced in smaller quantities by several fungi (Crowley et al. 1987). On the other hand, Holmström et al. (2004) identified the main siderophores in coniferous forest soils intensively colonized by ectomycorrhizal hyphae as ferrichrome and ferricrocin, with the former detected in nanomolar concentrations in humic layers overlying granitic rock and soils (Holmström et al. 2004). Ferricrocin is a widespread siderophore in forest soils that seems to be resistant to the proteases excreted by plants and

Gram-positive bacteria (Winkelmann 2007). It is now considered that organic non-siderophore ligands, as several amino acids and organic acids like citrate, can be exudated by plants and influence the iron availability. These ligands are ubiquitous in soil and might have a synergistic or inhibitory effect on the siderophores dissolution rates (Kraemer 2004).

Furthermore, Kraemer (2004) proposed that the compressive understanding of the role of siderophores in increasing iron oxide solubility and promoting dissolution in soils requires the consideration of the rates of various processes that occurred simultaneously. Thus, the siderophore exudation rates, the uptake, and the degradation rates, as well as the loss of siderophores by adsorption on other mineral surfaces, the partitioning of iron into humic substances, and the complexation of metal other than iron (which stability may be significant, specially for similar ions as Al(III) or for Ca(II) that is often present in much higher concentrations), should be considered. In addition, iron oxides in natural terrestrial environments are often coated with humic and fulvic acids, exo-polysaccharides, or biogenic low-molecular-weight organic acids, and the inhibitory, competitive, or synergistic effects of such substances on siderophore-controlled iron acquisition need to be investigated.

11.3 Microbial Siderophores

Microbial siderophores show great variability in their chemical structures. This may be due to genetic factor or biochemical

11.3.1 Chemical Structures

Siderophores are iron-chelating secondary metabolites with masses below 2,000 Da (Budzikiewicz 2010). Almost 500 siderophores with known structure have been reported (Boukhalfa and Crumbliss 2002), and several hundred active iron-chelator compounds have been characterized and purified (Hider and Kong 2010). Most, but not all, of siderophores are hexadentate ligands forming 1:1 complexes with Fe(III) (Kraemer 2004), and their capability to form stable complexes with Fe(II) is rather low (Miethke and Marahiel 2007).

The major Fe(III) ligand types are catecholates, hydroxamates, and alpha-hydroxycarboxylates and often are combined in the same molecule of siderophore (Budzikiewicz 2010). Carboxylate siderophores are produced by microorganisms that live in acidic environments, e.g., fungi, but these could not compete with stronger siderophores such as catecholates at physiological pH (Dertz and Raymond 2003), since catecholates have higher affinity for Fe(III). These ligands are supported in different chemical structures such as peptides, di- and tri-aminoalkanes, and siderophores based on citric acid along with miscellaneous

siderophores. The peptide chain carrying the ligand sites usually contains cyclic structures at the extremes that prevent their degradation by proteolytic enzymes. The peptidic siderophores are produced by fluorescent *Pseudomonas* (pyoverdines), as well as by species of the genera *Azotobacter*, *Mycobacterium*, *Rhodococcus*, and by many enterobacteria and by most of fungi. This also includes lipopeptide siderophores produced by species of the genera *Burkholderia*, *Nocardia*, and *Mycobacterium*. The siderophores based on di- and tri-aminoalkane skeletons are produced by few *rhizobia*, *Paracoccus*, *Burkholderia*, *Agrobacterium*, and several *Actinomycetes*. Siderophores based on citric acid are produced by bacteria from the genera *Bacillus*, *Acinetobacter*, *Arthrobacter*, *Ochrobactrum*, *Rhizobium*, *Synechococcus*, *Vibrio*, *Ralstonia*, *Staphylococcus*, and *Marinobacter* (Budzikiewicz 2010). Thus, the stability of Fe(III) siderophore complexes varies in a range about 30 orders of magnitude depending on the siderophore structure and on the ligand type. Also, the pH of the environment strongly influences the chelation efficiency (Miethke and Marahiel 2007). Although Gram-negative and Gram-positive bacteria have differences in their cell structure, they share some genes in common for both specific siderophores transport and iron-binding proteins (Clarke et al. 2000).

Many bacteria produce more than one type of siderophore or have more than one iron uptake system to take up multiple siderophores (Neilands 1981). Recently, other compounds able to bind iron with comparable affinity to the known bacterial siderophores have been reported. The degradation product of an acylhomoserine lactone (signal molecule in the Quorum Sensing system) produced by *Pseudomonas aeruginosa* possibly is an unrecognized mechanism for iron solubilization (Kaufmann et al. 2005). Recently detail description on types and chemistry of siderophores is reviewed by Desai and Archana (2011).

11.3.2 Biochemical and Genetic Determinants Involved in Bacterial Siderophores Production

Siderophores production as a response to iron limitation is widespread among aerobic microorganisms (Neilands et al. 1987). It has been reported that among 302 different fluorescent *Pseudomonas* strains isolated from soils, 297 produced detectable siderophores under iron deficiency (Cocozza and Ercolani 1997).

Although this iron-acquisition system is induced under iron-limiting conditions, other environmental factors such as pH, the presence of other trace elements, and the availability of carbon, nitrogen, and phosphorous sources also influence the siderophores production (Duffy and Defago 1999). This system involves several steps: intracellular biosynthesis of siderophores, exudation of siderophores in the extracellular space, iron mobilization by competitive complexation or dissolution of iron-bearing minerals, and recognition and uptake of ferric siderophore

complexes by highly efficient transport systems or liberation of iron from the siderophore complex and uptake of iron (Boukhalfa and Crumbliss 2002).

The system requires tightly regulated enzymes and transport systems that allow concerted siderophore biosynthesis, secretion, siderophore-delivered iron uptake, and iron release. In bacteria, gene regulation of siderophore utilization and iron homeostasis is mediated mainly at the transcriptional level by the ferric uptake repressor Fur (in Gram-negative and low mol % GC Gram-positive bacteria) or by the diphtheria toxin regulator DtxR (in Gram-positive high GC contents as streptomycetes and corynebacteria) (Hantke 2001). The synthesis of catecholates mostly depends on the nonribosomal peptide synthetases, whereas hydroxamate and carboxylate siderophores are assembled by diverse enzymes such as monooxygenases, decarboxylases, and aminotransferases (Miethke and Marahiel 2007).

In bacteria, the main route for the uptake of Fe complexed in siderophores is the import of the complex into the cytosol through specific transporters. Moreover, the organisms that can use exogenous siderophores (synthesized by other organisms) showed frequently a greater battery of Fe-siderophore importers than siderophore exporters (Miethke and Marahiel 2007). The iron release from the Fe(III) siderophore complex into the cytosol comprises either the reduction to Fe(II) by relatively unspecific ferric siderophore reductases or the hydrolysis of the complex by specific enzymes that liberate Fe(III) which is further reduced or complexed by other cellular iron components (Miethke and Marahiel 2007).

Moreover, the role of siderophores might not be limited to the iron chelation. The nitrogen-fixing bacterium *Azotobacter vinelandii* produces at least five different siderophores, where concentration increases sharply at low iron concentration in diazotrophic cultures although their production is not suppressed at high iron concentration (Bellenger et al. 2008). Kraepiel et al. (2009) suggested that *A. vinelandii* may produce siderophores to acquire molybdenum (Mo) and vanadium (V), two important metals required for nitrogen fixation, when these metals are limiting in diazotrophic cultures.

11.3.3 Siderophores Influence the Interaction Among Organisms

Siderophores production can modify the interaction among organisms in the environment leading to mechanisms of cooperation or competence.

The capability of sensing iron in the environment is an advantage by the siderophore-producing organism and may help other microorganisms that do not have this capability or that are not so competitive. Many microorganisms are able to utilize the Fe(III) complexes of siderophores which they have not synthesized. The persistence in soils of ferrichromes, the most common fungal siderophores, benefits other microorganisms that have the receptors for the uptake of these siderophores as

in case of several enterobacteria like *Pantoea*, *Enterobacter*, *Erwinia*, and *Yersinia* (Winkelmann 2007). Also, the uptake of bacterial siderophores by fungi, like *Saccharomyces* and *Aspergillus*, has been observed (Haas 2003¸ Heymann et al. 2000; Lesuisse et al. 1998), and the enterobactin, the predominant siderophore produced by enterobacteria, can also be utilized by *Saccharomyces*, a non-siderophore-producing microorganism (Winkelmann 2007).

In addition, it has been proposed that partial degradation of fungi siderophores or iron exchange between bacterial siderophores and phytosiderophores is involved in the iron nutrition of *Poaceae* plants (Yehuda et al. 1996; Winkelmann 2007). An indirect effect of cooperation has been postulated by Kraepiel et al. (2009) between non-nodulating plants and free-living diazotrophs inhabiting their rhizosphere. Besides iron, several metals are complexed and accumulated in plant leaves that when decomposed in topsoil constitute a source of essential minerals for the nitrogen fixation, from which the plants benefit. The diazotrophic bacteria extract these essential minerals through the excreted siderophores.

Competence among microorganisms is well illustrated by several examples and can benefit or be negative for the siderophore-producing microorganism. In general bacterial siderophores, though differing in their abilities to sequester iron, deprive pathogenic fungi of this essential element since the fungal siderophores have lower affinity for Fe(III). This constitutes one of the main mechanisms of biocontrol of plant pathogenic fungi (Loper and Henkels 1999). Their ability to use a large number of heterologous siderophores has been confirmed by the presence of many homologues of iron-siderophore receptor genes in their genomes (Cornelis and Matthijs 2002; Kaufmann et al. 2005). Conversely, siderophore producers can be invaded by nonproducing cheats from the same or different species that have the siderophore receptors. Siderophores production is metabolically expensive to individual producers but benefits all cells in the vicinity able to capture iron-siderophore complexes produced by other cells of the same species (Harrison et al. 2008). On the other hand, certain microorganisms synthesize structurally distinct siderophores apparently as a strategy to overcome the competition of cheaters. *Streptomyces* species produce two different siderophores with two independent uptake systems; whereas ferrioxamines can be taken by several organisms, the ferric coelichelin complex can be selectively absorbed into *Streptomyces coelicolor* cells through an independent uptake system (Challis and Hopwood 2003). Additionally, the capability of microorganisms to degrade siderophores in soil can modify the interaction established through siderophores production. It has been reported that bacteria of the genus *Azospirillum* in pure cultures are able to degrade ferrioxamines when present as iron-free compounds (Winkelmann et al. 1999).

A singular case may be the endophytic bacteria that colonize internal tissues of the plants and their relationship with the siderophores production. In Uruguay it has been shown that at the end of the cropping cycle, the leaves of three different rice varieties were colonized by high amounts of siderophore-producing bacteria (Fig. 11.1), with *Pantoea* and *Pseudomonas* as the predominant genera. Furthermore, the proportion of siderophore-producing bacteria to heterotrophic bacteria

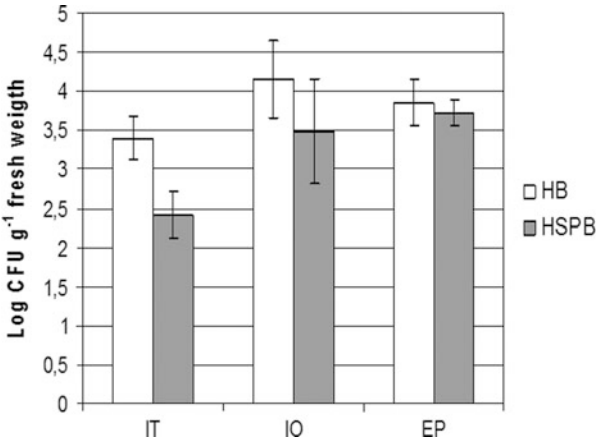


Fig. 11.1 Enumeration endophytic heterotrophic bacteria (HB) and endophytic heterotrophic siderophore-producing bacteria (HSPB) in leaves of three rice varieties cultivated in Uruguay at the end of the crop season. EP, El Paso 144; IT, INIA Tacuarí; IO, INIA Olimar. The values represent the mean of triplicate plots in a field experiment

augmented in leaves when the plant grew, and they increased in roots compared to rhizospheric soil after the flooding, when the environment becomes anoxic (Loaces et al. 2011). They remained strongly associated to the plant tissues although in vitro inhibition towards pathogenic fungi or PGPB was not observed. Apparently, siderophore-producing bacteria were selected into the plant tissues, though the benefit for the plant results is still unclear. Their role capturing Fe(III) generated by the oxidation of Fe(II) in oxic micro-niches into the plant or in the rhizosphere, increasing the iron availability locally, or reducing the Fe(II) toxicity towards the plant by accumulation of the sequestered metal into the bacterial cells should not be dismissed (Loaces et al. 2011).

Finally, the role of siderophore-producing bacteria as bacterial growth promoters should be also considered. The (until now) uncultured bacteria may be stimulated and become culturable in the presence of siderophore-producing bacteria. Recently D'Onofrio et al. (2010) have shown that previously uncultured isolates from marine sediment biofilm, grow on a Petri dish in the presence of cultured organisms from the same environment. This helper strain produces a grow factor identified as new acyl-desferrioxamine siderophore.

11.4 Siderophores Production in Plant Growth-Promoting Bacteria

Siderophores have been implicated for both direct and indirect enhancement of plant growth by rhizospheric microorganisms. The ecological significance of microbial siderophores in soil and plant surfaces has attracted the attention of workers.

11.4.1 Plant Growth-Promoting Bacteria: Mechanisms of Action

PGPB are a heterogeneous group of bacteria, such as the genera *Azotobacter*, *Azospirillum*, *Azoarcus*, *Herbaspirillum*, *Pseudomonas*, and *Rhizobium*, among others, that can be found in the rhizosphere at root surfaces and in association with inner root tissues and other habitats (Ahmad et al. 2008). The enhancement of plant growth using PGPB is well documented (Reed and Glick 2004; Bashan and de Bashan 2010), and these organisms have also been used to reduce plant stress associated with phytoremediation strategies for metal-contaminated soils (Reed and Glick 2005).

PGPB enhance plant growth through different mechanisms, such as (1) enhancing asymbiotic nitrogen fixation (Khan 2005) or indirectly affecting symbiotic N_2 fixation, nodulation, or nodule occupancy (Fuhrmann and Wollum 1989); (2) reducing ethylene production, allowing plants to develop longer roots, and better establishment during early stages of growth, due to the synthesis of 1-aminocyclopropane-1-carboxylate (ACC) deaminase which modulates the level of ethylene by hydrolyzing ACC, a precursor of ethylene, in ammonia and α-ketobutyrate (Glick et al. 1998); (3) production of hormones such as auxins, cytokinins, and gibberellins (Glick 1995; Ahmad et al. 2008); (4) raising the solubilization of nutrients with resulting increase in the supply of bioavailable phosphorous and other trace elements for plant nutrition (Glick 1995); and (5) synthesis of antibiotic and other pathogen-depressing substances such as siderophores, volatiles, and chelating agents that protect plants from that antagonize phytopatogens. (Kamnev and Lelie 2000; Tortora et al. 2011, 2012). These microorganisms can also increase plant tolerance to environmental stresses such as flooding (Grichko and Glick 2001), salt stress (Mayak et al. 2004a), and water deficiency (Mayak et al. 2004b). PGPB are not only significant from an agricultural point of view, as they can also play an important role in soil remediation strategies, not only by enhancing growth and successful establishment of plants in contaminated soils but also by increasing the availability of contaminants, as reported for heavy metals, namely, Zn and Ni, in *Thlaspi caerulescens* (Whiting et al. 2001) and in *Alyssum murale* and *Thlaspi goesingense* (Abou-Shanab et al. 2003; Idris et al. 2004). Recently Kumar et al. (2010) observed reduction of chemical fertilizer by using combination of root-nodulating *Sinorhizobium fredii* KCC5 and rhizospheric *Pseudomonas fluorescens* LPK2.

11.4.2 Siderophores as a Competitive Advantage for Plant Growth

Given that iron is an essential nutrient, plants have evolved strategies for its acquisition, which, in dicotyledonous plants such as cowpea (*Vigna unguiculata*),

is based on strategy I. Unlike strategy II found in grass monocotyledonous plants, strategy I does not involve the release of phytosiderophores. Rather, it is characterized by an enhanced Fe(III) reductase activity, release of reductants such as phenolics, and acidification of the rhizosphere (Römheld and Marschner 1986). Furthermore, in strategy I plants, microbial siderophores have been reported to promote plant growth under Fe deficiency (Crowley et al. 1991).

In a work about enhanced plant growth by siderophores produced by PGPB, specific strains of the *Pseudomonas fluorescens-putida* group have been used as seed inoculants on crop plants to promote growth and increase yields (Kloepper et al. 1980). Several workers observed that these bacteria rapidly colonized plant roots of potato, sugar beet, radish, and other crop plants, which caused statistically significant yield increases in field tests (Maheshwari 2011). These results prompted them to investigate the mechanism by which plant growth was enhanced. Most of these workers have concluded that these bacteria exerted their plant growth-promoting activity by depriving native microflora of iron as they were able to produce extracellular siderophores which efficiently complexed environmental iron, making it less available to certain native microflora (Kloepper et al. 1980).

Sharma and Johri (2003) reported about maize seeds inoculated with siderophore-producing pseudomonads with the aim to develop a system suitable for better iron uptake under iron-stressed conditions. They found that inoculation of maize seeds with fluorescent *Pseudomonas* spp. strains GRP3A and PRS showed significant increase in germination percentage and plant growth. Maximum shoot and root length and dry weight were observed with 10 μM Fe(III) along with bacterial inoculants, suggesting that application of siderophore-producing plant growth-promoting bacterial strains positively influences the crop productivity in calcareous soil system. Pandey et al. (2005) found *Pseudomonas aeruginosa* GRC1 having prolific production ability of hydroxamate siderophore in iron-deficient conditions. The siderophore of GRC1 was purified and characterized. The purified siderophore appeared to be of pyoverdine type with typical amino acid composition. In field trials, *P. aeruginosa* GRC1 enhanced the growth of *Brassica campestris* var Pusa Gold (Indian mustard).

Although extensive research has been directed to correct chlorosis (iron deficiency) by the application of available iron compounds to the soil and by selective plant breeding to produce Fe-chlorosis-resistant cultivars, during the last years, the possible implication of siderophores production by PGPB has been considered as a potential way to improve plant growth, nodulation, and N_2 fixation in iron-deficient conditions. The beneficial effect of using siderophore-producing strains of *Bradyrhizobium* sp. and *Rhizobium meliloti* was reported by O'Hara et al. (1988) and Gill et al. (1991), respectively. In addition, siderophore-producing ability might favor the persistence of rhizobia in iron-deficient soils (Lesueur et al. 1995).

11.4.3 Importance of Siderophores in Plant Protection Against Diseases

Nowadays, control of plant diseases is performed by the intensive use of chemical products that may cause environmental pollution, pathogen resistance, increase in production costs, and serious risks to the environment and human health. An alternative of crop protection against pathogens is the biological control exerted by some PGPB. Several factors can affect the efficacy of siderophores as control agents against plant pathogens, the most important among them being type of microorganism, target phytopathogen, and medium composition (Glick and Bashan 1997). Because of their catabolic versatility, their excellent root-colonizing abilities, and their capacity to produce a wide range of antifungal metabolites, the soil-borne fluorescent pseudomonads have received particular attention as efficient biological control agents (Nautiyal et al. 2003). They produce several siderophores such as pyoverdine, pyochelin, azotobactin, salicylic acid, and pseudomonine (Dave and Dube 2000; Mercado-Blanco et al. 2001; Labuschagne et al. 2010). All these siderophores contribute to disease suppression through the competition for iron.

However, siderophores production in the genus *Azospirillum*, an important member of PGPB, is a biocontrol mechanism that has been scarcely studied. Saxena et al. (1986) and Shah et al. (1992) reported the production of salicylic acid (SA) among siderophores produced by *Azospirillum lipoferum* under iron-starved conditions. Salicylic acid (SA) besides being a compound with siderophore activity (Visca et al. 1993) is a precursor in the biosynthesis of microbial catechol-type siderophores, such as yersiniabactin, pyoverdine, and pyochelin (Cox et al. 1981; Jones et al. 2007; Serino et al. 1995). Moreover, it was demonstrated to play a crucial role as an endogenous regulator of localized and systemic acquired resistance (SAR) against pathogen infection in many plants (Delaney et al. 1994). Therefore, SA-producing strains may increase defense mechanisms in plants. However, bacterial SA participation on plant-induced systemic resistance (ISR) is still controversial (Siddiqui and Shaukat 2005; Cornelis and Matthisj 2007). It was hypothesized that bacterial SA excreted to the medium was recognized by plant roots inducing signals for systemic resistance (Maurhofer et al. 1998), although in some interactions, it has been proposed that SA may not be the primary signal for ISR induction (Press et al. 1997), but other siderophores could be implicated (Siddiqui and Shaukat 2004).

Recently, it was reported that *A. brasilense* siderophores contain antifungal activity against *Colletotrichum acutatum*, the causal agent of anthracnose disease in strawberry crop (Tortora et al. 2011). They demonstrated that under iron-limiting conditions, different strains of *A. brasilense* produce siderophores, exhibiting different yields and rates of production according to their origin. The bacteria strains have also been isolated from rhizosphere or inner tissues of strawberry roots and stolons (Fig. 11.2).

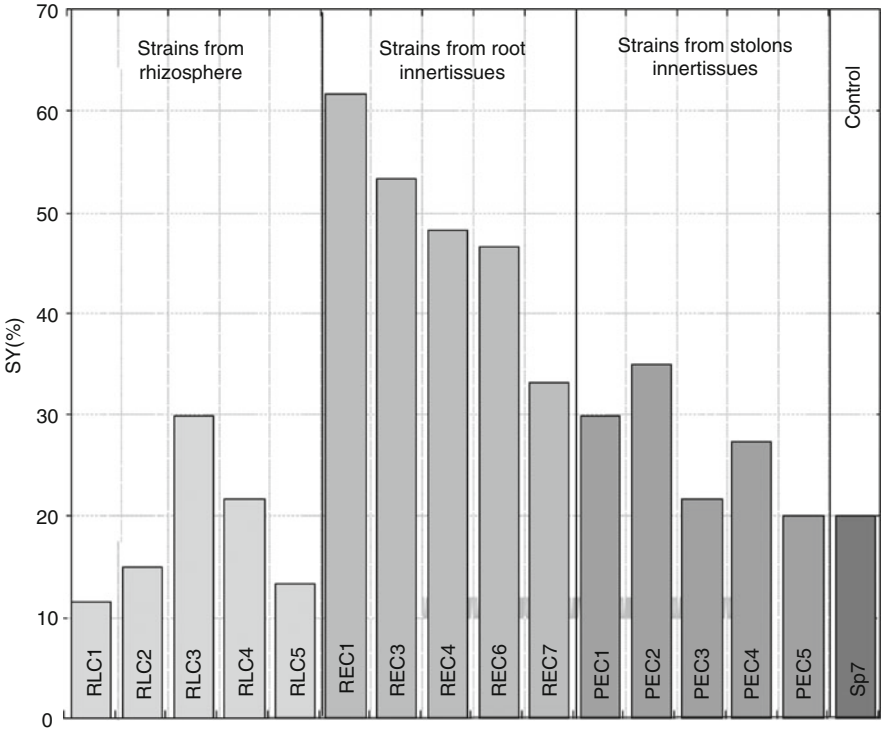


Fig. 11.2 Percent of siderophores yield production (SY %) of different *A. brasilense* strains isolated from strawberry plants, using CAS agar plates assay. Type strain *A. brasilense* Sp7 was used as the control for comparison of *A. brasilense* isolates tested. The control and rhizosphere (RLC), root endophytic (REC), and first stolon endophytic (PEC) strains were evaluated after 7 days of incubation at 30 °C

Chemical assays revealed that *A. brasilense* REC2 and REC3 secrete catechol-type siderophores, including SA, detected by TLC coupled with fluorescence spectroscopy and gas chromatography–mass spectrometry analysis. Siderophores produced by these strains showed in vitro antifungal activity against *C. acutatum* M11. Additionally, this later coincided with results obtained from phytopathological tests performed in plants, where reduction of anthracnose symptoms on strawberry plants previously inoculated with *A. brasilense* was observed. These outcomes suggested that some strains of *A. brasilense* could act as biocontrol agent preventing anthracnose disease in strawberry and involved siderophore. In recent work, the same authors provided evidence that endophytic root colonization of strawberry plants with *A. brasilense* strain REC3 confers systemic protection against *C. acutatum* M11 by the direct activation of some plant defense reactions and also primes the plant for a stronger defense reaction when exposed to further infection (Tortora et al. 2012). Defense mechanisms induced by *A. brasilense* REC3 included the reinforcement of plant cell wall by increasing the content of

total soluble phenolic compounds and callose depositions and the transient accumulation of SA. The latter brings about the upregulation of defense-related genes, such as those encoding pathogenesis-related proteins like PR1, chitinases, and glucanase. Therefore, the activation of a systemic defense response, together with the plant growth-promoting effect exerted by *A. brasilense* REC3 (Pedraza et al. 2010), could, in part, explain the increase of strawberry plants' tolerance to anthracnose disease caused by *C. acutatum* M11.

11.4.4 Biotechnological Application in Agriculture

In agriculture, the increasing introduction of new biotechnological products has allowed the achievement of higher yields in almost every present-day commercial crop, leading at the same time to a higher quality and minimizing ecological damage. In this context, agro-biotechnology may be used to develop environmentally safe and economically sound alternatives to chemical fertilizers and pesticides. New products are currently being developed through the stimulation of plant self-defense by the application of PGPB for biological control disease and as plant growth promoters (biofertilizers), applied as inoculants. In Table 11.1 are shown several examples of PGPB siderophore producers, some of them already used as inoculants.

Much research has been dedicated to the development of *Pseudomonas* inoculants and other biological products constituted by active metabolites such as antibiotics and siderophores as biocontrol agents (Mark et al. 2006). *Pseudomonas* spp. have been efficiently used for biocontrol in the past decade, and at present time, there are several commercial products already in the market. For example, there is a biological product constituted by antimicrobial metabolites such as siderophore pyoverdine and SA produced by *P. aeruginosa* PSS, very effective against *Peronospora tabacina* in tobacco culture, *Alternaria solani* in tomato, and *Pseudoperonospora cubensis* in cucumber (Díaz de Villegas 2007).

Microbe-assisted phytoremediation provides plants with natural metal-solubilizing chelators which do not represent a potential source of environmental pollution. At the same time as with microbial chelators, plant growth promotion can be enhanced through bacterially produced phytohormones (e.g., auxins). Recently, Dimkpa et al. (2008) studied the simultaneous production of siderophores and auxins by *Streptomyces* aiming for future application in plant growth and phytoremediation in a metal-contaminated soil. Standard auxin and siderophore detection assays indicated that different *Streptomyces* strains can produce these metabolites simultaneously. However, Al^{3+}, Cd^{2+}, Cu^{2+}, Fe^{3+}, and Ni^{2+} or a combination of Fe^{3+} and Cd^{2+} and Fe^{3+} and Ni^{2+} affected auxin production negatively, as revealed by spectrophotometry and gas chromatography–mass spectrometry. This effect was more dramatic in a siderophore-deficient mutant. In contrast, except for Fe, all the metals stimulated siderophores production. Mass spectrometry showed that siderophore and auxin-containing supernatants from a representative

Table 11.1 Examples of some siderophore producers within the plant growth-promoting bacteria (PGPB) and their main features

PGPB	Main features	References
Azotobacter vinelandii	Produces at least five different siderophores types	Bellenger et al. (2008)
Azotobacter vinelandii	May produce siderophores to acquire Mo and V for nitrogen fixation when these metals are limiting in diazotrophic cultures	Kraepiel et al. (2009)
Pseudomonas fluorescens *Pseudomonas putida*	Used as seed inoculants on crop plants to promote growth and increase yields	Kloepper et al. (1980)
Bradyrhizobium sp. *Rhizobium meliloti*	Improve nodulation and N_2 fixation in iron-deficient conditions	O'Hara et al. (1988), Gill et al. (1991)
Azospirillum lipoferum	Produces salicylic acid among other siderophores under iron-starved conditions	Saxena et al. (1986), Shah et al. (1992)
Azospirillum brasilense	Produces siderophores with antifungal activity against *Colletotrichum acutatum*, the causal agent of anthracnose disease in strawberry crop	Tortora et al. (2011)
Pseudomonas aeruginosa	Produces siderophore pyoverdine and salicylic acid; very effective against *Peronospora tabacina* in tobacco culture, *Alternaria solani* in tomato, and *Pseudoperonospora cubensis* in cucumber	Diaz de Villegas (2007)
Gluconacetobacter diazotrophicus	Nitrogen-fixing acetic acid bacterium producing hydroxamate-type siderophores	Logeshwaran et al. (2009)

Streptomyces species contain three different hydroxamate siderophores, revealing the individual binding responses of these siderophores to Cd^{2+} and Ni^{2+} and, thus, showing their auxin-stimulating effects. They concluded that siderophores promote auxin synthesis in the presence of Al^{3+}, Cd^{2+}, Cu^{2+}, and Ni^{2+} by chelating these metals. Chelation makes the metals less able to inhibit the synthesis of auxins and potentially increases the plant growth-promoting effects of auxins, which in turn enhances the phytoremediation potential of plants.

11.5 Concluding Remarks

Agrochemicals, including fertilizers and pesticides, are extensively used in agricultural production to control pests, diseases, and weeds, minimizing the yield losses and maintaining high product quality. The increasing cost and the negative impact of agrochemicals and their degradation products in the environmental are major ecological and health problems. Therefore, the use of PGPB as biofertilizers or biocontrol agents, most of which are siderophores producers, is quite promising to support an eco-friendly and sustainable agriculture.

Literature available revealed that siderophores production is not directly linked to the plant growth promotion neither to plant protection; siderophores are involved on iron availability in soil and in the interaction between plant and microorganisms in this habitat. The importance of siderophores is known since more than 30 years, and many siderophore-producing bacteria that benefit the crops, promote their growth, or protect them against pathogens have been reported. However, it is still not entirely known if this mechanism effectively operates in the interaction and whether it is the only one. Assuming that siderophore-producing microorganisms can obtain certain competitive advantages in the soil, where Fe(III) is not easily available, they are not the only attribute obtaining that benefit as siderophore–iron complexes may persist, be destroyed, or utilized by other organisms. Nevertheless, the role that siderophores can play as signal molecules or regulators in the microbe-plant interactions is evident and opens great perspectives for biotechnological applications in agriculture.

Acknowledgments The laboratory work of ROP was partially supported by CIUNT and ANPCyT grants (PICT 2007 N°472). The research of AFS was supported by CSIC (Comisión Sectorial de Investigación Científica, Universidad de la República), PEDECIBA (Programa de Desarrollo de Ciencias Básicas), and ANII (Agencia Nacional de Investigación e Innovación, Uruguay). The authors acknowledge the support of CYTED through the DIMIAGRI network project.

References

Abou-Shanab RI, Angle JS, Delorme TA, Chaney RL, van Berkum P, Moawad H, Ghanem K, Ghozlan HA (2003) Rhizobacterial effects on nickel extraction from soil and uptake by *Alyssum murale*. New Phytol 158:219–224

Ahmad F, Ahmad I, Khan MS (2008) Screening of free living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res 163:173–181

Andrews SC (1998) Iron storage in bacteria. Adv Microb Physiol 40:281–351

Bashan Y, de Bashan LE (2010) How the plant growth-promoting bacterium *Azospirillum* promotes plant growth – a critical assessment. Adv Agron 108:77–136

Becker M, Asch F (2005) Iron toxicity in rice—conditions and management concepts. J Plant Nutr Soil Sci 168:558–573

Bellenger JP, Wichard T, Kustka AB, Kraepiel AML (2008) Nitrogen fixing soil bacterium uses catechol siderophores for molybdenum and vanadium acquisition. Nat Geosci 1:243–246

Bergeron RJ, Wiegand J, Brittenham GM (1999) HBED: the continuing development of a potential alternative to deferoxamine for iron-chelating therapy. Blood 93:370–375

Bossier P, Hofte M, Verstraete W (1988) Ecological significance of siderophores in soil. Adv Microb Ecol 10:385–434

Boukhalfa H, Crumbliss AL (2002) Chemical aspects of siderophore mediated iron transport. Biometals 15:325–339

Budzikiewicz H (2010) Siderophores from bacteria and from fungi. In: Cornelis P, Andrews SC (eds) Iron uptake and homeostasis in microorganisms. Caister Academic, Norfolk, pp 1–16

Castignetti D, Smarrelli J (1986) Siderophores, the iron nutrition of plants and nitrate reductases. FEBS Lett 209:147–151

Challis G, Hopwood D (2003) Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces* species. Proc Natl Acad Sci 25:14555–14561

Clarke TE, Ku SY, Dougan DR, Vogel HJ, Tari LW (2000) The structure of the ferric siderophore binding protein FhuD complexed with gallichrome. Nat Struct Biol 7:287–291

Cocozza C, Ercolani GL (1997) Produzione di siderofori e caratteristiche associate in pseudomonadi fluorescenti rizosferiche e non rizosferiche. Annali di Microbiol Enzimol 47:17–28

Compant S, Duffy B, Nowak J, Clement C, Ait Barka E (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959

Cornelis P, Matthijs S (2002) Diversity of siderophore-mediated iron uptake systems in fluorescent pseudomonads: not only pyoverdines. Environ Microbiol 4:787–798

Cornelis P, Matthisj S (2007) *Pseudomonas* siderophores and their biological significance. In: Ajit V, Chincholkar SB (eds) Microbial siderophores, Soil biology. Springer, Berlin, pp 193–200

Cox CD, Rinehart KL, Moore ML, Cook JC (1981) Pyochelin: novel structure of an iron-chelating growth promoter for *Pseudomonas aeruginosa*. Proc Natl Acad Sci USA 78:4256–4260

Crichton RR, Charloteaux-Wauters M (1987) Iron transport and storage. Eur J Biochem 164:485–506

Crowley DE, Reid CPP, Szaniszlo PJ (1987) Microbial siderophores as iron sources for plants. In: Winkelmann G, van der Helm D, Neilands JB (eds) Iron transport in microbes. Plants and animals. VCH Publishers, New York, pp 375–386

Crowley DE, Wang YC, Reid CPP, Szaniszlo PJ (1991) Mechanisms of iron acquisition from siderophores by microorganisms and plants. Plant Soil 130:179–198

D'Onofrio A, Crawford JM, Stewart EJ, Witt K, Gavrish E, Epstein S, Clardy J, Lewis K (2010) Siderophores from neighboring organisms promote the growth of uncultured bacteria. Chem Biol 17:254–264

Dave BP, Dube HC (2000) Detection and chemical characterization of siderophores of rhizobacterial fluorescent pseudomonads. Ind Phytopathol 53:97–98

Delaney TP, Uknes S, Vernooij B, Friedrich L, Weymann K, Negrotto D, Gaffney T, Gut-Rella M, Kessmann H, Ward E, Ryals J (1994) A central role of salicylic acid in plant disease resistance. Science 266:1247–1250

Dertz EA, Raymond KN (2003) Siderophores and transferrins. In: Que I Jr, Tolman WB (eds) Comprehensive coordination chemistry II, vol 8. Elsevier, Philadelphia, PA, pp 141–168

Desai A, Archana G (2011) Role of siderophores in crop improvement. In: Maheshwari DK (ed) Bacteria in agrobiology: plant nutrient management. Springer, Heidelberg, pp 109–140

Díaz de Villegas ME (2007) Biotechnological production of siderophores. In: Varma A, Chincholkar SB (eds) Soil biology, microbial siderophores, vol 12. Springer, Berlin, pp 219–231

Dimkpa CO, Svatoš A, Dabrowska P, Schmidt A, Boland W, Kothe E (2008) Involvement of siderophores in the reduction of metal-induced inhibition of auxin synthesis in *Streptomyces* spp. Chemosphere 74:19–25

Duffy BK, Defago G (1999) Environmental factors modulating antibiotic and siderophore biosynthesis by *Pseudomonas fluorescens* biocontrol strains. Appl Environ Microbiol 65:2429–2438

Eissendle M, Oberegger H, Zadra I, Hass H (2003) The siderophore system is essential for viability of *Aspergillus nidulus*: functional analysis of two genes encoding l-ornithine monooxygenase (Sid A) and a non-ribosomal peptide synthesis (Sid C). Mol Microbiol 49:359–375

Fan TWM, Lane AN, Pedler J, Crowley D, Higashi RM (1997) Comprehensive analysis of organic ligands in whole root exudates using nuclear magnetic resonance and gas chromatography–mass spectrometry. Anal Biochem 251:57–68

Fuhrmann JJ, Wollum AG (1989) Nodulation competition among *Bradyrhizobium japonicum* strains as influenced by rhizosphere bacteria and iron availability. Biol Fertil Soil 7:108–112

Gill PR Jr, Barton LL, Scoble MD, Neilands JB (1991) A high affinity iron transport system of *Rhizobium meliloti* may be required for efficient nitrogen fixation in plants. Plant Soil 130:211–217

Glick BR (1995) The enhancement of plant growth by free living bacteria. Can J Microbiol 41:109–114

Glick BR, Bashan Y (1997) Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of phytopathogens. Biotechnol Adv 15:353–378

Glick BR, Penrose DM, Li J (1998) A model for the lowering of plant ethylene concentrations by plant growth promoting bacteria. J Theor Biol 190:63–68

Grichko VP, Glick BR (2001) Flooding tolerance of transgenic tomato plants expressing the bacterial enzyme ACC deaminase controlled by the 35S, rolD or PRB-1b promoter. Plant Physiol Biochem 39:19–25

Haas H (2003) Molecular genetics of fungal siderophore biosynthesis and uptake: the role of siderophores in iron uptake and storage. Appl Microbiol Biotechnol 62:316–330

Hantke K (2001) Iron and metal regulation in bacteria. Curr Opin Microbiol 4:172–177

Harrison F, Paul J, Massey R, Buckling A (2008) Interspecific competition and siderophore-mediated cooperation in *Pseudomonas aeruginosa*. ISME J 2:49–55

Heymann P, Ernst JF, Winkelmann G (2000) A gene of the major facilitator superfamily encodes a transporter for enterobactin (Enb1p) in *Saccharomyces cerevisiae*. Biometals 13:65–72

Hider RC, Kong X (2010) Chemistry and biology of siderophores. Nat Prod Rep 27:637–657

Holmström SJM, Lundström US, Finlay RD, Van Hees PAW (2004) Siderophores in forest soil solution. Biogeochemistry 71:247–258

Idris R, Trifonova R, Puschenreiter M, Wenzel WW, Sessitsch A (2004) Bacterial communities associated with flowering plants on the Ni hyperaccumulator *Thlaspi goesingense*. Appl Environ Microbiol 70:2667–2677

Jones AM, Lindow SE, Wildermuth MC (2007) Salicylic acid, yersiniabactin, and pyoverdin production by the model phytopathogen *Pseudomonas syringae* pv. *tomato* DC3000: synthesis, regulation, and impact on tomato and *Arabidopsis* host plants. J Bacteriol 189:6773–6786

Kamnev AA, Lelie D (2000) Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. Biosci Rep 20:239–258

Kaufmann GF, Sartorio R, Lee SH, Rogers CJ, Meijler MM, Moss JA, Clapham B, Brogan AP, Dickerson TJ, Janda KD (2005) Revisiting quorum sensing: discovery of additional chemical and biological functions for 3-oxo-N-acylhomoserine lactones. Proc Natl Acad Sci USA 102:309–314

Khan AG (2005) Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. J Trace Elem Med Biol 18:355–364

Kloepper JW, Leong J, Teintze M, Schroth MN (1980) Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286:885–886

Konigsberger LC, Konigsberger E, May PM, Hefter GT (2000) Complexation of iron (III) and iron (II) by citrate. Implications for iron speciation in blood plasma. J Inorg Biochem 78:175–184

Kraemer SM (2004) Iron oxide dissolution and solubility in the presence of siderophores. Aquat Sci 66:3–18

Kraepiel AML, Bellenger JP, Wichard T, Morel FMM (2009) Multiple roles of siderophores in free-living nitrogen-fixing bacteria. Biometals 22:573–581

Kumar H, Bajpai VK, Dubey RC, Maheshwari DK, Kang SC (2010) Wilt disease management and enhancement of growth and yield of *Cajanus cajan*(L) var. Manak by bacterial combinations amended with chemical fertilizer. Crop Prot 29(6):591–598

Labuschagne N, Pretorius T, Idris AH (2010) Plant growth promoting rhizobacteria as biocontrol agents against soil-born plant disease. In: Maheshwari DK (ed) Plant growth and health promoting bacteria, vol 18, Microbiology monographs. Springer, Heidelberg, pp 211–230. doi:10.1007/978-3-642-13612-2_9

Lesueur D, Carro del Rio M, Diem HG (1995) Modification of the growth and the competitiveness of *Bradyrhizobium* strain obtained through affecting its siderophore-producing ability. In: Abadia J (ed) Iron nutrition in soils and plants. Kluwer Academic, Dordrecht, pp 59–66

Lesuisse E, Simon-Casteras M, Labbe P (1998) Siderophore-mediated iron uptake in *Saccharomyces cerevisiae* encodes a ferrioxamine B permease that belongs to the major facilitator superfamily. Microbiology 144:3455–3462

Loaces I, Ferrando L, Fernández Scavino A (2011) Dynamics, diversity and function of endophytic siderophore-producing bacteria in rice. Microb Ecol 61:606–618

Logeshwaran P, Thangaraju M, Rajasundari K (2009) Hydroxamate siderophores of endophytic bacteria *Gluconacetobacter diazotrophicus* isolated from sugarcane roots. Australian J Basic Appl Sci 3:3564–3567

Loper JE, Henkels MD (1999) Utilization of heterologous siderophores enhances levels of iron available to *Pseudomonas putida* in the rhizosphere. Appl Environ Microbiol 65:5357–5363

Maheshwari DK (2011) Bacteria in agrobiology: plant growth responses. Springer, Heidelberg

Mark GL, Morrissey JP, Higgins P, O'Gara F (2006) Molecular-based strategies to exploit *Pseudomonas* biocontrol strains for environmental biotechnology applications. FEMS Microbiol Ecol 56:167–177

Matzanke BF, Muller-Matzanke G, Raymond KN (1989) Siderophore-mediated iron transport. In: Loehr TM (ed) Iron carriers and iron proteins, vol 5, Physical bioinorganic chemistry. VCH Publishers, New York, pp 1–121

Maurhofer M, Reimmann C, Schmidli-Sacherer P, Heeb S, Haas D, Defago G (1998) Salicylic acid biosynthetic genes expressed in *Pseudomonas fluorescens* P3 improve the induction of systemic resistance in tobacco against tobacco necrosis virus. Phytopathology 88:678–684

Mayak S, Tirosh T, Glick BR (2004a) Plant growth-promoting bacteria that confer resistance in tomato plants to salt stress. Plant Physiol Biochem 42:565–572

Mayak S, Tirosh T, Glick BR (2004b) Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Sci 166:525–530

Mercado-Blanco J, van der Drift KMGM, Olsson PE, Thomas-Oates JE, van Loon LC, Bakker PAHM (2001) Analysis of the *pmsCEAB* gene cluster involved in biosynthesis of salicylic acid and the siderophore pseudomonine in the biocontrol strain *Pseudomonas fluorescens* WCS374. J Bacteriol 183:1909–1920

Miethke M, Marahiel MA (2007) Siderophore-based iron acquisition and pathogen control. Microbiol Mol Biol Rev 71:413–451

Nautiyal CS, Johri JK, Singh HB (2003) Survival of the rhizosphere-competent biocontrol strain *Pseudomonas fluorescens* NBR 12650 in the soil and phytosphere. Can J Microbiol 48:588–601

Neilands JB (1957) Some aspects of microbial iron metabolism. Bacteriol Rev 21:101–111

Neilands JB (1981) Iron absorption and transport in microorganisms. Annu Rev Nutr 1:27–46

Neilands JB (1995) Siderophores: structure and function of microbial iron transport compounds. J Biol Chem 270:26723–26726

Neilands JB, Leong SA (1986) Siderophores in relation to plant growth and disease. Annu Rev Plant Physiol 37:187–208

Neilands JB, Konopka K, Schwyn B, Coy M, Francis RT, Paw BH, Bagg A (1987) Comparative biochemistry of microbial iron assimilation. In: Winkelmann G, Van der Helm D, Neilands JB (eds) Iron transport in microbes, plants and animals. VCH, Weinheim, pp 3–33

O'Hara GW, Dilworth MJ, Boonkerd N, Parkpian P (1988) Iron deficiency specifically limits nodule development in peanut inoculated with *Bradyrhizobium* sp. New Phytol 108:51–57

Pandey A, Bringel F, Meyer JM (1994) Iron requirement and search for siderophores in lactic acid bacteria. Appl Microbiol Biotechnol 40:735–739

Pandey P, Kang SC, Gupta CP, Maheshwari DK (2005) Rhizosphere competent *Pseudomonas aeruginosa* GRC1 produces characteristic siderophore and enhances growth of Indian mustard (*Brassica campestris*). Curr Microbiol 51:303–309

Pedraza RO, Motok J, Salazar SM, Ragout A, Mentel MI, Tortora ML, Guerrero Molina MF, Winik BC, Díaz Ricci JC (2010) Growth-promotion of strawberry plants inoculated with *Azospirillum brasilense*. World J Microbiol Biotechnol 26:265–272

Powell PE, Cline GR, Reid CPP, Szaniszlo PJ (1980) Occurrence of hydroxamate siderophore iron chelators in soils. Nature 287:833–834

Press CM, Wilson M, Tuzun S, Kloepper JW (1997) Salicylic acid produced by *Serratia marcescens* 90–166 is not the primary determinant of induced systemic resistance in cucumber or tobacco. Mol Plant Microbe Interact 10:761–768

Reed MLE, Glick BR (2004) Applications of free living plant growth-promoting rhizobacteria. Ant van Leeuwen 86:1–25

Reed MLE, Glick BR (2005) Growth of canola (*Brassica napus*) in the presence of plant growth-promoting bacteria and either copper or polycyclic aromatic hydrocarbons. Can J Microbiol 51:1061–1069

Römheld V (1991) The role of phytosiderophores in acquisition of iron and other micronutrients in graminaceous species: an ecological approach. Plant Soil 130:127–134

Römheld V, Marschner H (1986) Mobilization of iron in the rhizosphere of different plant species. In: Tinker B, Laüchi A (eds) Advances in plant nutrition, vol 2. Greenwood publishing, New York, NY, pp 155–204

Saxena B, Modi M, Modi VV (1986) Isolation and characterization of siderophores from *Azospirillum lipoferum* D-2. J Gen Microbiol 132:2219–2224

Serino L, Reimmann C, Baur H, Beyeler M, Visca P, Haas D (1995) Structural genes for salicylate biosynthesis from chorismate in *Pseudomonas aeruginosa*. Mol Gen Genet 249:217–228

Shah S, Karkhanis V, Desai A (1992) Isolation and characterization of siderophore, with antimicrobial activity from *Azospirillum lipoferum* M. Curr Microbiol 25:34–35

Sharma A, Johri BN (2003) Growth promoting influence of siderophore-producing *Pseudomonas* strains GRP3A and PRS_9 in maize (*Zea mays* L.) under iron limiting conditions. Microbiol Res 158:243–248

Siddiqui IA, Shaukat SS (2004) Systemic resistance in tomato induced by biocontrol bacteria against the root-knot nematode, *Meloidogyne javanica* is independent of salicylic acid production. J Phytopathol 152:48–54

Siddiqui IA, Shaukat S (2005) *Pseudomonas aeruginosa* mediated induction of systemic resistance in tomato against root knot nematode. J Phytopathol 4:21–25

Stumm W, Morgan JJ (1995) Aquatic chemistry. Wiley-Interscience, New York

Takagi SI (1976) Naturally occurring iron-chelating compounds in oat-root and rice-root washings. 1. Activity measurement and preliminary characterization. Soil Sci Plant Nutr 22:423–433

Tortora ML, Díaz Ricci JC, Pedraza RO (2011) *Azospirillum brasilense* siderophores with antifungal activity against *Colletotrichum acutatum*. Arch Microbiol 193:275–286

Tortora ML, Diaz Ricci JC, Pedraza RO (2012) Protection of strawberry plants (*Fragaria ananassa* Duch.) against anthracnose disease induced by *Azospirillum brasilense*. Plant Soil 356:279–290. doi:10.1007/s11104-011-0916-6

Vasil ML, Ochsner UA (1999) The response of *Pseudomonas aeruginosa* to iron: genetics, biochemistry and virulence. Mol Microbiol 34:399–413

Visca P, Ciervo A, San Filippo V, Orsi N (1993) Iron-regulated salicylate synthesis by *Pseudomonas* spp. J Gen Microbiol 139:1995–2001

Whiting SN, de Souza MP, Terry N (2001) Rhizosphere bacteria mobilize Zn for hyper accumulation by *Thlaspi caerulescens*. Environ Sci Technol 15:3144–3150

Winkelmann G (1992) Structures and functions of fungal siderophores containing hydroxamate and complexone type iron binding ligands. Mycol Res 96:529–534

Winkelmann G (2007) Ecology of siderophores with special reference to the fungi. Biometals 20:379–392

Winkelmann G, Busch B, Hartmann A, Kirchhof G, Sußmuth R, Jung G (1999) Degradation of desferrioxamines by *Azospirillum irakense*: assignment of metabolites by HPLC/electrospray mass spectrometry. Biometals 12:255–264

Yehuda Z, Shenker M, Romheld V, Marschner H, Hadar Y, Chen Y (1996) The role of ligand exchange in the uptake of iron from microbial siderophore by gramineous plants. Plant Physiol 112:1273–1280

Chapter 12
Role of Microbial Siderophores in Improving Crop Productivity in Wheat

Prashant Sarode, Makarand Rane, Meghraj Kadam, and Sudhir Chincholkar

12.1 Introduction

At present, approximately 30 plant species account for about 95 % of the world's food energy supply (Cakmak et al. 2004), wheat being the third largest food crop behind corn and rice. To satisfy the food demand, modern cropping systems have been implemented specifically for cereals and cash crops by using high-yielding cultivars. This resulted in a dramatic reduction in food diversity and reduction in micronutrient intake. A micronutrient-poor diet has resulted in two billion people suffering from micronutrient malnutrition in third world countries (Cakmak et al. 2010). Among the different elements, iron (Fe) plays an important role in plant growth and development because of its unique physico-chemical properties. In the same context, a marked iron deficiency in wheat growth and productivity was observed due to (1) changes in soil salinity, (2) changes in soil pH and (3) anti-nutritional compounds in the soil such as phytic acid and phenolic (Welch and Graham 2004). In general, plants employ two strategies for iron absorption. However, this chapter focuses on a third strategy, i.e. iron solubilization through microbial siderophores and its utilization by plants, with special reference to wheat in terms of productivity and value-addition of iron.

12.2 Iron and Its Availability to Plants

Iron, the second-most abundant metal and fourth-most abundant element, is the most important mineral for living organisms (Crichton et al. 2001). In absentia of di-oxygen, the redox properties of iron resulted in the availability of a soluble

P. Sarode • M. Rane • M. Kadam • S. Chincholkar (✉)
School of Life Sciences, North Maharashtra University, Jalgaon 425 001, Maharashtra, India
e-mail: sudhirchincholkar@gmail.com

D.K. Maheshwari et al. (eds.), *Bacteria in Agrobiology: Crop Productivity*,
DOI 10.1007/978-3-642-37241-4_12,

ferrous form, which became a crucial component of physiology. As this transition occurred through a period of a few 100 million years, all living organisms have evolved strategies for making soluble iron available from insoluble forms (Guerinot and Yi 1994; Briat et al. 1995; Castignetti and Smarrelli 1986). Due to the ability of biological iron complexes to transfer electrons, iron is ubiquitous in metabolic reactions (Chincholkar et al. 2000; Crichton et al. 2001). The most common sources of iron in soil are the ferric oxides, which are the most stable form of Fe at neutral to alkaline pH and less than 10^{-15} M, which is insufficient to meet plant needs (Schwertmann 1991). Thus, Fe deficiency often limits plant growth, causing agricultural problems and reduced crop yields.

12.2.1 Role of Iron in Plant Systems

Although, Fe acquisition by plants is challenging due to the low solubility of iron in soil (Guerinot and Yi 1994), iron is an essential element for all organisms (Briat and Lobréaux 1998). It is required for many vital enzymes, including the cytochromes of the electron transport chain, as well as a wide range of other biological functions (Mori 1999). Except in anaerobic life, the physiological importance of iron has remained prominent. The role of iron in plant metabolism is shown in Table 12.1 (Chincholkar et al. 2000; Hemantaranjan 1995).

12.2.2 Strategies for Iron Acquisition in Plants

In order to avoid iron deficiency, various graminaceous plants biosynthesize and excrete non-proteinaceous chelating agents through the roots and then take up iron as a Fe complex by a highly specific uptake system that is enhanced by Fe deficiency (strategy I plants). However, dicotyledonous plants follow a different strategy (strategy II) whereby release of protons and reducing substances is combined with enzymatic splitting of chelates as a mechanism of solubilizing soil Fe and/or taking up chelated Fe (Table 12.2; Marschner et al. 1986; Römheld and Marschner 1986).

12.2.3 Causes of Iron Deficiency

The concentration of iron in soil ranges from 1 to 6 % (Chincholkar et al. 2000; Scholtz 1983), which is extraordinarily high compared with other plant nutrients, yet iron-deficiency in plants is recurrent. Iron deficiency is often seen in high pH and calcareous soils in arid regions. The reasons for this discrepancy are: (1) excessive application of chemical fertilizers and pesticides, which include a high

Table 12.1 Role of iron in different plant biochemical processes

Metabolic pathway	Biosynthetic regulation	Enzyme cofactor
Nitrogen fixation	Chlorophyll	Peroxidase
Tri-carboxylic acid (TCA) cycle	Toxin	Superoxide dismutase
Electron transport chain	Vitamins	Nitrogenase
Oxidative phosphorylation	Antibiotic	Hydrogenase
Photosynthesis	Cytochrome	Glutamate synthase
Respiration	Pigment	Cytochrome oxidase

Table 12.2 Iron acquisition mechanisms in plants

Strategy	Mechanism	Example	Reference
Strategy I	Lowering the rhizospheric pH by H^+ exudation		
	Releasing the organic reductants	*Cucumis safivus*	Rabotti et al. (1995)
	Secretion of Fe-chelating phenolics	*Lycopersicon esculenrum*	Chaney et al. (1992)
	Iron acquisition through:	*Beta vulgaris*	Gonzalez-Vallejo et al. (2000)
Strategy II	Secretion and uptake of iron- chelating non-proteinaceous amino acids, i.e. phytosiderophores		
	Secretion and uptake of iron-chelating non-proteinaceous amino acids, i.e. phytosiderophores	Avenic acid in oat (*Avena sativa* L.)	Ma and Nomoto (1993)
		2-Deoxymugineic acid in rice (*Oryza sativa* L.)	Nishiyama et al. (2012)
		2-Deoxymugineic acid, mugineic acid in barley (*Hordeum vulgare* L.)	Kawai et al. (1988)
		Mugineic acid in wheat (*Triticum aestivum* L.)	Ma et al. (1999)

content of phosphates, NO_3, Mg, Mn, Cu, Zn, Co and Ni, (2) irrigation water and (3) elevated levels of carbonate in soil. All these factors adversely affect pH, salinity and the C:N ratio. Additionally, root pruning by nematodes, insects and fungal diseases also cause iron deficiency in plants (Chincholkar et al. 2000; Mohammad et al. 2009; Mozafar 1995).

12.2.4 Iron Deficiency Symptoms

Due to the role of iron in the development of chloroplasts, which harvest light energy and transport electrons from water to $NADP^+$ (Briat and Lobréaux 1998),

iron-limiting conditions lead to a decrease in the concentration of light-harvesting pigments, resulting in yellowing of pigments (i.e. chlorosis) (Zhang et al. 1991). Iron-deficient chlorosis leads to poor growth of seedlings and hence low productivity. Due to the integral role of photosystems I and II, iron-poor conditions lead to: (1) uncoupling of the light-harvesting complex I (LHCI) antenna from photosystem I, (2) irreversibly impairment of photosystem II (Bertamini et al. 2004), (3) activity loss of chlorophyll-biosynthesizing enzymes (Briat and Lobréaux 1998), (4) diminished protein concentration in the leaves (Bisht et al. 2002; Yousfi et al. 2007), (5) low photosynthetic rate, (6) poor stomatal conductance and (7) low transpiration rate (Bertamini et al. 2004).

12.3 Wheat (*Triticum aestivum* and *T. durum*)

Wheat is the third-most important food grain crop and, economically, the most important group of plants in agriculture. In India it contributed about 37 % (72.06 million metric tonnes) of total food grain production in 2008. The area under production of wheat has increased from a mere 12.93 million ha in 1960–1961 to about 27 million ha in 2006–2007, with an increase in production of 11 an 76.37 million metric tonnes in 1960–61 and 1999–2000, respectively. A step down in production of around 65.1 metric tonnes due to reduced fertilizer consumption, changed fertilizer policy, poor variety development, global economic compulsions and trade readjustment was observed in 2003–2004, raising questions about the reliability of the food security system (Nagarajan 2005). Thus, determining and evaluating the factors affecting production and remediating them has become of high importance. Almost all biotic and abiotic stresses create micronutrient depletion due to changes in the ionic state, resulting in insolubility.

12.3.1 Role of Metal Ions in Wheat

Metals ions in plants are indispensable for healthy growth and productivity. The unavailability of metal ions (as well as toxicity due to the presence of excess) is coped with by metal homeostasis, which involves coordination of metal ion transporters for uptake, translocation and compartmentalization. Due to complexing with oxides, metal ions lack free ion conformation (i.e. solubility). Table 12.3, reviews the requirement of different metal ions and their deficiency syndromes in wheat plants.

Table 12.3 Metal ions and their deficiency symptoms in wheat

Metal ion	Concentration required	Deficiency symptom	Reference
N %	3.7–4.2 %	Pale yellow older leaves and poor growth	Hu and Schmidhalter (2005)
P %	0.2–0.5 %	Dark green plants, often with purple color; oldest leaves may be dark yellow to orange turning to brown	Neumann and Römheld (1999)
K %	1.5 %	Pale green plants with a limp or wilted appearance; bright yellow chlorosis turning brown along the margins of the oldest leaves	Pettigrew (2008)
S %	0.15 %	Pale yellow plants; uniformly yellow leaves without necrosis	Spencer and Freney (1980)
Ca%	0.2 %	Distorted growth	Ehret et al. (1990)
Mg%	0.15 %	Green yellow plants with yellow interveinal chlorosis turning to brown necrosis on the middle leaves	Chatterjee et al. (1994)
Cu	5–10 mg kg^{-1}	Male sterility in wheat plants	Graham (1975)
Zn	20–70 mg kg^{-1}	Stunted, pale green plants with localized white to pale yellow chlorosis, turning to brown or gray necrotic lesions	Yilmaz et al. (1998)
Mn	35–100 mg kg^{-1}	Green yellow plants with yellow interveinal chlorosis turning to brown necrosis on the middle leaves	Chatterjee et al. (1994)
Fe	50–180 mg kg^{-1}	Yellow leaves with prominently green veins	Zhang et al. (1991)
Mo	0.05–0.1 mg kg^{-1}	Paler green leaves	Modi and Cairns (1994)
B	6–10 mg kg^{-1}	Poor growth and male sterility	Rerkasem and Jamjod (1997)

12.3.2 Use of Iron Chelators for Increasing Wheat Productivity in the Presence of Iron Deficiency

Several conventional methods are used to manage iron deficiency problems. These include avoiding mis-management of nutrient imbalance and over-irrigation, the use of ferrous compounds as a foliar spray and application of iron chelates such as Fe-EDTA (ethylenediaminetetraacetic acid), Fe-EDDHA (ethylenediaminedi-Q-hydroxyphenlyacetic acid), Fe-DTPA (diethylenetriaminepentaacetic acid), FeIDHA (iminodisuccinic acid) and Fe-citrate through drip irrigation (Wallace 1995).

However, the effect of any chelators depends upon: (1) temperature (elevated temperature reduces stability), (2) dielectric point (increased dielectric point reduces stability), (3) ionic strength (increased ionic strength reduces stability), (4) competing complexes and (5) pH (at acidic pH complexation is lower). Along with this, the effectiveness of chelated Fe compounds in overcoming Fe-deficiency chlorosis is highly variable depending on the penetration ability through the leaf

cuticle and the mobility/translocation following diffusion into leaf tissue (Schonherr et al. 2005; Fernandez et al. 2006; Rodriguez-Lucena et al. 2010; Aciksoz et al. 2011). Biofortification using ferric–chelator complexes has shown elevated crop productivity (Aciksoz et al. 2011).

In a similar approach, Rawat et al. (2011) have demonstrated siderophore production by all the rhizospheric bacteria of a 120-day wheat crop. In the same experiment, they found dominance of *Bacillus* microflora in the rhizosphere (37.5 %) and rhizoplane (49 %) of a 30-day crop. Similarly, there was dominance of *Pseudomonas* population (29.09 %) in the rhizosphere (62.5 %) and rhizoplane (40 %) of a 90-day crop. Both bacterial genera showed siderophore production. This suggests metal dissolution through microbial siderophores. The role of ligand exchange in the uptake of iron from microbial siderophores by gramineous plants (strategy II) has been postulated (Fig. 12.1) and the ability of groundnut, cotton, sorghum, sunflower and cucumber (strategy I plants) to acquire Fe from microbial siderophores has been well documented, although the mechanism is unclear (Beard and Stoltzfus 2001). This discussion gives the impression that plants rely largely on microbes for iron. The mutualistic relationship in plant microbe interaction, i.e. PGPR (plant-growth promoting rhizobacteria) screens out undesirable and/or harmful intruders/opportunists (Wardle 1992; Nguyen 2003).

The effect of PGPR on plants is mediated by direct or indirect mechanisms (Glick 1995). Direct mechanisms include fixation of atmospheric nitrogen (Bakker et al. 1991), solubilization of phosphorous (Linderman 1992; Han and Lee 2005), potassium (Han and Lee 2005), zinc (Saravanan et al. 2007) and iron (Chincholkar et al. 2000, 2005), enhanced uptake of magnesium and calcium in plants (Lippmann et al. 1995) and synthesis of phyto-hormones (Frankenberger and Arshad 1995; Glick 1995). Indirect mechanisms include protection of the plant from pathogens, which is achieved through antagonism (Rosas 2007), deprivation of space and nutrient source (Sorensen 1997), iron (Chincholkar et al. 2007), parasitism towards phytopathogens (Rosas 2007; Rane et al. 2007) and induction of systemic resistance in the host (Hofte and Bakker 2007; Bloemberg and Lugtenberg 2001; Persello-Cartieaux et al. 2003).

12.4 Siderophore-Mediated Iron Nutrition of Plants

Although plants have evolved special mechanism for iron nutrition, they often fail to accumulate sufficient iron to meet nutritional requirements. In Sect. 12.2.2, two strategies have been mentioned for iron utilization by plants; however, Loper and Buyer (1991) have proposed a third strategy for iron uptake by plants, i.e. uptake of microbial Fe(III) siderophores. The role of microbial siderophores have been previously described in plant pathology as: (1) determinants of biocontrol activity, (2) virulence factors or ecological determinants and (3) factors influencing the iron nutrition of plants (Leong 1986; Neilands and Leong 1986). Table 12.4 illustrates

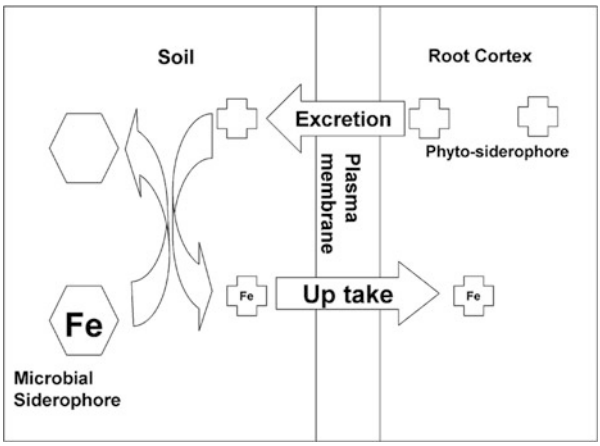


Fig. 12.1 Hypothetical mechanism of exchange of iron between microbial and phytosiderophores for observed iron nutrition of plant

the activity of siderophoregenic microorganisms and their role as growth promoters in different plants.

12.4.1 Siderophores and Their Plant Growth-Promoting Potential

Kloepper et al. (1980) have proved the plant growth-promoting activity of siderophores produced by strains of the *Pseudomonas fluorescens-putida* group because of antagonism to potentially deleterious rhizoplane fungi and bacteria, as well as improved iron nutrition to the plant. Siderophores produced by microorganisms were found in soil solutions at concentrations that may influence the Fe nutrition of plants (Rroco et al. 2003), which suggests the need for soil microbial activity along with phytosiderophores for satisfactory Fe supply in sorghum. Similarly, the fluorescent siderophore pyoverdin has been reported for its role in plant growth stimulation (Hofte et al. 1991). Table 12.4 clearly shows the influence of different plant growth-promoting organisms. Similar observations were also reported for the active ingredient, i.e. siderophore (Table 12.5).

12.4.2 Siderophoregenic Microbes and Siderophore-Mediated Induced Systemic Resistance

Disease can be reduced if defense mechanisms are triggered by leaf-necrosis pathogens or rhizobacteria prior to infection and these phenomena are commonly known as systemic acquired resistance (SAR) and induced systemic resistance (ISR), respectively (Bakker et al. 2003). Various non-pathogenic rhizobacteria

Table 12.4 Siderophoregenic microorganisms having PGPR activity

Siderophoregenic microorganism	Plant	Growth effects	Reference
Pseudomonas fluorescens PGPR1	Pea nut	Increase in pod yield, nodule dry weight, root length and nodule number	Dey et al. (2004)
Pseudomonas strains GRP3A and PRS9	Maize	Increase in germination percentage, shoot and root length and dry weight	Sharma and Johri (2003)
Bradyrhizobium japonicum	Soybean	Increase in the percentage of germination, nodulation, chlorophyll, oil and protein content and in number of pods and shoot length and number of branches and root length	Khandelwal et al. (2002), Khandelwal (2001)
Penicillium chrysogenum[a]	Cucumber, maize	Increased chlorophyll content	Hordt et al. (2000)
Kluyvera ascorbata	Tomato, canola and Indian mustard	Decreased heavy metal toxicity	Burd et al. (2000)
Pseudomonas B10	Potato	Increased growth	Buyer and Leong (1986)
Pseudomonas aeruginosa[b]	Groundnut	Improved percentage germination, root ramification, nodulation, height, foliage and chlorophyll content	Manwar et al. (2004)

[a]Ferreted siderophore mixture used under hydroponic conditions
[b]Increase in nutritional values of groundnut has also been reported

Table 12.5 Ferric–pyoverdin complex and its plant growth-promoting effect on different plants

Bacterial strain	Plant	Effect on plant	References
P. putida	*Arachis hypogeae* L.[a]	Enhanced chlorophyll content	Jurkevitch et al. (1988)
P. putida P3	*Arachis hypogeae* L.[b]; *Gossypium hirsutum* L.[b]	Enhanced chlorophyll content; presence of ^{59}Fe in roots	Bar-Ness et al. (1991)
P. putida	*Dianthus caryophyllus* L.[b]	Enhanced chlorophyll content; ferric reductase activity	Duijff et al. (1994a)
P. putida WC358	*Hordeum vulgare* L.[b]	Enhanced chlorophyll content; presence of ^{59}Fe in the roots	Duijff et al. (1994b)
P. putida P3	*Sorghum bicolor* L.[b]	Enhanced chlorophyll content; uptake of ^{59}Fe by host plant	Bar-Ness et al. (1991)
P. putida	*Zea mays* L.[b]	Presence of ^{55}Fe in the roots	Bar-Ness et al. (1992)

[a]Growing plants in calcareous soil
[b]Growing plants in nutrient solution

have been shown to induce systemic resistance in plants and thereby provide protection against a broad spectrum of phyto-pathogenic fungi, bacteria and viruses (Table 12.6). Several bacterial determinants have been claimed to produce systemic resistance, including siderophores, salicylic acid and the *O*-antigenic side chain of the bacterial outer membrane protein lipopolysaccharide (LPS). Colonization of tobacco roots by *Pseudomonas fluorescens* CHA0 reduces leaf necrosis caused by tobacco necrosis virus (TNV) and induces physiological changes in the plant (e.g. an increase in salicylic acid and pathogen-related proteins in the leaves). A pyoverdin-negative mutant of CHA0 could only partially induce resistance against TNV (Notz 2002; Uknes et al. 1993). Because bacterial treatments protected potato tubers from subsequent infections by *P. solanacearum,* the concept that biocontrol agents might induce resistance in the host was suggested (Kempe and Sequeira 1983). Similar observations were noted for active biomolecules (i.e. siderophores), as shown in Table 12.7.

12.5 Siderophore and Siderophorogenic PGPR in Wheat Productivity

A strong competition in the rhizosphere was exhibited for acquiring iron, and the high-affinity stability of ferric–siderophore (pyoverdin) chelate and pyoverdin-producing bacteria were tested for antagonistic activity against phytopathogens, iron nutrition and growth of wheat plants (Sarode 2007). *Pseudomonas putida* was isolated from black cotton soils having siderophoregenic plant growth-promoting potential (Sarode et al. 2007) and tried for its effect on wheat productivity. For evaluating amplified productivity, the performance of pyoverdin, a fluorescent siderophore isolated from *P. putida*, was evaluated at three scales: (1) plate assay, (2) pot assay and (3) field trial.

12.5.1 Antagonistic Effect of Pyoverdin Biosynthesized by P. putida *Against Phytopathogens*

The presence of siderophoregenic rhizobacteria around the root zone of plants is known to protect the plant from phytopathogen infestations by competing with them for iron nutrition (O'Sullivan and O'Gara 1992). In vitro antifungal performance of siderophore-rich supernatants produced by *P. putida* under iron stress conditions in desferri (pyoverdin) and ferri (Fe + pyoverdin) states have been determined in standard optimal conditions with different phytopathogens. Table 12.8 shows the higher inhibitory potency of the supernatant in the absence of iron than in presence of iron, thus proving the siderophore-induced resistance to these types of fungal phytopathogens.

Table 12.6 Siderophoregenic microorganisms reported for biocontrol activity

Siderophore producer strain	Organism controlled by	Plant disease	Reference
Pseudomonas fluorescens EPS62e	*Erwinia amylovora*	Fire blight of pear trees and many plants	Cabrefiga et al. (2007)
P. fluorescens	*A. niger* and *S. rolfsii*	Collar rot and stem rot of peanut	Dey et al. (2004)
Pseudomonas fluorescens Pf4-99	*Macrophomina phaseolina*	Charcoal rot of chickpea	Kumar et al. (2007)
Rhizobium meliloti	*Macrophomina phaseolina*	Charcoal rot in groundnut	Arora et al. (2001)
Enterobacter cloacae	*Aspergillus niger, Aspergillus flavus, Fusarium oxysporum* and *Alternaria* spp.	–	Naphade (2002)
Pseudomonas fluorescens	*Rhizoctonia solani*	Rice sheath blight	Nagarajkumar et al. (2004)
Pseudomonas spp. GRP3A, PRS9	*Colletotrichum dematium, Rhizoctonia solani* and *Sclerotium rolfsii*	Maize	Sharma and Johri ((2003)
Pseudomonas sp. EM85	*Macrophomina phaseolina, Fusarium moniliforme* and *Fusarium graminearum*	Maize root diseases	Pal et al. (2001)
Proteus sp.	*Fusarium oxysporum*	Mungo beans	Barthakur (2000)
Rhodotorulla strains	*Botrytis cinerea*	Grey mould on a wide variety of host plants	Calvente et al. (2001)
P. aeruginosa (GRC1)	*Macrophomina phaseolina, Fusarium oxysporum*	–	Gupta et al. (1999)
P. aeruginosa	*Aspergillus niger, Aspergillus flavus, Aspergillus oryzae, Fusarium oxysporum, Sclerotium rolfsii* and *Alternaria alternata*	–	Manwar (2001)
P. aeruginosa 7NSK2	*Pythium*	Damping-off in tomato	Buysens et al. (1996)
P. putida WCS358	*Botrytis cinerea*	Grey mould in eucalyptus	Ran et al. (2005)
P. fluorescens 2-79RN 10	*G. graminis* var. *tritici*	Take-all in wheat	Weller et al. (1988)
Cumulative effect of *P. fluorescens* and *P. putida*	*Verticillium dahliae*	Wilt in olive	Mercado-Blanco et al. (2004)

Table 12.7 Siderophores or iron-regulated compounds in induced systemic resistance

Siderophore or iron-regulated compound	Producer strains	Pathogen	Plant	Reference
2,4-Diacetylphloroglucinol	*Pseudomonas fluorescens* CHA0	*Peronospora parasitica*	Arabidopsis (downy mildew)	Iavicoli et al. (2003)
Catechol-type siderophore	*Serratia marcescens* 90-166	*Colletotrichum orbiculare*	Cucumber	Press et al. (2001)
Lipo-polysaccharides	*Pseudomonas fluorescens* WCS417	*Pseudomonas syringae* pv. *tomato*	*Arabidosis thaliana* (bacterial speck)	Van Wees et al. (1997)
N-Trialkylated benzylamine	*Pseudomonas putida* BTP1	*Pythium aphandermatumi*	Cucumber (root rot)	Ongena et al. (2005)
Pseudobactin	*Pseudomonas putida* WCS358	*Botrytis cinerea*	Tomato	Meziane et al. (2005)
Pyocyanin	*Pseudomonas aeruginosa* 7NSK2	*Magnaporthe grisea*	Rice	De Vleesschauwer et al. (2006)
Pyoverdin	*Pseudomonas fluorescens* CHA0	*Tobacco mosaic virus*	Tobacco (tobacco necrosis)	Maurhofer et al. (1994)
Salicylic acid	*Pseudomonas aeruginosa* 7NSK2	*Colletotrichum lindemuthianum, Botrytis cinerea*	Bean	Bigirimana and Höfte (2002), De Meyer and Höfte (1997)
Pseudobactin, LPS	*Pseudomonas fluorescens WCS374*	*Fusarium oxysporum*	Radish (Wilt)	Leeman et al. (1996)

Table 12.8 Influence of siderophore strains against phytopathogens

	Inhibition (%)	
Phytopathogen	Absence of iron (0 μM)	Presence of iron (50 μM)
Aspergillus niger	80.00	72.00
Aspergillus flavus	32.14	17.86
Fusarium oxysporum	47.67	20.93
Colletotrichum capsicum	50.98	31.37

Values are averages from results obtained in triplicates

12.5.2 Wheat Germination: Plate Assay

Plate experiments were performed to study the germination ability of wheat variety Chandosi as a function of various treatments, i.e. pyoverdin, *Pseudomonas putida* and *Pseudomonas aeruginosa* (pyoverdin-producing organism), EDTA and untreated control. The treated seeds of wheat after 5 days were found to show more vigorous emergence/germination over untreated controls.

Although, the rate of seed germination was 100 % in all treatments, the number of roots and intensity of rootlets (Fig. 12.2) was higher for seeds treated with siderophore and siderophoregenic bacterial strains than for EDTA-treated seeds and untreated controls, confirming that microbial siderophore alone or in production by microbes had better impact than synthetic chelators. Table 12.9 supports the visual observation, whereby 100 % germination was seen in all the conditions; however, the average shoot and root length with vigor of germination seemed to be maintained by the siderophore. The results of siderophore (pyoverdin) treatment were similar to those for pyoverdin-producing strains, i.e. *P. aeruginosa* and *P. putida*.

12.5.3 Pot Assay for Siderophore-Incorporated Soil

The activity of these iron-chelating biomolecules in a pot assay (i.e. in soil conditions) revealed the performance of siderophores in the bio-geo state present around the plant. A pot assay is the laboratory step prior to field application. Under the natural conditions (i.e. pH 7.5) of black soil for the same variety of wheat (Chandosi) and with addition of purified pyoverdin (20 mg kg^{-1} soil), plants showed vigorous growth in comparison to controls (without addition of siderophore). After 8 days of growth, no significant difference was observed in percentage germination and shoot height comparing control with siderophore-treated seeds, whereas rootlet growth and iron content were improved in roots as well as in leaves compared to control (Fig. 12.3). However, marked differences in the iron content in leaves and roots were noted. Thus, along with antagonism, siderophores increase value-addition of iron in plants. Thus, the pyoverdin-

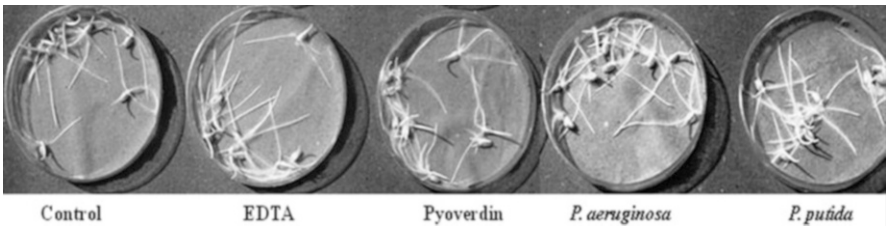


Fig. 12.2 Influence of various chelators on wheat germination

Table 12.9 Comparison of plate assays of seeds treated with iron and its chelates after 5 days of incubation

Sample	Total number of seeds	Rate of germination (%)	Average shoot length (cm)	Number of roots	Average root length (cm)	Rootlet intensity
Control (with iron)	10	100	5.5 (0.93)	5 (0.00)	6.5 (1.71)	+
Synthetic chelator with iron (Fe-Na_2EDTA)	10	100	6.2 (0.67)	5 (0.00)	6.5 (0.47)	++
Pyoverdin (siderophore) with iron	10	100	6.2 (0.86)	6 (0.58)	6.3 (0.79)	+++
Pseudomonas aeruginosa	10	100	6.5 (0.51)	6 (0.43)	6.5 (0.28)	+++
Pseudomonas putida	10	100	6.4 (0.63)	6 (0.00)	6.5 (0.37)	+++

+ indicates the intensity of appearance of rootlets on the roots. Values in parenthesis give standard deviation

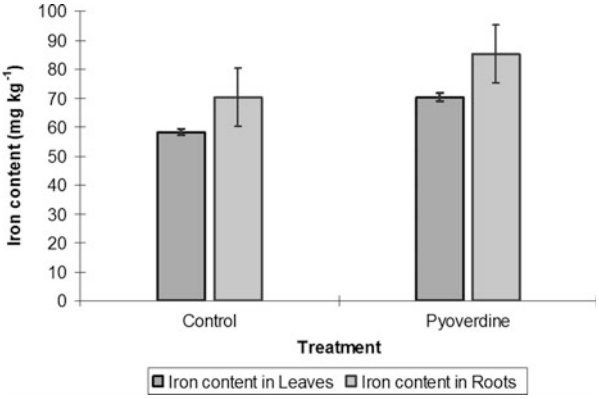


Fig. 12.3 Effect of siderophore on iron addition in plant leaves and roots in a pot assay

mediated iron nutrition of wheat can be an efficient way for value-added plant growth.

Sections 12.5.1, 12.5.2 and 12.5.3 described the third strategy of iron acquisition by plants, i.e. microbial siderophore-mediated iron acquisition. Similarly, the role

of siderophore (pyoverdin) in (1) mobilization of iron from phytopathogens, (2) increased plant vigor and (3) value-addition of iron in plant systems (i.e. in wheat) has been shown. Thus, use of a siderophoregenic bioinoculant certainly improves the productivity of wheat.

12.5.4 Field Application (Variety: Chandosi)

The conclusion of Sect. 12.5.3 has been tested in experimental field trials to study the reliability of siderophoregenic PGPR, here *P. aeruginosa* and *P. putida* treatments in field conditions. Root colonization by many *Pseudomonas* strains is well established, whereby the siderophore plays a crucial role (Lugtenberg et al. 2001). The wheat seed bacterization by *P. aeruginosa* and *P. putida* strains from two diverse habitats was tested for their effect on wheat in terms of healthier germination and productivity. It was revealed that percentage germination in *P. aeruginosa* and *P. putida* (Fig. 12.4) was 85 % and 80 %, respectively, whereas 70 % was recorded in controls (Fig. 12.5). The treatment was found to produce a significant ($P < 0.05$) increase in shoot height, root length after 25 days, chlorophyll content, weight of spikelets (Fig. 12.6), grain yield and iron content in grain. As shown in Table 12.10, seeds treated with *P. aeruginosa* gave maximum production, followed by *P. putida* and least production for controls (no seed treatment). *Pseudomonas* was found to work consistently, which was reflected by an increase in vegetative vigor, i.e. shoot and root length. Physiological studies indicated that plants treated with *Pseudomonas* had increased chlorophyll content. The overall increase in the grain yield (i.e. productivity) was 13.09 % and 18.27 % with *P. putida* and *P. aeruginosa*, respectively, over control without inoculation. Apart from these, the iron content of grain was found to be increased over control. As depicted in Table 12.10, in the field study it was found that in comparison with untreated control, *P. aeruginosa* was capable of exerting its maximum effect, leading to more than 27 % rise in iron content of wheat grains; *P. putida* was also instrumental in giving more than 18 % rise in iron content.

12.6 Conclusion

Although, there are no in-vivo studies to show that plants have the ability to take up iron from iron–siderophore complexes, there are number of in-vitro studies showing that uptake of iron by plants like groundnut, cotton, sorghum, sunflower and cucumber from a ferric–siderophore complex chelate has been observed (Beard and Stoltzfus 2001). Synthetic iron-chelating agents and their effect in terms of productivity have been described here. Based on the observations and experimental results, siderophores and siderophoregenic Pseudomonads have been found to: (1) improve the productivity of wheat in terms of grain yield by 13–18 %, (2) increase

Fig. 12.4 Control field of wheat (variety: Chandosi)

Fig. 12.5 Test field of wheat (variety: Chandosi)

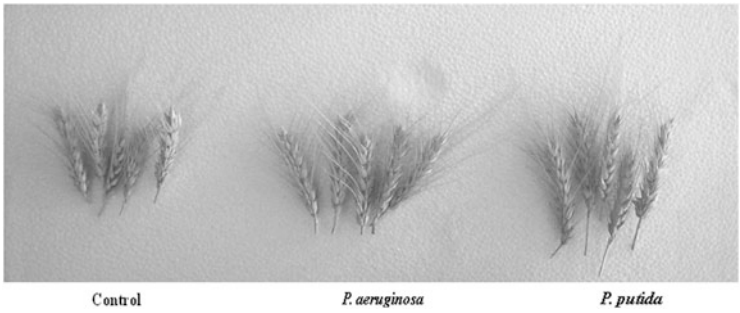


Fig. 12.6 Wheat quality (variety: Chandosi)

value-addition of iron in wheat by 18–27 % of total iron content, (3) improve the vigor of plant growth by means of plant shoot, root length and chlorophyll content by 12–16 %, 6–11 % and 34–60 %, respectively and (4) induce systemic resistance. Thus, a profound role in increasing wheat productivity has been established for siderophores.

Table 12.10 Field trials of wheat (variety: Chandosi)

	After 25 days		After 50 days			After harvesting		
Treatment and statistics	Shoot height (cm)	Root length (cm)	Shoot height (cm)	Root length (cm)	Chlorophyll (μg/g)	Weight of spikelets (gm/100)	Grain yield (Q/ha)	Iron content in grains (ppm)
Pseudomonas putida	28.22	8.81	78.87	14.41	11.84	321.07	25.03	27.45
Pseudomonas aeruginosa	30.85	8.89	82.00	15.11	14.07	330.00	26.17	29.66
Control	25.44	6.68	70.41	13.50	8.78	255.33	22.13	23.19
F	9.46	12.60	16.10	1.84	376.44	1,477.35	36.95	1,727.60
P value	0.0139[a]	0.0071[a]	0.0038[a]	0.2381[b]	4.94×10^{-7}[a]	8.32×10^{-9}[a]	0.0004[a]	5.21×10^{-9}[a]

Each value represents the mean of seven replicates

[a]The difference in the mean values between the treatment groups (*P. putida* versus *P. aeruginosa*) is greater than would be expected by chance; $P < 0.05$ (F_{crit} 5.14) indicates a statistically significant difference

[b]Not significant

References

Aciksoz SB, Yazici A, Ozturk L, Cakmak I (2011) Biofortification of wheat with iron through soil and foliar application of nitrogen and iron fertilizers. Plant Soil 349:215–225

Arora NK, Kang SC, Maheshwari DK (2001) Isolation of siderophore producing strains of *Rhizobium meliloti* and their biocontrol potential against *Macrophomina phaseolina* that causes charcoal rot of groundnut. Curr Sci 81:673–677

Bakker PAH, Van Peer R, Schippers B (1991) Suppression of soil borne plant pathogens by fluorescent Pseudomonad: mechanism and prospect. In: Beemester ABR, Bollen GJ, Gerlach M, Russien MA, Schippers B, Temple A (eds) Development in agriculturally managed forest ecology. Elsevier, Amsterdam, pp 217–230

Bakker PAHM, Ran LX, Pieterse CMJ, van Loon LC (2003) Understanding the involvement of rhizobacteria mediated induced resistance in biocontrol of plant diseases. Can J Plant Pathol 25:5–9

Bar-Ness E, Chen Y, Hadar Y, Marschner H, Römheld V (1991) Siderophores of *Pseudomonas putida* as an iron source for dicot and monocot plants. Plant Soil 130:231–241

Bar-Ness E, Hadar Y, Chen Y, Romheld V, Marschner H (1992) Short-term effects of rhizosphere microorganisms on Fe uptake from microbial siderophores by maize and oat. Plant Physiol 100:451–456

Barthakur M (2000) Plant growth promotion and fungicidal activity in a siderophore-producing strain of *Proteus* sp. Folia Microbiol 45:539–543

Beard J, Stoltzfus R (2001) Iron-deficiency anaemia: reexamining of the nature and magnitude of the public health problem. In: Beard J, Stoltzfus R (eds) Proceedings of the Belmont conference. J Nutr 131(2SII):563S–703S

Bertamini M, Nedunchezhian N, Borghi B (2004) Effect of iron deficiency induced changes on photosynthetic pigments, ribulose-1, 5-bisphoshpate carboxylase, and photosystem activities in field grown grapevine (*Vitis vinifera* L. cv. Pinot Noir) leaves. Photosynthetica 39:59–65

Bigirimana J, Höfte M (2002) Induction of systemic resistance to *Colletotrichum lindemuthianum* in bean by a benzothiadiazole derivative and rhizobacteria. Phytoparasitica 30:159–168

Bisht SS, Nautiyal BD, Sharma CP (2002) Biochemical changes under iron deficiency and recovery in tomato. Plant Physiol 7:183–186

Bloemberg GV, Lugtenberg BJJ (2001) Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Curr Opin Plant Biol 4:343–350

Briat JF, Lobréaux S (1998) Iron storage and ferritins in plants. In: Sigel A, Sigel H (eds) Metal ions in biological systems. Dekker, New York, pp 563–584

Briat JF, Fobis-Loisy I, Grignon N, Lobréaux S, Pascal N, Savino G, Thoiron S, von Wirén N, Van Wuytswinkel O (1995) Cellular and molecular aspects of iron metabolism in plants. Biol Cell 84:69–81

Burd GI, Dixon DG, Glick BR (2000) Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. Can J Microbiol 46:237–245

Buyer JS, Leong J (1986) Iron transport-mediated antagonism between plant growth-promoting and plant-deleterious *Pseudomonas* strains. J Biol Chem 261:791–794

Buysens S, Heungenes K, Poppe J, Hofte M (1996) Involvement of pyochelin and pyoverdin in suppression of *Pythium*-induced damping-off of tomato by *Pseudomonas aeruginosa* 7NSK2. Appl Environ Microbiol 62:865–871

Cabrefiga J, Bonaterra A, Montesinos E (2007) Mechanisms of antagonism of *Pseudomonas fluorescens* EPS62e against *Erwinia amylovora*, the causal agent of fire blight. Int Microbiol 10:123–132

Cakmak I, Torun A, Millet E, Feldman M, Fahima T, Korol A, Nevo E, Braun HJ, Ozkan H (2004) *Triticum dicoccoides*: an important genetic resource for increasing zinc and iron concentration in modern cultivated wheat. Soil Sci Plant Nutr 50:1047–1054

Cakmak I, Pfeiffer WH, McClafferty B (2010) Biofortification of durum wheat with zinc and iron. Cereal Chem 87:10–20

Calvente V, de Orellano ME, Sansone G, Benuzzi D, Sanz de Tosetti MI (2001) Effect of nitrogen source and pH on siderophore production by *Rhodotorula* strains and their application to biocontrol of phytopathogenic moulds. J Ind Microbiol Biotechnol 26:226–229

Castignetti D, Smarrelli J (1986) Siderophores, the iron nutrition of plants and nitrate reductases. FEBS Lett 209:147–151

Chaney RL, Chen Y, Green CE, Holden MJ, Bell PF, Luster DG, Angle JS (1992) Root hairs on chlorotic tomatoes are an effect of chlorosis rather than part of the adaptive Fe-stress response. J Plant Nutr 15:1857–1875

Chatterjee C, Nautiyal N, Agarwala SC (1994) Influence of changes in manganese and magnesium supply on some aspects of wheat physiology. Soil Sci Plant Nutr 40:191–197

Chincholkar SB, Chaudhari BL, Talegaonkar SK, Kothari RM (2000) Microbial iron chelators: a sustainable tool for the biocontrol of plant disease. In: Upadhyay RK, Mukerji KG, Chamola PC (eds) Biocontrol potential and its exploitation in sustainable agriculture. Kluwer, New York, pp 49–70

Chincholkar SB, Rane MR, Chaudhari BL (2005) Siderophores: their biotechnological applications. In: Podila GK, Varma A (eds) Biotechnological applications: microbes; microbiology series. I K International, New Delhi, pp 177–198

Chincholkar SB, Chaudhari BL, Rane MR, Sarode PD (2007) Fungal phytopathogen suppression using siderophoregenic bio-inoculants. In: Chincholkar SB, Mukerji KG (eds) Biological control of plant diseases: current concepts. Haworth, USA, pp 401–417

Crichton R, Boelaert JR, Braun V, Hantke K, Marx M, Santos M, Ward R (2001) Solution chemistry of iron in biological media. In: Crichton R (ed) Inorganic biochemistry of iron metabolism. John Wiley, New York, pp 1–14

De Meyer G, Höfte M (1997) Salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 induces resistance to leaf infection by *Botrytis cinerea* on bean. Phytopathology 87:588–593

De Vleesschauwer D, Cornelis P, Hofte M (2006) Redox active pyocyanin secreted by *Pseudomonas aeruginosa* 7NSK2 triggers systemic resistance to *Magnaporthe grisea* but enhances *Rhizoctonia solani* susceptibity in rice. Mol Plant Microbe Interact 19:1406–1419

Dey R, Pal KK, Bhatt DM, Chauhan SM (2004) Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. Microbiol Res 159:371–394

Duijff BJ, Bakker PAHM, Schippers B (1994a) Ferric pseudobactin 358 as an iron source for carnation. J Plant Nutr 17:2069–2078

Duijff BJ, De Kogel WJ, Bakker PAHM, Schippers B (1994b) Influence of pseudobactin 358 on the iron nutrition of barley. Soil Biol Biochem 26:1681–1994

Ehret DL, Redmann RE, Harvey BL (1990) Cipywnyk A salinity-induced calcium deficiencies in wheat and barley. Plant Soil 128:143–151

Fernandez V, Rio V, Abadia J, Abadia A (2006) Foliar iron fertilization of peach (*Prunus persica* (L.) Batsch): effects of iron compounds, surfactants and other adjuvants. Plant Soil 289:239–252

Frankenberger WTJ, Arshad M (1995) Phytohormones. In: Frankenberger WT, Arshad M (eds) Soil in microbial production and functions. Dekker, New York, pp 503–518

Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117

Gonzalez-Vallejo EB, Morales F, Cistue L, Abadia A, Abadia J (2000) Iron deficiency decreases the Fe(III)-chelate reducing activity of leaf protoplasts. Plant Physiol 122:337–344

Graham RD (1975) Male sterility in wheat plants deficient in copper. Nature 254:514–515

Guerinot ML, Yi Y (1994) Iron: nutritious, noxious, and not readily available. Plant Physiol 104:815–820

Gupta CP, Sharma A, Dubey RC, Maheshwari DK (1999) *Pseudomonas aeruginosa (GRC1)* as a strong antagonist of *Macrophomina phaseolina* and *Fusarium oxysporum*. Cytobios 99:183–189

Han HS, Lee KD (2005) Phosphate and potassium solubilizing bacteria effect on mineral uptake, soil availability and growth of eggplant. Res J Agric Biol Sci 1:176–180

Hemantaranjan A (1995) Physiological and biochemical aspects of iron nutrition. In: Hemantaranjan A (ed) Advancements in iron nutrition research. Scientific Publishers, Jodhpur, pp 63–108

Hofte M, Bakker P (2007) Competition for iron and induced systemic resistance by siderophores of plant growth promoting bacteria. In: Verma A, Chincholkar SB (eds) Microbial siderophores. Soil biology, vol 12. Springer, Berlin, pp 121–133

Hofte M, Seong KY, Jurkevitch E, Verstraete W (1991) Pyoverdin production by the plant growth beneficial *Pseudomonas* strain 7SNK2: ecological significance in soil. Plant Soil 130:249–257

Hordt W, Romheld V, Winkelmann G (2000) Fusarinines and dimerum acid, mono- and dihydroxamate siderophores from *Penicillium chrysogenum*, improve iron utilization by strategy I and strategy II plants. Biometals 13:37–46

Hu Y, Schmidhalter U (2005) Drought and salinity: a comparison of their effects on mineral nutrition of plants. J Plant Nutr Soil Sci 168:541–549

Iavicoli A, Boutet E, Buchala A, Métraux JP (2003) Induced systemic resistance in *Arabidopsis thaliana* in response to root inoculation with *Pseudomonas fluorescens* CHA0. Mol Plant Microbe Interact 16:851–858

Jurkevitch E, Hadar Y, Chen Y (1988) Involvement of bacterial siderophores in the remedy of lime-induced chlorosis in peanut. Soil Sci Soc Am J 52:1032–1037

Kawai S, Itoh K, Takagi S (1988) Studies on phytosiderophores: biosynthesis of mugineic acid and 2′-deoxymugineic acid in *Hordeum vulgare* L. var. Minorimugi. Tetrahedron Lett 29:1053–1056

Kempe J, Sequeira L (1983) Biological control of bacterial wilt of potatoes: attempts to induce resistance by treating tubers with bacteria. Plant Dis 67:499–503

Khandelwal SR (2001) Studies on the siderophores of *Rhizobium* sp., and its applicability in plant growth promotion and disease suppression. PhD Thesis, North Maharashtra University, Jalgaon, India

Khandelwal SR, Manwar AV, Chaudhari BL, Chincholkar SB (2002) Siderophoregenic bradyrhizobia boost yield of soybean. Appl Biochem Biotechnol 102:155–168

Kloepper JW, Leong J, Teintze M, Schroth MN (1980) Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286:885–886

Kumar V, Kumar A, Kharwar RN (2007) Antagonistic potential of fluorescent pseudomonads and control of charcoal rot of chickpea caused by *Macrophomina phaseolina*. J Environ Biol 28:15–20

Leeman M, Den Ouden FM, Van Pelt JA, Dirkx FPM, Steijl H, Bakker PAHM, Schippers B (1996) Iron availability affects induction of systemic resistance against fusarium wilt of radish by *Pseudomonas fluorescens*. Phytopathology 86:149–155

Leong J (1986) Siderophores: their biochemistry and possible role in the biocontrol of plant pathogens. Annu Rev Phytopathol 24:187–209

Linderman RG (1992) Vesicular arbuscular mycorrhizae and soil microbial interactions, in Mycorrhizae. In: Bethelenfalvay GJ, Linderma RG (eds) Sustainable agriculture. American Society of Agronomy, Madison, pp 45–70

Lippmann B, Leinhos V, Bergmann H (1995) Influence of auxin producing rhizobacteria on root morphology and nutrient accumulation of crops. Changes in root morphology and nutrient accumulation in maize (*Zea mays* L.) caused by inoculation with indol-3 acetic acid (IAA) producing Pseudomonas and Acinetobacter strains of IAA applied exogenously. Angew Bot 69:31–36

Loper JE, Buyer JS (1991) Siderophores in microbial interactions on plant surfaces. Mol Plant Microbe Interact 4:5–13

Lugtenberg BJ, Dekkers L, Bloemberg GV (2001) Molecular determinants of rhizosphere colonization by Pseudomonas. Annu Rev Phytopathol 39:461–490

Ma JF, Nomoto K (1993) Two related biosynthetic pathways of mugineic acids in gramineous plants. Plant Physiol 102:373–378
Ma JF, Taketa S, Chang Y, Takeda K, Matsumoto H (1999) Biosynthesis of phytosiderophores in several *Triticeae* species with different genomes. J Exp Bot 50:723–726
Manwar AV (2001) Application of microbial iron chelators (siderophores) for improving yield of groundnut. PhD Thesis, North Maharashtra University, Jalgaon, India
Manwar AV, Khandelwal SR, Chaudhari BL, Meyer JM, Chincholkar SB (2004) Siderophore production by a marine *Pseudomonas aeruginosa* and its antagonistic action against phytopathogenic fungi. Appl Biochem Biotechnol 118:243–251
Marschner H, Romheld V, Kissel V (1986) Different strategies in higher-plants in mobilization and uptake of iron. J Plant Nutr 9:695–713
Maurhofer M, Hase C, Meuwly P, Métraux J-P, Défago G (1994) Induction of systemic resistance of tobacco to tobacco necrosis virus by the root-colonizing *Pseudomonas fluorescens* strain CHA0: influence of the gacA gene and of pyoverdine production. Phytopathology 84:139–146
Mercado-Blanco J, Rodriguez-Jurado D, Hervas A, Jimenez-Diaz RM (2004) Suppression of *Verticillium* wilt in olive planting stocks by root-associated fluorescent *Pseudomonas* spp. Biol Contr 30:474–486
Meziane H, Van der Sluis I, Van Loon LC, Höfte M, Bakker PAHM (2005) Determinants of *Pseudomonas putida* WCS358 involved in inducing systemic resistance in plants. Mol Plant Pathol 6:177–185
Modi AT, Cairns ALP (1994) Molybdenum deficiency in wheat results in lower dormancy levels via reduced ABA. Seed Sci Res 4:329–333
Mohammad R, Pahlavan R, Mohammad P (2009) Response of wheat plants to zinc, iron, and manganese applications and uptake and concentration of zinc, iron, and manganese in wheat grains. Commun Soil Sci Plant Anal 40:1322–1332
Mori S (1999) Iron acquisition by plants. Curr Opin Plant Biol 2:250–253
Mozafar A (1995) Is direct physical contact between plant roots and soil a prerequisite for iron mobilization? A review. In: Hemantaranjan A (ed) Advancements in iron nutrition research. Scientific Publishers, Jodhpur, pp 177–189
Nagarajan S (2005) Can India produce enough wheat even by 2020? Curr Sci 89:1467–1471
Nagarajkumar M, Bhaskaran R, Velazhahan R (2004) Involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen. Microbiol Res 159:73–81
Naphade BS (2002) Studies on application of siderophoregenic *Enterobacter cloacae* as biocontrol agent. PhD Thesis, North Maharashtra University, Jalgaon, India
Neilands JB, Leong SA (1986) Siderophores in relation to plant growth and disease. Annu Rev Plant Physiol 37:187–208
Neumann G, Römheld V (1999) Root excretion of carboxylic acids and protons in phosphorus-deficient plants. Plant Soil 211:121–130
Nguyen C (2003) Rhizodeposition of organic C by plants: mechanisms and controls. Agronomie 23:375–396
Nishiyama R, Kato M, Nagata S, Yanagisawa S, Yoneyama T (2012) Identification of Zn-nicotianamine and Fe-2'-Deoxymugineic acid in the phloem sap from rice plants (*Oryza sativa* L.). Plant Cell Physiol 53:381–390
Notz Regina E (2002) Biotic factors affecting 2, 4-diacetylphloroglucinol biosynthesis in the model biocontrol strain *Pseudomonas fluorescens* Cha0. PhD Thesis, Swiss Federal Institute of Technology, Zurich, Switzerland
O'Sullivan DJ, O'Gara F (1992) Traits of fluorescent *Pseudomonas* sp. involved in suppression of plant root pathogens. Microbiol Rev 56:662–676
Ongena M, Jourdan E, Schäfer M, Kech C, Budzikiewicz H, Luxen A, Thonart P (2005) Isolation of an N-alkylated benzylamine derivative from *Pseudomonas putida* BTP1 as elicitor of induced systemic resistance in bean. Mol Plant Microbe Interact 18:562–569

Pal KK, Tilak KV, Saxena AK, Dey R, Singh CS (2001) Suppression of maize root diseases caused by *Macrophomina phaseolina, Fusarium moniliforme* and *Fusarium graminearum* by plant growth promoting rhizobacteria. Microbiol Res 156:209–223

Persello-Cartieaux F, Nussaume L, Robaglia C (2003) Tales from the underground: molecular plant-rhizobacteria interactions. Plant Cell Environ 26:189–199

Pettigrew WT (2008) Pale green plants with a limp or wilted appearance; bright yellow chlorosis turning brown along the margins of the oldest leaves. Physiol Plant 133:670–681

Press CM, Loper JE, Kloepper JW (2001) Role of iron in rhizobacteria mediated induced systemic resistance of cucumber. Phytopathology 91:593–598

Rabotti C, Nisi P, Zocchi C (1995) Metabolic implications in the biochemical responses to iron deficiency in cucumber (*Cucumis safivus* L.) roots. Plant Physiol 107:1195–1199

Ran LX, Li ZN, Wu GJ, Van Loon LC, Bakker PAHM (2005) Induction of systemic resistance against bacterial wilt in *Eucalyptus urophylla* by fluorescent *Pseudomonas* sp. Eur J Plant Pathol 113:59–70

Rane MR, Sarode PD, Chaudhari BL, Chincholkar SB (2007) Detection, isolation and identification of phenazine-1-carboxylic acid produced by biocontrol strains of *Pseudomonas aeruginosa*. J Sci Ind Res 66:627–631

Rawat S, Izhari A, Khan A (2011) Bacterial diversity in wheat rhizosphere and their characterization. Adv Appl Sci Res 2:351–356

Rerkasem B, Jamjod S (1997) Boron deficiency induced male sterility in wheat (*Triticum aestivum* L.) and implications for plant breeding. Euphytica 96:257–262

Rodriguez-Lucena P, Ropero E, Apaolaza-Hernandez L, Lucena JJ (2010) Iron supply to soybean plants through the foliar application of IDHA/Fe^{3+}: effect of plant nutritional status and adjuvants. J Sci Food Agric 90:2633–2640

Römheld V, Marschner H (1986) Evidence for a specific uptake system for iron phytosiderophores in roots of grasses. Plant Physiol 80:175–180

Rosas S (2007) Role of rhizobacteria in biological control of plant diseases. In: Chincholkar SB, Mukerji KG (eds) Biological control of plant diseases: current concepts. Haworth, USA, pp 75–102

Rroco ER, Kosegarten H, Harizaj F, Imani J, Mengel K (2003) The importance of soil microbial activity for the supply of iron to sorghum and rape. Eur J Agron 19:487–493

Saravanan VS, Kalaiarasan P, Madhaiyan M, Thangaraju M (2007) Solubilization of insoluble zinc compounds by *Gluconacetobacter diazotrophicus* and the detrimental action of zinc ion (Zn) and zinc chelates on root knot nematode *Meloidogyne incognita*. Lett Appl Microbiol 44:235–241

Sarode PD (2007) Studies on the role of microbial siderophores in iron nutrition and growth promotion in plants. PhD Thesis, North Maharashtra University, Jalgaon, India

Sarode PD, Rane MR, Chaudhari BL, Chincholkar SB (2007) Screening for siderophore producing PGPR from black cotton soils of North Maharashtra. Curr Trends Biotechnol Pharm 1:96–105

Scholtz G (1983) A report on iron uptake by higher plants. Biol Zbl 102:65–75

Schonherr J, Fernandez V, Schreiber L (2005) Rates of cuticular penetration of chelated Fe-III: role of humidity, concentration, adjuvants, temperature, and type of chelate. J Agric Food Chem 53:4484–4492

Schwertmann U (1991) Solubility and dissolution of iron oxides. Plant Soil 130:1–25

Sharma A, Johri BN (2003) Growth promoting influence of siderophore-producing *Pseudomonas* strains GRP3A and PRS9 in maize (*Zea mays* L.) under iron limiting conditions. Microbiol Res 158:243–248

Sorensen J (1997) The rhizosphere as a habitat for soil microorganisms. In: Van Elsas JD, Trevors JT, Wellington EMH (eds) Modern soil microbiology, vol 2. Dekker, New York, pp 1–45

Spencer K, Freney JR (1980) Assessing the sulfur status of field-grown wheat by plant analysis. Agronomy J 72:469–472

Uknes S, Winter AM, Delaney T, Vernooij B, Morse A, Friedrich L, Nye G, Potter S, Ward E, Ryals J (1993) Biological induction of systemic acquired resistance in *Arabidopsis*. Mol Plant Microbe Interact 6:692–698

Van Wees SCM, Pieterse CMJ, Trijssenaar A, Van Westende Y, Hartog F, Van Loon LC (1997) Differential induction of systemic resistance in *Arabidopsis* by biocontrol bacteria. Mol Plant Microbe Interact 10:716–724

Wallace A (1995) Soil and plant procedures to control iron deficiency. In: Hemantaranjan A (ed) Advancements in iron nutrition research. Scientific Publishers, Jodhpur, pp 1–20

Wardle DA (1992) A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biol Rev 67:321–342

Welch RM, Graham R (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. J Exp Bot 55:353–364

Weller DM, Howie WJ, Cook RJ (1988) Relationship between in vitro inhibition of *Gaeumannomyces graminis* var. tritici and suppression of take-all of wheat by fluorescent pseudomonads. Phytopathology 78:1094–1100

Yilmaz A, Ekiz H, Gültekin I, Torun B, Barut H, Karanlik S, Cakmak I (1998) Effect of seed zinc content on grain yield and zinc concentration of wheat grown in zinc-deficient calcareous soils. J Plant Nutr 21:2257–2264

Yousfi S, Wissal M, Mahmoudi H, Abdelly C, Gharsalli M (2007) Effect of salt on physiological responses of barley to iron deficiency. Plant Physiol Biochem 45:309–314

Zhang F, Römheld SV, Marschner H (1991) Diurnal rhythm of release of phytosiderophores and uptake rate of zinc in iron-deficient wheat. Soil Sci Plant Nutr 37:671–678

Chapter 13
Induction of Plant Defense Response and Its Impact on Productivity

Louis-Philippe Hamel and Nathalie Beaudoin

13.1 Introduction

Plants are sessile organisms that perpetually encounter a vast array of environmental conditions and biotic agents, such as insects, nematodes, bacteria, fungi, and viruses. As some of these encounters may be harmful, plants have evolved a wide range of adaptations to prevent pathogen invasion and disease while maintaining growth, development, and reproduction to ensure plant survival. Hence, disease will only occur in compatible interaction, where the pathogen is able to overcome the multiple layers of plant defense (Coll et al. 2011). In the battle against invading microorganisms, such as fungi and bacteria, the first line of plant defense involves preformed barriers that are mostly directed at preventing physical entry of the pathogen, such as cell walls that may be embedded or not with lignin or suberin, waxy cuticles, trichomes, thorns, and bark. In addition to these constitutive defenses, plants are able to detect invading pathogens and activate complex defense-signaling cascades that lead to the production of cell wall reinforcement molecules, toxic compounds, and pathogen-degrading enzymes, thus providing basal resistance to the infected tissues (Chisholm et al. 2006; Jones and Dangl 2006; Van Loon et al. 2006). Specific recognition of pathogens in incompatible interactions may further activate a form of programmed cell death that restricts pathogen growth at the site of infection, the so-called hypersensitive response (HR) (Coll et al. 2011). While generally quite efficient at preventing pathogen spread and invasion, these inducible defenses represent energy-demanding processes that may impend on plant growth, development, and reproduction (Walters and Heil 2007). Hence, there is increasing evidence that the evolution of defense responses and developmental processes must have been tightly coordinated to ensure that fitness

L.-P. Hamel • N. Beaudoin (✉)
Département de biologie, Université de Sherbrooke, 2500 Boul. Université, Sherbrooke, QC, Canada J1K 2R1
e-mail: Nathalie.Beaudoin@USherbrooke.ca

D.K. Maheshwari et al. (eds.), *Bacteria in Agrobiology: Crop Productivity*,
DOI 10.1007/978-3-642-37241-4_13,

and productivity are maintained even when the plant arsenal is deployed to fight invaders (Alcázar et al. 2011).

In the first part of this chapter, we will present the various defense responses that are activated in response to pathogens with a special attention to the molecular mechanisms involved in the detection of bacterial pathogens and the induction of plant defense. The second part will highlight the current knowledge on how these processes may affect plant productivity and fitness and how this should be taken into account in the development of strategies to enhance disease resistance in plants.

13.2 Induction of Plant Innate Immunity

In most cases, interactions between plants and microorganisms do not lead to disease. This is mainly due to the induction of defense response and basal resistance, or plant innate immunity, which may be activated as a general response to most pathogens (Chisholm et al. 2006; Jones and Dangl 2006). Typical defense includes the fortification of the cell wall, the production of reactive oxygen species (ROS), the induction of defense gene expression (e.g., *PAL*, *LOX*, *CHS*, *PR-1*, defensin), and the synthesis of antimicrobial molecules (e.g., phytoalexins, other secondary metabolites). This also coincides with the synthesis of salicylic acid (SA), jasmonic acid (JA), ethylene, and additional long-distance signals that are further involved in regulating local as well as systemic defense responses (Chisholm et al. 2006; Jones and Dangl 2006; Van Loon et al. 2006; Ahmad et al. 2010).

On the other hand, some pathogens have evolved specific mechanisms to repress or avoid these induced plant defense mechanisms, leading to a successful infection. To win this battle, plants have also developed ways of specifically recognizing pathogen invaders in a process that detects microbial effectors within the plant cell (Chisholm et al. 2006; Jones and Dangl 2006). As a result, the plant develops a specific resistance to the invading pathogen, a process that is frequently associated with the HR (Coll et al. 2011). As described below, both recognition mechanisms activate overlapping signaling cascades that will lead to the induction of plant immunity and, under specific conditions, to the HR.

13.2.1 MAMP-Triggered Immunity

Induction of plant defense in response to microorganisms relies on the ability of plants to recognize microbe-derived molecules called microbe-associated molecular patterns (MAMPs, also known as PAMPs for pathogen-associated molecular patterns) (Chisholm et al. 2006; Jones and Dangl 2006; Boller and Felix 2009; Zipfel 2009; Millet et al. 2010). Detection of MAMPs by pattern-recognition

receptors (PRRs) activates the plant innate immune response in a process referred to as MTI for MAMP-triggered immunity (MTI) (Jones and Dangl 2006; Zipfel 2009; Millet et al. 2010) (Fig. 13.1). MAMPs that have been identified in bacteria comprise the flagellin (Felix et al. 1999; Zipfel et al. 2004), the elongation factor Tu (EF-Tu) (Kunze et al. 2004), lipopolysaccharides (Newman et al. 1995; Meyer et al. 2001), and peptidoglycans (Felix and Boller 2003; Gust et al. 2007).

PRRs are transmembrane receptors that include members of the leucine-rich repeats receptor-like kinase (LRR-RLK) protein family (Chisholm et al. 2006; Coll et al. 2011), such as the *Arabidopsis thaliana* FLS2 receptor that recognizes bacterial flagellin (Gómez-Gómez and Boller 2000; Chinchilla et al. 2006) and EFR that binds to a conserved 18-amino-acid peptide found in EF-Tu (Zipfel et al. 2006) (see Fig. 13.1a for details). As for many PRRs, each of FLS2 and EFR works with a coreceptor known as BAK1, which is required for stimulus-induced heterodimerization with PRR and activation of MTI (Chinchilla et al. 2007, 2009; Schulze et al. 2010). In addition to *Arabidopsis*, homologs of these types of receptors have been identified in a variety of plant species, including rice, tomato, and poplar (Boller and Felix 2009).

Upon recognition of MAMPs, the concentration of cytosolic calcium ions (Ca^{2+}) is rapidly elevated, which triggers the oxidative burst characterized by the generation of signaling molecules, mainly reactive oxygen species (ROS) and nitric oxide (NO) (Schwessinger and Zipfel 2008; Mazars et al. 2010) (Fig. 13.1b). The Ca^{2+} signature is also monitored by a group of Ca^{2+}-binding proteins, including calmodulins (CaMs) and Ca^{2+}-dependent protein kinases (CDPKs) (Boudsocq et al. 2010). Activation of PRRs also results in the activation of mitogen-activated protein kinase (MAPK) cascades that use three levels of interacting kinases: the MAPK themselves, whose activity is induced upon phosphorylation by upstream MAPK kinases (MAP2Ks), which are in turn activated upon phosphorylation by upstream MAPKK kinases (MAP3Ks). These proteins operate as signal transmission modules that amplify stimuli from upstream receptors into appropriate downstream intracellular responses. These include the activation of transcription factors and a massive transcriptional reprogramming of regulatory and defense-associated genes (Asai et al. 2002; Katagiri 2004; Mészáros et al. 2006; Zipfel et al. 2006; Fiil et al. 2009). This basal and large-spectrum resistance is generally sufficient to prevent most pathogen invasions (Ahmad et al. 2010).

Plants also establish beneficial interactions with microorganisms, such as plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi. These interactions contribute to the acquisition of nutriments from the soil and stimulate plant growth. However, the expression of plant defense responses can inhibit the colonization of plant tissues by beneficial microbes, thus perturbing plant growth and development (Walters and Heil 2007; Cipollini and Heil 2010). Since MAMPs from beneficial microbes also have the potential to trigger plant defense responses, some of these organisms have evolved new molecules and mechanisms that suppress plant defense and ensure efficient interactions with their hosts (Soto et al. 2009; Hamel and Beaudoin 2010; Van Hulten et al. 2010).

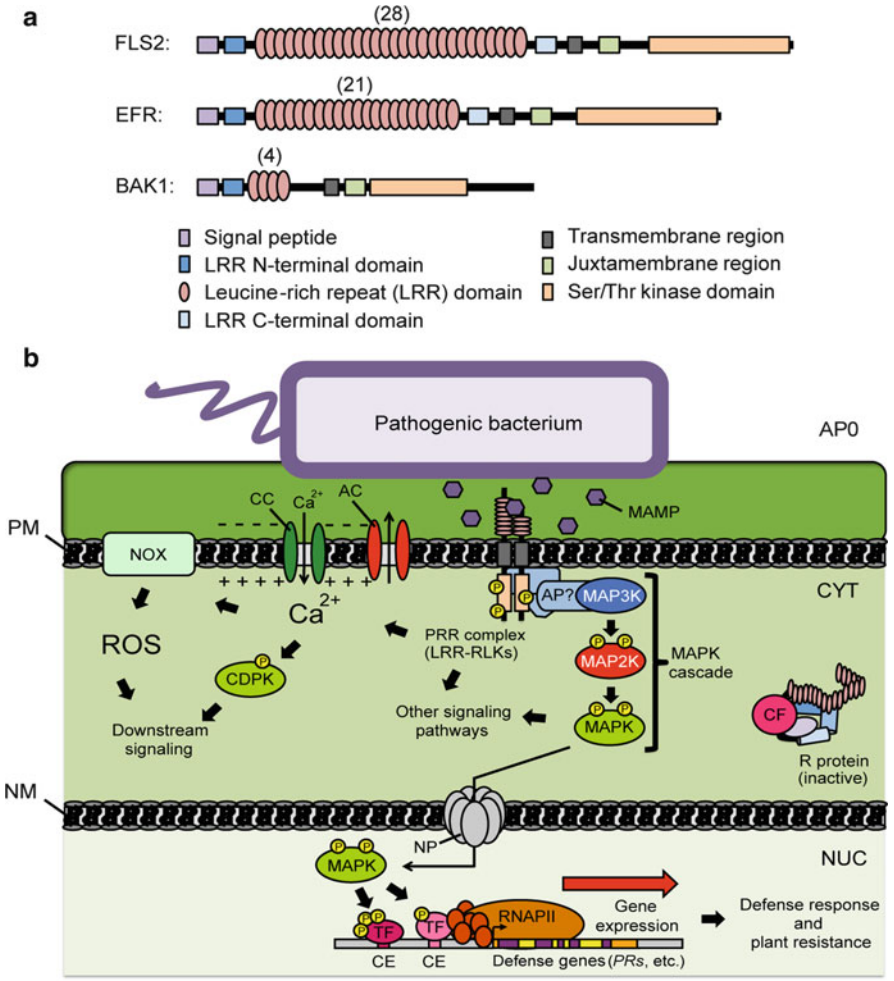


Fig. 13.1 MAMP-triggered immunity (MTI) in plants. (**a**) Schematic view of the conserved domains found in prototypical pattern-recognition receptors (PRRs) from *Arabidopsis thaliana*. FLS2 recognizes flagellin, the main building block of the bacterial flagellum. EFR recognizes the bacterial elongation factor Tu (EF-Tu), which mediates the entry of the aminoacyl-tRNA into a free site of the ribosome. BAK1 functions as a coreceptor required for full activation of PRRs. The number of LRR motifs is indicated in parentheses. (**b**) During attempted colonization of plant tissues, bacteria release MAMPs that bind to the extracellular portion of PRRs. Many PRRs controlling defense against bacteria belong to the leucine-rich repeats receptor-like kinase (LRR-RLK) family. Following MAMP recognition, LRR-RLKs heterodimerize with a coreceptor, leading to ion fluxes, membrane depolarization, calcium signaling, and generation of reactive oxygen species (ROS). Perception of MAMPs also alters protein phosphorylation (P) status, including the activation of mitogen-activated protein kinase (MAPK) cascades. Activated MAPKs signal in the cytoplasm (CYT) but also travel to the nucleus (NUC) where they phosphorylate transcription factors (TFs) controlling expression of defense-related genes. *Abbreviations*: *AC* anion channel, *AP* adaptor protein, *APO* apoplast, Ca^{2+} calcium ion, *CC* calcium channel, *CDPK* calcium-dependent protein kinase, *CE cis* element, *CF* cofactor protein, *NM* nuclear membrane, *NOX* NADPH oxidase, *NP* nuclear pore, *PM* plasma membrane, *PR* pathogenesis related, *R* resistance, *RNAPII* RNA polymerase II

13.2.2 Effector-Triggered Immunity

During evolution, some plant pathogens have developed specific mechanisms to evade or suppress the first line of detection that leads to basal resistance as a result of MTI. In the case of phytopathogenic bacteria, this suppression activity involves proteinaceous secretion systems that allow injection of virulence effectors in the plant cells (Jones and Dangl 2006; Grant et al. 2006; Block et al. 2008; Boller and He 2009) (Fig. 13.2b).

To counter such strategy, plants have evolved a sophisticated surveillance mechanism called effector-triggered immunity (ETI), which specifically detects the presence of microbial effectors within the plant cells using intracellular innate immune receptors (Jones and Dangl 2006) (Fig. 13.2). Pathogen recognition leads to the inhibition of pathogen growth and often triggers the HR. This local programmed cell death proceeds according to the *gene-for-gene* model, in which a specific avirulence protein (Avr), the effector, is recognized by a host-specific receptor protein or resistance (R) protein (Dangl and Jones 2001). However, while induction of HR is generally associated with ETI, recent results point to the occasional activation of HR in the case of MTI and in response to particular environmental stress (Thomma et al. 2011).

Most receptors involved in ETI are members of the nucleotide-binding and leucine-rich repeat (NB-LRR) disease resistance protein family (Eitas and Dangl 2010) (see Fig. 13.2a for details). Detection of a specific bacterial effector may occur by direct binding to the NB-LRR or may be indirect via an intermediary protein (Coll et al. 2011) (Fig. 13.2b). This indirect recognition can be explained by the guard hypothesis where the effectors modify specific host proteins (guardees or cofactors) that are monitored for alterations by a particular NB-LRR (guard) (Dangl and Jones 2001). Alternatively, the guardee can also be used as a molecular decoy that fools the effector because it is homologous to one of its actual host target protein (Van der Hoorn and Kamoun 2008). Whether Avr detection goes through the guard or the decoy hypothesis model, the molecular events that lead to ETI and HR activation partly overlap those described for MTI (Maleck et al. 2000; Tao et al. 2003; Navarro et al. 2004; Coll et al. 2011). These include a rise in intracellular calcium levels, production of ROS and NO, activation of MAPK cascades, transcriptional reprogramming of defense-related genes, and synthesis of antimicrobial compounds. However, it has been proposed that these events would occur more rapidly and more strongly during ETI when compared to MTI, which could explain the frequent incidence of HR as a result of ETI (Jones and Dangl 2006; Coll et al. 2011). Nonetheless, genetic evidence suggests that ETI-mediated specific resistance can, at least in some instances, be uncoupled from the induction of HR cell death (Coll et al. 2011). In these cases, the purpose of HR cell death would not be to inhibit pathogen growth but may rather occur as a result of a rise in toxic intermediates aimed at killing pathogens. Alternatively, it was proposed to serve as an adaptive process for the generation of long-range signals (mediated by ROS or

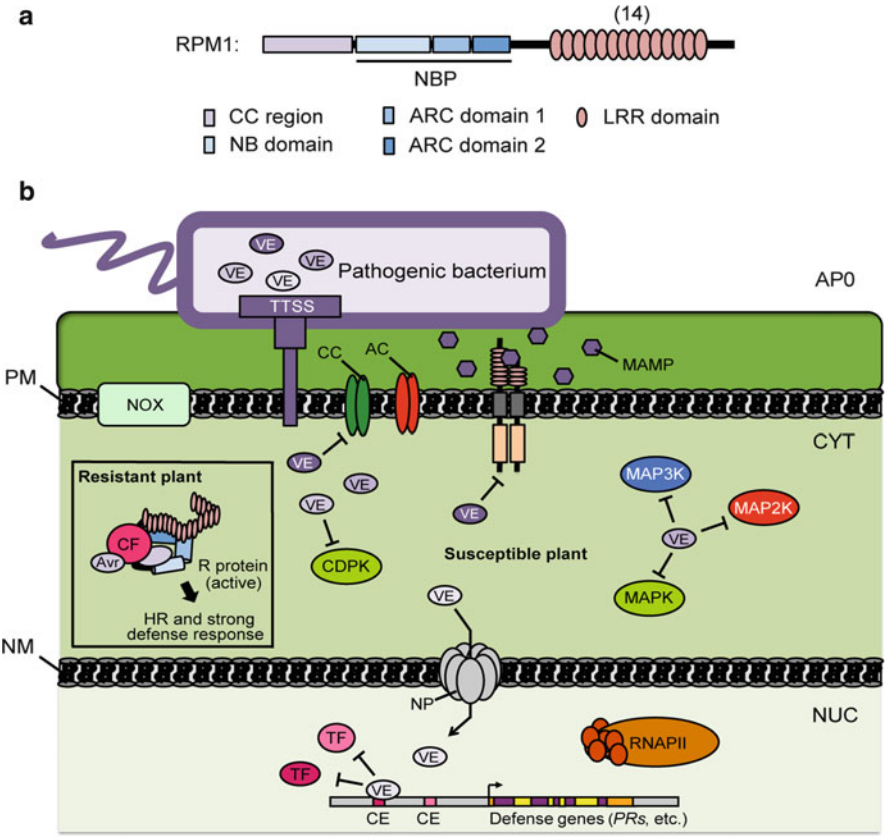


Fig. 13.2 Effector-triggered susceptibility (ETS) and effector-triggered immunity (ETI). (**a**) Schematic view of a prototypical resistance (R) protein and organization of its conserved domains. R proteins are defined by a central nucleotide-binding (NB) domain and C-terminal leucine-rich repeats (LRR). Located between the NB and LRR domains lie two regions known as the Apaf-1, R proteins, and CED4 (ARC) homology domains. Together with the NB, ARC domains constitute a nucleotide-binding pocket (NBP) that controls activation of the R (or NB-LRR) protein. Depending on the N-terminus region, NB-LRRs fall into two broad classes: those with a Toll and interleukin-1 receptor (TIR) domain are known as the TIR-NB-LRRs (not shown here), while those without a TIR most often display a coiled-coil (CC) region and are referred to as the CC-NB-LRRs. In *Arabidopsis*, R protein RPM1 confers resistance against strains of *Pseudomonas syringae* carrying the avirulence (Avr) gene *avrRpm1*. (**b**) Susceptible plant: specialized microbes have the ability to suppress plant defense through the use of virulence effectors (VE). Phytopathogenic bacteria rely on proteinaceous channels, including the type three secretion system (TTSS), to inject a collection of VE into the cytoplasm (CYT) of host cells. VE can then target host proteins and modify their functions in order to compromise signaling pathways that regulate activation of plant defense. Resistant plant: to overcome hijacking of defense systems, plants have evolved intracellular R proteins that function as receptors against VE. R proteins usually monitor the integrity of host proteins, also called cofactors (CF), that are targeted by VE. The CF can be an actual virulence target of the effector (guard hypothesis) or may simply look like one (decoy model). Activation of R proteins leads to a strong defense response that is often accompanied by a form of localized programmed cell death, the so-called hypersensitive response

SA) that induce systemic acquired resistance (SAR) to prime plants for secondary infection (Coll et al. 2011) (see Sect. 13.2.3).

The main drawback of ETI is that it is only effective against specific races of biotrophic pathogens, which rapidly become under high selective pressure to break ETI (Ahmad et al. 2010). Hence, some pathogens can overcome ETI by producing new effectors that suppress ETI or that are no longer recognized by NB-LRR disease resistance proteins. Some pathogens have also evolved new effectors that inhibit HR cell death in a mechanism that remains to be elucidated (Coll et al. 2011). This ongoing evolution of plants and pathogens can be viewed as an everlasting arms race where defeat on one side or the other will promote the development of new weapons (Jones and Dangl 2006).

13.2.3 Priming for Defense

Localized pathogen attack can trigger the induction of a systemic resistance called SAR that is associated with the induction of defense responses in distant organs or even at the whole plant level (Ryals et al. 1996; Sticher et al. 1997; Durrant and Dong 2004). SAR depends on the synthesis of SA which would participate in the induction of SAR and of a large set of genes encoding pathogenesis-related (PR) proteins with antimicrobial activities (Dong 2001; Durrant and Dong 2004; Van Loon et al. 2006). Root colonization by some nonpathogenic microbes, such as PGPR, can often activate another form of systemic resistance which is called induced systemic resistance (ISR) (Van Loon et al. 1998; Pieterse et al. 1998; Conrath et al. 2006; Van Loon 2007; Van Wees et al. 2008; Conrath 2009). ISR does not rely on SA synthesis but is characterized by an increased sensitivity to JA and ethylene (Pieterse et al. 1998; Conrath 2009). In contrast to SAR, ISR is generally not associated with the upregulation of *PR* genes. Activation of systemic defenses by SAR and ISR as well as other selected molecules can also potentiate the activation of basal resistance in a process called priming (Conrath et al. 2006; Conrath 2009). Priming agents include plant- or pathogen-derived molecules and biologically active chemicals, such as SA, JA, vitamin B, cytokinins, and β-aminobutyric acid (BABA) (Walters and Heil 2007; Conrath 2009; Ahmad et al. 2010).

After priming, defense response and disease resistance become apparent only when the primed tissues are challenged by a secondary pathogen infection. In the primed state, plants can respond faster or more strongly to a secondary infection and activate amplified defense responses that frequently provide enhanced basal

Fig. 13.2 (continued) (HR). *Abbreviations*: *AC* anion channel, *AP* adaptor protein, *APO* apoplast, Ca^{2+} calcium ion, *CC* calcium channel, *CDPK* calcium-dependent protein kinase, *CE cis* element, *MAMP* microbe-associated molecular pattern, *MAPK* mitogen-activated protein kinase, *NM* nuclear membrane, *NOX* NADPH oxidase, *NP* nuclear pore, *NUC* nucleus, *PM* plasma membrane, *PR* pathogenesis related, *RNAPII* RNA polymerase II, *TF* transcription factor

resistance not only against the invader but against a broad spectrum of virulent pathogens and pests (Conrath et al. 2006; Conrath 2009). Interestingly, very little changes in the expression of defense-related genes or resistance traits are usually detected as a result of priming alone (Zimmerli et al. 2008). However, a secondary infection in primed plants generally triggers a strong expression of defense-related genes that is significantly higher than that occurring as a result of direct induction of defense. Experimental evidence has shown that mutant plants with defective pathogen defense are frequently compromised in gene priming, while mutants with permanently enhanced immunity are often constitutively primed for enhanced activation of defense (Jaskiewicz et al. 2011). These results suggest that priming may enhance the expression of basal resistance that is mediated by MTI (Ahmad et al. 2010). It was also shown that induction of SAR and ISR that lead to priming can also reduce lesion formation caused by avirulent pathogens, suggesting that priming also increases ETI mechanisms. Nevertheless, direct induction of defense by ETI still provides a more efficient protection against one specific pathogen or pathogen race that cannot be attained by priming (Ahmad et al. 2010).

Until recently, very few studies had examined the actual molecular mechanisms that occur during priming. Recent reports have however unraveled some of the key events that prepare plants for defense during priming (Conrath 2011; Jaskiewicz et al. 2011). Using the plant model *Arabidopsis*, it was found that priming with the SA analog and SAR activator benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) increased the expression of two members of the MAPK defense-signaling cascades, MPK3 and MPK6. These MAPKs accumulate in a dormant form in primed tissues (Beckers et al. 2009). Upon secondary pathogen challenge, both kinases were activated in primed plants to a level that was significantly higher than that found in non-primed plants. This increased activity was also associated with enhanced expression of *PAL* and *PR1* defense genes and SAR in primed plants. Interestingly, priming and SAR were not induced in *mpk3* mutants but were only partially compromised in *mpk6* mutants, suggesting that MPK3 would play a major role in priming with a minor (but essential) contribution from MPK6 (Beckers et al. 2009; Conrath 2011).

Modifications in chromatin structure have also been associated with the induction of priming. In the nucleus, genomic DNA is organized in nucleosomes, where 147 base pairs of DNA are wrapped around an octamer core of two copies of histones H2A, H2B, H3, and H4. Genes found in nucleosomes that are further packed by linker histone H1 are transcriptionally inactive. However, covalent modifications of DNA and histones, such as methylation, acetylation, ubiquitination, and poly-ADP-ribosylation can alter chromatin architecture and regulate gene transcription. Active gene expression is generally associated with an "open" chromatin state in which chromatin modifications facilitate DNA access to the general transcriptional machinery and to specific transcription factors and co-activators (Conrath 2011). Recent data suggest that priming activates chromatin remodeling at defense gene promoters by inducing marks, such as histone methylation and acetylation, which are generally associated with gene activation (Alvarez-Venegas et al. 2007; Bruce et al. 2007; March-Diaz et al. 2008; Van den Burg and

Takken 2009; Alvarez et al. 2010; Jaskiewicz et al. 2011). While priming does not directly induce the expression of these marked defense genes, a subsequent stress or infection can substantially increase their expression. It has been proposed that chromatin remodeling during priming would help recruit the transcriptional machinery to defense gene promoters, thus providing a rapid and strong defense gene expression upon secondary infection (Conrath 2011).

13.3 Costs and Benefits of Induced Defense on Plant Productivity and Fitness

In the fight against pathogens, the induction of plant innate immunity or basal resistance is much more efficient in protecting from disease than constitutive preformed defenses. This inducible mechanism has also the advantage of directing energy toward defense only when it is needed. However, this process implies that there is some time gap between pathogen recognition and the onset of defense response that may allow pathogen invasion into plant tissues (Bolton 2009). Moreover, in the absence of pathogen, several studies have shown that plant productivity and fitness (growth, seed set, and yield) are perturbed when defense is induced (reviewed in Walters and Heil 2007; Bolton 2009). These negative effects may be attributed to the toxicity of the plant's own defense response and to hormonal imbalances or may occur if defenses alter interaction with beneficial organisms, such as PGPR and mycorrhizal fungi (Heil and Baldwin 2002). Most importantly, the full development of an inducible defense remains an energy-demanding process that involves the intense expression of an important number of plant defense genes, antimicrobial compounds, and defense signals which can divert resources (e.g., assimilates) away from growth and yield (Walters and Heil 2007; Bolton 2009). As an example, it was found that synthesis of the SA-dependent defense protein PR1 is induced in infected leaf tissues to a level that represents up to 1 % of all soluble proteins and total PR proteins may constitute up to 10 % (Heil and Bostock 2002; Bolton 2009). Obviously, all nitrogen and carbon atoms used for PR synthesis as a defense strategy cannot be used for primary metabolism (Walters and Heil 2007). The mechanisms deployed by plants to manage this important redistribution of energy toward defense are just beginning to be unraveled.

13.3.1 Costs of Induced Immunity

While the induction of plant defense seems to represent the best strategy to survive in a pathogen-infested environment, induced plant defense and resistance clearly impose a cost on plant growth and development in the absence of challenge. The

costs of induced immunity are difficult to evaluate in natural environments, as pleiotropic factors may affect the outcome of plant–pathogen interactions (Bolton 2009). The amplitude and efficiency of defense responses are not only specific to each plant genotype and pathogen but are also modulated by environmental conditions (light intensity, temperature, humidity, etc.) and the developmental stage of the plants (Walters and Heil 2007; Bolton 2009; Ahmad et al. 2010; Cipollini and Heil 2010; Alcázar et al. 2011). Light itself is required for the development of a resistance response in a number of plant–pathogen interactions but can also be required for pathogen virulence and the development of disease symptoms (Bolton 2009; Roden and Ingle 2009). Moreover, light can stimulate the oxidative burst and is necessary for the execution of HR in response to several pathogens (Bolton 2009). The developmental stage also influences the plant immune response. For example, induction of flowering in mature *Arabidopsis* plants is associated with the development of a general resistance (the so-called age-related resistance or ARR) that inhibits the growth of a variety of virulent pathogens (Rusterucci et al. 2005; Carviel et al. 2009). However, genetic perturbations of ARR can delay flowering time, suggesting that both defense and developmental mechanisms are tightly linked in this type of resistance (Carviel et al. 2009; Balazadeh et al. 2010).

To minimize the effects on plant productivity that may be caused by environmental variations, most studies on plant defense and resistance mechanisms have been performed in controlled environments. Data obtained in those conditions largely support the view that induction of plant defense and resistance can reduce plant productivity and fitness. For instance, it has been found that constitutive expression of defense responses in various mutants generally has a negative impact on plant growth and yield (Heil and Baldwin 2002; Bolton 2009). In particular, most *Arabidopsis* mutants in which SA signaling and SA-inducible defense are constitutively enhanced display growth and developmental defects (Bolton 2009) (Fig. 13.3). This is the case for *cpr* (*constitutive expresser of PR genes*) mutants, which exhibit enhanced PR gene expression and resistance to pathogens but show reduced growth and compromised seed production (Bowling et al. 1997; Clarke et al. 1998). The *dnd1* (*defense no death 1*) mutation, which causes elevated levels of SA and constitutive systemic resistance, is another example where constitutive defense also leads to a dwarfed morphology in mature plants (Clough et al. 2000; Genger et al. 2008). Similarly, both *ssi1* and *ssi2* (*suppressor of salicylic acid insensitivity of npr1-5*) mutants, which are affected in SA-dependent signaling, are dwarf and display spontaneous cell death lesions (Shah et al. 1999, 2001). Constitutive expression of JA and ethylene-dependent defense responses has a similar effect on plant growth, as illustrated by the *Arabidopsis cev1* (*constitutive expression of VSP1*) mutant which exhibits reduced stature when compared to WT (Ellis and Turner 2001). Other studies have also demonstrated that R gene-mediated resistance can also be a costly process that negatively impacts plant yield, although the cost may vary depending on the R genes (Brown 2002, 2003). For example, it was found that transgenic *Arabidopsis* plants expressing the gene *RPM1*, which confers resistance to *Pseudomonas syringae* pv. *maculicola*, have smaller shoots

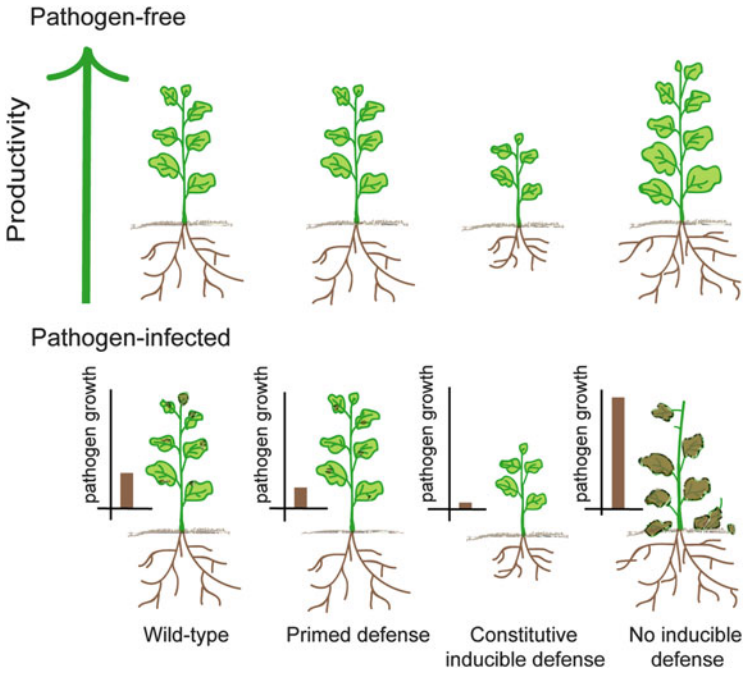


Fig. 13.3 The delicate balance between plant growth and defense. Predictions on the effects of induced defense on plant productivity in pathogen-free environment (*upper panel*) or on pathogen growth in pathogen-infected plants (*lower panel*). It is assumed that environmental conditions are controlled and do not alter plant growth and development or the expression of defense responses. In the absence of pathogen infection, plant productivity is predicted to be similar in wild-type (WT) and primed plants, reduced in mutants expressing constitutive inducible defense responses, and increased in mutants suppressed for inducible defense responses. During pathogen infection and disease, WT plants induce basal resistance mechanisms, which considerably reduce pathogen growth. In primed plants, basal resistance is enhanced during the secondary infection, thus drastically reducing disease symptoms and inhibiting pathogen growth. Mutants expressing constitutive defense responses display complete resistance to the pathogen. In mutants suppressed for inducible defense responses, pathogens grow freely and infect all plant tissues

and reduced seed yield per plant when compared to susceptible plants (Tian et al. 2003). In contrast, it was shown that a mutant that is no longer able to induce defense and resistance, such as the *Arabidopsis ein2* (*ethylene-insensitive 2*) mutant that is defective for ethylene signaling, can exhibit an increase fitness characterized by increased growth and seed set (Thomma et al. 1999; Geraats et al. 2003) (Fig. 13.3). Likewise, the growth-promoting effects of beneficial microorganisms have also been attributed, at least partially, to their ability to suppress inducible plant defense responses (Van Hulten et al. 2010).

Results obtained from transcriptomics analyses performed in plants infected by pathogens demonstrate that the induction of defense involves an important reprogramming of gene expression (Katagiri 2004; Somssich 2007; Major et al. 2010). In addition to defense-related genes, a significant proportion of genes

involved in primary metabolism and various biosynthetic processes are differentially expressed by pathogen infection (Berger et al. 2004; Bolton 2009). Although some of these changes can occur as a direct response to pathogen attack, there is growing evidence that several of them are necessary to reallocate energy and resources to defense. In most plant–pathogen compatible interactions, the induction of defense is accompanied by downregulation of several photosynthesis-related genes, a trend that is also correlated with a local reduction in photosynthetic rates (Bolton 2009). Reduction of photosynthesis may be a plant strategy to shift resources to defense (Major et al. 2010). Decreased photosynthesis and increasing demand for energy may in turn initiate the translocation of carbohydrates to infected tissues. Accordingly, some of the genes involved in the transition from source-to-sink status, such as genes encoding cellular invertases, are also upregulated during the induction of defense (Roitsch et al. 2003; Bolton 2009; Major et al. 2010). Similarly, an important number of genes involved in plant respiration that converts nutrients to energy, including genes encoding enzymes of glycolysis and of the tricarboxylic acid (TCA) cycle, are upregulated during the induction of defense. This also correlates with the observed stimulation of respiration that generally occurs during the resistance response (Bolton 2009; Major et al. 2010).

Interestingly, it was recently shown that it is possible to unlink the constitutive expression of defense and its negative impact on plant growth and development. The *Arabidopsis* mutant *cdd1* (*constitutive defense without growth defect1*) is characterized by enhanced disease resistance that is associated with constitutive SA-signaling (Swain et al. 2011). However, in contrast with mutants described before, expression of constitutive defense in *cdd1* has no detectable impact on its growth and development. These results suggest that plants have the potential to induce immune responses without perturbing plant growth and development. This finding will certainly contribute to the development of new strategies that may constitutively enhance disease protection in crops species without compromising productivity.

13.3.2 Benefits of Priming

As mentioned earlier, priming for defense provides several advantages over the direct induction of defense responses by pathogens (Fig. 13.3). Firstly, primed plants can rapidly activate defense responses upon pathogen challenge, thus reducing the time lag that occurs between pathogen detection and the direct induction of defenses. Moreover, defense responses induced in primed plants are stronger, thus providing enhanced resistance to several pathogens. Finally, since priming potentiates cellular defense responses rather than directly upregulating defense mechanisms, this may considerably decrease the energy costs associated with defense (Conrath et al. 2006; Van Hulten et al. 2006, 2010; Walters and Heil 2007; Conrath 2009; Ahmad et al. 2010). This last advantage has been corroborated

in the plant model *Arabidopsis*, where a first group of plants was treated with low doses of BABA for priming and a second group was treated with higher doses for the activation of inducible defense responses (Van Hulten et al. 2006). In the absence of pathogen, priming caused minor reductions in plant growth but did not alter seed set, while both characteristics were significantly affected as a result of direct induction of defense response in plants treated with high doses of BABA. When infected by virulent pathogens, the growth of primed plants was significantly less affected than that of control or primarily infected plants. The authors concluded that the costs of priming for defense in *Arabidopsis* are outweighed by its benefits under relatively high disease pressure (Van Hulten et al. 2006). However, while priming may represent some minor costs under conditions of low disease pressure, it is still less costly than direct induction of defense (Van Hulten et al. 2006). Interestingly, priming can also enhance defense against herbivorous insects and abiotic stress and thus contributes to maintaining optimal plant growth and development even under harsh or pest-infested environments (Ton et al. 2005; Beckers and Conrath 2007; Conrath 2009).

Very recently, two different groups have used *Arabidopsis* to demonstrate that the effects of priming can also be inherited by a plant progeny and protect descendants from pathogen attack (Luna et al. 2012; Slaughter et al. 2012). In both studies, *Arabidopsis* plants were primed either with BABA or the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*). Descendants of primed plants that were challenged with *Pst* or with the (hemi-)biotrophic pathogen *Hyaloperonospora arabidopsidis* exhibited enhanced SA-inducible and defense-related gene expression as well as increased resistance to both pathogens. While the primed state was lost in the next generation in one study (Slaughter et al. 2012), transgenerational priming was maintained over one stress-free generation when priming was performed with recurrent pathogen stress rather than single inoculations (Luna et al. 2012). This shows that durability of transgenerational priming, at least partly, relies on the intensity of the initial priming stress applied on the parental generation. It was found that the transgenerational primed state is associated with changes in the chromatin structure and DNA methylation patterns, suggesting that some of the epigenetic-related changes induced by first-generation priming can be transmitted to the offspring to maintain the primed state and enhanced resistance. Moreover, no effects on plant growth, development, or seed set were observed in the transgenerational primed plants, suggesting that no additional fitness costs are imposed in the primed progeny (Luna et al. 2012). These results demonstrate that the benefits of priming can be maintained in future generations, which makes priming a very promising strategy to increase plant protection against a wide range of pathogens.

13.4 Conclusion

Plant diseases cause important reduction in productivity and decrease food supply. It is estimated that 31–42 % of agricultural production is lost worldwide because of pests and disease (FAO 2005). With the increasing demand for food supply, the impact of rapid climate change, and the pressure to restrict pesticides use, there is an urgent need for the development of high-yield crops with inherent and sustainable disease protection. Recent studies have shown that plants usually need to recognize pathogen invaders with specialized receptors before they can induce efficient defense responses and disease resistance. Genetic and molecular analyses have provided a detailed characterization of the molecular events that occur in the complex signaling cascades that link pathogen detection and defense responses. Using this information, different strategies have been tested to enhance disease protection in plants. However, constitutive expression of inducible defense as well as enhanced R gene-mediated resistance have generally not proven to be suitable strategies to enhance disease protection in plants as they frequently lead to a reduction in growth and yield. While usually quite effective against pathogens, the deployment of inducible defenses represents an energy intensive process that can divert energy and resources from plant growth, thus reducing productivity and fitness. For this reason, plants will generally induce defense only if the benefits outweigh the cost. Nonetheless, the activation of defense response remains almost always more beneficial in plants under attack when compared with plants that offer no resistance. Consequently, strategies directed at enhancing disease protection while sustaining productivity must take into account the intrinsic relationships that connect the expression of plant defenses and plant growth.

More recently, priming plants for defense has emerged as a promising approach that may efficiently provide enhanced resistance against various pathogens as well as other stress without significantly compromising productivity and fitness (Van Hulten et al. 2006). Since priming can be induced as a result of root interactions with beneficial rhizobacteria or mycorrhizal fungi, this strategy could also limit the need for chemical inducers or pesticides (Van Wees et al. 2008; Van Hulten et al. 2010). However, the few studies that have looked at some of the molecular mechanisms and effects of priming have been performed with plant model species such as *Arabidopsis*. Future research will be necessary to determine whether priming can also lead to the expression of durable and energy-efficient disease resistance in major crop species. Finally, a better understanding of the molecular events that prepare plants for defense during priming should also provide new tools for the design of plants that are able to face the future challenges of rapidly changing environments.

Acknowledgments L.-P. Hamel is the recipient of a postdoctoral fellowship from the Fonds Québécois de la Recherche sur la Nature et la Technologie (FQRNT). We apologize to our colleagues whose work could not be cited here due to space limitations.

References

Ahmad S, Gordon-Weeks R, Pickett J, Ton J (2010) Natural variation in priming of basal resistance: from evolutionary origin to agricultural exploitation. Mol Plant Pathol 11:817–827

Alcázar R, Reymond M, Schmitz G, de Meaux J (2011) Genetic and evolutionary perspectives on the interplay between plant immunity and development. Curr Opin Plant Biol 14:378–384

Alvarez ME, Nota F, Cambiagno DA (2010) Epigenetic control of plant immunity. Mol Plant Pathol 11:563–576

Alvarez-Venegas R, Abdallat AA, Guo M, Alfano JR, Avramova Z (2007) Epigenetic control of a transcription factor at the cross section of two antagonistic pathways. Epigenetics 2:106–113

Asai T, Tena G, Plotnikova J, Willmann MR, Chiu WL, Gomez-Gomez L, Boller T, Ausubel FM, Sheen J (2002) MAP kinase signalling cascade in *Arabidopsis* innate immunity. Nature 415:977–983

Balazadeh S, Siddiqui H, Allu AD, Matallana-Ramirez LP, Caldana C, Mehrnia M, Zanor MI, Kohler B, Mueller-Roeber B (2010) A gene regulatory network controlled by the NAC transcription factor ANAC092/AtNAC2/ORE1 during salt-promoted senescence. Plant J 62:250–264

Beckers GJ, Conrath U (2007) Priming for stress resistance: from the lab to the field. Curr Opin Plant Biol 10:425–431

Beckers GJ, Jaskiewicz M, Liu Y, Underwood WR, He SY, Zhang S, Conrath U (2009) Mitogen-activated protein kinases 3 and 6 are required for full priming of stress responses in *Arabidopsis thaliana*. Plant Cell 21:944–953

Berger S, Papadopoulos M, Schreiber U, Kaiser W, Roitsch T (2004) Complex regulation of gene expression, photosynthesis and sugar levels by pathogen infection in tomato. Physiol Plant 122:419–428

Block A, Li G, Fu ZQ, Alfano JR (2008) Phytopathogen type III effector weaponry and their plant targets. Curr Opin Plant Biol 11:396–403

Boller T, Felix G (2009) A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu Rev Plant Biol 60:379–406

Boller T, He SY (2009) Innate immunity in plants: an arms race between pattern recognition receptors in plants and effectors in microbial pathogens. Science 324:742–744

Bolton MD (2009) Primary metabolism and plant defense–fuel for the fire. Mol Plant Microbe Interact 22:487–497

Boudsocq M, Willmann MR, McCormack M, Lee H, Shan L, He P, Bush J, Cheng SH, Sheen J (2010) Differential innate immune signalling via Ca^{2+} sensor protein kinases. Nature 464:418–422

Bowling SA, Clarke JD, Liu Y, Klessig DF, Dong X (1997) The *cpr5* mutant of *Arabidopsis* expresses both NPR1-dependent and NPR1-independent resistance. Plant Cell 9:1573–1584

Brown JK (2002) Yield penalties of disease resistance in crops. Curr Opin Plant Biol 5:339–344

Brown JK (2003) A cost of disease resistance: paradigm or peculiarity? Trends Genet 19:667–671

Bruce TJA, Matthes MC, Napier JA, Pickett JA (2007) Stressful "memories" of plants: evidence and possible mechanisms. Plant Sci 173:603–608

Carviel JL, Al-Daoud F, Neumann M, Mohammad A, Provart NJ, Moeder W, Yoshioka K, Cameron RK (2009) Forward and reverse genetics to identify genes involved in the age-related resistance response in *Arabidopsis thaliana*. Mol Plant Pathol 10:621–634

Chinchilla D, Bauer Z, Regenass M, Boller T, Felix G (2006) The *Arabidopsis* receptor kinase FLS2 binds flg22 and determines the specificity of flagellin perception. Plant Cell 18:465–476

Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nurnberger T, Jones JD, Felix G, Boller T (2007) A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. Nature 448:497–500

Chinchilla D, Shan L, He P, de Vries S, Kemmerling B (2009) One for all: the receptor-associated kinase BAK1. Trends Plant Sci 14:535–541

Chisholm ST, Coaker G, Day B, Staskawicz BJ (2006) Host-microbe interactions: shaping the evolution of the plant immune response. Cell 124:803–814
Cipollini D, Heil M (2010) Costs and benefits of induced resistance to herbivores and pathogens in plants. CAB Rev Perspect Agric Vet Sci Nutr Nat Res 5:1–25
Clarke JD, Liu Y, Klessig DF, Dong X (1998) Uncoupling PR gene expression from NPR1 and bacterial resistance: characterization of the dominant *Arabidopsis cpr6-1* mutant. Plant Cell 10:557–569
Clough SJ, Fengler KA, Yu IC, Lippok B, Smith RK Jr, Bent AF (2000) The *Arabidopsis dnd1* "*defense*, *no death*" gene encodes a mutated cyclic nucleotide-gated ion channel. Proc Natl Acad Sci USA 97:9323–9328
Coll NS, Epple P, Dangl JL (2011) Programmed cell death in the plant immune system. Cell Death Differ 18:1247–1256
Conrath U (2009) Priming of induced plant defense responses, Chap 9. In: Loon LCV (ed) Advances in botanical research. Academic, London, pp 361–395
Conrath U (2011) Molecular aspects of defence priming. Trends Plant Sci 16:524–531
Conrath U, Beckers GJ, Flors V, Garcia-Agustin P, Jakab G, Mauch F, Newman MA, Pieterse CM, Poinssot B, Pozo MJ, Pugin A, Schaffrath U, Ton J, Wendehenne D, Zimmerli L, Mauch-Mani B (2006) Priming: getting ready for battle. Mol Plant Microbe Interact 19:1062–1071
Dangl JL, Jones JD (2001) Plant pathogens and integrated defence responses to infection. Nature 411:826–833
Dong X (2001) Genetic dissection of systemic acquired resistance. Curr Opin Plant Biol 4:309–314
Durrant WE, Dong X (2004) Systemic acquired resistance. Annu Rev Phytopathol 42:185–209
Eitas TK, Dangl JL (2010) NB-LRR proteins: pairs, pieces, perception, partners, and pathways. Curr Opin Plant Biol 13:472–477
Ellis C, Turner JG (2001) The *Arabidopsis* mutant *cev1* has constitutively active jasmonate and ethylene signal pathways and enhanced resistance to pathogens. Plant Cell 13:1025–1033
FAO (2005) Status of research and application of crop biotechnologies in developing countries. FAO, Rome. http://www.fao.org/docrep/008/y5800e/y5800e00.htm
Felix G, Boller T (2003) Molecular sensing of bacteria in plants. The highly conserved RNA-binding motif RNP-1 of bacterial cold shock proteins is recognized as an elicitor signal in tobacco. J Biol Chem 278:6201–6208
Felix G, Duran JD, Volko S, Boller T (1999) Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. Plant J 18:265–276
Fiil BK, Petersen K, Petersen M, Mundy J (2009) Gene regulation by MAP kinase cascades. Curr Opin Plant Biol 12:615–621
Genger RK, Jurkowski GI, McDowell JM, Lu H, Jung HW, Greenberg JT, Bent AF (2008) Signaling pathways that regulate the enhanced disease resistance of *Arabidopsis* "*defense*, *no death*" mutants. Mol Plant Microbe Interact 21:1285–1296
Geraats BP, Bakker PA, Lawrence CB, Achuo EA, Hofte M, van Loon LC (2003) Ethylene-insensitive tobacco shows differentially altered susceptibility to different pathogens. Phytopathology 93:813–821
Gómez-Gómez L, Boller T (2000) FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis*. Mol Cell 5:1003–1011
Grant SR, Fisher EJ, Chang JH, Mole BM, Dangl JL (2006) Subterfuge and manipulation: type III effector proteins of phytopathogenic bacteria. Annu Rev Microbiol 60:425–449
Gust AA, Biswas R, Lenz HD, Rauhut T, Ranf S, Kemmerling B, Gotz F, Glawischnig E, Lee J, Felix G, Nurnberger T (2007) Bacteria-derived peptidoglycans constitute pathogen-associated molecular patterns triggering innate immunity in *Arabidopsis*. J Biol Chem 282:32338–32348
Hamel LP, Beaudoin N (2010) Chitooligosaccharide sensing and downstream signaling: contrasted outcomes in pathogenic and beneficial plant-microbe interactions. Planta 232:787–806

Heil M, Baldwin IT (2002) Fitness costs of induced resistance: emerging experimental support for a slippery concept. Trends Plant Sci 7:61–67
Heil M, Bostock RM (2002) Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. Ann Bot 89:503–512
Jaskiewicz M, Conrath U, Peterhansel C (2011) Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response. EMBO Rep 12:50–55
Jones JD, Dangl JL (2006) The plant immune system. Nature 444:323–329
Katagiri F (2004) A global view of defense gene expression regulation–a highly interconnected signaling network. Curr Opin Plant Biol 7:506–511
Kunze G, Zipfel C, Robatzek S, Niehaus K, Boller T, Felix G (2004) The N terminus of bacterial elongation factor Tu elicits innate immunity in *Arabidopsis* plants. Plant Cell 16:3496–3507
Luna E, Bruce TJA, Roberts MR, Flors V, Ton J (2012) Next-generation systemic acquired resistance. Plant Physiol 158:844–853
Major IT, Nicole MC, Duplessis S, Seguin A (2010) Photosynthetic and respiratory changes in leaves of poplar elicited by rust infection. Photosynth Res 104:41–48
Maleck K, Levine A, Eulgem T, Morgan A, Schmid J, Lawton KA, Dangl JL, Dietrich RA (2000) The transcriptome of *Arabidopsis thaliana* during systemic acquired resistance. Nat Genet 26:403–410
March-Diaz R, Garcia-Dominguez M, Lozano-Juste J, Leon J, Florencio FJ, Reyes JC (2008) Histone H2A.Z and homologues of components of the SWR1 complex are required to control immunity in *Arabidopsis*. Plant J 53:475–487
Mazars C, Thuleau P, Lamotte O, Bourque S (2010) Cross-talk between ROS and calcium in regulation of nuclear activities. Mol Plant 3:706–718
Mészáros T, Helfer A, Hatzimasoura E, Magyar Z, Serazetdinova L, Rios G, Bardóczy V, Teige M, Koncz C, Peck S, Bögre L (2006) The *Arabidopsis* MAP kinase kinase MKK1 participates in defence responses to the bacterial elicitor flagellin. Plant J 48:485–498
Meyer A, Puhler A, Niehaus K (2001) The lipopolysaccharides of the phytopathogen *Xanthomonas campestris* pv. *campestris* induce an oxidative burst reaction in cell cultures of *Nicotiana tabacum*. Planta 213:214–222
Millet YA, Danna CH, Clay NK, Songnuan W, Simon MD, Werck-Reichhart D, Ausubel FM (2010) Innate immune responses activated in *Arabidopsis* roots by microbe-associated molecular patterns. Plant Cell 22:973–990
Navarro L, Zipfel C, Rowland O, Keller I, Robatzek S, Boller T, Jones JD (2004) The transcriptional innate immune response to flg22. Interplay and overlap with Avr gene-dependent defense responses and bacterial pathogenesis. Plant Physiol 135:1113–1128
Newman MA, Daniels MJ, Dow JM (1995) Lipopolysaccharide from *Xanthomonas campestris* induces defense-related gene expression in *Brassica campestris*. Mol Plant Microbe Interact 8:778–780
Pieterse CM, van Wees SC, van Pelt JA, Knoester M, Laan R, Gerrits H, Weisbeek PJ, van Loon LC (1998) A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. Plant Cell 10:1571–1580
Roden LC, Ingle RA (2009) Lights, rhythms, infection: the role of light and the circadian clock in determining the outcome of plant-pathogen interactions. Plant Cell 21:2546–2552
Roitsch T, Balibrea ME, Hofmann M, Proels R, Sinha AK (2003) Extracellular invertase: key metabolic enzyme and PR protein. J Exp Bot 54:513–524
Rusterucci C, Zhao Z, Haines K, Mellersh D, Neumann M, Cameron RK (2005) Age-related resistance to *Pseudomonas syringae* pv. *tomato* is associated with the transition to flowering in *Arabidopsis* and is effective against *Peronospora parasitica*. Physiol Mol Plant Pathol 66:222–231
Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner HY, Hunt MD (1996) Systemic acquired resistance. Plant Cell 8:1809–1819

Schulze B, Mentzel T, Jehle A, Mueller K, Beeler S, Boller T, Felix G, Chinchilla D (2010) Rapid heteromerization and phosphorylation of ligand-activated plant transmembrane receptors and their associated kinase BAK1. J Biol Chem 26:9444–9451

Schwessinger B, Zipfel C (2008) News from the frontline: recent insights into PAMP-triggered immunity in plants. Curr Opin Plant Biol 11:389–395

Shah J, Kachroo P, Klessig DF (1999) The *Arabidopsis ssi1* mutation restores pathogenesis-related gene expression in *npr1* plants and renders defensin gene expression salicylic acid dependent. Plant Cell 11:191–206

Shah J, Kachroo P, Nandi A, Klessig DF (2001) A recessive mutation in the *Arabidopsis SSI2* gene confers SA- and NPR1-independent expression of PR genes and resistance against bacterial and oomycete pathogens. Plant J 25:563–574

Slaughter A, Daniel X, Flors V, Luna E, Hohn B, Mauch-Mani B (2012) Descendants of primed *Arabidopsis* plants exhibit resistance to biotic stress. Plant Physiol 158:835–843

Somssich IE (2007) Networks of transcriptional regulation underlying plant defense responses toward phytopathogens. In: Grasser KD (ed) Annual plant reviews, vol 29, Regulation of transcription in plants. Blackwell, Oxford, pp 266–284

Soto MJ, Dominguez-Ferreras A, Perez-Mendoza D, Sanjuan J, Olivares J (2009) Mutualism versus pathogenesis: the give-and-take in plant-bacteria interactions. Cell Microbiol 11:381–388

Sticher L, Mauch-Mani B, Metraux JP (1997) Systemic acquired resistance. Annu Rev Phytopathol 35:235–270

Swain S, Roy S, Shah J, Van Wees S, Pieterse CM, Nandi AK (2011) *Arabidopsis thaliana cdd1* mutant uncouples the constitutive activation of salicylic acid signalling from growth defects. Mol Plant Pathol 12:855–865

Tao Y, Xie Z, Chen W, Glazebrook J, Chang HS, Han B, Zhu T, Zou G, Katagiri F (2003) Quantitative nature of *Arabidopsis* responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae*. Plant Cell 15:317–330

Thomma BP, Eggermont K, Tierens KF, Broekaert WF (1999) Requirement of functional *ethylene-insensitive 2* gene for efficient resistance of *Arabidopsis* to infection by *Botrytis cinerea*. Plant Physiol 121:1093–1102

Thomma BP, Nurnberger T, Joosten MH (2011) Of PAMPs and effectors: the blurred PTI-ETI dichotomy. Plant Cell 23:4–15

Tian D, Traw MB, Chen JQ, Kreitman M, Bergelson J (2003) Fitness costs of R-gene-mediated resistance in *Arabidopsis thaliana*. Nature 423:74–77

Ton J, Jakab G, Toquin V, Flors V, Iavicoli A, Maeder MN, Metraux JP, Mauch-Mani B (2005) Dissecting the beta-aminobutyric acid-induced priming phenomenon in *Arabidopsis*. Plant Cell 17:987–999

Van den Burg HA, Takken FL (2009) Does chromatin remodeling mark systemic acquired resistance? Trends Plant Sci 14:286–294

Van der Hoorn RA, Kamoun S (2008) From guard to decoy: a new model for perception of plant pathogen effectors. Plant Cell 20:2009–2017

Van Hulten M, Pelser M, van Loon LC, Pieterse CM, Ton J (2006) Costs and benefits of priming for defense in *Arabidopsis*. Proc Natl Acad Sci USA 103:5602–5607

Van Hulten M, Ton J, Pieterse CMJ, Wees SCM (2010) Plant defense signaling from the underground primes aboveground defenses to confer enhanced resistance in a cost-efficient manner. In: Baluška F, Ninkovic V (eds) Plant communication from an ecological perspective. Springer, Berlin, pp 43–60

Van Loon L (2007) Plant responses to plant growth-promoting rhizobacteria. Eur J Plant Pathol 119:243–254

Van Loon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol 36:453–483

Van Loon LC, Rep M, Pieterse CM (2006) Significance of inducible defense-related proteins in infected plants. Annu Rev Phytopathol 44:135–162

Van Wees SC, Van der Ent S, Pieterse CM (2008) Plant immune responses triggered by beneficial microbes. Curr Opin Plant Biol 11:443–448

Walters D, Heil M (2007) Costs and trade-offs associated with induced resistance. Physiol Mol Plant Pathol 71:3–17

Zimmerli L, Hou BH, Tsai CH, Jakab G, Mauch-Mani B, Somerville S (2008) The xenobiotic beta-aminobutyric acid enhances *Arabidopsis* thermotolerance. Plant J 53:144–156

Zipfel C (2009) Early molecular events in PAMP-triggered immunity. Curr Opin Plant Biol 12:414–420

Zipfel C, Robatzek S, Navarro L, Oakeley EJ, Jones JD, Felix G, Boller T (2004) Bacterial disease resistance in *Arabidopsis* through flagellin perception. Nature 428:764–767

Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JD, Boller T, Felix G (2006) Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. Cell 125:749–760

Chapter 14
Plant Growth-Promoting Rhizobacteria for Plant Immunity

Marilyn Sumayo and Sa-Youl Ghim

14.1 Introduction

Plants possess an array of mechanisms to defend themselves against attack by different biotic and abiotic stresses. Some of these defenses are innate, while others become activated only upon interaction with different plants and soil-associated organisms. The rhizosphere is the soil-plant root interphase consists of the soil adhering to the root besides the loose soil surrounding it (Babalola 2010) and is a zone for many diverse and complex microbial-plant interactions. These interactions can be deleterious or can help improve fitness and soil quality that is essential for plant growth and development.

Rhizosphere bacteria known as rhizobacteria can utilize the metabolites secreted by the plant roots as nutrients and can beneficially influence the plant by improving the extent or quality of plant growth directly or indirectly. The direct promotion by PGPR involves either by providing plant growth-promoting substances that are synthesized by the bacterium or by facilitating the uptake of certain plant nutrients from the environment. The indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effect of one or more phytopathogenic microorganisms (Ahmad et al. 2006). PGPR suppress plant pathogens through competition for nutrients and niche (Elad and Baker 1985; Elad and Chet 1987), antibiosis by producing antibiotics (Pierson and Thomashow 1992), and production of siderophores which limit iron availability needed for the growth of pathogens (Kloepper et al. 1980). Other mechanisms include the production of lytic enzymes such as chitinase and glucanase, volatile production of HCN, and degradation of toxins produced by the pathogen (Ramamoorthy et al. 2001).

M. Sumayo • S.-Y. Ghim (✉)
School of Life Science, Kyungpook National University, Daehak-ro 80, Buk-gu, Daegu, 702-701, Korea
e-mail: ghimsa@knu.ac.kr

D.K. Maheshwari et al. (eds.), *Bacteria in Agrobiology: Crop Productivity*,
DOI 10.1007/978-3-642-37241-4_14,

To survive plants respond adequately by activating appropriate defense responses (Jones and Dangl 2006). Plants can activate a line of defense referred to as induced resistance defined as an enhancement of the plant's defensive capacity, which acts systematically throughout the plant and is effective against a broad range of plant attackers (Walters et al. 2007). Induced defense responses involve fitness cost. Fitness cost must be minimized while optimal level of resistance is achieved. An important effect that is usually associated with direct resistance induction is the growth reduction that results from the toxicity of the resistance-inducing compounds or from the fitness costs associated with resistance (Heil and Baldwin 2002). Plants have the ability to acquire this induced resistance after exposure to biotic stimuli provided by PGPR. This PGPR-plant association elicits a steady state of defense in plant referred to as rhizobacteria-mediated induced systemic resistance (ISR) (Choudhary and Johri 2008). ISR is phenotypically similar to systemic acquired resistance (SAR). SAR is evident by direct antibiosis between the inducing bacterium and the challenging pathogen (Fig. 14.1), and a maximum level of SAR is expressed when the inducing organism causes necrosis (Cameron et al. 1994), whereas ISR by rhizobacteria typically do not cause any necrotic symptoms on the host plants (Van Loon et al. 1998). The utilization of SAR-inducing organisms has not been successful under field conditions, and generally, the duration of protection is less following induction of a pathogen than that with rhizobacteria-mediated ISR (Wei et al. 1991). Reports have been published on PGPR as elicitors of tolerance also to abiotic stresses, such as drought, salt, and nutrient deficiency or excess (Ashraf et al. 2004; Mayak et al. 2004a, b; Figueiredo et al. 2008). PGPR strains utilized as inducers of defense responses of plants may increase their applicability and other practical and environment-friendly ways to deliver plant immunization. This chapter focuses on PGPR-mediated ISR for plant immunity against biotic and abiotic stresses minimizing the fitness cost entailed upon defense reaction, therefore without compromising the overall wellness of the plant.

14.2 Broad Spectrum of PGPR-Mediated Induced Systemic Resistance

Plant diseases caused by seed and soilborne pathogens are often controlled by using resistant plants and chemicals. However, resistance does not occur against all diseases and producing resistant plants takes many years (Lugtenberg and Kamilalova 2009). Application of chemicals to enhance plant growth or induce resistance is limited because of the adverse effects of chemical treatment as well as the difficulty to determine the optimal concentrations to benefit plants (Ryu et al. 2005). PGPR strains initiate ISR against a wide array of plant pathogens causing fungal, bacterial, and viral diseases (Ramamoorthy et al. 2001).

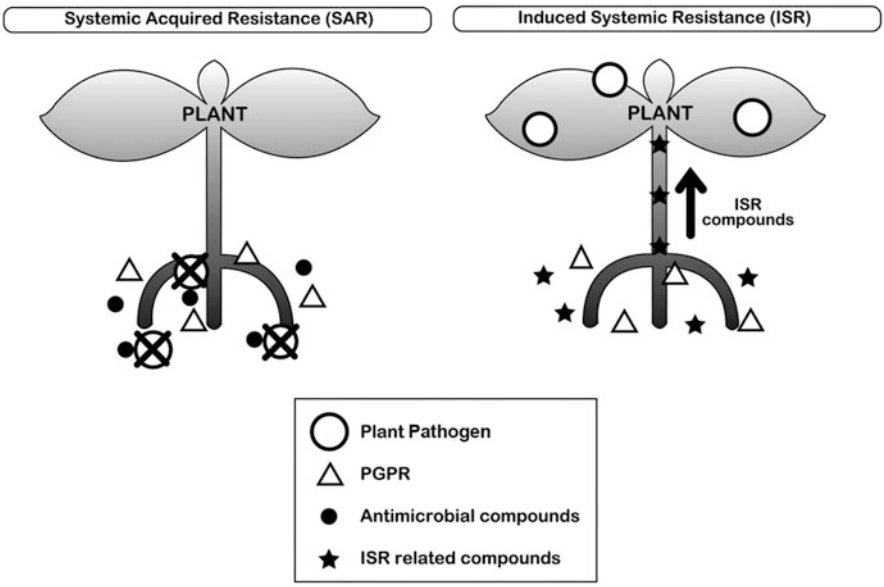


Fig. 14.1 Illustration of systemic acquired resistance and induced systemic resistance for biological control of plant diseases by plant growth-promoting rhizobacteria (PGPR). (SAR) The bacterium produces some antibiotic molecules killing the pathogens via direct antagonism. (ISR) Inducing PGPR and pathogen are spatially separated. ISR-related compounds produced by the bacterium induce systemic signaling resulting to protection of the whole plant

PGPR can induce systemic resistance against bacterial, fungal, and viral diseases, and manifestation of ISR is dependent on the combination of host plant and bacterial strain (Van Loon et al. 1998). Cucumber seeds treated with *Pseudomonas putida* 89B-27, *Flavimonas oryzihabitans* INR-5, *Serratia marcescens* 90–166, and *Bacillus pumilus* INR-7 reduced the lesion diameter of angular leaf spot caused by *Pseudomonas syringae* pv. *lachrymans* (Liu et al. 1995b; Wei et al. 1996). Beans were protected against halo blight disease caused by *Pseudomonas syringae* pv. *phaseolicola* by seed treatment with *P. fluorescens* 97. Application of PGPR strains as seed treatment significantly reduced anthracnose caused by *Colletotrichum orbiculare* in cucumber (Wei et al. 1991, 1996). In rice, seed treatment followed by root dipping and a foliar spray with *P. fluorescens* Pf1 and FP7 showed higher induction of ISR against the sheath blight pathogen, *Rhizoctonia solani* (Vidhyasekaran and Muthamilan 1999). *Pseudomonas* sp. WCS417r protected carnation plants systemically against *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *dianthi* (Van Peer et al. 1991). In sugarcane, PGPR-mediated ISR was observed against *Colletotrichum falcatum* causing red rot disease (Viswanathan and Samiyappan 1999). *Pseudomonas putida* BTP1 enhanced the resistance of cucumber to root rot caused by *Pythium aphanidermatum* and of bean to *Botrytis cinerea* (Ongena et al. 1999, 2002). *Enterobacter* species isolated from the rhizosphere of *Gramineae* plants induced systemic resistance of pepper against

gray leaf spot in pepper caused by *Stemphylium solani* (Son et al. 2012). Soil application of *P. fluorescens* CHAO induced systemic protection against necrosis virus (TNV) in tobacco (Maurhofer et al. 1994). Seed treatment with *P. fluorescens* 89B-27 and *S. marcescens* 90–166 consistently reduced the number of cucumber mosaic virus (CMV)-infected plants and delayed the development of symptoms in cucumber and tomato (Raupach et al. 1996). *Bacillus subtilis* IN937b, *B. pumilus* SE34, and *B. amyloliquefaciens* IN937a protected tomato plants against leaf spot caused by cucumber mosaic virus (Zehnder et al. 2000) and against tomato mottle virus (ToMoV) (Murphy et al. 2000).

PGPR can mediate ISR against various pathogens in different plants (Wei et al. 1996; Ramamoorthy et al. 2001). The same PGPR strain can induce resistance against multiple pathogens in the same crop or against a wide range of plant diseases (Table 14.1). *P. fluorescens* WCS417 protected radish not only against the fungal root pathogen *F. oxysporum* f. sp. *raphani* but also against the avirulent bacterial leaf pathogen *P. syringae* pv. *tomato* and fungal leaf pathogens *Alternaria brassicicola* and *F. oxysporum* (Hoffland et al. 1996). *Pseudomonas fluorescens* Pf1 induces resistance against *Colletotrichum falcatum* in sugarcane (Viswanathan 1999) and *Pythium aphanidermatum* in tomato (Ramamoorthy et al. 1999). *Paenibacillus polymyxa* mediate ISR against *E. carotovora* and *P. syringae* pv. *maculicola* in *A. thaliana* and against bacterial spot pathogen *Xanthomonas axonopodis* pv. *vesicatoria* in pepper (Ryu et al. 2003; Timmusk and Wagner 1999). Also, *P. polymyxa* E681 reduced the incidence of damping-off disease caused by *Pythium ultimatum*, *R. solani*, and *F. oxysporum* in cucumber (Ryu et al. 2005). *S. marcescens* strain 90–166 has shown ISR in cucumber against anthracnose, cucumber mosaic virus, bacterial angular leaf spot, and cucurbit wilt diseases (Kloepper et al. 1993; Liu et al. 1995a, b). *Ochrobactrum lupini* KUDC1013 induced systemic resistance of tobacco against leaf soft rot caused by *E. carotovora* subsp. *carotovora* and of pepper against *Xanthomonas axonopodis* pv. *vesicatoria* (Ham et al. 2009).

PGPR used singly or in mixtures can induce systemic resistance. Mixtures of PGPR strains *Bacillus pumilus* INR7, *Bacillus subtilis* GBO3, and *Curtobacterium flaccumfaciens* ME1 provided control against three cucumber pathogens—*Colletotrichum orbiculare* (anthracnose), *Pseudomonas syringae* pv. *lachrymans* (angular leaf spot), and *Erwinia tracheiphila* (cucurbit wilt) (Raupach and Kloepper 1998). *Ochrobactrum lupini* KUDC1013, either applied singly or in combination with other PGPR strains, induced systemic resistance of pepper against *Xanthomonas axonopodis* pv. *vesicatoria* under greenhouse and field conditions (Hahm et al. 2012). Combinations of PGPR strains were tested for induced resistance activity against multiple plant diseases: bacterial wilt in tomato caused by *Ralstonia solanacearum*, anthracnose in long cayenne pepper caused by *Colletotrichum gloeosporioides*, damping-off disease on green kuang futsoi caused by *Ralstonia solani*, and cucumber mosaic virus on cucumber. Most mixtures provided greater disease suppression than individual PGPR strains indicating that PGPR mixtures can elicit ISR against fungal, bacterial, and viral phytopathogens (Jetiyanon and Kloepper 2002). These studies evidenced that PGPR-mediated ISR

Table 14.1 Broad spectrum of PGPR-mediated induced systemic resistance

Bacterial strain	Plant host: disease	Reference
Ochrobactrum lupini KUDC 1013	Tobacco: soft rot Pepper: bacterial leaf spot	Ham et al. (2009)
Serratia marcescens strain 90-166	Cucumber: anthracnose, cucumber mosaic virus, bacterial angular leaf spot, and cucurbit wilt diseases	Kloepper et al. (1993), Liu et al. (1995a, b)
Paenibacillus polymyxa E681	Cucumber: damping-off disease	Ryu et al. (2005)
Pseudomonas fluorescens strain Pf1	Sugarcane: red rot disease Tomato: damping-off disease	Ramamoorthy et al. (1999)
P. fluorescens strain WCS 417	Radish: *Fusarium* wilt, bacterial speck, and fungal leaf spot	Hoffland et al. (1996)
Bacillus subtilis IN937b, *B. pumilus* SE34, and *B. amyloliquefaciens* IN937a	Tomato: leaf spot (cucumber mosaic virus and tomato mottle virus)	Zehnder et al. (2000), Murphy et al. (2000)

has a broad spectrum for disease protection, and selection of strains with potential ISR activity against multiple pathogens and the method of PGPR application are important in establishing a good biological control agent.

14.3 Mechanisms of Induced Systemic Resistance

Rhizobacteria-mediated ISR resembles the pathogen-induced SAR (Van Loon et al. 1998). Many PGPR eliciting ISR can also directly inhibit pathogen growth suggesting that their disease control activity may involve more than one mechanism. Therefore, PGPR-mediated ISR has been proven to be induced and truly systematic by spatially separating the pathogen and PGPR in plants (Fig. 14.1). Other criteria useful in comparing the characteristics of rhizobacteria-mediated ISR to SAR were presented by Van Loon et al. (1998).

14.3.1 Modification in Cell Wall Structural Components in the Host Plant

PGPR are also known to induce modifications in the plant cell wall as a response to pathogen attack (Benhamou et al. 1996, 1998; M'Piga et al. 1997). Treatment of tomato with *B. pumilus* strain SE 34 induced strengthening of the cell walls against *F. oxysporum* f. sp. *radicis-lycopersici* which protects the fungal site of entry and therefore delaying the infection process (Benhamou et al. 1998). Lignification of bean cell walls was observed after treatment with PGPR. Roots of beans inoculated with *Pseudomonas putida* had higher lignin contents compared with uninoculated seedlings. Plants with roots colonized by *P. putida* gained more weight after

inoculation with *F. solani* f. sp. *phaseoli* compared with plants grown without *P. putida*. Foliar wilting and onset of lesion formation were delayed in plants inoculated with both *P. putida* and *Fusarium*. Protection may involve alteration of the plant's defense potential through an increase in lignin in the root tissues (Anderson and Guerra 1985). Thickening of the tomato cortical cell walls was observed in reaction to colonization of epidermal or hypodermal cells by *P. fluorescens* WCS417 (Duijff et al. 1997). Pre-inoculation of pea roots with *P. fluorescens* 63-28R resulted cell wall appositions and papillae upon challenge inoculation with either *P. ultimum* or *F. oxysporum* f. sp. *pisi* (Benhamou et al. 1996).

14.3.2 Role of Microbial Population and Quorum Sensing

Application of PGPR changes the microbial density and diversity in the rhizosphere and leads to the alteration of the physiology and the response of the plant host. Microbial products trigger changes in the exudation from plant roots (Phillips et al. 2004). PGPR-mediated ISR can reduce symptoms without affecting or reducing the pathogen population (Van Loon 2007). Prior treatment of ACC deaminase containing *Pseudomonas fluorescens* CHAO leads to the reduced disease damage in cucumber against *P. ultimum* cucumbers and in potato against *E. carotovora* pv. *carotovora*. ACC deaminase lowers ethylene level in the plant therefore acting together with other mechanisms to reduce symptoms without affecting the population density of the pathogen. Changes in microbial population can possibly affect the population as well as the activity of ISR-inducing PGPR. Quorum sensing (QS) is population density-dependent regulation of gene expression in bacteria, and bacterial infection of plants often depends on quorum sensing signals (Miller and Bassler 2001). A quorum of bacteria is present when the signal concentration reaches a level capable of triggering changes in the gene expression (Bauer and Mathesius 2004). In QS, there is exchange of small signal molecules between bacterial cells. Changes in the population also change the concentration of the QS signals which affects the QS-regulated bacterial behavior and triggers a diverse response in plant. Studies have established that plants can detect physiological levels of bacterial QS signal (*N*-acyl homoserine lactones) AHLs (Bauer and Mathesius 2004). Moreover, plants also secrete compounds that mimic the bacterial signals, thereby affecting QS regulation in bacteria (Givskov et al. 1996). Further investigations are needed for better understanding of the effects of microbial population and diversity on the metabolic and ISR-inducing activity of rhizobacteria.

14.3.3 Biochemical and Physiological Changes in the Host Plant

Expression of PGPR-mediated ISR can involve different physiological mechanisms (Van Loon 2007). The role of plant hormones in regulating induced resistance is well established. A major distinction between ISR and SAR is the dependence of the SAR on the accumulation of salicylic acid (SA) (Pettersson and Bååth 2004). An impressive number of reviews were made on the involvement of SA and JA/ET pathways on induction of systemic resistance by PGPR (Vallad and Goodmand 2004; Van Loon et al. 1998). ISR activated by PGPR is independent of the SA pathway and rather involves the jasmonate and ethylene (JA/ET) pathways (Pieterse et al. 1998). Increase sensitivity to jasmonate and ethylene leads to the activation of defense genes (Hase et al. 2003; Pieterse and Van Loon 1999). SAR is dependent of the phytohormone salicylate (salicylic acid) and is associated with the accumulation of pathogenesis-related (PR) proteins. PGPR-mediated ISR is dependent on ethylene and jasmonate (jasmonic acid) and is not associated with accumulation of PR proteins (Vallad and Goodmand 2004; Van Loon et al. 1998).

14.3.4 Insect–Plant–Rhizobacteria (Tri-Trophic) Interactions

Plants function in a complex multitrophic environment. The spatial and temporal dynamics of above- and belowground herbivores, plant pathogens, and their antagonists can differ in space and time. This affects the temporal interaction strengths and impacts of above- and belowground higher trophic level organisms on plants (Van der Putten et al. 2001). There is a new insight of understanding the tri-trophic (insect–plant–PGPR) interactions and its role in inducing systemic resistance which indicated that plant-mediated aboveground to belowground communication and vice versa is common (Yang et al. 2011; Yi et al. 2011). The effects of aboveground (AG) insect-elicited plant defense on the resistance expression in roots and the belowground (BG) microbial community were investigated. Symptom development caused by the leaf pathogen *Xanthomonas axonopodis* pv. *vesicatoria* (AG) and soilborne *Ralstonia solanacearum* (BG) in peppers was significantly reduced in whitefly (*Bemisia tabaci*)-exposed plants as compared to controls. An increased expression of plant genes associated with induced resistance (*Capsicum annuum pathogenesis-related protein* (*CaPR*) *1*, *CaPR4*, *CaPR10*, and *Ca protease inhibitor II*) was observed after whitefly exposure indicating that AG whitefly infestation elicited SA and jasmonic acid signaling in AG and BG. Whitefly feeding reduced plant size, which usually occurs as a consequence of the high costs of direct resistance induction. On the other hand, infestation of the leaves manipulated the population of BG Gram-positive bacteria and fungi which may cause plant growth-promoting benefits and induction of systemic resistance on the plant (Yang et al. 2011). Defense responses can occur via interactions among organisms; however,

much more knowledge about the AG-BG interactions, specifically on insect-plant-PGPR interactions, is required in order to improve our understanding on its role in inducing plant resistance.

14.4 Determinants Involved in Induction of Systemic Resistance

Induced systemic resistance mimics the original resistance usually regulated by dominant single gene. A series of researchers to elucidate the mechanism of ISR have given valuable insight into its mechanisms.

14.4.1 Salicylic Acid and Other Siderophores

Under iron-limited conditions, several PGPR can produce siderophores. Siderophore-mediated Fe(III) acquisition is essential for the survival of microorganisms in the environment as well as for the ability of pathogens to establish and maintain infection (Weinberg 1978). Suppression of *Fusarium* wilt in carnation by *Pseudomonas* spp. was mediated by the production of siderophore (Duijff et al. 1993). *P. fluorescens* CHA0, which produces a pyoverdine siderophore and SA in culture (Meyer et al. 1992), suppressed necrosis caused by tobacco necrosis virus (TNV) (Maurhofer et al. 1994). Salicylic acid (SA) is an intermediate in the biosynthesis of pyochelin siderophores (Ankenbauer and Cox 1988). Several ISR-eliciting bacteria are known to produce salicylic acid (SA) bacteria under iron-limited conditions (Meyer et al. 1992; Leeman et al. 1996; De Meyer and Höfte 1997). Some rhizobacteria trigger an SA-dependent signaling pathway by producing nanogram amounts of SA in the rhizosphere. Leeman et al. (1996) suggested that the ISR provided by an SA producer *P. fluorescens* strain WCS374 in radish to *F. oxysporum* was due to bacterial SA production. However, Press et al. (1997) reported that SA produced by *Serratia marcescens* 90–166 is not the primary determinant of ISR in cucumber or tobacco. Mini-Tn*5phoA* mutants, which did not produce detectable amounts of SA, retained ISR activity in cucumber against *Colletotrichum orbiculare* at levels not significantly different from those of the SA^+ parental strain. The ISR-mutant that no longer induced resistance in cucumber produced SA at concentrations not significantly different from those of the wild-type strain. Furthermore, results with transgenic tobacco support the conclusion that microbial SA production by 90–166 was not involved in induced resistance, since this strain was able to induce NahG-10 tobacco, which expresses salicylate hydroxylase. For tomato, it was established that it is not the SA which is produced by this strain that triggers ISR but synthesis of the SA-containing siderophore pyochelin and the antibiotic pyocyanin. In combination, pyochelin and pyocyanin induce the formation of oxygen free radicals in the roots, which triggers SA production in the plant and subsequent activation of an SA-dependent enhanced resistance (Audenaert et al. 2002).

14.4.2 Lipopolysaccharides

Lipopolysaccharides (LPS) can be recognized by plants to directly trigger some plant defense-related responses and can also alter the response of plants to subsequent bacterial inoculation. This may allow the expression of resistance in the absence of catastrophic tissue damage (Dow et al. 2000). LPS present in the outer membrane of PGPR are the major determinants of ISR in certain PGPR strains. LPS from plant growth-promoting *Pseudomonas* spp. strain WCS417r induce resistance in carnation to *Fusarium* wilt caused by *F. oxysporum* f. sp. *dianthi* (Van Peer and Schippers 1992). The LPS of *Pseudomonas fluorescens* induced systemic resistance against *Fusarium wilt* of radish *F. oxysporum* f. sp. *raphani*. Mutant bacteria lacking the O-antigen side chain are defective in inducing resistance suggesting that the O-antigen of the LPS is responsible for ISR (Leeman et al. 1995). *P. fluorescens* WCS417 and LPS derived from this strain are both active in *Arabidopsis thaliana* as in carnation and radish. O-antigen lacking mutant of this strain still elicited ISR in *A. thaliana*, which suggests that the O-antigen cannot be the sole determinant of the response (Van Wees 1999). Bacterial LPS aids in PGPR colonization, creates a favorable microenvironment, acts a barrier to plant-defensive compounds, and modulates host reactions (Newman et al. 1995). Tobacco plants inoculated with purified LPS from PGPR strains prevented the hypersensitive response (HR) to pathogens and reduced disease symptoms (Graham et al. 1977; Maurhofer et al. 1994).

14.4.3 Flagellin Perception

Bacterial flagellin is the principal structural component of flagella. In plants, bacterial flagellins are recognized by surface receptors containing extracellular leucine-rich repeat (LRR) domain FLS2 (Meindl et al. 2000). Stimulation of the receptor by flagellin results in induction of an oxidative burst, callose deposition, and ethylene production, thus inducing defense-related genes such as *PR1*, *PR5*, *PAL 1*, and *GST1* (Gómez-Gómez et al. 1999; Asai et al. 2002). Bacterial flagellin can act as elicitors of defense response in *Arabidopsis*. Treatment with a peptide flg22 that represents the elicitor-active epitope of flagellin induced resistance of *Arabidopsis* wild plants against pathogenic bacteria. Mutation in the flagellin receptor gene *FLS2* showed that plants lacking flagellin perception are more susceptible to pathogenic bacteria carrying flagellin (Zipfel et al. 2004). Also, analysis of the mutant and transgenic lines revealed that the induced resistance seems independent of SA, JA, and ET signaling. *FLS2* mutant plants are more susceptible to the pathogen *Pseudomonas syringae* pv. *tomato* DC3000 when it is sprayed on the leaf surface. Thus, flagellin perception restricts bacterial invasion, probably at an early step, and contributes to the plant's disease resistance. Plant's recognition system for flagellin is highly inclined of animals' innate immunity

response. Flagellin together with other molecules recognized by toll-like receptor (TLR) called pathogen-associated molecular patterns (PAMPs) might serve as an important signal indicating the presence of foreign or nonself organisms (Gómez-Gómez and Boller 2002). PAMPs should be investigated on their similar or complementary functions in controlling and inducing resistance against pathogens.

14.4.4 Volatile Organic Compounds

Volatile organic compounds (VOCs) serve as signaling molecules mediating plant-microbe interaction. Some PGPR release a blend of volatile components, and plant growth is stimulated by differences in these volatile blends. PGPR strains *Bacillus subtilis* GB03 and *Bacillus amyloliquefaciens* IN937a released volatile components 2,3-butanediol and acetoin. Chemical and biochemical studies showed that 2,3-butanediol is an essential bacterial component responsible for airborne chemical signaling triggering growth promotion in *Arabidopsis*. Mutants blocked in 2,3-butanediol and acetoin synthesis were devoid in this growth-promotion capacity released from promoting the growth of *Arabidopsis* (Ryu et al. 2003). VOCs not only promote growth but also can serve as agents for triggering defense responses in plants. In *Arabidopsis* seedlings exposed to bacterial volatile blends from *Bacillus subtilis* GB03 and *Bacillus amyloliquefaciens* IN937a, disease severity by the bacterial pathogen *Erwinia carotovora* subsp. carotovora was significantly reduced compared with seedlings not exposed to bacterial volatiles before pathogen inoculation. The major components detected from GBO3 and IN937a were 3-hydroxy-2-butanone (acetoin) and (2R,3R)-(_)-2,3-butanediol (Ryu et al. 2004). Han et al. (2006) also reported that the GacS-dependent production of (2R, 3R)-butanediol by *Pseudomonas chlororaphis* O6 is a major determinant for eliciting systemic resistance against *E. carotovora* in tobacco. Transgenic lines of *B. subtilis* emitting reduced levels of 2,3-butanediol and acetoin have reduced seedling protection compared with those exposed to VOCs from wild-type bacteria. Mutant and transgenic plant lines were exposed to bacteria emission containing VOCs in order to determine the signaling pathways involved. GBO3 triggered ISR independent of the SA, NPR1, and JA signaling pathways but is mediated by ET. IN937 activates ISR independent of all of these pathways suggesting that ISR triggered by VOCs involved other pathways (Ryu et al. 2004). The involvement of PGPR, their volatile production, and its role in elicitation of ISR should be further exploited. Volatiles from PGPR-induced plants can possibly elicit airborne induction of plant resistance. Yi et al. (2009) have studied that lima beans exposed to the air released from plants that had been spray inoculated with avirulent *P. syringae* strain 61–18 were found to have induced resistance against *P. syringae* pv. syringae. No resistance induction in plants that had been exposed to the air was released from treatment with SAR inducer BTH (Fig. 14.2).

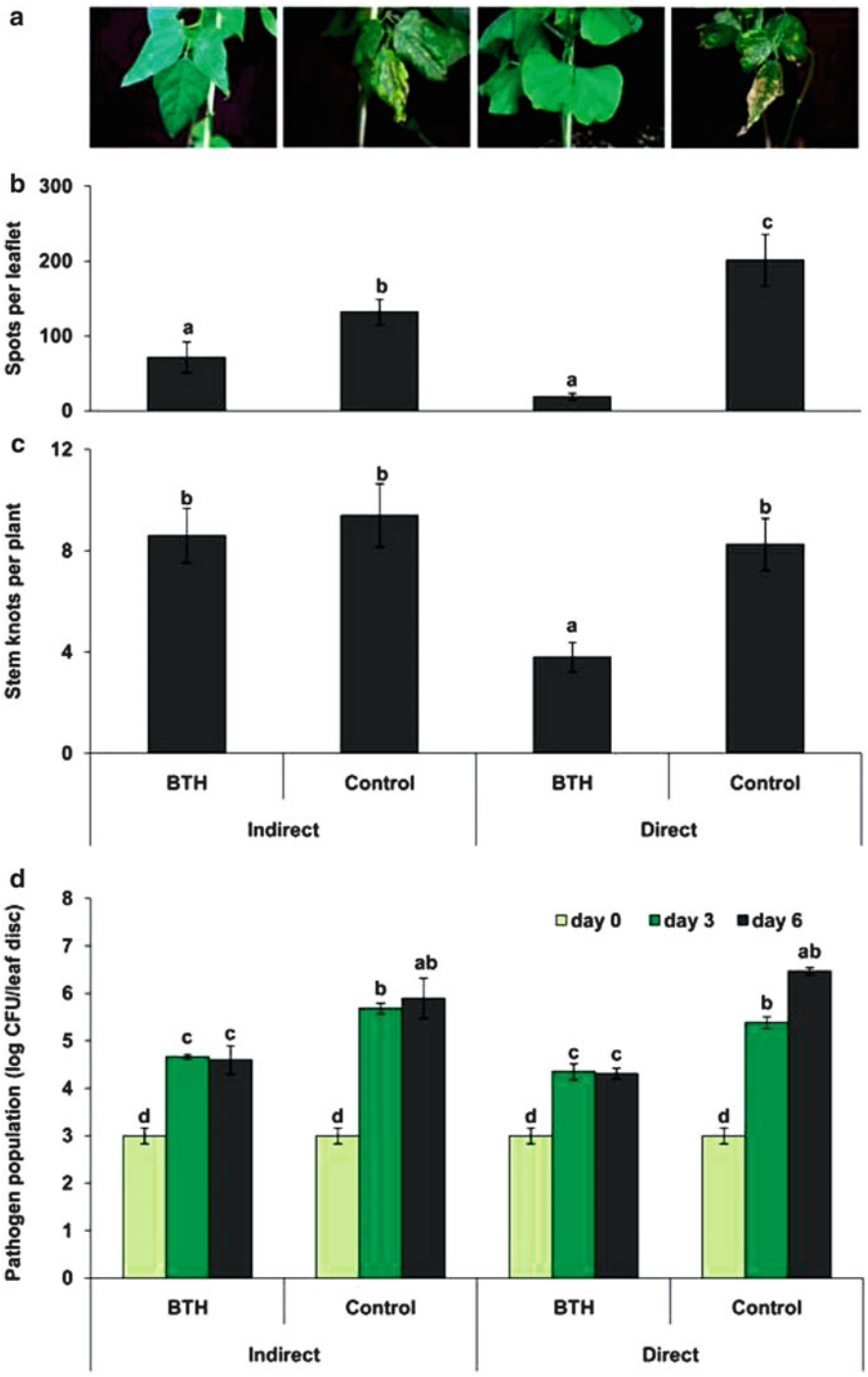


Fig. 14.2 Induction of systemic resistance via airborne signaling (Yi et al. 2009)

14.4.5 Secondary Metabolites

PGPR produce a wide range of secondary metabolites that help defend plants against pathogens. Park et al. (2008) identified an ISR metabolite from *P. chlororaphis* O6 against wildfire pathogen in tobacco *P. syringae* pv. *tabaci* (Table 14.2). Based on the spectroscopy data, the metabolite was identified 4-(aminocarbonyl) phenylacetate (4-ACPA). 4-ACPA applied at 68.0 mM exhibited ISR activity at a level similar 1.0 mM salicylic acid. Also, production of phenazines by *Pseudomonas chlororaphis* O6 induced systemic resistance of tobacco against *E. carotovora* subsp. *carotovora* (Kang et al. 2007). Earlier Kang et al. (2004) isolated an antifungal secondary metabolite 2-hydroxy-methyl-chroman-4 produced by endophytic plant growth-promoting *Burkholderia* MSSP. *P. putida* BTP1 produces an *N*-alkylated benzylamine derivative that induces systemic resistance in bean, tomato, and cucumber (Ongena et al. 2005, 2007). Butyl 2-pyrrolidone-5-carboxylate was reported as metabolite of *Klebsiella oxytoca* C1036 that caused ISR against leaf infections by *P. carotovorum* subsp. *carotovorum* (SCC1) in tobacco. Recently, the biosurfactant massetolide A from *P. fluorescens* SS101 (Tran et al. 2007), a surfactant lipoprotein produced by *Bacillus subtilis* (Ongena et al. 2007), other rhizobacteria produced compound such as *N*-acyl-L-homoserine lactone (Schuhegger et al. 2006) were shown to induce resistance in several host plants against their pathogens. These secondary metabolites produced by PGPR involved in ISR are good candidates for formulation of effective disease control and plant growth-promotion products. More work should be devoted in identifying more ISR-related metabolites and in identification of genes and the study of environmental factors and pathways regulating the production of these metabolites.

14.5 Induction of Systemic Resistance Against Abiotic Stresses

PGPR can augment plant productivity and immunity as well as elicit tolerance to salt and drought. *Burkholderia phytofirmans* PsJN increased growth and physiological activity of grapevine at a low temperature (Barka et al. 2006). Yang et al. (2011) proposed the term induced systemic tolerance (IST) for PGPR-induced physical and chemical changes in plants resulting to an enhanced tolerance against abiotic stresses.

Table 14.2 Secondary metabolites involved in induction of systemic resistance

ISR determinant	PGPR	Host plant	Pathogen	Reference
N-alkylated benzylamine derivative	*Pseudomonas putida* BTP1	Bean	*Botrytis cinerea*	Ongena et al. (2005)
4-(aminocarbonyl) phenylacetate	*Pseudomonas chlororaphis* O6	Tobacco	*Pseudomonas syringae* pv. *tabaci*	Park et al. (2008)
BPC (butyl 2-pyrrolidone-5-carboxylate)	*Klebsiella oxytoca* C1036	Tobacco	*Pectobacterium carotovorum* subsp. *carotovorum* SCC1	Park et al. (2009)

14.5.1 Increased Drought Tolerance upon PGPR Application

Water restriction is one of the abiotic stresses that have a substantial impact on wild plants and crops yield. Maize seedlings inoculated with plant growth-promoting *Bacillus* strains have increased plant biomass, relative water content, leaf water potential root adhering soil/root tissue ratio, aggregate stability, and decreasing leaf water loss. There were physiological responses on maize seedlings inoculated with *Bacillus* spp. that could lessen the negative effects of drought stress (Vardharajula et al. 2010). Ali et al. (2009) identified a thermotolerant strain of *Pseudomonas* sp. AKM-P6 possessing PGPR activities from the rhizosphere of pigeon pea grown under semiarid conditions in India. The colonization of plants with rhizobacteria *Pseudomonas chlororaphis* 06 induces tolerance to drought stress (Cho et al. 2008). Inoculation by the PGPR *Paenibacillus polymyxa* can protect *A. thaliana* against a bacterial pathogen and drought stress in a gnotobiotic system. Increased expression of plant genes associated with abiotic stress (*PR-1*, *HEL*, and *ATVSP*) and genes associated with biotic stress (*ERD15* and *RAB18*) was induced upon inoculation (Timmusk and Wagner 1999). The plant hormone ethylene regulates plant growth under stressful conditions including drought. Ethylene inhibits root elongation and nodulation, speeds aging, and promotes senescence and abscission. The ethylene precursor ACC can be degraded by bacterial ACC deaminase that can help rescue plants back to normal growth (Glick et al. 2007). The *Achromobacter piechaudii* ARV8 containing ACC deaminase was found to be both capable of lowering ethylene production in tomato and pepper and of ameliorating some of the effects of drought stress (Mayak et al. 2004a). Also, drought stress in the common bean (*Phaseolus vulgaris* L.) was alleviated by co-inoculation with *Paenibacillus polymyxa* and *Rhizobium tropici* (Figueiredo et al. 2008). *Pseudomonas fluorescens Pf1* increased the vigor index, fresh weight, and dry weight of green gram (*Vigna radiata*) seedlings in vitro. Greater accumulation of proline and greater activity of stress-related enzymes catalase and peroxidase were found in plants treated with *P. fluorescens* Pf1 against water stress compared with uninoculated plants (Saravanakumar et al. 2011).

14.5.2 Resistance Against Salt Stress

Plant growth and development are adversely affected by salinity which limits agricultural production. Salinity affects both vegetative and reproductive development of plants in different ways such as osmotic effects, specific ion toxicity, and/or nutritional disorders (Lauchli and Epstein 1990). Field salinization is a growing problem worldwide. It was estimated that 10 % of the world's cropland and as much as 27 % of the irrigated land may be already affected by salinity (Shannon 1997). PGPR (*Staphylococcus* sp. strain I26, *Bacillus* sp. strain L81, *Curtobacterium* sp. strain M84, and *Arthrobacter oxidans* strain BB1) isolated from *Pinus* sp. enhanced the protection of *Arabidopsis thaliana* against the foliar pathogen *Pseudomonas syringae* DC3000 and, except strain M84, increased plant tolerance to salt stress (Barriuso et al. 2008). Bano and Fatima (2009) had studied the effect of co-inoculation of PGPR strains that resulted in some positive adaptive responses of maize plants under high salinity. Inoculation of chickpeas and faba beans with *Azospirillum brasilense* significantly reduces the negative effects on plant growth caused by irrigation with saline water (Hamaoui et al. 2001).

The bacterium *Achromobacter piechaudii* significantly increased the fresh and dry weights of tomato seedlings grown in the presence of up to 172 mM NaCl salt. Ethylene production by tomato seedlings which is supposed to increase upon challenged with increasing salt concentrations was reduced. Sodium content was not reduced; however, there was a slight increase in the uptake of phosphorus and potassium, which possibly contributes in the alleviation of the effect of salt (Mayak et al. 2004b). Increase in salinity level decreased the growth of maize seedlings, but this effect was reduced by inoculation with rhizobacterial strains. *Pseudomonas syringae* S5, *Enterobacter aerogenes* S14, and S20 *Pseudomonas fluorescens* S20 were effective in promoting the growth and yield of maize, even at high salt stress. Arora et al. (2006) observed salinity-induced accumulation of poly-β-hydroxybutyrate in rhizobia indicating its role in cell protection. The relatively better salt tolerance of inoculated plants was associated with a high K^+/Na^+ ratio as well as high relative water and chlorophyll and low proline contents (Nadeem et al. 2007). Exopolysaccharide-producing bacteria could be useful in alleviating salinity stress in salt-sensitive plants.

Ashraf et al. (2004) reported that inoculation with *Aeromonas hydrophila/caviae* and *Bacillus insolitus* restricted Na^+ uptake by roots. The decreased Na^+ uptake by roots of inoculated than uninoculated plants was not attributable to the binding of Na^+ by the RS, or to the ameliorative effects of Ca^{2+} under salinity but is probably caused by a reduced passive (apoplasmic) flow of Na^+ into the stele due to the higher proportion of the root zones covered with soil sheaths in inoculated treatments. Dimpka et al. (2009) did a comprehensive review about the mechanisms of increased tolerance to abiotic stress by bacterial colonization. Inoculation of plants with PGPR not only induces plant responses to biotic stress but also contributes in alleviating the negative effects of abiotic stresses suggesting that PGPR conferred a cross protection between biotic and abiotic stresses (Timmusk

and Wagner 1999; Dimpka et al. 2009). The precise mechanisms on the use of plant growth-promoting bacteria that decrease damage on plants under abiotic stress are a potentially important adjuvant to agricultural practices. The plant growth-promoting activity of PGPR and their ability to induce abiotic stress resistance are important tools in developing abiotic management strategies. Recently, Maheshwari (2011) has highlighted source of the important issues on PGPR in plant stress management.

14.6 Concluding Remarks and Future Perspectives

This chapter has shown that PGPR can augment plant immunity through induction of systemic resistance (ISR). PGPR are considered environmentally friendly, unlike the overuse of chemical fertilizers. Chemical fertilizers increase yield in agriculture but are expensive and harmful to the environment. They deplete nonrenewable energy via side effects, such as leaching out and polluting water basins, destroying microorganisms and friendly insects, making the crop more susceptible to the attack of diseases, and reducing soil fertility, thereby causing irreparable damage to the overall system. PGPR-elicited ISR can aid the growth of plants in environmentally unfavorable conditions and upon attack by pathogens. Some PGPR are even capable of inducing resistance in both biotic and abiotic stresses such as drought and high salinity. The role of PGPR in inducing plant defenses under greenhouse and field conditions has been established; moreover, a number of PGPR have been commercially available. Various PGPR factors were shown to elicit ISR. These ISR determinants from PGPR are diverse ranging from cellular components such as LPS and flagella to secondary metabolites and VOCs. The mechanisms and pathways in which PGPR mediates ISR against pathogens are well studied. However, it is more beneficial to also elucidate the signal transduction pathways engaged by PGPR under abiotic stress conditions. Plants are often exposed to biotic and abiotic stress at the same time; therefore, studies on genes involved in ISR induction during both biotic and abiotic stress are essential in further understanding and maximizing the potential of PGPR in aiding plant immunity.

References

Ahmad F, Ahmad I, Khan MS (2006) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res 163:173–181

Ali SZ, Sandhya V, Grover M, Kishore N, Venkateshwar Rao L, Venkateswarlu B (2009) *Pseudomonas* sp. strain AKM-P6 enhances tolerance of sorghum seedlings to elevated temperatures. Biol Fertil Soils 46:45–55

Anderson AS, Guerra D (1985) Responses of bean to root colonization with *Pseudomonas putida* in a hydroponic system. Phytopathology 75:992–995

Ankenbauer RG, Cox CD (1988) Isolation and characterization of *Pseudomonas aeruginosa* mutants requiring salicylic acid for pyochelin biosynthesis. J Bacteriol 170:5364–5367

Arora NK, Singhal V, Maheshwari DK (2006) Salinity-induced accumulation of poly-β - hydroxybutyrate in rhizobia indicating its role in cell protection. World J Microbiol Biotechnol 22:603–606

Asai T, Tena G, Plotnikova J, Willman MR, Chiu WL, Gomez-Gomez L, Boller T, Ausubel FM, Sheen J (2002) MAP kinase signalling cascade in *Arabidopsis* innate immunity. Nature 415:977–983

Ashraf M, Hasnain S, Berge O, Mahmood T (2004) Inoculating wheat seedlings with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. Biol Fertil Soils 40:157–162

Audenaert K, Pattery T, Cornelis P, Höfte M (2002) Induction of systemic resistance to *Botrytis cinerea* in tomato by *Pseudomonas aeruginosa* 7NSK2: role of salicylic acid, pyochelin, and pyocyanin. Mol Plant Microbe Interact 15:1147–1156

Babalola OO (2010) Beneficial bacteria of agricultural importance. Biotechnol Lett 32:1559–1570

Bano A, Fatima M (2009) Salt tolerance in *Zea mays* (L.) following inoculation with *Rhizobium* and *Pseudomonas*. Biol Fertil Soils 45:405–413

Barka EA, Nowak J, Clement C (2006) Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting rhizobacterium, *Burkholderia phytofirmans* strain PsJN. Appl Environ Microbiol 72:7246–7252

Barriuso J, Solano BR, Gutiérrez Mañero FJ (2008) Protection against pathogen and salt stress by four plant growth-promoting rhizobacteria isolated from *Pinus* sp. on *Arabidopsis thaliana*. Phytopathology 98:666–672

Bauer WD, Mathesius U (2004) Plant responses to bacterial quorum sensing signals. Curr Opin Plant Biol 7:429–433

Benhamou N, Kloepper JW, Quadt-Hallman A, Tuzun S (1996) Induction of defense-related ultrastructural modifications in pea root tissues inoculated with endophytic bacteria. Plant Physiol 112:919–929

Benhamou N, Kloepper JW, Tuzun S (1998) Induction of resistance against *Fusarium* wilt of tomato by combination of chitosan with an endophytic bacterial strain: ultrastructure and cytochemistry of the host response. Planta 204:153–168

Cameron RK, Dixon R, Lamb C (1994) Biologically induced systemic acquired resistance in *Arabidopsis thaliana*. Plant J 5:715–725

Cho SM, Kang BR, Han SH, Anderson AJ, Park JY, Lee YH, Cho BH, Yang KY, Ryu CM, Kim YC (2008) 2R,3R-Butanediol, a bacterial volatile produced by *Pseudomonas chlororaphis* 06, is involved in induction of systemic tolerance to drought in *Arabidopsis thaliana*. Mol Plant Microbe Interact 21:1067–1075

Choudhary DK, Johri BN (2008) Interactions of *Bacillus* spp. and plants – with special reference to induced systemic resistance (ISR). Microbiol Res 164:493–513

De Meyer G, Höfte M (1997) Salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 induces resistance to leaf infection by *Botrytis cinerea* on bean. Phytopathology 87:588–593

Dimpka C, Weinand T, Asch F (2009) Plant-rhizobacteria interactions alleviate abiotic stress conditions. Plant Cell Environ 32:1682–1694

Dow M, Newman MA, Von Roepenack E (2000) The induction and modulation of plant defense responses by bacterial lipopolysaccharides. Annu Rev Phytopathol 38:241–261

Duijff BJ, Meijer JW, Bakker PAHM, Schippers B (1993) Siderophore-mediated competition for iron and induced resistance in the suppression of *Fusarium* wilt of carnation by fluorescent *Pseudomonas* spp. Neth J Plant Pathol 99:277–289

Duijff BJ, Gianinazzi-Pearson V, Lemanceau P (1997) Involvement of the outer membrane lipopolysaccharides in the endophytic colonization of tomato roots by biocontrol *Pseudomonas fluorescens* strain WCS417r. New Phytol 135:325–334

Elad Y, Baker R (1985) Influence of trace amounts of cations and siderophore-producing pseudomonads on chlamydiospore germination of *Fusarium oxysporum*. Phytopathology 75:1047–1052

Elad Y, Chet I (1987) Possible role of competition for nutrient in biocontrol of *Pythium* damping-off by bacteria. Phytopathology 77:190–195

Figueiredo M, Burity H, Martinez C, Chanway C (2008) Alleviation of drought stress in the common bean (*Phaseolus vulgaris* L.) by co-inoculation with *Paenibacillus polymyxa* and *Rhizobium tropici*. Appl Soil Ecol 40:182–188

Givskov M, Nys RD, Manefield M, Gram L, Maximilien R, Eberl L, Molin S, Steinberg PD, Kjelleberg S (1996) Eukaryotic interference with homoserine lactone-mediated prokaryotic signaling. J Bacteriol 178:6618–6622

Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B (2007) Promotion of plant growth by bacterial ACC deaminase. Crit Rev Plant Sci 26:227–242

Gómez-Gómez L, Boller T (2002) Flagellin perception: a paradigm for innate immunity. Trends Plant Sci 7:251–256

Gómez-Gómez L, Felix G, Boller T (1999) A single locus determines sensitivity to bacterial flagellin in *Arabidopsis thaliana*. Plant J 18:277–284

Graham LT, Sequeira L, Huang TSR (1977) Bacterial lipopolysaccharides as inducers of disease resistance in tobacco. Appl Environ Microbiol 34:424–432

Hahm MS, Sumayo M, Hwang YJ, Jeon SA, Park SJ, Lee JY, Ahn JH, Kim BS, Ryu CM, Ghim SY (2012) Biological control and plant growth promoting capacity of rhizobacteria on pepper under greenhouse and field conditions. J Microbiol 50:380–385

Ham MS, Ym P, Sung HR, Sumayo M, Ryu CM, Park SH, Ghim SY (2009) Characterization of rhizobacteria isolated from family *Solanaceae* plants in Dokdo island. Kor J Microbiol Biotechnol 37:110–117

Hamaoui B, Abbadi JM, Burdman S, Rashid A, Sarig S, Okon Y (2001) Effects of inoculation with *Azospirillum brasilense* on chickpeas (*Cicer arietinum*) and faba beans (*Vicia faba*) under different growth conditions. Agronomie 21:553–560

Han SH, Lee SJ, Moon JH, Park KH, Yang KY, Cho BH, Kim KY, Kim YW, Lee MC, Anderson AJ, Kim YC (2006) GacS dependent production of (2*R*, 3*R*)-butanediol by *Pseudomonas chlororaphis* O6 is a major determinant for eliciting systemic resistance against *Erwinia carotovora* but not against *Pseudomonas syringae* pv. *tabaci* in tobacco. Mol Plant Microbe Interact 19:924–930

Hase S, Van Pelt JA, Van Loon LC, Pieterse CMJ (2003) Colonization of *Arabidopsis* roots by *Pseudomonas fluorescens* primes the plant to produce higher levels of ethylene upon pathogen infection. Physiol Mol Plant Pathol 62:219–226

Heil M, Baldwin IT (2002) Fitness costs of induced resistance: emerging experimental support for a slippery concept. Trends Plant Sci 2:61–67

Hoffland E, Hakulinem J, Van Pelt JA (1996) Comparison of systemic resistance induced by avirulent and nonpathogenic *Pseudomonas* species. Phytopathology 86:757–762

Jetiyanon K, Kloepper JW (2002) Mixtures of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. Biol Contr 24:285–291

Jones JDG, Dangl JL (2006) The plant immune system. Nature 444:323–329

Kang JG, Shin SY, Kim MJ, Bajpai V, Maheshwari DK, Kang SC (2004) Isolation and anti-fungal activities of 2-hydroxy-methyl-chroman-4-one produced by *Burkholderia* sp. MSSP J Antibiot 57:726–731

Kang BR, Han SH, Zdor RE, Anderson AJ, Spencer M, Yang KY, Kim YH, Lee MC, Cho BH, Kim YC (2007) Inhibition of seed germination and induction of systemic disease resistance by *Pseudomonas chlororaphis* O6 requires phenazine production regulated by the global regular GacS. J Microbiol Biotechnol 17:586–593

Kloepper JW, Leong J, Teintze M, Schroth MN (1980) Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286:885–886

Kloepper JW, Tuzun S, Liu L, Wei G (1993) Plant growth-promoting rhizobacteria as inducers of systemic disease resistance. In: Lumsden RD, Waughn JL (eds) Pest management: biologically based technologies. American Chemical Society Books, Washington, DC, pp 156–165

Lauchli AE, Epstein E (1990) Plant responses to saline and sodic conditions. In: Tanji KK (ed) Agricultural salinity assessment and management. ASCE manuals and reports on engineering practice, vol 71. ASCE, New York, pp 113–137

Leeman M, Van Pelt JA, Denouden FM, Heinsbroek M, Bakker P, Schippers B (1995) Induction of systemic resistance against *Fusarium* wilt of radish by lipopolysaccharides of *Pseudomonas fluorescens*. Phytopathology 85:1021–1027

Leeman M, Den Ouden FM, Van Pelt JA, Dirkx FPM, Steijl H, Bakker PAHM, Schippers B (1996) Iron availability affects induction of systemic resistance to *Fusarium* wilt of radish by *Pseudomonas fluorescens*. Phytopathology 86:149–155

Liu L, Kloepper JW, Tuzun S (1995a) Induction of systemic resistance in cucumber against bacterial leaf spot by plant growth promoting rhizobacteria. Phytopathology 85:843–847

Liu L, Kloepper JW, Tuzun S (1995b) Induction of systemic resistance in cucumber against *Fusarium* wilt by plant growth promoting rhizobacteria. Phytopathology 85:695–698

Lugtenberg B, Kamilalova S (2009) Plant growth promoting rhizobacteria. Annu Rev Microbiol 63:541–556

M'Piga P, Belanger RR, Paulitz TC, Benhamou N (1997) Increased resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato plants treated with the endophytic bacterium *Pseudomonas fluorescens* strain 63–28. Physiol Mol Plant Pathol 50:301–320

Maheshwari DK (2011) Bacteria in agrobiology: stress management. Springer, Heidelberg, Germany

Maurhofer M, Hase C, Meuwly P, Metraux JP, Defago G (1994) Induction of systemic resistance of tobacco to tobacco necrosis virus by the root-colonizing *Pseudomonas fluorescens* strain CHAO: influence of the *gacA* gene and of pyoverdine production. Phytopathology 84:139–146

Mayak S, Tirosh T, Glick B (2004a) Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Sci 166:525–530

Mayak S, Tirosh T, Glick B (2004b) Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol Biochem 42:565–572

Meindl T, Boller T, Felix G (2000) The bacterial elicitor flagellin activates its receptor in tomato cells according to the address-message concept. Plant Cell 12:1783–1794

Meyer JM, Azelvandre P, Georges C (1992) Iron metabolism in *Pseudomonas*: salicylic acid, a siderophore of *Pseudomonas fluorescens* CHA0. Biofactors 4:23–27

Miller MB, Bassler BL (2001) Quorum sensing in bacteria. Annu Rev Microbiol 55:165–199

Murphy JF, Zehnder GW, Schuster DJ, Sikora EJ, Polston JE, Kloepper JW (2000) Plant growth-promoting rhizobacterial mediated protection in tomato against tomato mottle virus. Plant Dis 84:779–784

Nadeem SM, Zahir ZA, Naveed M, Arshad M (2007) Preliminary investigations on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC deaminase activity. Can J Microbiol 53:1141–1149

Newman MA, Daniels MJ, Dow JM (1995) Lipopolysaccharide from *Xanthomonas campestris* induces defense-related gene expression in *Brassica campestris*. Mol Plant Microbe Interact 8:778–780

Ongena M, Daayf F, Jacques P, Thonart P, Benhamou N, Paulitz TC, Corne'lis P, Koedam N, Be'langer RR (1999) Protection of cucumber against *Pythium* root rot by fluorescent pseudomonads: predominant role of induced resistance over siderophores and antibiosis. Plant Pathol 48:66–76

Ongena M, Giger A, Jacques P, Jacques D, Thonart P (2002) Study of Bacterial Determinants involved in the induction of systemic resistance in bean by *Pseudomonas putida* BTP1. Eur J Plant Pathol 108:187–196

Ongena M, Jourdan E, Schäfer M, Kech C, Budzikiewicz H, Luxen A, Thonart P (2005) Isolation of an N- alkylated benzylamine derivative from *Pseudomonas putida* BTP1 as elicitor of induced systemic resistance in bean. Mol Plant Microbe Interact 18:562–569

Ongena M, Jourdan E, Adam A, Schäfer M, Budzikiewicz H, Thonart P (2007) Amino acids, iron, and growth rate as key factors influencing production of the *Pseudomonas putida* BTP1 benzylamine derivative involved in systemic resistance induction in different plants. Microb Ecol 55:282–292

Park MR, Kim YC, Park JY, Han SH, Kim KY, Lee SW, Kim IS (2008) Identification of an ISR-related metabolite produced by *Pseudomonas chlororaphis* 06 against the wildfire pathogen *Pseudomonas syringae* pv. *tabaci* in tobacco. J Microbiol Biotechnol 18:1659–1662

Park MR, Kim YC, Lee SW, Kim IS (2009) Identification of an ISR-related metabolite produced by rhizobacterium *Klebsiella oxytoca* C1036 active against soft-rot disease pathogen in tobacco. Pest Manag Sci 65:1114–1117

Pettersson M, Bååth E (2004) Effects of the properties of the bacterial community on pH adaptation during recolonization of a humus soil. Soil Biol Biochem 36:1383–1388

Phillips DA, Fox TC, King MD, Bhuvaneswari TV, Teuber LR (2004) Microbial products trigger amino acid exudation from plant roots. Plant Physiol 136:2887–2894

Pierson LS III, Thomashow LS (1992) Cloning and heterologous expression of the phenazine biosynthetic locus from *Pseudomonas aureofaciens* 30–84. Mol Plant Microbe Interact 5:330–339

Pieterse CMJ, Van Loon LC (1999) Salicylic acid-independent plant defense pathways. Trends Plant Sci 4:52–58

Pieterse CMJ, Van Wees SCM, Van Pelt JA, Knoester M, Laan R, Gerrits H, Weisbeek PJ, Van Loon LC (1998) A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. Plant Cell 10:1571–1580

Press CM, Wilson M, Tuzun S, Kloepper JW (1997) Salicylic acid produced by *Serratia marcescens* 90–166 is not the primary determinant of induced systemic resistance in cucumber or tobacco. Mol Plant Microbe Interact 10:761–768

Ramamoorthy V, Raguchander T, Samiyappan R (1999) Isolation, characterization and screening fluorescent pseudomonads for managing damping-off disease of major vegetable crops. In: Symposium on plant disease management for sustainable agriculture. Indian Phytopathological Society, Southern Zone held at C.P.C.R.I. Kayankulam, Kerala, India, p 26

Ramamoorthy V, Viswanathan R, Raguchander T, Prakasan V, Samiyappan R (2001) Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. Crop Prot 20:1–11

Raupach GS, Kloepper JW (1998) Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology 88:1158–1164

Raupach GS, Liu L, Murphy JF, Tuzun S, Kloepper JW (1996) Induced systemic resistance in cucumber and tomato against cucumber mosaic cucumovirus using plant growth promoting rhizobacteria (PGPR). Plant Dis 80:891–894

Ryu CM, Farag MA, Hu CH, Reddy MS, Wei HX, Pare' PW, Kloepper JW (2003) Bacterial volatiles promote growth in *Arabidopsis*. Proc Natl Acad Sci USA 100:4927–4932

Ryu CM, Farag MA, Hu CH, Reddy MS, Kloepper JW, Pare' PW (2004) Bacterial volatiles induce systemic resistance in *Arabidopsis*. Plant Physiol 134:1017–1026

Ryu CM, Kim J, Choi O, Park SY, Park SH, Park CS (2005) Nature of a root-associated *Paenibacillus polymyxa* from field-grown winter barley in Korea. J Microbiol Biotechnol 15:984–991

Saravanakumar D, Kavino M, Raguchander T, Subbian P, Samiyappan R (2011) Plant growth promoting bacteria enhance water stress resistance in green gram plants. Acta Physiol Plant 33:203–209

Schuhegger R, Ihring A, Gantner S, Bhanweg G, Knappe C, Hartmann A, Langebartels C (2006) Induction of systemic resistance in tomato by N-acyl-L-homoserine lactone producing rhizosphere bacteria. Plant Cell Environ 29:909–918

Shannon M (1997) Adaptation of plants to salinity. Adv Agron 60:75–120
Son JS, Sumayo M, Kang HU, Kim BS, Kwon DK, Ghim SY (2012) Induction of systemic resistance against gray leaf spot in pepper by *Enterobacter* species isolated from *Gramineae* plants in Dokdo. Kor J Microbiol Biotechnol 135–143
Timmusk S, Wagner EGH (1999) The plant-growth-promoting rhizobacterium *Paenibacillus polymyxa* induces changes in *Arabidopsis thaliana* gene expression: a possible connection between biotic and abiotic stress responses. Mol Plant Microbe Interact 12:951–959
Tran H, Ficke A, Asiimwe T, Hofte M, Raaijmarkers JM (2007) Role of the cyclic lipopeptide massetolide A in biological control of *Phytophthora infestans* and in colonization of tomato plants by *Pseudomonas fluorescens*. New Phytol 175:731–742
Vallad GE, Goodmand RM (2004) Systemic acquired resistance and induced systemic resistance in conventional agriculture. Crop Sci 44:1920–1934
Van der Putten WH, Vet LEM, Harvey JA, Wackers FL (2001) Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. Trends Ecol Evol 16:547–554
Van Loon LC (2007) Plant responses to plant growth-promoting rhizobacteria. Eur J Plant Pathol 119:243–254
Van Loon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol 36:453–483
Van Peer R, Schippers B (1992) Lipopolysaccharides of plant growth promoting *Pseudomonas* spp. strain WCS417r induce resistance in carnation to *Fusarium* wilt. Neth J Plant Pathol 98:129–139
Van Peer R, Niemann GJ, Schippers B (1991) Induced resistance and phytoalexin accumulation in biological control of *Fusarium* wilt of carnation by *Pseudomonas* sp strain WCS 417r. Phytopathology 81:728–734
Van Wees SCM (1999) Rhizobacteria-mediated induced systemic resistance in *Arabidopsis*; signal transduction and expression. Ph.D thesis. Utrecht University, The Netherlands, p 137
Vardharajula S, Ali SZ, Grover M, Reddy G, Bandi V (2010) Drought-tolerant plant growth promoting *Bacillus* spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. J Plant Interact 6:1–14
Vidhyasekaran P, Muthamilan M (1999) Evaluation of powder formulation of *Pseudomonas fluorescens* Pf1 for control of rice sheath blight. Biocontrol Sci Technol 9:67–74
Viswanathan R (1999) Induction of systemic resistance against red rot disease in sugarcane by plant growth promoting rhizobacteria. Ph.D. Thesis, TNAU, Coimbatore, India, p 175
Viswanathan R, Samiyappan R (1999) Induction of systemic resistance by plant growth promoting rhizobacteria against red rot disease caused by *Colletotrichum falcatum* went in sugarcane. In: Proceedings of the sugar technology association of India, vol 61. Sugar Technology Association, New Delhi, pp 24–39
Walters D, Newton A, Lyon G (2007) Induced resistance for plant defence: a sustainable approach to crop protection. Blackwell, Oxford
Wei G, Kloepper JW, Tuzun S (1991) Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacteria. Phytopathology 81:1508–1512
Wei L, Kloepper JW, Tuzun S (1996) Induced systemic resistance to cucumber diseases and increased plant growth by plant growth promoting rhizobacteria under field conditions. Phytopathology 86:221–224
Weinberg ED (1978) Iron and infection. Microbiol Rev 42:45–66
Yang JW, Yi HS, Kim H, Lee B, Lee S, Ghim SY, Ryu CM (2011) Whitefly infestation of pepper plants elicits defence responses against bacterial pathogens in leaves and roots and changes the below-ground microflora. J Ecol 99:46–56
Yi HS, Heil M, Adame-Álvarez RM, Ballhorn DJ, Ryu CM (2009) Airborne induction and priming of plant defenses against a bacterial pathogen. Plant Physiol 4:2152–2161

Yi HS, Yang JW, Ghim SY, Ryu CM (2011) A cry for help from leaf to root Aboveground insect feeding leads to the recruitment of rhizosphere microbes for plant self-protection against subsequent diverse attacks. Plant Signal Behav 6:1192–1194

Zehnder GW, Yao C, Murphy JF, Sikora EJ, Kloepper JW (2000) Induction of resistance in tomato against cucumber mosaic cucumovirus by plant growth-promoting rhizobacteria. BioControl 45:127–137

Zipfel C, Robatzek S, Navarro L, Oakeley EJ, Jones JDG, Felix G, Boller T (2004) Bacterial disease resistance in Arabidopsis through flagellin perception. Nature 428:764–767

Chapter 15
Integrated Diseases Management in Groundnut for Sustainable Productivity

Urja Pandya and Meenu Saraf

15.1 Introduction

The food legume crops play an important role in terms of food and nutritional security and are an important pillar of sustainable food production globally. Food legume crops are important components of cropping systems and provide an opportunity to produce diversified food products for human consumption. Inclusion of these crops into agricultural production systems increases the profitability of cropping systems globally (Al-Tawaha et al. 2010). Legumes, broadly defined by their unusual flower structure, podded fruit, and the ability of 88 % of the species examined to date for nodulation formation with rhizobia (Graham and Vance 2003), are second only to the *Gramineae* in their importance to humans. Increased cultivation of legumes is essential for the regeneration of nutrient-deficient soils and for providing needed protein, minerals, and vitamins to humans and livestock (Dubey and Maheshwari 2011). Legumes can be a means of improving the livelihoods of smallholder farmers around the world. Three oilseed crops, i.e., groundnut, soybean, and rapeseed/mustard, together account for over 80 % aggregate of cultivated oilseed outputs. World's largest edible oil consuming countries are the USA, China, Brazil, and India. India contributes about 8 % of the world oilseed production and about 6 % of the global production of oils and fats and currently is the fifth largest vegetable oil economy in the world, after the USA, China, Brazil, and Argentina (Ramesh and Hegde 2010). Legumes belong to the taxonomic family *Fabaceae*, containing over 18,000 species divided into the three subfamilies *Mimosoideae*, *Caesalpinioideae*, and *Papilionoideae*. Legume species have been cultivated for millennia all over the world because of the nutritional value of their seeds. Among different legumes, soybean (*Glycine max* L.), chickpea

U. Pandya • M. Saraf (✉)
Department of Microbiology, University School of Sciences, Gujarat University, Ahmedabad 380 009, Gujarat, India
e-mail: sarafmeenu@gmail.com

D.K. Maheshwari et al. (eds.), *Bacteria in Agrobiology: Crop Productivity*,
DOI 10.1007/978-3-642-37241-4_15,

(*Cicer arietinum* L.), common bean (*Phaseolus vulgaris* L.), groundnut or peanut (*Arachis hypogaea* L.), cowpea (*Vigna unguiculata* L.), and pigeon pea (*Cajanus cajan* L.) contribute significantly to the diets of large numbers of people in Asia, Africa, and South America (Varshney et al. 2007).

Legumes play a vital role as more predominant in the years to come under climate change due to changes in planting environments, increased levels of abiotic stresses, increased demand on these crops in consumption, etc. The present level of productivity of these crops is low due to major constraints as mentioned below (1) greater susceptibility to biotic and abiotic stresses, (2) they are cultivated on marginal lands under poor environments, (3) low inputs are often applied to them by farming communities during the cropping season, (4) they have indeterminate growth habit and poor genetic yield potential in comparison to cereals and (5) there is often non-adoption of integrated crop production and management technologies by the legume growers (Al-Tawaha et al. 2010). Thus, a big challenge is visible to increase the production/productivity of these crops in future particularly when changing climates are overlain on their production areas. There is a great need for focus on developing the role of legumes and their contribution to both the sustainable intensification of production and the livelihoods of smallholder farmers in many parts of the world. Therefore, it will be more appropriate to adopt the simplest improved approaches which can be implemented in many countries by the farming communities without much deviation in the existing cropping system to mitigate the climatic changes. The aim of this chapter is to discuss the potential role of rhizobacteria as biofertilizer and biocontrol agent for groundnut production for sustainable productivity.

15.1.1 World Groundnut Production

Groundnut also called peanut is one of the principal oilseed as well as economic crops of the world. Groundnut is grown on a large scale in almost all the tropical and subtropical countries of the world. The most important groundnut growing countries are India, China, Nigeria, Sudan, and USA. It is grown over an area of 24.7 million hectares with a total production of 33 million tonnes in the whole world. World peanut production totals approximately 35.7 million tonnes during 2011, with India being the world's second largest producer after China (Fig. 15.1). Worldwide peanut exports are approximately 2.75 million metric tonnes during 2011. The cultivated peanut (*Arachis hypogaea* L.) originated in South America (Bolivia and adjoining countries) (Stalker and Simpson 1995). This crop was grown widely by native peoples of the New World at the time of European expansion in the sixteenth century and was subsequently taken to Europe, Africa, Asia, and the Pacific Islands. Peanut was introduced to the present Southeastern United States during colonial times. It is currently grown throughout the tropical and warm temperate regions of the world, with 35.7 million tonnes of worldwide production in 2006 (FAO Food Outlook 2007).

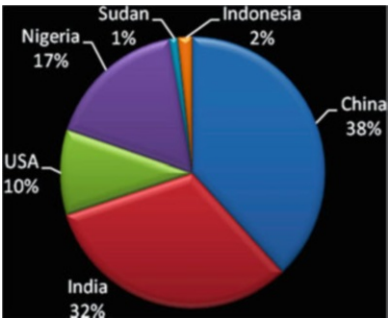


Fig. 15.1 Groundnut seed yield in different countries around the world according to 2010 groundnut cultivation (FAO 2010)

Among oilseeds, groundnut (*Arachis hypogaea* L.) "king of oilseed crops" is a rainfed crop grown in India mainly during kharif/rainy season. It is also grown during rabi/summer either under assured irrigation or residual moisture. Among oilseed crops in India, groundnut accounts for about 50 % of area and 45 % of oil production. India occupies the first place in acreage and second in production. Groundnut is cultivated in more than 60 countries of the world. The total production of groundnut has been inconsistent during the past few years. According to the reports of FAO (2010), the yield was 10,073 hg ha^{-1} from an area of 5,470,000 ha in 2009, whereas it was 11,440 hg ha^{-1} from an area of 4,930,000 ha in 2010 from a comparatively less area (Fig. 15.2). A gradual increase in the area harvested since 2007 was not reciprocated in the yield/production. In India, it is grown in 11 states in an area of 8 million hectares producing over 9 million tonnes of pods. About 88 % area of cultivation and production are confined mainly to Andhra Pradesh, Karnataka, Maharashtra, and Gujarat states (Arsule and Pande 2012). Gujarat accounts to 36 % of total production of groundnut in India, and it is the largest producer in India (Table 15.1). Patil et al. (2009) made an attempt to know the trends in area, production, and productivity of groundnut crop in Maharashtra.

15.1.2 Importance of Groundnut Cultivation

Seeds yield a nondrying, edible oil used in cooking, margarines, salads, canning, for deep frying in pastry and bread. Seeds are eaten raw, whole roasted and salted, or chopped in confectioneries, or ground into peanut butter. Young pods may be consumed as a vegetable. Young leaves and tips are suitable as a cooked green vegetable. Other products include ice cream, massage oil, and peanut milk. Groundnut (*Arachis hypogaea* L.) is an important oilseed and food crop. Its seeds are important source of oil (50 %) and a valuable source of proteins (24.7 %) to improve human nutrition (Rivlin 2001; Van Damme et al. 1993).

Groundnut oil is also used for pharmaceuticals, soaps, cold creams, cosmetics, dyes, paints, pomades and lubricants, and emulsions for insect control. Peanut hulls are used for furfural, fuel, and as filler for fertilizers. Groundnut shell (GS), a

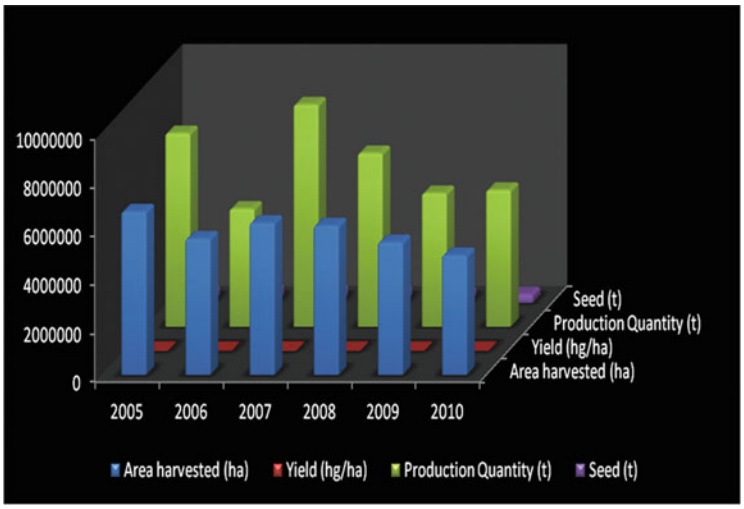


Fig. 15.2 Report of groundnut cultivation from 2005 to 2010 in India (FAO 2010)

Table 15.1 State-wise groundnut production in 2011–2012 (Chennakrishnan 2012)

States	2011–2012 ('000 tonnes)	2010–2011 ('000 tonnes)	Per cent change (%)
Gujarat	17.75	18.7	–5.08
Andhra Pradesh	5.5	6	–8.33
Tamil Nadu	3.5	2	75
Maharashtra	1.8	2	–10
Madhya Pradesh	1.8	1.5	20
Punjab, Haryana, and UP	0.8	0.8	–
Others	10.6	10	6

residue after separation of pod, is available in copious amount in the world. The crop residue is of low economic value and generally used in burning in gasifiers as a fuel source or sometimes as manure to increase the soil conditions. The residue containing a total of 54.4 % total carbohydrate content (dry weight) in its cell wall makes it an appropriate substrate for bioconversion to fuel ethanol (Gajula et al. 2011). Groundnuts are sold in the local market as boiled unshelled and shelled roasted nuts, while some are sold in the confectionery trade (Kiriro 1993). The haulms are either fed to livestock or used in compost or left in the fields as crop residue (Kiriro and Rachier 1989). As a legume, groundnuts improve soil fertility in the farming systems by fixing atmospheric nitrogen and also as trap-catch crop in the management of Striga weed in cereal crop (Kiriro 1993).

15.2 Factors Affecting Groundnut Production

Throughout the cultivation of groundnut, from planting to storage, different types of biotic and abiotic factors form major threats. The abiotic factors include physiological and environmental stresses such as temperature, salinity, and drought, and the biotic threats include insects, fungi, bacteria, virus, nematodes, etc.

15.2.1 Abiotic Stress

That includes major factors like drought, temperature, and salinity that directly affect on groundnut production.

15.2.1.1 Drought

Drought stress is particularly critical during flowering and pod-filling stage for yield and agronomic characters. This would result in drastic reduction in crop yield, and magnitude of reduction would depend on groundnut varieties. Due to drought stress, both the yield and product of groundnut are decreased. Pot experiments were conducted to evaluate four groundnut varieties (TAG-24, TG-26, WEST-20, and WEST-44) under four regimes of water (100 %, 80 %, 60 %, and 40 %). Under increasing moisture deficit, dry weight per plant of all varieties was decreased. At higher water stress level, the variety TG-26 showed maximum reduction in dry weight 64.37 %, followed by 54.59 % in WEST-20, 42.08 % in WEST-44, and 25.36 % in TAG-24 (Shinde and Laware 2010). Khan et al. (2011) investigated the physiological and biochemical traits of groundnut cultivar Swat Phalli-96 under drought stress. Results showed that drought stress significantly reduced flowers (8 %), fruits (6 %), and pod yield (46 %) as compared to control. Sharad and Nail (2011) studied the effect of water deficit on early growth and biochemical constituents of groundnut (K-1375 and R-9251). Drought-induced decrease in relative water content (44 %), chlorophyll content (35.26 %), and total protein content (38 %) was significantly higher in R-9251 than K-1375. Recently, Hamidou et al. (2012) observed assessment of groundnut under combine heat and drought stress. Results showed that pod yield decrease due to drought stress was 72 % at high temperature and 55 % at moderate temperature. Pod yield under well-watered (WW) conditions did not decrease under high temperature conditions. Haulm yield decrease due to water stress (WS) was 34 % at high temperature and 42 % under moderate temperature.

15.2.1.2 Temperature

After groundnut seeds are sown, germination and emergence are primarily determined by the temperature and soil moisture in the seeding zone. At the optimum range of temperature and soil moisture, both germination and emergence take place at a maximum rate. Between their minimum threshold and lower optimum values, the rates of germination and emergence increase with the increase in temperature and soil moisture. Above their optimum range, these processes are progressively slowed down until they completely stop at their respective maximum threshold values (damaging thresholds). Awal and Ikeda (2002) and Prasad et al. (2006) reported that base temperature for germination of groundnut is approximately 10 °C and the optimum temperature for emergence is between 25 and 30 °C.

Groundnut is cultivated between latitudes 40 °N and 40 °S. About 95 % of the cultivated area is in the semiarid tropics (FAO 2000) where daytime temperatures often exceed 35 °C during flowering. Such high temperature episodes are likely to increase in frequency as a result of anthropogenic causes (Houghton et al. 2001). High-temperature studies conducted so far in groundnuts have shown that temperatures greater than 34 °C during the reproductive period severely reduce both peg and pod number (Ketring 1984; Wheeler et al. 1997; Prasad et al. 1999a, b, 2006). The reduction in peg and pod number has been attributed to fewer pollen grains and poorer pollen viability (Prasad et al. 1999b). Genotypes were found to range from most tolerant to most susceptible based on both pollen characters and membrane thermostability. Mean cardinal temperatures (T_{min}, T_{opt} and T_{max}) averaged over 21 genotypes were 14.1, 30.1, and 43.0 °C for percentage pollen germination and 14.6, 34.4, and 43.4 °C for maximum pollen tube length. The genotypes 55-437, ICG 1236, TMV 2, and ICGS 11 can be grouped as tolerant to high temperature and genotypes Kadiri 3, ICGV 92116, and ICGV 92118 as susceptible genotypes, based on the cardinal temperatures as reported by Kakani et al. (2002).

15.2.1.3 Salinity

Soil salinity, saline irrigation water, and also the heavy use of fertilizers salts can severely restrict plant growth, responsible for foliage damage and even death of the plants (Taffouo et al. 2010). Saline soil, which causes reductions in yield, is one of the important abiotic constraints to groundnut production. Pulses in general are sensitive and have inadequate control over ion uptake, which leads to high internal salt concentrations and results in plant injury. However, tremendous variability exists regarding salt tolerance among different species/cultivars in all pulses (Chauhan and Singh 2000). Mensah et al. (2006) examined effect of different salinity concentration (0.015, 1.50, 2.60, 4.68, 8.90, and 17 ms cm^{-1}) on five groundnut genotypes. The results revealed that salinity significantly delayed germination and also reduced the final percentages at electrical conductivities greater

than 2.60 mS cm^{-1}. Seedling emergence, radical elongation, plant height, and dry matter weight also tended to decrease with increasing salinity in all five genotypes. Gajera et al. (2009) reported effect of chloride-dominant salt stress (0, 20, 40, 80 meq L^{-1}) on groundnut genotypes. Chloride-based salinity decreased the seedling vigor index of all groundnut genotypes, and the decreases were found more in GG-7, GG-20, and GG-2 genotypes (susceptible group) at 4 and 8 DAS. With increasing salinity regimes, various metabolites like free amino acid, total sugars, free fatty acids, and free proline contents were deposited at higher rate in seedlings of JL-24, GAUG-10, and GG-13 genotypes (tolerant group) compared to susceptible ones for better osmotic adjustment. Mineral nutrient status and morphological characteristics changes in peanut (*Arachis hypogaea* L.) cultivars under salt stress were studied by Desire et al. (2010) under pot experiments. The results showed that the salt stress reduced significantly ($p < 0.05$) the plant height in Pyrieur cultivar from 40.49 to 21.45 cm, the number of leaves from 11.2 to 7.0, the dry weight of roots from 0.15 to 0.11 g per plant, the dry weight of stems from 0.37 to 0.15 g per plant, and the dry weight of leaves from 0.46 to 0.19 g per plant. As salinity increases, nutrients like K, Mg, Ca, P, N, K, Na, and Ca/Na uptake by peanut were reduced in Pyrieur, Vanda, and MbiaH cultivars.

15.2.2 Biotic Stress

Among the biotic stresses, the foliar fungal (early leaf spot, late leaf spot, rust), viral (peanut bud necrosis, stem necrosis), and soil-borne (stem rot, collar rot, and pod rot complexes) diseases and the insect pests like defoliators (*Spodoptera*, *Helicoverpa*, red hairy caterpillar, and leaf miner), and sucking pests (Jassids, Aphids, Thrips) are the major ones that limit groundnut production and productivity. In addition, the pre and postharvest aflatoxin contamination in the kernels and meal also reduces the quality as well as export value, as shown in Table 15.2.

The major foliar fungal diseases, which are early leaf spot, late leaf spot, and rust, have magnitude of yield loss that is very high and ranged from 10 to 70 % all over the world but vary considerably from place to place and between seasons (Ghewande 1983, 1985, 1990; Subrahmaniyam and Mc Donald 1983). The seed and seedling diseases (collar rot, stem rot, and root rot) of groundnut cause severe seedling mortality, resulting in patchy crop stand, and have a devastating effect on the prospects of a successful groundnut crop. Collar rot is reported to cause 40 % loss in the crop establishment and yield in Punjab (Chohan 1973). Pande and Narayana Rao (2000) have observed up to 30 % reductions in plant stand due to collar rot and estimated 20 % pod yield reduction in the farmers' fields in the states of Andhra Pradesh, Karnataka, and Tamil Nadu. Stem rot caused up to 27 % loss in Uttar Pradesh and in the Deccan Plateau (Singh and Mathur 1953; Pande and Narayana Rao 2000). Approximately 5–15 % loss in the initial crop stand is due to seed rot and seedling collapse. Additionally, pod deterioration caused by the soil-borne pathogenic fungi has been reported to be potentially serious in several

Table 15.2 List of major disease of groundnut and effects on its productivity

Type of disease	Causal organisms	Symptoms	Reference
Foliar fungal disease, Early leaf spot, Late leaf spot, Rust	*Cercospora arachidicola Hori*, *Phaeoisariopsis personata* (*Berk.* and *Curt.*) *v. Arx*, *Puccinia arachidis Speg*	Early leaf spot causes light to dark-brown lesions on the upper surface of the leaflets with a chlorotic halo surrounding the lesions. Infected leaves finally necrotize and defoliate. Late leaf spot symptoms occur as circular, dark lesions on the lower leaflet surface but without the halo formation. The leaves defoliate after necrosis. During severe infections, oval elongate lesions are formed on the petioles and stems. Leaf rust of groundnut causes orange-colored pustules on the lower surface and, in severe cases, on the upper surface of leaflets	Subrahmanyam et al. (1997), Pande et al. (2001), Ambang et al. (2011), Bdliya and Alkali (2010), Khedikar et al. (2010)
Bacterial disease, Bacterial wilt	*Pseudomonas solanacearum* (*Smith*)	Young infected plants show sudden wilting of stem and foliage with leaves on dead plants remaining green. Dying branches often curl to form a "shepherd's crook." The disease can be identified by dark-brown spots in the xylem and pith	Hong et al. (1999)
Soil-borne fungal disease, Brown blotch, Damping off, Root rot, Crown rot, Stem rot	*Colletotrichum capsici*, *Pythium* spp., *Phytophthora* spp., *Fusarium solani* (*Mart*) *Sacc*, *Aspergillus niger*, *Sclerotium rolfsii*	Brown blotch diseases starts as a tiny, light brown spot on the upper leaf surface. Under humid conditions, the spots enlarge very fast and coalesce, and the entire leaf surface might become blotched without necessarily resulting in defoliation. In damping-off disease, stem is affected and that portion becomes constricted and weak, incapable of bearing the load of the upper portion. As a result, the seedlings topple down and die. The affected tissues die, decompose to greater extent, and turn brown. In root rot, water-soaked necrotic spots appear on the stem just above the ground level. Pod infection leads to blackening of the shells, and sclerotia can be seen inside the shells. In crown rot, large lesions develop on the stem below the soil and spread upwards along the branches causing drooping of leaves, wilting and death of the plants. In stem rot, lesions on the developing pegs can retard pod development and seeds in the infected pods show a characteristic bluish-gray discoloration	Obagwu (2001), Ihejirika et al. (2010), Ahmed and Zaman (2012), Rasheed et al. (2004), Ganesan et al. (2007)

Viral disease, Bud necrosis disease	*Bud necrosis virus*	Disease first appears on young leaflets as chlorotic spots or mottling that may develop into chlorotic and necrotic rings and streaks. Terminal bud necrosis occurs when temperature is relatively high. As plant matures, it becomes stunted with short internodes and proliferation of axillary shoots	Gopal et al. (2010, 2011), Gupta and Shukla (2011)
Nematode disease, Root-knot disease	*Meloidogyne* spp.	Galls develop into various sizes resulting from an internal swelling from the root tissue. Infected pods develop knobs, protuberances, or small warts	Eisenback et al. (2003)
Insects, Red hairy caterpillars	*Amsacta albistriga*, *A. moorei*	Caterpillars cause defoliation of the crop—all the leaves eaten away leaving the main stem alone	Ghewande and Nandgopal (2009)
Groundnut leaf miner	*Aproaerema modicella*	Young larvae initially mine into the leaflets, feed on the mesophyll, and form small brown blotches on the leaf. Later stages larvae web the leaflets together and feed on them, remaining within the folds. Severely attacked field looks "burnt" from a distance	Wightman and Rao (1993)
Bihar hairy caterpillar	*Spilosoma* (*Diacrisia*) *obliqua*	Young larvae feed gregariously mostly on the under surface of the leaves, feed on leaves and cause loss by way of defoliation. In severe cases, only stems are left behind	Wightman and Rao (1993)
Gram pod borer	*Helicoverpa armigera*	Larvae feed on the foliage and prefer flowers and buds. When tender leaf buds are eaten, symmetrical holes or cuttings can be seen upon unfolding of leaflets	Wightman and Rao (1993)
Groundnut bud borer	*Anarsia ephippias*	The larva bores into the terminal buds and shoots and tip of the stem. The tender leaflets emerging from central spindle will show shot-hole symptoms initially. In severe infestation, emerging leaflets will have only the midribs or several oblong feeding holes	Wightman and Rao (1993)
Aphids	*Aphis craccivora*	Wilting of tender shoots during hot weather. Stunting and distortion of the foliage and stems. They excrete honeydew on which sooty molds flow forming a black coating. Act as vector for peanut stripe virus and groundnut rosette virus complex	Wightman and Rao (1993)

farmers' fields in Andhra Pradesh, Tamil Nadu, and Karnataka (Pande and Narayana Rao 2000). In India, bud necrosis disease caused yield losses up to 50 % (Chohan 1978). In the case of late infection caused by clump disease, losses up to 60 % have been recorded (Ghanekar 1980). The root-knot nematodes (*Meloidogyne* spp.) are the most important nematode species causing damage ranging from 20 to 90 % in infested fields of groundnut (Rodrigues-Kabana 1984).

15.3 Microbial Interactions with Groundnut Crop

Soil bacteria are very important in biogeochemical cycles and have been used for crop production for decades. Plant–microbe interactions in the rhizosphere are the determinants of plant health and soil fertility. Interaction of plant growth-promoting rhizobacteria (PGPR) with host plants is an intricate and interdependent relationship involving not only the two partners but other biotic and abiotic factors of the rhizosphere region (Jha et al. 2012). Plant growth-promoting rhizobacteria (PGPR) are free-living soil bacteria that can either directly or indirectly facilitate rooting and growth of plants. PGPR can affect the plant growth directly by (1) production or changing the concentration of phytohormones such as IAA, gibberellic acid, cytokinins, and ethylene; (2) solubilization of mineral phosphates and other nutrients; and (3) asymbiotic N_2 fixation and indirectly by enhancing plant growth via suppression of phytopathogens by a variety of mechanisms. These include the ability to produce siderophores that chelate iron, making it unavailable to pathogens; the ability to synthesize antifungal metabolites such as antibiotics, fungal cell wall-lysing enzymes, or production of volatiles such as hydrogen cyanide, which suppress the growth of fungal pathogens; the ability to successfully compete with pathogens for nutrients or specific niches on the root; and the ability to induced systemic resistance (ISR) (Saraf et al. 2010).

Based on their above-mentioned attributes, Somers et al. (2004) classified PGPR as biofertilizers (increasing the availability of nutrients to plant), phytostimulators (plant growth promoting, usually by the production of phytohormones), rhizoremediators (degrading organic pollutants), and biopesticides (controlling diseases, mainly by the production of antibiotics, antifungal metabolites, lytic enzymes producers, etc.). Biofertilizers are preparations containing live microorganisms that help in nutrient availability through fixation, solubilization, or mobilization. There are many biofertilizers for application in agricultural crop production. Their importance can be realized from the fact that more than 43 million ha under paddy, 35 million ha under coarse cereals, 23 million ha under pulses, 25 million ha under groundnut, and 4 million ha under soybean can be benefited by using one or other types of biofertilizers. Biofertilizers benefit the crop by way of increased N fixation, enhanced availability of nutrients through solubilization, or increased absorption and stimulation of plant growth through phytohormones production like IAA, GA, etc. (Desai et al. 2012).

15.3.1 Rhizobium *and* Bradyrhizobium

Bradyrhizobium and *Rhizobium* spp. showed two modes of root colonization in leguminous plants: one by root hair entry and infection thread formation and another by crack entry and intercellular spreading (Boogerd and van Rossum 1997). Root hair entry has received the majority of study and takes place in most temperate and some subtropical legumes (*Medicago*, *Trifolium*, *Pisum*, *Phaseolus*, *Lotus*, *Glycine* spp., etc.). The crack entry/intercellular spreading infection mode was reported to occur in a few subtropical and tropical legumes, including *Arachis*, *Sesbania*, *Stylosanthes*, *Neptunia*, and *Aeschynomene*. Root colonization of groundnut by rhizobia is characterized by crack entry and intercellular spreading, and no infection threads are formed. *Bradyrhizobium* spp. penetrates into the roots by breaching the epidermal barrier where lateral roots come off instead of entering through curled root hairs. After entry, *Bradyrhizobium* cells occupy the space between epidermal and cortical cells and further spread through the root cortex in an intercellular matrix. This matrix consists of broken plant cell wall fragments and bacterial exopolysaccharides. It was proposed that during this process, groundnuts secrete a variety of compounds that are engaged in the plant-defensive response to the invading organism (Azpilicueta et al. 2004).

The use of *Rhizobium* spp. inoculants for groundnut is a common practice for groundnut production. Also, co-inoculation of *Rhizobium* with other plant growth-promoting bacteria received considerable attention in legume growth promotion. Co-inoculation of *Thiobacillus* sp. strain LCH (applied at 60 kg ha^{-1}) with *Rhizobium* sp. strain TNAU14 under field condition of groundnut recorded significantly higher nodule number, nodule dry weight, and plant biomass 136.9 per plant, 740.0 mg per plant, and 15.0 g per plant, respectively, on 80 DAS and enhanced the pod yield by 18 %. Also inoculation of S-oxidizing bacteria increased the soil available S from 7.4 to 8.43 kg ha^{-1}. These results suggest that inoculation of S-oxidizing bacteria along with rhizobia results in synergistic interactions promoting the yield and oil content of groundnut, in S-deficit soils (Anandham et al. 2007). Associative effects of VAM with rhizobia have been well documented by Tilak (1993). Devi and Reddy (2001) observed the influence of VAM fungus and *Rhizobium* inoculation on groundnut growth response in relation to growth, nodulation, *phosphorus* content, and phosphatase activity in pot culture studies. The amount of phosphorus and activity of acid and alkaline phosphatases increased significantly with dual inoculation than with individual inoculations.

15.3.2 Mycorrhiza

Role of mycorrhiza to influence plant growth, water, and nutrient content has been widely reported over the years. The mycorrhiza has a high-affinity P uptake mechanism that enhances P nutrition in plants. The contribution of indigenous

arbuscular mycorrhiza (AM) on phosphorus (P) uptake by groundnut was examined by Rakshit and Bhadoria (2008) in a low P field soil. Results showed that phosphorus supply affected percentage of root infected by AM which was 40 % of the roots at P-0 and decreased to around 30 % and 10 % at P-50 and P-400. Doley and Jite (2012) reported that the effect of mycorrhizal fungus *Glomus fasciculatum* on vegetative growth parameters of groundnut such as leaf number, shoot length, root length, fresh weight, dry weight, pod number, and nodule number was significantly increased as compared to control under pot experiments. Peanuts and mycorrhizal association, increasing dry matter yield, phosphorus (P) uptake, and stimulation of root and shoot growth as a result (Rao et al. 1990; Bergero et al. 2003). Al-Khaliel (2010) studied two mycorrhizal species (*G. mosseae* and *G. fasciculatum*) on peanut production. Results revealed that *G. mosseae* was the more effective fungus in enhancing peanut growth when compared with *G. fasciculatum*.

15.3.3 Pseudomonads, Bacillus*, and Others*

Members of the genus Pseudomonads showed remarkable metabolic and physiologic versatility, implementing colonization of diverse terrestrial and aquatic habitats and are of great interest because of their importance in plant and human disease and their growing potential in biotechnological applications. Many Pseudomonads interact with plants and several species contribute to plant health by antagonizing plant pathogens by biocontrol mechanisms and directly plant growth promotion as rhizosphere colonizers (Silby et al. 2011). Earlier attempts for selection of PGPR for groundnut growth promotion in soils identified an increase in pod yield following seed treatment with *Pseudomonas* sp. (Pal et al. 2000). Strains of the genus *Bacillus* are another most commonly reported PGPR (Compant et al. 2005; Vessey 2003). *B. licheniformis* strain MML2501 has efficient spermosphere colonization with maximum IAA producing attribute that isolated from groundnut rhizosphere showed the significant seed germination and other growth parameters such as number of gynophores (91), number of pods (56), pod weight (41.5), and kernel weight (28.0), and it also increased the root nodules (234) and nodule weight (1.25 g) under in vitro conditions (Prashanth and Mathivanan 2010). Phylloplane isolates of *P. aeruginosa* GPS 55 and GPS 21 showed similar increase in the growth and yield of groundnut when applied as seed treatment (Kishore et al. 2005a). *B. subtilis* strains showed the increased emergence vigor and yield in Florunner groundnuts as reported by Jaks et al. (1985). Efficient germination, emergence, and increased nodulation by groundnut *Rhizobium* and *B. subtilis* were reported by Turner and Backman (1991). Our experiments performed with groundnut seedlings with PGPR strains revealed that enhanced root length, fresh root mass, fresh shoot mass, dry root mass, dry shoot mass, number of leaf, chlorophyll content, and biomass over control after 60 DAS (Unpublished results) (Fig. 15.3).

Fig. 15.3 Effect of PGPR strains on groundnut growth as compared to control

Siderophore-producing PGPR play a vital role in Fe nutrition of plants and therefore in plant growth promotion leading to healthy plants which are vital for increasing crop/food yield (Bloemberg and Lugtenberg 2001). Sayyed et al. (2010) found the optimization of siderophoregenesis of *A. faecalis* BCCM 2374 enhanced seed germination (8.75 %), root length (9.35 %), shoot length (16 %) and chlorophyll (8 %) in *Arachis hypogaea* under pot culture experiments. Taurian et al. (2010) reported the endophytic isolate such as *Pantoea* from root nodules has significant increased plant biomass of groundnut as compared to the effects of rhizobacterial isolates. Methylotrophic bacteria with growth-promoting and nitrogen-fixing ability have been isolated from the rhizosphere and phyllosphere of groundnut (Madhaiyan et al. 2006, 2010). The *Methylobacterium* sp. PPFM-Ah was originally isolated from groundnut leaves and applied through seed imbibitions to stimulate germination and plant growth under greenhouse conditions (Madhaiyan et al. 2006). *Rhizobium meliloti* RMP3 and RMP5 showed siderophore production on CAS agar medium and 72 % and 75 % inhibition of *M. phaseolina* in in vitro conditions, whereas the two strains' disease incidence reduces to a mere 7.9 % and 3.5 % at treated groundnut plant (Arora et al. 2001).

15.3.4 Plant Growth-Promoting Fungi

Similar to PGPR, some rhizosphere fungi able to promote plant growth upon root colonization are functionally designated as "plant growth-promoting fungi (PGPF)." PGPF may make use of one of the several mechanisms to promote plant growth like production of phytohormones, solubilization of minerals, and

antagonism to phytopathogens (Pandya and Saraf 2010). PGPF have been reported to produce substances such as plant hormones to allow plants to utilize decomposing organic matter through mineral solubilization and to suppress plant pathogens in the rhizosphere by antagonistic mechanisms, such as the production of hydrolytic enzymes, aggressive mycoparasitism, competition for saprophytic colonization, and the induction of plant systemic resistance (Masunaka et al. 2011). Phosphorus (P) is a vital plant nutrient, available to plant roots only in soluble forms that are in short supply in the soil. A wide range of soil fungi are reported to solubilize insoluble phosphorous. Strains of *Aspergillus niger* and *Penicillium* are the most common fungi capable of phosphate solubilization. *A. niger* and *Penicillium notatum* were studied for their efficacy to solubilize tricalcium phosphate (TCP) in vitro as well as their effect in vivo to promote the growth of groundnut (*Arachis hypogaea*) plants grown in soil amended with TCP. The results showed that pot experiment showed that the dual inoculation of phosphate-solubilizing fungi (*A. niger* and *P. notatum*) significantly increased dry matter and yield of groundnut plants as compared to the control soil. These treatments also showed significant increased in percentage of protein and oil content and as well as increased percentage of N and P content of the plant (Malviya et al. 2011).

15.4 Disease Management for Sustainable Productivity of Groundnut

The success and management of diseases by Integrated Diseases Management (IDM) strategies includes a combination of chemical, cultural, and biological practices.

15.4.1 Integrated Disease Management

Although chemical pesticides have played an important role in increasing groundnut production, their indiscriminate use has led to several environmental problems, such as the development of pesticide resistance in pest populations, pesticide residues, and the destruction of beneficial organisms (parasites and predators) with soil nutrient fertility loss. IDM is currently defined as: "A sustainable approach to managing diseases by combining biological, cultural, physical and chemical tools in a way that minimizes economic, health and environmental risks." This concept evolved from the original IPM definition after responding to today's call for ecologically based pest management. Biological tools in this definition include host plant resistance as well as microbial agents for disease control (El Khuoury and Makkouk 2010). Opportunities for more sustainable use are offered by an integrated approach based on Integrated Disease Management (IDM). Given the

costs of production and pest losses, it could be most economic and feasible to develop an IDM program for groundnut (Ghewande and Nandangopal 1997).

There are three main components of IDM: Firstly, the diseases and pest are managed effectively so that the presence of pathogens/pests remains below the economic thresholds for the crop and the market. Secondly, a variety of pests (ranging from microorganisms to insects and large weeds) are managed and thirdly, the produce must meet the prescribed quality standards with respect to the extent of damage caused by the pathogen/pest and pesticide residues (lowest possible or nil pesticide residues). IDM offers immense long-term advantages through the use of fewer pesticides and better maintenance of environment leading to sustainability. Therefore, any ideal IDM programs must include one or more of the following management methods (Charaya and Mehrotra 2004):

1. Proper identification of the pathogen
2. Using the knowledge of the environment (manipulating the environment to reduce disease incidence)
3. Use of natural enemies of pests (biological control agents) and selective biopesticides
4. Selective minimum properly planned spray of broad spectrum biopesticides
5. Choosing cultivars resistant to or tolerant to pests/pathogens
6. Appropriate cultural techniques (like time of planting, crop rotation, sanitation, and adequate plant nutrition)

The changing production system scenario demands for cost-effective, easily adaptable, and ecofriendly tools for the efficient management of the major diseases. Disease management requires judicious adoption of the several management tools. The important among them are discussed below.

15.4.2 Host Plant Resistance

Host plant resistance is an important tool to control diseases of major food crops in developing countries, especially wheat, rice, potato, cassava, chickpea, peanuts, and cowpea. The use of resistant varieties is very much welcomed by resource poor farmers because it does not require additional cost and environment-friendly. A large collection of the world germplasm has been screened against leaf spots and rust under laboratory and field conditions at ICRISAT, and the lines showing resistance have been identified (Mehan et al. 1996; Subrahmanyam et al. 1980). Reddy and Wightman (1988) reported peanut varieties resistant to peanut virus disease in Africa. Peanut cultivars (ICGV 89104 and ICGV 91114) yield 55–60 % more than local cultivar and also lower disease severity of late leaf spot and rust in India (Pande et al. 2001). ICGV 50 line, ICGV 86031, and ICGV 86699 cultivars were developed at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) highly resistant to leaf miners (Ghewande and Nandangopal 1997).

15.4.3 Cultural Practices

Cultural control methods not only serve in promoting the healthy growth of the crop but are also effective in directly reducing inoculum potential (pruning, roguing, crop rotation, ploughing, etc.) and in enhancing the biological activities of antagonists in the soil (solarization, crop rotation, mulching, etc.). Intercropping with pigeon pea, black gram, pearl millet, sorghum, and other crops reduced the intensity of foliar diseases. Deep ploughing of fields and rotation with gram and wheat reduced collar rot disease in groundnut (Sathiyanarayanmurthy et al. 1988). Application of neem cake and farmyard manure to soil gave good control of collar rot (Karthikeya 1996). Foliar disease management was established by Ghewande et al. (2002) by deep burying of crop residues, destruction of crop debris by burning, removal of affected groundnut plants, early planting, and wider row spacing and intercropping of pearl millet, sorghum, and pigeon pea.

15.4.4 Chemical Control

For many decades fungicides played an important role in disease control. The use of synthetic insecticides is inevitable in the management of devastating insect pests in agriculture. The use of synthetic insecticides is associated with population resurgence, outbreaks of secondary pests, and the development of insecticide resistance in insect populations. Although insecticides are expensive and hazardous to apply, farmers are inclined to use them because the obvious quick knock-down effect of synthetic insecticides is convincing to them (Ghewande and Nandangopal 1997). Several systemic and nonsystemic fungicides were tried for reducing the severity of major foliar diseases, viz., early leaf spot, late leaf spot, and rust of groundnut. Akgul et al. (2011) investigated the effects and the possibility of using some systemic fungicides (Fludioxonil, Azoxystrobin, Metalaxyl-M, Tolclofos-Methyl, Thiram, Carboxin) as seed treatments with different active ingredients against stem rot caused by *Sclerotium rolfsii* in peanuts. Results showed that fungicides such as Fludioxonil 100 g L^{-1}, Azoxystrobin + Fludioxonil + Metalaxyl-M, Carboxin + Thiram, and Tolclofos-Methyl + Thiram were the most effective fungicides in decreasing stem rot on peanuts under pot experiments. Field experiments were carried out to evaluate the effects of variety and fungicidal rate on *Cercospora* leaf spot disease of groundnut by Muhammad and Bdliya (2011). Results showed that 2 kg ha^{-1} of fungicide mancozeb and either RRB or ICGV-86024 appeared to be more promising against this disease. Naab et al. (2005) also reported that application of foliar sprays of fungicide in Ghana was effective in controlling *Cercospora* leaf spot and improved groundnut biomass and pod yield by 39 % and 75 %, respectively, when averaged across cultivars and years. Tebuconazole and azoxystrobin are highly active against both foliar and soil-borne diseases; however, they both have site-specific modes of action and, therefore, a greater risk for

resistance development (Bertrand and Padgett 1997). Culbreath et al. (2001) also evaluated the efficacy of various alternations and combinations of chlorothalonil and benomyl for managing benomyl-resistant *C. arachidicola* and *C. personatum* populations. Results of that study showed that full-season tank mixes of the compounds provided leaf spot control comparable to the standard chlorothalonil program, suggesting that tank mixing is a valid resistance management tool where fungicide resistance is already a problem.

15.4.5 Biological Control

Biological control of plant diseases is an important area, which needs attention since most of the hazardous inputs added into the agricultural system are in the form of plant protection chemicals. Detailed studies aimed at replacing chemical pesticides with environmentally safer methods are currently being a greater importance at this juncture. The biological control of soil-borne pathogens with antagonistic bacteria belonging to plant growth-promoting rhizobacteria has received prominent attention because of the dual role of these bacteria in plant growth promotion and disease control (Basha et al. 2012). Biological control of plant diseases has been considered as a viable alternative method to manage plant diseases. Biological control is the inhibition of growth, infection, or reproduction of one organism using another organism. Biocontrol is environmentally safe and in some cases is the only option available to protect plants against pathogens. Fungal plant diseases are considered the most important microbial agents that cause serious loss of agricultural crops annually (Heydari and Pessarakli 2010).

Extracellular chitinolytic enzymes of microorganisms have a potential to suppress the activities of the pathogens by degrading the chitin in their cell walls and thus protect the plant from disease (Unpublished data). Chitinolysis plays an important role in biological control of plant diseases and has been substantiated with increased disease control by chitin-supplemented application of chitinolytic biocontrol agents (Zhang and Yuen 2000; Manjula and Podile 2001), greater field efficiency of chitinase preparations in disease control (Shternshis et al. 2002), and enhanced biocontrol potential of genetically engineered organisms for chitinase overproduction (Limon et al. 1999). *Bacillus subtilis* AF 1, a potent chitinolytic bacterial isolate, had a broad spectrum antifungal activity. The bacterium was an effective biocontrol agent of watery rot of papaya fruits (Kumar et al. 1988), crown rot of groundnut, and *Fusarium* wilt of pigeon pea (Manjula and Podile 2001). Severity of groundnut early leaf spot caused by *Cercospora arachidicola* was reduced by foliar application of a chitinolytic *B. cereus* (Kokalis-Burelle et al. 1992). Manjula et al. (2004) studied partially purified β-1, 4-*N*-acetylglucosaminidase (NAGase) of a biocontrol strain *Bacillus subtilis* AF 1 for control of rust in groundnut (caused by *Puccinia arachidis*). In the presence of NAGase, germination of urediniospores of *P. arachidis* was reduced by 96 % compared with the control. In a detached leaf bioassay, NAGase reduced the rust lesion frequency by >60 %. Foliar application of chitinolytic

Serratia marcescens resulted in decrease in the incidence of late leaf spot disease in groundnut (Kishore et al. 2005b). Senthilraja et al. (2010) reported field study of talk-based formulation of *Beauveria* and *Pseudomonas* strain mixture (with and without chitin) significantly suppressed the leaf miner and collar rot incidence of groundnut. The maximum control of leaf miner (94.84 % and 93.96 %) and collar rot (95.47; 94.52 %) was observed in plots that received the mixture with and without chitin amendment, respectively.

Some *Pseudomonas* species and strains have been reported for their ability to control stem rot disease of groundnut caused by *S. rolfsii* (Ganesan and Gnanamanickam 1987; Tonelli et al. 2011). Pseudomonads are well known for the production of a diverse array of antifungal compounds, including 2,4-diacetylphloroglucinol, pyrrolnitrin, pyoluteorin, rhizoxins, phenazines (PHZ), and lipopeptides (LPs) (Raaijmakers et al. 2002, 2009, 2010; Haas and Defago 2005; Gross and Loper 2009; D'aes et al. 2010). Le et al. (2011) studied the phenazine and lipopeptide producing *Pseudomonas* spp. inhibited hyphal growth of *S. rolfsii* and significantly reduced stem rot disease of groundnut in greenhouse and field experiments. *Pseudomonas aeruginosa* GRC1 showed 97 % reduction in stem rot of peanut in *S. sclerotiorum*-infested soil (Gupta et al. 2006). Kishore et al. (2006) also found that biocontrol agent *Pseudomonas aeruginosa* GSE 18 reduced the preemergence of groundnut rotting by 60 % in *A. niger*-infested potting mixture. Bhatia et al. (2008) reported increased seed germination, growth promotion, and suppression of charcoal rot due to *M. phaseolina* with fluorescent pseudomonads, *P. fluorescens* for groundnut vegetative growth and in biocontrol of late leaf spot caused by *C. personatum* in groundnut (Meena et al. 2006). Our experiments performed with biocontrol agents and PGPR strains under in vitro conditions revealed that SEM images of MBCU2 (*Bacillus* spp.) showed aberrant features such as hyphal perforation, lysis, fragmentation, and degradation of mycelia of *M. phaseolina* after five days of incubation (Fig. 15.4a, b), whereas Fig. 15.5 showed inhibition of various fungal pathogens by PGPR strains (unpublished results).

Trichoderma spp. is effective in control of soil/seed-borne fungal diseases in several crop plants. Species of this genus are well reported as biocontrol agents against several fungal pathogens through mechanisms such as mycoparasitism (mycelial coiling), antibiosis, cell wall-degrading enzymes, and induced resistance in host plant against diseases by altering plant gene expression (Pandya and Saraf 2010; Alfano et al. 2007). Gajera and Vakharia (2012) observed that biocontrol agent *T. viride* 60 has a significant role in the control of collar rot disease by reducing the virulence of *A. niger* (86.2 %) in the peanut with lytic enzymes like chitinase, β-1,3-glucanase, and protease production as compared to control. *Trichoderma harzianum* (Th3) was used against groundnut varieties (GG-10, GG-20, M-13, and local varieties) to reduce the yield loss by root rot disease during the year 2009 and 2010 in farmers' fields in twelve villages in the Jaipur district of Rajasthan. Maximum values of yield (39.17 Q ha^{-1}), R.C. Index (0.15), C.F.U. (38.5×10^6), and lowest root rot incidence (14.03%) were recorded in the Th3-treated groundnut crops (Sharma et al. 2012). *T. harzianum* reduced the seedling

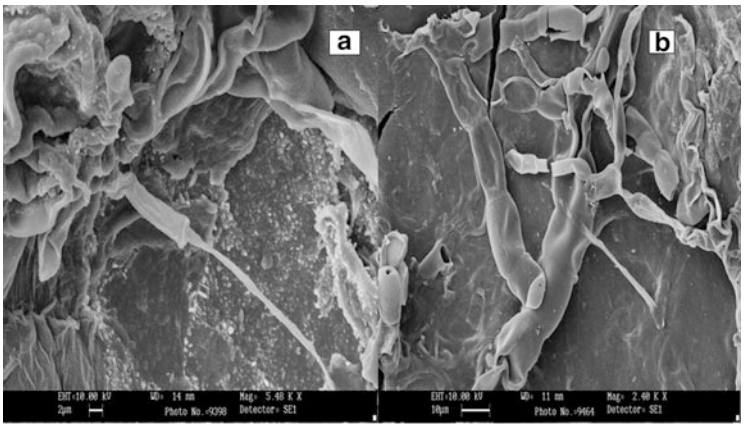


Fig. 15.4 SEM photographs showing deformalities in fungal mycelia during interaction between *Macrophomina phaseolina* and MBCU2 (*Bacillus* spp.). (**a**) Hyphal shriveling and (**b**) hyphal lysis and fragmentation

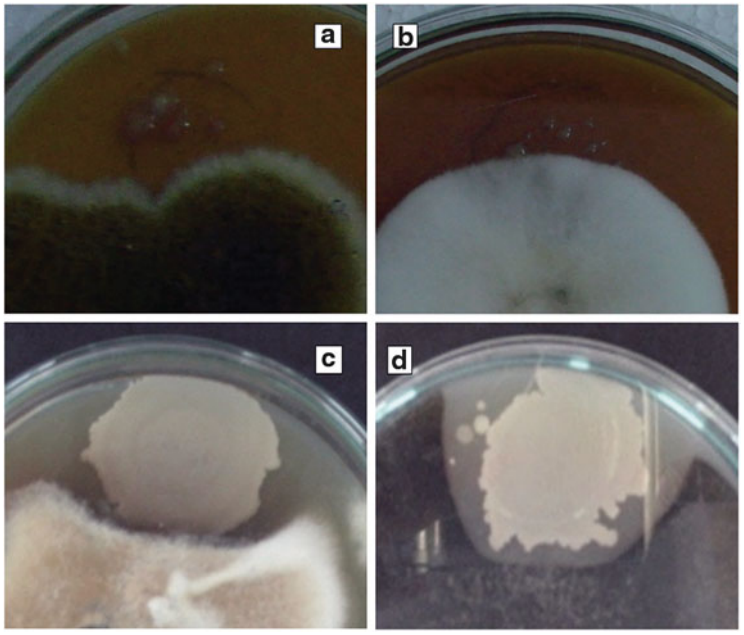


Fig. 15.5 In vitro growth inhibition of fungal phytopathogens like *A. nidulance* (**a**), *A. versicolor* (**c**), *F. oxysporum* (**b**), *M. phaseolina* (**d**) by PGPR strains

mortality of groundnut due to collar rot, and soil drenching was more effective than seed treatment (Kulkarni et al. 1995). Groundnut seed rot and collar rot were reduced when *Trichoderma* isolates were incorporated into the soil under

greenhouse experiments (Prabha and Urs 1998). The *Trichoderma* spores remained viable up to nine weeks of storage at room temperature when groundnut seed coated and reduced the stem rot incidence (Biswas et al. 2000).

Rhizobacteria-induced systemic resistance (ISR) is a type of systemically enhanced resistance against a broad spectrum of pathogens that is triggered upon root colonization by selected strains of non-pathogenic bacteria. Pathogenesis-related proteins are generally used as ISR markers (Heil and Bostock 2002), but increased activities and accumulation of these proteins depend mainly on the inducing agent as well as plant genotype, physiological condition, and pathogen (Madhaiyan et al. 2006). PGPR strains like *S. marcescens* GPS5 and *P. aeruginosa* GSE18 reduced late leaf spot disease of peanut by defense-related enzymes like chitinase, β-1,3-glucanase, PO (peroxidase), and PAL (phenylalanine ammonia-lyase) (Kishore et al. 2006). Madhaiyan et al. (2006) observed ISR activity in peanut against rot pathogens inoculated in leaves by methylotrophic bacteria that showed significant higher activities of PAL, β-1,3-glucanase, and PO when treated with *A. niger* or *S. rolfsii*. Zhang et al. (2001) examined the induction in peanut of systemic resistance to late leaf spot disease (caused by *Cercosporidium personatum*) by PGPR like *Bacillus* and *Paenibacillus* that elicited ISR in other plants and by the addition of chemical elicitors. *Bacillus* sp. CHEP5 and *Pseudomonas* sp. BREN6 induce ISR against root and stem wilt caused by *S. rolfsii* disease by activation of β-1,3-glucanase, PAL, and PO (Tonelli et al. 2011).

Recently, Anil and Podile (2012) reported the chlorothalonil-resistant chitinolytic *B. thuringiensis* which genetically engineered to secrete the harpinPss (heat stable, glycine, and leucine-rich with no cysteine protein with its resistance to denaturation by heat), and this was studied to promote the growth of groundnut. Results showed that seed treated with Bt-pss (chlorothalonil-tolerant *B. thuringiensis* SFC24 from soil and genetically engineered Bt-pss for extracellular secretion of harpinPss) suggested the additive effect of harpinPss on the growth and vigor possibly due to better health of groundnut plants. Moreover, modification of chlorothalonil-tolerant strains for dual benefit of growth promotion and disease control is relatively a new strategy that could make biocontrol as the major component of IDM.

15.5 Conclusion

Diseases pose a major threat to the production of peanuts each year, and prevention of disease in peanut is a major concern for producers. It is evident from the literature that there is great potential for integration of disease control measures on groundnut. A cost-effective and highly efficient IDM program should include components such as use of biocontrol agents must be considered for these components to be highly successful. Biological control is an emerging technology to control insect pests, diseases, and weeds. Genetic engineering will play a vital role in production of transgenic biocontrol agents having biocontrol potential and

ecological acceptability. Impact of the environmental conditions on the expression of biocontrol potentialities needs to be addressed. Much emphasis needs to be given to the interdisciplinary approach in groundnut IDM programs involving field scouting for insect pests, plant diseases, nematodes, and weeds. IDM needs to be strengthened on a large scale for economically important pests of groundnut.

Acknowledgement We gratefully acknowledge financial support by Department of Science and Technology (DST), New Delhi, under Women Scientist Scheme (WOS-A).

References

Ahmed S, Zaman N (2012) Evaluation of groundnut germplasm against root rot disease in agro-ecological conditions of district Chakwal, Punjab-Pakistan. Afr J Microbiol Res 6:5862–5865

Akgul DS, Ozgonen H, Erkilic A (2011) The effects of seed treatments with fungicides on stem rot caused by *Sclerotium rolfsii* sacc in peanut. Pak J Bot 43:2991–2996

Alfano G, Lewis Ivey ML, Cakir C, Bos JIB, Miller SA, Madden LV, Kamoun S, Hoitink HAJ (2007) Systemic modulation of gene expression in tomato by *Trichoderma hamatum* 382. Phytopathology 97:429–437

Al-Khaliel AS (2010) Effects of arbuscular mycorrhization in sterile and non-sterile soils. Trop Life Sci Res 21:55–70

Al-Tawaha AR, McNeil DL, Yadav SS, Turk M, Ajlouni M, Abu-Darwish MS, Al-Ghzawi ALA, Al-udatt M, Aladaileh S (2010) Integrated legume crops production and management technology. In: Yadav SS, Redden R (eds) Climate change and management of cool season grain legume crops. Springer, Dordrecht, pp 325–349

Ambang Z, Ndongo B, Essono G, Ngoh JP, Kosma P, Chewachong GM, Asanga A (2011) Control of leaf spot disease caused by *Cercospora* sp on groundnut (*Arachis hypogaea*) using methanolic extracts of yellow oleander (*Thevetia peruviana*) seeds. Aust J Crop Sci 5:227–232

Anandham R, Sridar R, Nalayini P, Poonguzhali S, Madhaiyan M, Sa T (2007) Potential for plant growth promotion in groundnut (*Arachis hypogaea* L.) cv. ALR-2 by co-inoculation of sulfur-oxidizing bacteria and *Rhizobium*. Microbiol Res 162:139–153

Anil K, Podile AR (2012) HarpinPss-mediated enhancement in growth and biological control of late leaf spot in groundnut by a chlorothalonil-tolerant *Bacillus thuringiensis* SFC24. Microbiol Res 167:194–198

Arora NK, Kang SC, Maheshwari DK (2001) Isolation of siderophore producing strains of *R. meliloti* and their biocontrol potential against *M. phaseolina* that causes charcoal rot of groundnut. Curr Sci 81:673–677

Arsule CS, Pande BN (2012) Aerobiological and epidemiological approach to some groundnut diseases. Asian J Exp Biol Sci 3:118–123

Awal MA, Ikeda T (2002) Effects of changes in soil temperature on seedling emergence and phenological development in field-grown stands of peanut (*Arachis hypogaea*). Environ Exp Bot 47:101–113

Azpilicueta CE, Myriam S, Zawoznik MS, Tomaro ML (2004) Phytoalexins synthesis is enhanced in groundnut plants inoculated with Bradyrhizobium sp. (Arachis). Crop Prot 23:1069–1074

Basha ST, Radhaiah A, Devamma MN, Reddy NPE (2012) Biocontrol potential of indigenous *Pseudomonas* spp against *Sclerotium rolfsii* causing stem rot of groundnut. Int J Food Agric Vet Sci 2:134–141

Bdliya BS, Alkali G (2010) Cost-benefit of using some plant extracts in controlling Cercospora leaf spot of groundnut in the Sudan savanna of Nigeria. Arch Phytopathol Plant Prot 43:95–104

Bergero R, Harrier LA, Franken P (2003) Reporter genes: applications to the study of arbuscular mycorrhizal (AM) fungi and their symbiotic interactions with plant roots. Plant Soil 255:143–155

Bertrand PF, Padgett GB (1997) Fungicide resistance management. Univ GA Coop Ext Serv College of Agric Environ Sci Bull 1132

Bhatia S, Maheshwari DK, Dubey RC, Arora DS, Bajpai VK, Kang SC (2008) Beneficial effects of fluorescent pseudomonads on seed germination, growth promotion, and suppression of charcoal rot in groundnut (*Arachis hypogaea* L). J Microbiol Biotechnol 18:1578–1583

Biswas KK, Duncan GH, Welsely SV, Naidu RA, Mayo MA (2000) Virus like particles assemble in plants and bacteria expressing the coat protein gene of peanut clump virus. J Gen Virol 81:267–272

Bloemberg GV, Lugtenberg BJJ (2001) Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Curr Opin Plant Biol 4:343–350

Boogerd FC, van Rossum D (1997) Nodulation of groundnut by *Bradyrhizobium*: a simple infection process by crack entry. FEMS Microbiol Rev 21:5–27

Charaya M, Mehrotra RS (2004) Integrated plant disease management: recent approaches. In: Tilak KVBR, Mukadam DS, Deshpande J, Mayee CD (eds) Biotechnological approaches for the integrated management of crop diseases. Daya Publishing House, Delhi, pp 74–87

Chauhan MP, Singh IS (2000) Variability estimates and identifying chick pea genotypes for yield and yield attributes in salt affected soil. Legumes Res 23:199–200

Chennakrishnan P (2012) Groundnuts oiling the economy. Market Surv 8–12

Chohan JS (1973) Recent advances in diseases of groundnut. In: Rayachaudhary SP, Verma JP (eds) Plant pathology. SN Das Gupta Birthday celebration committee, Lucknow, pp 171–184

Chohan JS (1978) Diseases of oilseed crops, future plans and strategy for control under smallholdings. Indian Phytopathol 31:1–15

Compant S, Duffy B, Nowak J, Clement C, Barka EA (2005) Use of plant growth promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action and future prospects. Appl Environ Microbiol 71:4951–4959

Culbreath AK, Stevenson KL, Brenneman TB (2001) Management of late leaf spot of peanut with benomyl and chlorothalonil: a study in preserving fungicide utility. Plant Dis 86:349–355

D'aes J, Maeyer KD, Pauwelyn E, Höfte M (2010) Biosurfactants in plant *Pseudomonas* interactions and their importance to biocontrol. Environ Microbiol Rep 2:539–572

Desai S, Grover M, Amalraj EL, Kumar GP, Ahmed SKM (2012) Exploiting plant growth promoting rhizomicroorganisms for enhanced crop productivity. In: Satyanarayana T, Johri BN, Prakash A (eds) Microorganisms in sustainable agriculture and biotechnology. Springer, New York, pp 227–241

Desire TV, Liliane T, Le Prince NM, Jonas PI, Akola A (2010) Mineral nutrient status, some quality and morphological characteristics changes in peanut (*Arachis hypogaea* L.) cultivars under salt stress. Afr J Environ Sci Technol 4:471–479

Devi MC, Reddy MN (2001) Growth response of groundnut to VAM fungus and *Rhizobium* inoculation. Plant Pathol Bull 10:71–78

Doley K, Jite PK (2012) Response of groundnut ('JL-24') cultivar to mycorrhiza inoculation and phosphorous application. Not Sci Biol 4:118–125

Dubey RC, Maheshwari DK (2011) Role of PGPR in integrated nutrient management of oil seed crops. In: Maheshwari DK (ed) Bacteria in agrobiology: plant nutrient management. Springer, Berlin, pp 1–15

Eisenback JD, Bernard EC, Starr JL, Lee TA, Tomaszewski EK (2003) *Meloidogyne haplanaria* n. sp. (Nematoda: *Meloidogynidae*), a root-knot nematode parasitizing peanut in Texas. J Nematol 35:395–403

El Khuoury W, Makkouk K (2010) Integrated plant disease management in developing countries. J Plant Pathol 92:35–42

FAO (Food and Agricultural Organisation) (2000) http://www.fao.org

FAO (Food and Agricultural Organization of the United Nations) (2007) Food outlook: global market analysis. http://www.fao.org/docrep/010/ah864e/ah864e07.htm
Food and Agriculture Organization of the United Nations (2010) FAOSTAT http://faostat.fao.org/site/567
Gajera HP, Vakharia DN (2012) Production of lytic enzymes by *Trichoderma* isolated during in vitro antagonism with *Aspergillus niger*, the causal agent of collar rot of peanut. Braz J Microbiol 43–52
Gajera HP, Patel SV, Kanani JK, Golakiya BA (2009) Alterations in metabolites during germination and seedling growth of groundnut (*Arachis hypogaea* L.) genotypes in response to chloride based salt stress. Int J Plant Sci 4:586–592
Gajula SS, Chelasani VK, Panda AK, Mantena VLNR, Savaram RR (2011) Effect of supplemental inorganic Zn and Mn and their interactions on the performance of broiler chicken, mineral bioavailability and immune response. Biol Trace Elem Res 139:177–187
Ganesan P, Gnanamanickam SS (1987) Biological control of *Sclerotium rolfsii* Sacc. in peanut by inoculation with *Pseudomonas fluorescens*. Soil Biol Biochem 19:35–38
Ganesan S, Kuppusamy RG, Sekar R (2007) Integrated management of stem rot disease (*Sclerotium rolfsii*) of Groundnut (*Arachis hypogaea* L.) using *Rhizobium* and *Trichoderma harzianum* (ITCC - 4572). Turk J Agric For 31:103–108
Ghanekar AM (1980) Groundnut virus research at ICRISAT. In: Proceedings of international workshop on groundnuts, Patancheru, AP, India
Ghewande MP (1983) Effect of cultural practices on the disease (bud necrosis, collar rot, stem rot) incidence and yield of groundnut. Indian Bot Rep 2:176–177
Ghewande MP (1985) Seed and seedling diseases of groundnut deserve attention for better productivity. Seeds Farms 11:9–12
Ghewande MP (1990) Diseases of groundnut and their management. J Oil Seeds Res 7:79–97
Ghewande MP, Nandangopal V (1997) Integrated pest management in groundnut (*Arachis hypogaea* L.) in India. Int Pest Manage Rev 2:1–15
Ghewande MP, Nandgopal V (2009) Integrated pest management in groundnut (*Arachis hypogaea* L.). In: Upadhyay RK, Mukerji HG, Chamola BP, Dubey OP (eds) Integrated pest and disease management. APH Publishing Corporation, New Delhi, pp 275–302
Ghewande MP, Desai S, Basu MS (2002) Diagnosis and management of major diseases of Groundnut. In: NRCG Technical Bulletin, pp 3–12
Gopal K, Muniyappaa V, Jagadeeshwar R (2010) Management of peanut bud necrosis disease in groundnut through intercropping with cereal and pulse crops. Arch Phytopathol Plant Prot 43:883–891
Gopal K, Muniyappaa V, Jagadeeshwar R (2011) Management of peanut bud necrosis disease in groundnut by botanical pesticides. Arch Phytopathol Plant Prot 44:1233–1237
Graham PH, Vance CP (2003) Legumes: importance and constraints to greater use. Plant Physiol 131:872–877
Gross H, Loper JE (2009) Genomics of secondary metabolite production by *Pseudomonas* spp. Nat Prod Rep 26:1408–1446
Gupta UP, Shukla LK (2011) Groundnut necrosis disease caused by a strain of groundnut necrosis virus. Indian J Res 5:46–50
Gupta CP, Kumar CP, Dubey RC, Maheshwari DK (2006) Chitinase-mediated destructive antagonistic potential of *Pseudomonas aeruginosa* GRC1 against *Sclerotinia sclerotiorum* causing stem rot of peanut. BioControl 51:821–835
Haas D, Defago G (2005) Biological control of soil borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3:307–319
Hamidou F, Halilou O, Vadez V (2012) Assessment of groundnut under combined heat and drought stress. J Agro Crop Sci. doi:10.1111/j.1439-037X.2012.00518.x
Heil M, Bostock R (2002) Induced systemic resistance (ISR) against pathogens in the context of induced plant defenses. Ann Bot 89:503–512

Heydari A, Pessarakli M (2010) A review on biological control of fungal plant pathogens using microbial antagonists. J Biol Sci 10:273–290

Hong NX, Mehan VK, Lieu NV, Yen NT (1999) Identification of groundnut genotypes resistant to bacterial wilt in Vietnam. Int J Pest Manage 45:239–243

Houghton JT, Ding Y, Griggs DJ, Noguer M, Linden PJ, Maskell K, Johnson CA (2001) Climate change 2001: the scientific basis (Contribution of working group to the third assessment report to the Intergovernmental Panel on Climate Change (IPCC)). Cambridge University Press, Cambridge

Ihejirika GO, Nwufo MI, Obiefuna JC, Ibeawuchi II (2010) Evaluation of some fungal diseases and yield of groundnut in groundnut based cropping systems. Arch Phytopathol Plant Prot 43:1044–1049

Jaks AJ, Smith DH, Davis RE, Dolton BD (1985) Effects of *Bacillus subtilis* on seedling emergence and pod yield on Spanish market type cultivars and 'Florunner'. Proc Am Peanut Res Educ Soc 17:45 (Abstr)

Jha CK, Patel B, Saraf M (2012) Stimulation of the growth of *Jatropha curcas* by the plant growth promoting rhizobacterium *E. cancerogenus* MSA2. World J Micorbiol Biotechnol 28:891–899

Kakani VG, Prasad PVV, Craufurd PQ, Wheeler TR (2002) Response of in vitro pollen germination and pollen tube growth of groundnut (*Arachis hypogaea* L.) genotypes to temperature. Plant Cell Environ 25:1651–1661

Karthikeya A (1996) Effect of organic amendments, antagonist *Trichoderma viridae* and fungicides on seed and collar rot of groundnut. Plant Dis Res 11:72–74

Ketring DL (1984) Temperature effects on vegetative and reproductive development of peanut. Crop Sci 24:877–882

Khan A, Bakht J, Bano A, Malik NJ (2011) Effect of plant growth regulators and drought stress on groundnut (*Arachis hypogaea* L) genotypes. Pak J Bot 43:2397–2402

Khedikar YP, Gowda MVC, Sarvamangala C, Patgar KV, Upadhyaya HD, Varshney RK (2010) A QTL study on late leaf spot and rust revealed one major QTL for molecular breeding for rust resistance in groundnut (*Arachis hypogaea* L.). Theor Appl Genet 121:971–984

Kiriro FH (1993) Groundnuts in western Kenya Region: a status report presented to ICRISAT Regional Office. Nairobi

Kiriro FH, Rachier GO (1989) Groundnut (*Arachis hypogaea* L) production in western Kenya (Status report). ICRISAT Regional Office, Nairobi

Kishore GK, Pande S, Podile AR (2005a) Phylloplane bacteria increase seedling emergence, growth and yield of field-grown groundnut (*Arachis hypogaea* L.). Lett Appl Microbiol 40:260–268

Kishore GK, Pande S, Podile AR (2005b) Chitin-supplemented foliar application of *Serratia marcescens* GPS 5 improves control of late leaf spot disease of groundnut by activating defense-related enzymes. J Phytopathol 153:169–173

Kishore GK, Pande S, Podile AR (2006) *Pseudomonas aeruginosa* GSE 18 inhibits the cell wall degrading enzymes of *Aspergillus niger* and activates defense-related enzymes of groundnut in control of collar rot disease. Aust Plant Pathol 35:259–263

Kokalis-Burelle N, Backman PA, Rodriguez-Kabana R, Ploper LD (1992) Potential for biological control of early leafspot of peanut using *Bacillus cereus* and chitin as foliar amendments. Biol Control 2:321–328

Kulkarni SA, Srikant K, Kulakrni S (1995) Effect of organic amendments and green manuring on the survival of collar rot fungus of groundnut. Curr Res Univ Agric Sci 24:135–136

Kumar BSD, Podile AR, Dube HC (1988) Antagonistic activity of *Bacillus subtilis* towards *Rhizopus nigricans*. J Biocontrol 2:4–8

Le CN, Kruijit M, Raaijmakers JM (2011) Involvement of phenazines and lipopeptides in interactions between *Pseudomonas* species and *Sclerotium rolfsii*, causal agent of stem rot disease on groundnut. J Appl Microbiol 112:390–403

Limon MC, Pintor-Toro JA, Benitez T (1999) Increased antifungal activity of *Trichoderma harzianum* transformants that overexpress a 33-kDa chitinase. Phytopathology 89:254–261

Madhaiyan M, Suresh Reddy BV, Anandham R, Senthilkumar M, Poonguzhali S, Sundaram SP, Tongmin S (2006) Plant growth promoting *Methylobacterium* induces defense responses in groundnut (*Arachis hypogaea* L.) compared with rot pathogens. Curr Microbiol 53:270–276

Madhaiyan M, Poonguzhali S, Lee J, Saravanan VS, Lee K, Santhanakrishnan P (2010) *Enterobacter arachidis* sp. nov., a plant-growth-promoting diazotrophic bacterium isolated from rhizosphere soil of groundnut. Int J Syst Evol Microbiol 60:1559–1564

Malviya J, Singh K, Joshi V (2011) Effect of phosphate solubilizing fungi on growth and nutrient uptake of ground nut (*Arachis hypogaea*) Plants. Adv Biores 2:110–113

Manjula K, Podile AR (2001) Chitin-supplemented formulations improve biocontrol and plant growth promoting efficiency of *Bacillus subtilis* AF 1. Can J Microbiol 47:618–625

Manjula K, Kishore GK, Podile AR (2004) Whole cells of *Bacillus subtilis* AF 1 proved more effective than cell-free and chitinase-based formulations in biological control of citrus fruit rot and groundnut rust. Can J Microbiol 50:737–744

Masunaka A, Hyakumachi M, Takenaka S (2011) Plant growth promoting fungus, *T. koningii* suppresses isoflavonoid phytoalexin vestitol production for colonization on/in the roots of *Lotus japonicus*. Microb Environ 26:128–134

Meena B, Marimuthu T, Velazhahan R (2006) Role of fluorescent pseudomonads in plant growth promotion and biological control of late leaf spot of groundnut. Acta Phytopathol Entomol Hung 41:203–212

Mehan VK, Reddy PM, Subrahmanyam P, Mc Donald D, Singh AK (1996) Identification of new sources of resistance to rust and late leafspot in peanut. Int J Pest Manage 42:267–271

Mensah JK, Akomeah PA, Ikhajiagbe B, Ekpekurede EO (2006) Effects of salinity on germination, growth and yield of five groundnut genotypes. Afr J Biotechnol 5:1973–1979

Muhammad AS, Bdliya BS (2011) Effects of variety and fungicidal rate on *Cercospora* leaf spots disease of groundnut in the Sudan Savanna. Niger J Basic Appl Sci 19:135–141

Naab JB, Tsigbey FK, Prasad PVV, Boote KJ, Bailey JE, Brandenburg RL (2005) Effects of sowing date and fungicide application on yield of early and late maturing peanut cultivars grown under rainfed conditions in Ghana. Crop Prot 24:325–332

Obagwu J (2001) Evaluation of fungicides for control of brown blotch of Bambara Groundnut (*Vigna Subterranea* (l.) Verdc) Caused by *Colletotrichum Capsici* (syd) Butler and Bisby in Nigeria. Arch Phytopath Pflanz 34:353–360

Pal KK, Dey R, Bhatt DM, Chauhan SM (2000) Plant growth promoting fluorescent pseudomonads enhanced peanut growth, yield and nutrient uptake. In: Proceedings of the fifth international PGPR workshop, Cordoba, Argentina

Pande S, Narayana Rao J (2000) Changing scenario of groundnut diseases in Andhra Pradesh, Karnataka and Tamil Nadu states of India. Int Arachis Newslet Ter 20:42–44

Pande S, Narayana Rao J, Upadhyaya HD, Lennxe JM (2001) Farmers' participatory integrated management of foliar diseases of groundnut. Int J Pest Manage 47:121–126

Pandya U, Saraf M (2010) Application of fungi as a biocontrol agent and their biofertilizer potential in agriculture. J Adv Dev Res 1:90–99

Patil BN, Bhonde SR, Khandikar DN (2009) Trends in area, production and productivity of groundnut in Maharashtra. Finan Agric 41:118–123

Prabha KS, Urs SD (1998) Efficiency of bioagents for management of collar rot of groundnut caused by *Aspergillus niger*. Curr Res Univ Agric Sci 27:114–115

Prasad PVV, Craufurd PQ, Summerfield RJ (1999a) Sensitivity of peanut to timing of heat stress during reproductive development. Crop Sci 39:1352–1357

Prasad PVV, Craufurd PQ, Summerfield RJ (1999b) Fruit number in relation to pollen production and viability in groundnut exposed to short episodes of heat stress. Ann Bot 84:381–386

Prasad PVV, Boote KJ, Thomas JMG, Allen LH Jr, Gorbet DW (2006) Influence of soil temperature on seedling emergence and early growth of peanut cultivars in field conditions. J Agron Crop Sci 192:168–177

Prashanth S, Mathivanan N (2010) Growth promotion of groundnut by IAA producing rhizobacteria *Bacillus licheniformis* MML2501. Arch Phytopathol Pflanz 43:191–208

Raaijmakers JM, Vlami M, de Souza JT (2002) Antibiotic production by bacterial biocontrol agents. Antonie Van Leeuwenhoek 81:537–547

Raaijmakers JM, Paulitz T, Steinberg C, Alabouvette C, Moënne-Loccoz Y (2009) The rhizosphere: a playground and battlefield for soil borne pathogens and beneficial microorganisms. Plant Soil 321:341–361

Raaijmakers JM, de Bruijn I, Nybroe O, Ongena M (2010) Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. FEMS Microbiol Rev 34:1037–1062

Rakshit A, Bhadoria PBS (2008) Indigenous arbuscular mycorrhiza is more important for early growth period of groundnut (*Arachis hypogeal L.*) for P influx in an oxisol. Acta Agric Slov 91:397–406

Ramesh P, Hegde DM (2010) Directorate of oil seed research. News Lett 16:1–8

Rao PSK, Tilak KVBR, Arunachalam V (1990) Genetic variation for VA mycorrhiza dependent phosphate mobilization in groundnut (*Arachis hypogaea* L.). Plant Soil 122:137–142

Rasheed S, Dawar S, Ghaffar A, Shaukat SS (2004) Seed borne mycoflora of groundnut. Pak J Bot 36:199–202

Reddy DVR, Wightman JA (1988) Tomato spotted wilt virus: thrips transmission in disease vector research. Springer, New York, pp 203–219

Rivlin RS (2001) Historical perspective on the use of garlic. J Nutr 131:951–954

Rodrigues-Kabana R (1984) Root knot nematodes. In: Porter DM, Smith DH, Rodrigues R (eds) Compendium of peanut diseases. American Phytopathological Society, St. Paul, MN, pp 38–41

Sathiyanarayanamurthy GV, Ramasubbiah K, Rama Subba Rao K, Krishnamurthy MM (1988) Efficacy of certain synthetic pyrithroids and in combination against thrips and leaf miner of groundnut. J Agric Res Andhra Pradesh Agric Univ 16:16–19

Saraf M, Jha CK, Patel D (2010) The role of ACC deaminase producing PGPR in sustainable agriculture. In: Maheshwari DK (ed) Plant growth and health promoting bacteria. Springer, Berlin, pp 365–385

Sayyed RZ, Gangurde NS, Patel PR, Joshi SA, Chincholkar SB (2010) Siderophore production by *Alcaligenes faecalis* and its application for growth promotion in *Arachis hypogaea*. Indian J Biotechnol 9:302–307

Senthilraja G, Anand T, Durairaj C, Raguchander T, Samiyappan R (2010) Chitin based bioformulation of *Beauveria bassiana* and *Pseudomonas fluorescens* for improved control of leafminer and collar rot in groundnut. Crop Prot 29:1003–1010

Sharad P, Nail GR (2011) Physiological and biochemical responses of groundnut genotypes to drought stress. World J Sci Technol 1:60–66

Sharma P, Saini MK, Deep S, Kumar V (2012) Biological control of groundnut root rot in farmer's field. J Agric Sci 4:48–59

Shinde BM, Laware SL (2010) Effect of drought stress on agronomic and yield contributing characters in Groundnut (*Arachis Hypogaea* L.). Asian J Exp Biol Sci 1:968–971

Shternshis MV, Beljaev AA, Shpatova TV, Bokova JV, Duzhak AB (2002) Field testing of BACTICIDE®, PHYTOVERM® and CHITINASE for control of the raspberry midge blight in Siberia. Biocontrol 47:697–706

Silby MW, Winstanley C, Godfrey SAC, Levy SB, Jackson RW (2011) *Pseudomonas* genomes: diverse and adaptable. FEMS Microbiol Rev 35:652–680

Singh B, Mathur SC (1953) Sclerotial root-rot disease of groundnut in Uttar Pradesh. Curr Sci 22:214–215

Somers E, Vanderleyden J, Srinivasan M (2004) Rhizosphere bacterial signalling: a love parade beneath our feet. Crit Rev Microbiol 30:205–240

Stalker HT, Simpson CE (1995) Genetic resources in *Arachis*. In: Stalker HT (ed) Advances in peanut science. American Peanut Research Education Society, Stillwater, OK, pp 14–53

Subrahmaniyam P, Mc Donald D (1983) Rust diseases of groundnut. Information Bulletin No.13. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, AP

Subrahmanyam P, Mehan VK, Neville DJ, McDonald D (1980) Research on fungal diseases of groundnut at ICRISAT. In: Proceedings of international workshop on groundnut, Patancheru, AP, India, 13–18 Oct 1980, pp 193–198

Subrahmanyam P, Van Wyk PS, Kisyombe CT, Cole DL, Hildebrand GL, Chiyembekeza AJ, Van Der Merwe PJA (1997) Diseases of groundnut in the Southern African Development Community (SADC) region and their management. Int J Pest Manage 43:261–273

Taffouo VD, Wamba FO, Youmbi E, Nono GN, Amougou A (2010) Growth, yield, water status and ionic distribution response of three bambara groundnut (*Vigna subterranea* (L.) Verdc.) landraces grown under saline conditions. Int J Bot 6:53–58

Taurian T, Anzuay MS, Angelini J, Tonelli ML, Luduenã L, Pena D, Ibáněz F (2010) Phosphate-solubilizing peanut associated bacteria: screening for plant growth-promoting activities. Plant Soil 329:421–431

Tilak KVBR (1993) Associative effects of vesicular arbuscular mycorrhiza with nitrogen fixers. Proc Indian Natl Sci Acad 1359:325–332

Tonelli ML, Furlan A, Taurain T, Castro S, Fabra A (2011) Peanut priming induced by biocontrol agents. Physiol Mol Plant Pathol 75:100–105

Turner JT, Backman PA (1991) Factors relating to peanut yield increases after seed treatment with *Bacillus subtilis*. Plant Dis 75:347–353

Van Damme EJM, Willems P, Torrekens S, Van Leuven F, Peumans WJ (1993) Garlic (*Allium sativum*) chitinase: characterization and molecular cloning. Plant Physiol 87:177–186

Varshney RK, Hoisington DA, Upadhyaya HD, Gaur PM, Nigam SN, Saxena K, Vadez V, Sethy NK, Bhatia S, Aruna R, Gowada MVC (2007) Molecular genetics and breeding of grain legume crops for the semi arid tropics. In: Varshney RK, Tuberosa R (eds) Genomics assisted crop improvement, vol 2, Genomics application in crops. Springer, New York, pp 207–241

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586

Wheeler TR, Chatzialioglou A, Craufurd PQ, Ellis RH, Summerfield RJ (1997) Dry matter partitioning in peanut exposed to high temperature stress. Crop Sci 37:1507–1513

Wightman JA, Rao GVR (1993) A groundnut insect identification handbook for India. ICROST Information Bulletin No. 39, Patancheru, pp 1–56

Zhang Z, Yuen GY (2000) The role of chitinase production by *Stenotrophomonas maltophilia* strain C3 in biological control of *Bipolaris sorokiniana*. Phytopathology 90:384–389

Zhang S, Reddy M, Kokalis-burelle N, Wells L, Nightengale S, Kloepper J (2001) Lack of induced systemic resistance in peanut to late leaf spot disease by plant growth-promoting rhizobacteria and chemical elicitors. Plant Dis 85:879–884

Chapter 16
The Effects of Volatile Metabolites from Rhizobacteria on *Arabidopsis thaliana*

Katrin Wenke and Birgit Piechulla

16.1 Introduction

In the eukaryotic world the release and detection of volatile substances, often referred to as odorous compounds, is a well-known and effective way to send messages and to gain information. Just think of the wonderful, classic fragrances released by blossoms to attract bees from a distance. People are also strongly attracted—or repulsed—by many odors or scents of blossoms. The food and perfume industries take advantage of the human (olfactory) sense of smell, for example, the aroma of cheese or wine or the scent of a favorite deodorant. The great advantage of such substances, called volatiles by scientists, is the very long distance over which especially the sessile plants can attract or repulse their interaction partners. Volatiles are characterized by their lipophilicity, a low molecular weight of less than 300 Da, a high vapor pressure above 0.01 kPa (at 20 °C), and low boiling points. These properties cause the compounds to evaporate or vaporize. Most of the volatiles described up to now are aromatic compounds, derivatives of fatty acids, and terpenoids.

Microorganisms, in particular about 350 bacterial species studied to date, are important producers of volatile substances. Many commonly used and well-known aromas and odors such as those of cheese and wine (e.g., Urbach 1997; Schreier 1980) have their origin in the prokaryotic world, also the earthy smell in a forest after a rain shower caused by the geosmin released by actinomycetes (Gerber and Lechevalier 1965). The qualitative and quantitative distribution of individual compounds sometimes extremely complex mixtures of volatiles is mainly determined by the metabolic capabilities and capacities of the bacterial species involved and the availability of nutrients in line with respective growth conditions (Stotzky

K. Wenke • B. Piechulla (✉)
Institute for Biological Sciences, University of Rostock, Albert-Einstein-Straße 3, 18059 Rostock, Germany
e-mail: birgit.piechulla@uni-rostock.de

D.K. Maheshwari et al. (eds.), *Bacteria in Agrobiology: Crop Productivity*,
DOI 10.1007/978-3-642-37241-4_16,

and Schenck 1976; Fiddaman and Rossall 1994). Anywhere from a dozen to nearly 100 compounds are emitted, among others, by *Chondromyces crocatus*, *Carnobacterium divergens* 9P, *Streptomyces* sp. GWS-BW-H5, and *Serratia odorifera* 4Rx13 (Schulz et al. 2004; Ercolini et al. 2009; Dickschat et al. 2005; Kai et al. 2010). At present, nearly 770 bacterial volatiles have been identified as belonging to 48 different classes of compounds (Wenke et al. 2012b).

The quantitatively dominant compounds are alkenes, ketones, and terpenoids with 120–190 compounds each, followed by acids, benzenoids, esters, and pyrazines (60–80 compounds), and 30–40 are representatives of the aldehydes, ether compounds, and lactones. Bacterial species investigated so far, which emits volatile mixtures, is far below the number of microorganisms present on earth. As a result, the analysis and identification of bacterial volatiles continues to be an interesting and multifaceted field of research. A number of techniques have been developed to collect and detect volatile substances (e.g., the open VOC (volatile organic compound) collection system, Kai et al. 2007; the closed-loop-stripping device, Boland et al. 1984; solid-phase microextraction (SPME), Arthur and Pawliszyn 1990; gas chromatography/mass spectrometry (GC/MS); proton transfer reaction/MS (PTR/MS), Mayr et al. 2003, etc.). Each technique has its merits but only reveals part of the volatile spectrum in the form of actual quality and quantity (summarized in Wenke et al. 2012b).

16.2 Volatiles as Infochemicals in Soil

It is interesting and important not only to identify novel volatiles from bacteria, but in fact there is always the question of the potential ecological and/or physiological function of the emitted compounds. For their producers, the release of volatile metabolites means a “loss” of essential carbon, in part as extremely energy-rich compounds. The buzzword “talking tree” (Baldwin et al. 2006) makes clear in an exemplary way the ecophysiological potential of volatile emission. Up to now, the focus of research in this area was and is what happens above the soil surface. This has made it possible to accumulate an extensive knowledge of the effects of airborne signals in the atmosphere (e.g., van Dam et al. 2010; Müller and Hilker 2000; Piechulla and Pott 2003; Zangerl and Berenbaum 2009). For the most part, findings that volatile substances are also produced, released, and detected below the soil surface have been neglected (summarized in Wenke et al. 2010). The zone surrounding the roots, i.e., the rhizosphere (Barber and Martin 1976), is an attractive environment for numerous types of organisms such as microbes, arthropods, nematodes, amebas, and ciliates. This is due to the energy-rich root exudates (Wenke et al. 2010).

The major producers of volatile metabolites are roots of plants. Typical plant volatiles in the soil are 1,8-cineol, γ-terpinene, β-myrcene, α-pinene, β-phellandrene, and β-caryophyllene, of which some are of major ecological significance (summarized in Kai et al. 2009b; Wenke et al. 2010). Rasman and

colleagues (2005) described the emission of β-caryophyllene by the roots of maize (*Zea mays*) induced by feeding damage, which consequently attracted the nematode *Heterorhabditis megidis* to directly ward off the Western corn rootworm *Diabrotica virgifera virgifera*. This example shows that release of volatile chemicals is dependent on the physiological state of the plants, as was demonstrated using carrots (*Daucus carota spp. sativa*) that were undamaged or damaged mechanically or by feeding (Weissteiner and Schütz 2006). In addition to plants, soil fungi and rhizobacteria emit all sorts of volatiles such as fungal alcohols octanol, ethanol, β-phenylethanol, 1-octen-3-ol, and octenal, as well as the bacterial metabolites trimethylamine, cyclohexanol, dimethyl disulfide, 2,3-butanediol, geosmin, ethylene, and 2,5-dimethyl pyrazine, but also simple, inorganic compounds such as carbon dioxide, ammonia, and hydrogen cyanide (summarized in Kai et al. 2009b, 2010; Blom et al. 2011; Bernier et al. 2011). Fungal volatiles play a significant role not only in intraspecies communication, such as attracting mating partners, but also in attraction or repulsion of other fungal or plant species. In the latter case, this led to the use of the term "burned area" referring to the zone surrounding the host plants of truffles, where growth of herbaceous plants is most likely suppressed by the fungal volatiles (Pacioni 1991). With regard to the significance of rhizobacterial volatiles, it is assumed that they play a role in inter- and intraspecies communication or as signals between cells, which serve the disposal of excess carbon compounds, and may even affect the growth of other organisms (plants, bacteria, fungi, nematodes, amebas, ciliates). These effects may be positive as well as negative (Kai et al. 2009a). Around 40 years ago, Stall and colleagues (1972) demonstrated that the ammonia produced by *Xanthomonas vesicatoria* is involved in necrosis of infected pepper. On the other hand, hydrogen cyanide enables pseudomonads to impair root growth of *Lactuca sativa* seedlings (Alström and Burns 1989). The most recent data have demonstrated an ammonia-induced change in antibiotic resistance in gram-negative and gram-positive bacteria (Bernier et al. 2011).

16.3 Dual Culture Tests to Study the Effects of Volatile Compounds

The so-called dual culture system established in recent years to study the effects of bacterial volatiles on other organisms was used in this study because of its simplicity (Wenke et al. 2012a). In a two-chamber Petri dish, the rhizobacteria are separated from their respective interaction partner by a plastic barrier, which only permits exchange of volatile metabolites. Nonspecific binding of gaseous substances is ensured by adding active charcoal, in order to observe significant effects on reduction of growth (Vespermann et al. 2007). The advantages of this system, i.e., dual cultures in partitioned Petri dishes, is the use of synthetic growth media, and reduction to only two interaction partners, serve to minimize any

variables. This ensures a good reproducibility and evaluation of test results, both indispensable for transcriptome analysis. The test system used represents one segment of the spectrum of natural conditions. In-depth studies of volatile-induced inhibition of plants are still in its infancy; hence, a simple system is required to understand the basic relationships.

Initially the test system of Ryu and colleagues (2003) was employed, who described the strong growth-promoting impact of rhizobacterial volatile metabolites on plants. The studies revealed an enlargement of the leaf surface in *Arabidopsis thaliana* in response to volatiles from the species *Bacillus*, *Serratia*, *Pseudomonas*, and *Escherichia*. The single active substance was identified as 2,3-butanediol. Further research has also demonstrated a variety of positive effects of 2,3-butanediol or of other complex mixtures of bacterial volatiles on plants (1) initiation or increase of plant resistance to biotic (*Erwinia carotovora*, *Pseudomonas syringae*) and abiotic stress (salinity, desiccation, or osmotic stress); (2) physiological, metabolic, and morphological changes (cell-wall expansion, more chloroplasts, increased photosynthetic capacity, accumulation of starch and iron, changes in volatile emission, increased production of essential oils, alteration in primary metabolism); and (3) involvement of plant hormones (auxin, abscisic acid, ethylene) (Ryu et al. 2004; Zhang et al. 2007, 2008a, b, 2009, 2010; Cho et al. 2008; Xie et al. 2009; Banchio et al. 2009; Rudrappa et al. 2010; Kwon et al. 2010; Ezquer et al. 2010).

When evaluating the plant growth-promoting effects mentioned, it should be taken into account that most of the data was collected in a closed system. Kai and Piechulla (2009) were able to identify a clear correlation between growth promotion and CO_2 enrichment during cocultivation of plants and bacteria in a closed system. In open dual cultures, there was a possibility of indirect promotion of plant fitness by volatiles emitted due to inhibition of plant pathogenic fungi such as *Rhizoctonia solani* (Kai et al. 2007). *Serratia plymuthica* HRO-C48 and *Stenotrophomonas maltophilia* R3089 turned out to be two of the most effective organisms in the dual culturing system. At the same time, volatile mixtures from *S. plymuthica* HRO-C48 and *S. maltophilia* R3089 showed significant effect on *A. thaliana* seedlings (Vespermann et al. 2007).

Once the dual culturing system was established for in-depth investigations, the bacteria and plants were each applied in two equidistant straight lines. A given number of seedlings and a defined bacterial cell count of 10^7 were chosen at zero point in time. This corresponds to the bacterial concentration of *S. plymuthica* HRO-C48 and *Stenotrophomonas maltophilia* found on 1 g of fresh roots from strawberry or rapeseed plants, respectively, under field conditions (Kurze et al. 2001; Berg et al. 1996). At the beginning of the experiment, 10^7 cells were applied accordingly, whereas when between 10^5 and 10^7 colony-forming units of *S. plymuthica* HRO-C48 were applied in preliminary experiments, there were no clear differences in cotyledon or root length. In general, rhizobacteria reached values of up to 10^8 CFU/g fresh weight of strawberry, potato, and rapeseed roots (Berg et al. 2002). The formation of biofilms on root surfaces with a very high

localized bacterial density also has been described by number of researchers (Bloemberg et al. 2000; Walker et al. 2004).

16.4 Inhibitory Effects of Volatiles from *S. plymuthica* HRO-C48 and *S. maltophilia* R3089 on *A. thaliana*

The observation that *S. plymuthica* HRO-C48 and *S. maltophilia* R3089 inhibited *A. thaliana* seedling growth under dual culture conditions has raised many questions. First and foremost, it is interesting to find out which signaling pathways are involved and whether they are similar to plant responses to pathogens, whether these effects at physiological and molecular levels are dependent on the bacterial species, or whether the toxicity of mixtures of volatiles is nonspecific.

16.4.1 Morphological and Physiological Changes in A. thaliana *Under Dual Culture Conditions*

In response to bacterial volatiles, the wild-type seedlings of *A. thaliana* visibly showed a marked reduction in early vegetative growth and a distinct paling of the leaves. Determination of chlorophyll and carotenoid contents showed an earlier drop in carotenoid content, in comparison to chlorophyll content, in response to both volatile mixtures. Measurement of cotyledon and root lengths confirmed a significant inhibition after 2–3 days of exposure to volatiles *S. plymuthica* HRO-C48 and *S. maltophilia* R3089, whereas in both cases the underground portion of the plant was affected earlier or to a greater degree (Wenke et al. 2012a). This could be caused by more rapid growth of the roots. However, the possibility of qualitative and quantitative differences in the distribution of volatile substances between plant medium and airspace—due to divergent polarities and volatilities of individual components—should also be taken into account. Another source of speculation would be the recognition of effective volatiles via the roots, which has yet to be clarified.

As to growth and leaf pigmentation, the response to *S. maltophilia* volatiles was delayed by about one day. There have been reports that volatile emission is quantitatively dependent on the bacterial growth phase (Bunge et al. 2008; Kai et al. 2010). Bacterial growth in dual cultures was checked, and in both cases the bacteria were growing exponentially after 6 and 12 h and had entered the stationary phase with 10^{11} cells after 24 h (Wenke et al. 2012a). A comparison of the exact number of cells in correlation with the kinetics of morphological effects revealed that the viable cell count of *S. maltophilia* was lower after 6 and 12 h than that of *S. plymuthica*. Experiments with 10^0–10^7 *S. plymuthica* cells revealed that reduction of leaf and root length depends to a certain extent on the number of bacteria

used (Wenke et al. 2012b). The more cells applied initially, the earlier were significant effects detectable, whereas differences were minimal at around 10^5 bacterial cells or more (unpublished data). Since all of the experiments for this report began with 10^7–10^8 cells of *S. plymuthica* HRO-C48 or *S. maltophilia* R3089 (time zero), it can be assumed that slight differences in viable cell counts of both rhizobacterial species during the first few days had no significant effect on inhibition of *A. thaliana*. It is more likely that species-specific differences in the bacterial volatile mixtures (Kai et al. 2007) were responsible for kinetic differences in growth inhibition.

The complete loss of pigmentation by the *A. thaliana* seedlings within a few days led to the assumption that the rhizobacterial volatiles were lethal to the plants. Evans blue, a dye that penetrates dead cells (Kim et al. 2003), was used to recognize the time and location of cell death events in the cotyledons. A pale, randomly distributed blue staining of cotyledon cells was observed after 3 days in response to *S. maltophilia* R3069 and *S. plymuthica* HRO-C48 exposure. After 5 days, dye penetrated the cells to a very high degree (Wenke et al. 2012b). There were no species-specific differences in the effect caused by the bacterial volatiles, namely, the complete death of the seedlings in dual cultures after a continuous 5-day cocultivation of the plants with volatile infochemicals.

In an experiment in which the bacteria were removed from the coculture after different time periods, there was an enormous reduction in growth inhibition when the plants were only exposed to the volatile metabolites for up to 36 h (Wenke et al. 2012b). This implies that signaling pathways leading to a drastic inhibition of growth and to cell death had not been activated within this incubation period.

The type of stress caused by confrontation of plants with bacterial volatiles is probably similar to the effect of plant-pathogen interaction involving direct contact. So the previously reported (a)biotic stress associated with hydrogen peroxide accumulation was investigated by using diaminobenzidine (DAB) staining (Thordal-Christensen et al. 1997). High concentrations of hydrogen peroxide in the cotyledons could be detected after 2 or 3 days in dual cultures with *S. plymuthica* HRO-C48 and *S. maltophilia* R3089, respectively. The one-day delay in the response to *S. maltophilia* R3089 went along with differences in kinetics of growth impairment (Wenke et al. 2012a). This kinetic relationship, the lack of inhibitory effects under coculturing for less than 36 h, and the fact that a high formation of reactive oxygen species under stress conditions has an extremely detrimental effect on cells suggest that impairment of growth and cell death are closely linked to formation of hydrogen peroxide. It should also be taken into consideration that hydrogen peroxide is considered a nonspecific signaling molecule in stress situations. Its formation has been described as one of the initial responses of pathogen defense (Lamp and Dixon 1997) and to many abiotic stimuli as well (Neill et al. 2002; Mittler et al. 2004). Therefore, potentially specific signals can be expected earlier than 2 or 3 days in dual cultures, and later effects (formation of hydrogen peroxide, inhibition and killing of seedlings) can be interpreted as a nonspecific result of volatile exposure. At present it remains speculative to what

extent this hydrogen peroxide formation reflects a similarity to biotic or abiotic forms of stress.

16.4.2 Specific Changes in Gene Expression

Current data show that bacterial volatiles cause death of *A. thaliana* seedlings in dual culture within 5 days. This is accompanied by a marked impairment of leaf and root growth and a systemic accumulation of hydrogen peroxide after 2–3 days. None of these were observed when the seedlings were exposed to volatiles from *S. plymuthica* for a maximum of 1.5 days. It is, therefore, concluded that an exposure time of less than 36 h is especially suitable for analysis of gene expression in order to detect any specific plant responses not solely linked to the dying process.

First indications of transcriptional changes in dual cultures came from established lines of *A. thaliana* in which stress- and pathogen-induced promoter elements control the *uidA* (β-glucuronidase, GUS) gene (Rushton et al. 2002). In the course of these experiments, GUS activity in the cotyledons was detected histochemically using 5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc) and quantified fluorometrically using 4-methylumbelliferyl-β-D-glucuronide (MUG). Two transgenic lines of plants led to the decisive discovery of lines containing (1) an S box and (2) a Gst1 box. The S box is involved in the regulation of gene expression in response to fungal elicitors in parsley (*Petroselinum crispum*) (Kirsch et al. 2001) and apparently plays a special role in nonhost interaction with pathogens (Rushton et al. 2002). The S box is apparently regulated by APETELA 2/ethylene-responsive element-binding ERF (AP2/ERF) transcription factors (Rushton et al. 2002). In dual cultures with *S. plymuthica* HRO-C48 as well as with *S. maltophilia* R3089, the S box exhibits a volatile-dependent induction within 18 h, as determined histochemically and spectrophotometrically (Wenke et al. 2012b).

During the same time period, the specific activation of the Gst1 box in response to both mixtures of volatiles was detected (Wenke et al. 2012a). This element is already well known as the *gst1* gene of the potato (Strittmatter et al. 1996) and contains both S box and W box. So the Gst1 element is involved in responses to pathogens and in senescence (aging) processes. It is also regulated, in addition to the AP2/ERF proteins, by WRKYs. In conclusion, there is a new realization that WRKY- and AP2/ERF-regulated signaling pathways are activated in less than 18 h, without kinetic differences in the responses to *S. plymuthica* HRO-C48 and *S. maltophilia* R3089.

In order to gain more detailed knowledge of underlying signaling processes, early changes in *A. thaliana* wild-type seedlings at the transcriptome level were analyzed after 6, 12, and 24 h by microarray analysis. Responses to volatile mixtures from *S. plymuthica* HRO-C48 and *S. maltophilia* R3089 were analyzed independently by using ATH1 gene chips (Hennig et al. 2003). Many individuals from five dual cultures each were combined to form a biological replicate; this resulted in well reproducible data with duplicates, which were verified by real-time

PCR analysis as biologically independent on the basis of selected marker genes (Wenke et al. 2012a).

In response to volatiles of *S. plymuthica* HRO-C48 and *S. maltophilia* R3089, either 889 or 655 genes, respectively, underwent an at least twofold transcriptional change compared to the control (Wenke et al. 2012a). In both experiments, considerably more genes were repressed (turned off) than were expressed (turned on). On the other hand, the transcriptome changes induced by both bacteria had kinetically opposing effects. Whereas the volatiles of *S. plymuthica* HRO-C48 induced regulatory responses in most genes after 24 h, much of the response to cocultivation with *S. maltophilia* R3089 could be detected within hours. These kinetics are diametrically opposed to later, perhaps indirect, effects. This supports the assumption that early volatile-induced responses of *A. thaliana* are specifically adapted to the various elicitors and also need to be looked at separately from nonspecific effectors.

A direct comparison (Venn diagram) of the list of genes regulated by both treatments revealed that 162 genes were changed by *S. plymuthica* HRO-C48 and *S. maltophilia* R3089 volatiles at the expression level throughout the whole experiment (Wenke et al. 2012a). The remaining 727 or 493 genes were regulated specifically by volatiles of *S. plymuthica* HRO-C48 or *S. maltophilia* R3089, respectively. It can be assumed that the 162 genes independent of the bacterial species contain, among others, signaling elements responsible for growth inhibition of the plants at a later time. Transcription factor activity is located on 21 of these 162 genes, including three APETELA-2 proteins, six MYB factors, and WRKY18. It would be interesting to hypothesize whether members of these three protein families directly interact with each other in response to rhizobacterial volatiles.

MapMan (Thimm et al. 2004) is a good software tool for summary visualizations of functions of regulated genes. Both data sets of *S. plymuthica* HRO-C48 and *S. maltophilia* R3089 volatile-specific regulated genes were presented in an "Overview of biological stress responses" (Wenke et al. 2012a). A strong involvement of typical responses to pathogens can easily be recognized in dual cultures with *S. plymuthica* HRO-C48. Receptors involved include At5g45070 and At1g65390, which are essential for pathogen defense and the immune system of plants (e.g., Meyers et al. 2002). Also involved are peroxidases, glutathione-S-transferases, and enzymes of secondary metabolism and hormonal signaling pathways (salicylic acid, SA; abscisic acid, ABA; auxin; ethylene), as well as pathogenesis-related (PR) genes, including seven members of the Toll/interleukin1 receptor/nucleotide binding site/leucine-rich repeat (TIR-NBS-LRR) class of proteins, which are essential for pathogen recognition (Dangl and Jones 2001). Considerably, fewer pathogen-response-associated genes were regulated in response to *S. maltophilia* R3089 volatiles, although four proteins of the TIR–NBS–LRR class were involved. Other common features of the specific response to both volatiles were changes in proteolytic processes and cell wall metabolism as possible mechanical defense mechanisms. In addition, several representatives of the family of pathogen defense-associated transcription factors seemed to play a bacterial-nonspecific role in mediating volatile-induced responses: ERF, basic leucine zipper (bZIP),

MYB, and WRKY. Once again, the transcriptional changes of the cell wall stood out in the MapMan visualization of the "metabolic overview" (Wenke et al. 2012a). At the same time, the samples treated with *S. maltophilia* R3089 revealed an inactivation of genes coding for components of the mitochondrial electron transport chain and the photosystems. In part, these may be the cause of the one-day delay of hydrogen peroxide accumulation in response to *S. maltophilia* R3089 volatiles in comparison to the response induced by *S. plymuthica* HRO-C48.

In order to verify a correlation with certain functional activities, 727 or 493 of the volatile-specific genes and the 162 jointly regulated genes were assigned to functional categories according to the gene ontology (GO) annotation of TAIR (Swarbreck et al. 2008; Berardini et al. 2004) (Wenke et al. 2012a). In the set of *S. plymuthica* HRO-C48 specific genes, the categories "DNA, RNA, and protein metabolism" and "cell organization and biogenesis" were significantly underrepresented, also genes with functional roles in mitochondria and ribosomes, in the cytoplasm, and at the plasma membrane. On the other hand, there were significantly more genes of the "transcription" categories and genes involved in general stress responses and in extracellular processes.

With regard to the GO categories, the specific responses to *S. plymuthica* HRO-C48 had little in common with those induced by *S. maltophilia* R3089. The *S. maltophilia* R3089 specific genes had a higher incidence in the categories "cell organization and biogenesis," "mitochondria," and "cytosol." However, the categories "transport," "kinase activity," "nucleic acid binding," "transferase activity," and "developmental processes" are definitely overrepresented. On the basis of the functional categories, the differences revealed between the two responses investigated suggest the involvement of different effectors in the volatile mixtures, which trigger the specific responses in *A. thaliana* within 24 h.

When we consider the functional classification of the 162 genes that respond nonspecifically, it seems that general stress responses as well as transcription factors (TF) are increasingly regulated. In fact, 21 TF that are quite significantly overrepresented ($p \leq 1.3 \times 10^{-42}$) were identified. Some of these proteins have been described as having functions in developmental processes (e.g., two BTB domain scaffold proteins, BT2 and BT4: Robert et al. 2009) or in stress responses (e.g., C2H2-type ZAT10 family protein or WRKY18: Sakamoto et al. 2000; Rossel et al. 2007; Wang et al. 2008). On the other hand, genes of the "transport" and "protein metabolism" groups were significantly less regulated on average.

In order to better assess the type of plant response to rhizobacterial volatiles, the data sets were compared with published microarray data from biotic and abiotic stress experiments, hormone treatments, and the response to growth-enhancing GB03 volatiles (Kilian et al. 2007; Goda et al. 2008; Wanke et al. 2009; Zhang et al. 2007). Surprisingly, this resulted in a relatively uniform picture for the 727 and 493 nonspecific genes and the 162 specific genes (Wenke et al. 2012a). All three data sets revealed a slight overlap with respect to various biotic stresses. As already revealed by MapMan, it was confirmed that the responses to *S. plymuthica* HRO-C48 and to pathogens and the so-called gene-to-gene resistance have somewhat more in common. As for the transcriptional regulation of hormonal

signaling pathways, only abscisic acid and methyl jasmonate are of importance in the nonspecific as well as specific response.

Of all three sets of data, the closest similarity appeared in the responses to abiotic stress with the highest hypergeometric probabilities. Especially those genes were involved that are regulated by cold, osmotic, and salt stress as well as UV-B radiation. Then again, there proved to be very little in common with the dual cultures with *B. subtilis* GB03. This underlines the specificity of changes in plants as an adaptation to volatiles of various bacterial species. The low degree of similarity to oxidative stress was also interesting. This supports the notion that the systematic accumulation of hydrogen peroxide is not a typical response to reactive oxygen species, which includes the classic programmed cell death and appropriate signaling pathways upstream, as is known for defense responses upon pathogen challenge (Neill et al. 2002; Desikan et al. 1998).

The results to date suggest that key factors are present in the 162 generally regulated genes in response to two different mixtures of volatiles, those for inhibition of growth and for chlorosis. Due to the large number of transcription factors (TF) of these 162 genes, many other nonspecifically responding genes might be regulated by these TF. Analysis of *cis*-regulatory elements using the Athena database (O'Connor et al. 2005) revealed that 12 TF-binding motifs are highly enriched ($p \leq 10^{-3}$) in promoters of the 162 genes (Wenke et al. 2012a). Except for the TATA-box motif, all of the elements of stress responses are involved, especially those of biotic and abiotic stimuli, abscisic acid signaling, and light stress. The W-box motif TTGACY is of special interest. It was present in 124 of the 162 genes and also in the GST1-box, which had previously led to a highly volatile-dependent GUS activity in the promoter-GUS test. Moreover, W-box-WRKY interaction is known to play an essential role in important plant processes (Rushton et al. 2010).

16.4.3 Involvement of WRKY Transcription Factors in the Mediation of Volatile-Induced Changes

The higher frequency of the W box in the 162 nonspecifically regulated genes and its presence in the volatile-activated GST1 box directed more attention toward testing of W-box-WRKY interaction. Only AtWRKY18 (At4g31800) was induced to a considerable degree in both volatile treatments (Wenke et al. 2012a). AtWRKY18 has been described as a signal in pathogen defense (Pandey et al. 2010). It belongs to the IIa group of WRKY-proteins and is functionally redundant with its *Arabidopsis* paralogs AtWRKY40 and AtWRKY60 (Xu et al. 2006; Shen et al. 2007; Mangelsen et al. 2008). AtWRKY40 (At1g80840) was also induced in response to *S. plymuthica* HRO-C48 volatiles (Wenke et al. 2012a). WRKY40, among other things, is involved in the regulation of RRTF1 (redox responsive transcription factor) and JAZ8 (jasmonate ZIM-domain), both AP2 proteins (Pandey et al. 2010). Neither RRTF1 nor JAZ8 were regulated in the dual cultures.

To test the hypothesis that WRKY transcription factors are involved in volatile-mediated effects, *WRKY18*, *WRKY40,* and *WRKY60* single mutants were tested in tricultures with *S. plymuthica* HRO-C48 or *S. maltophilia* R3089 and wild-type seedlings of *A. thaliana*. Only the response of *WRKY18* mutants to volatiles of both bacterial species was significantly weaker compared to the wild type. These mutants had almost twice the fresh weight and significantly more total chlorophyll after 3 days in triculture. After 10 days, the relative fresh weight was increased by 300 % (Wenke et al. 2012a). Nevertheless, these *WRKY18* mutants were not capable of surviving. After 10 days they also became extremely chlorotic. DAB assays revealed no clear differences in hydrogen peroxide accumulation between the mutant and the wild type. This supports the hypothesis that seedling death is causally linked to chlorosis but may be indirectly related to accumulation of reactive oxygen species. Furthermore, it can be seen that the volatile-induced responses of *Arabidopsis* include an early WRKY18-dependent signaling pathway but also a later WRKY18-independent pathway involving hydrogen peroxide. With respect to fresh weight, chlorophyll content, and hydrogen peroxide content, both *WRKY40* and *WRKY60* mutants had no significant phenotypic changes compared to the wild type in tricultures with *S. plymuthica* HRO-C48 and *S. maltophilia* R3089.

WRKY18/40 plays an antagonistic role in ABA-dependent signal transduction (Chen et al. 2010; Shang et al. 2010), and WRKY18 positively regulates the JA signals (Wang et al. 2008; Pandey et al. 2010). A comparison of the volatile-regulated data sets with hormonal treatments revealed a large overlap of the ABA and JA effects. However, none of the known ABA or MeJA marker genes were changed in the dual cultures with *S. plymuthica* HRO-C48 or *S. maltophilia* R3089. It remains to be seen whether the ABA- and JA-dependent genes are direct target genes of WRKY18. Pandey and colleagues (2010) reported that the expression of NPR1 (non-expressor of PR genes), the SA marker, remains unchanged in the *WRKY18/40* double mutant. This goes along with the fact that both mixtures of volatiles caused no transcriptional changes in NPR1 or other SA marker genes (Wenke et al. 2012a).

In order to identify potential candidates for the WRKY18-dependent signaling cascade, a comparison was made of all volatile-dependent genes with the 165 genes that are deregulated in the *WRKY18/40* double mutant (Pandey et al. 2010). It turned out that there is an overlap of 70 genes (Wenke et al. 2012a). Of these 70 genes, 41 belong to the *S. plymuthica* HRO-C48 specific data set ($p \leq 1.4 \times 10^{-39}$), 10 to the *S. maltophilia* R3089 specific data set ($p \leq 3.0 \times 10^{-11}$), and the remaining 19 to the 162 genes regulated independent of the bacterial species. In turn, 10 of the 41 genes of the *S. plymuthica* HRO-C48 response are involved in the ethylene signaling pathway: ethylene biosynthesis (1-aminocyclopropane-1-carboxylic acid synthase 6), signal transduction (mitogen-activated protein kinase 9), and signal integration (5 ERFs). Participation of ERFs could already be assumed, based on the volatile-dependent activation of S and GST1 boxes. This was confirmed by transcriptome analysis of cocultures with *S. plymuthica* HRO-C48. In addition, 54 W boxes were located in the 19 genes that reacted to the volatiles in a bacterial-nonspecific manner and to the wrky18/40 double mutation, which means an average of 2.8 W boxes per promoter. This value leaves open the question whether

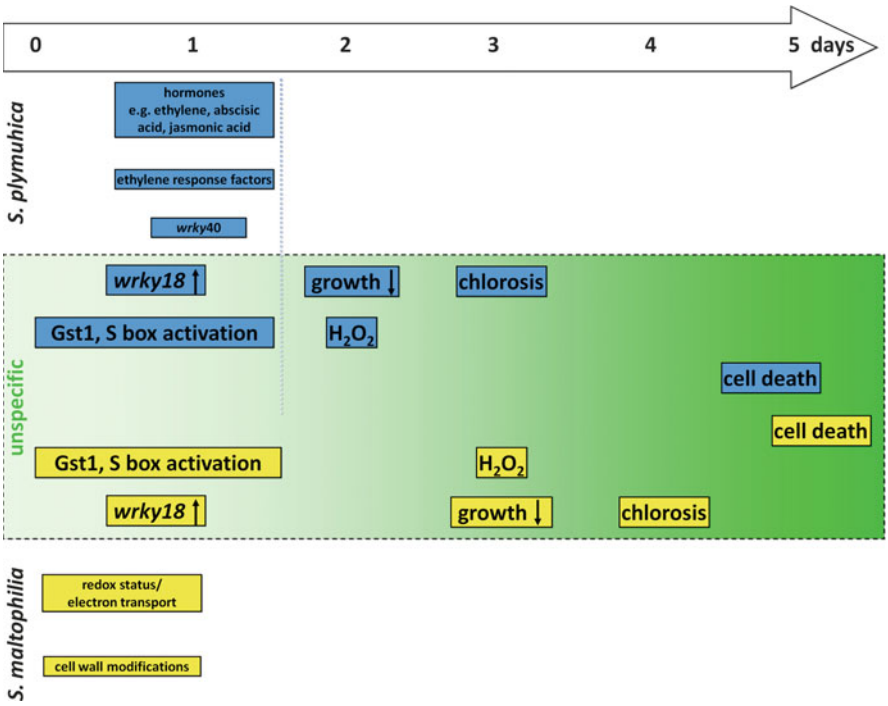


Fig. 16.1 Overview of transcriptional and morphological as well as physiological alterations in *Arabidopsis thaliana* in response to rhizobacterial volatiles

the 19 genes are under the direct control of WRKY18 and/or WRKY40. Aside from WRKY18, there are eight other TF among the 19 genes: two AP2/ERF, two MYB factors, two BT proteins (BTB and TAZ domains), SZF1 (salt-tolerance zinc finger 1), and ZAT10 (zinc finger, C2H2 type). These all play a role in stress responses and/or in hormone signal transduction.

A time schedule of events that occur in *A. thaliana* at the morphological, physiological, and transcriptional level in dual culture assays with both rhizobacterial strains is summarized in Fig. 16.1.

The observations are divided into strain-specific (in the white/green box in the middle) and strain-unspecific effects as well as into *S. plymuthica* (blue boxes) and *S. maltophilia* (yellow boxes) volatile-induced effects. The gray, dotted line represents the time of no return for the response to the *S. plymuthica* volatiles.

16.5 Which Type of Stress Is Induced by Rhizobacterial Volatiles?

The triggering of specific plant responses to airborne substances is one aspect of plant-pathogen interaction. That largely has not been taken into account. Biotic, as well as abiotic, exogenous or endogenous elicitors (pathogen- or microbe-associated

molecular patterns PAMPs/MAMPs, effectors, and damage-associated molecular patterns: DAMPs) initiate in plants a number of finely balanced and coordinated mechanisms. These elicit local or systemic resistance to pathogens or wound-healing responses following mechanical stress (summarized in, e.g., Chisholm et al. 2006; Lotze et al. 2007; Boller and Felix 2009). Plants have various means of signal transduction to elicit such specific responses: ionic currents, formation of reactive oxygen species, mitogen-activated protein kinase cascades, hormones (SA, JA, ET), other protein kinases, and phosphatases (summarized in Hématy et al. 2009). Up to now, the potential of volatile-induced specific responses had not been taken into account with regard to signal transduction under stress conditions. Therefore, a new term for volatile interspecies-active elicitors should be introduced at this point: "microbial volatile-associated molecular patterns" (mVAMPs).

This work focuses on the effects of a mixture of a number of potentially active volatiles. It could be shown with two rhizobacterial species that different volatile mixtures are capable of initiating specific responses in *A. thaliana* at an early point in time. These responses revealed factors such as WRKY18 that play an important role in the classical response to pathogens. An overall comparison of the various stress transcriptomes of *Arabidopsis* revealed that the two responses studied in dual cultures are more similar to abiotic stress responses than to those of pathogen defense. The involvement of several classical transducers of pathogen defense, in particular with regard to the transcription factors, demonstrates that a considerable number of PAMP responses up to now may have been induced by volatile metabolites. On the other hand, the absence of essential PAMP- and DAMP-regulated genes makes clear that mVAMP-induced stress is a new type of stress.

On the basis of present data, a model was developed that summarizes the new findings on the responses of plants to bacterial volatiles (Wenke et al. 2012a). Within the scope of this work, apparently general as well as bacterial species-specific mVAMP-induced changes in the gene expression of *A. thaliana* in dual culture with *S. plymuthica* HRO-C48 or *S. maltophilia* R3089 were discovered. The bacterial species-specific responses at the transcript level entailed signal transduction via the hormones ethylene, ABA, and JA after 24 h with *S. plymuthica* HRO-C48, whereby the corresponding upstream regulators of the ERF and WRKY40 groups are involved. In contrast, there is the very rapid, specific response to the *S. maltophilia* R3089 volatiles with respect to regulation of the redox potential and the electron transport chain. This was followed by very early transcriptional changes in the cell wall, which may serve to strengthen the mechanical barrier against the volatiles. This specificity is likely a unique characteristic of the differing volatile compositions (Kai et al. 2007).

In both treatments there were various indications that the family of plant-specific WRKY-TF plays an important role. The essential factor is apparently WRKY18, regardless of the type of volatile mixture. This factor has been described in the literature as a negative transcription regulator, which at the same time has an antagonistic effect on expression of the paralog factor WRKY40 (Xu et al. 2006; Shen et al. 2007; Chen et al. 2010; Pandey et al. 2010). *WRKY18* mutants had a significantly weaker inhibited phenotype in direct comparison with the wild type.

Therefore, the hypothesis of a double-negative cascade can be proposed. A yet unknown gene is repressed via WRKY18. The inactivated target gene itself codes directly or indirectly for an inactivator of cell death. In *WRKY18* mutants, this can extend the viable phase of the seedlings, since the WRKY18-dependent inactivation of the cell death repressor has been turned off. This makes it possible for such mutants to grow significantly better under cocultivation. As to the accumulation of hydrogen peroxide, it remained unchanged in the *WRKY18* mutants, but in the wild type in dual cultures with *S. plymuthica* HRO-C48 or *S. maltophilia* R3089, there were kinetic differences. These in turn can be explained by bacterial species-specific responses. These facts as a whole suggest a specific stimulation of hydrogen peroxide formation leading to cell death, independent of WRKY18.

This study on the effects of two different mixtures of rhizobacterial volatile metabolites has revealed a new group of stressors (elicitors), the mVAMPs (microbial volatile-associated molecular patterns). The responses of plants to these mVAMPs include general as well as bacterial strain-specific changes. At the same time, the regulation of classical stress marker genes described previously for stress situations (MAMP, PAMP, DAMP dependent) was not detected, which underlines the novelty of the mVAMP-dependent stress situation.

16.6 Potentially Biologically Active Individual Substances

Despite the important differences found using gene expression data, cocultivation with *S. plymuthica* HRO-C48 as well as *S. maltophilia* R3089 causes a considerable impairment of plant development accompanied by complete chlorosis and a systemic hydrogen peroxide accumulation leading to systemic cell death. It can, therefore, be concluded that identical or very similar single compounds are responsible for these nonspecific changes. The identification of single active substances, including their effective concentrations in dual cultures, is very complicated and difficult. In order to identify potentially active substances in the Petri-dish setups, known and accessible compounds were tested that might play an important role in cocultures with *S. plymuthica* HRO-C48 and *S. maltophilia* R3089, including 2-phenylethanol, dimethyl disulfide (DMDS), HCN, and NH_3. Plants as well as bacterial and fungal microorganisms emit 2-phenylethanol, the odor of roses.

This compound has antimicrobial properties because of its ability to alter plasma membrane permeability as well as amino acid and sugar transport (Etschmann et al. 2002). In dual cultures, 20 µmol 2-phenylethanol proved capable of inhibiting *A. thaliana* growth by 50 % (Wenke et al. 2012a). It has been seen that another bacterial volatile, dimethyl disulfide, has a similar affect in dual cultures (Kai et al. 2010). In addition, dimethyl disulfide has insecticidal properties due to its ability to inhibit cytochrome oxidase of the mitochondrial electron transport chain and the potassium channel (Dugravot et al. 2003; Gautier et al. 2008). HCN is a much-discussed compound in connection with volatile-induced inhibition of plants. It is produced by *Pseudomonas*, *Chromobacterium*, and *Rhizobium* (Blumer and Haas

2000; Kai et al. 2010; Blom et al. 2011). Even 1 μmol HCN causes a 400 % decrease in fresh mass (Blom et al. 2011). In several studies with noncyanogenic wild-type strains or HCN-negative mutants and HCN-producing bacteria, a correlation could be established between HCN production and inhibition of plant growth (fresh mass, root length) (Blumer and Haas 2000; Blom et al. 2011; Wenke et al. 2012b). During the studies of Blom and colleagues, it was shown that *Serratia* species are not capable of producing/releasing hydrogen cyanide, similar to that of *S. odorifera* 4Rx13 (Kai et al. 2010). They demonstrated that neither *S. plymuthica* HRO-C48 nor *S. maltophilia* R3089 emit HCN in Petri dishes on NB medium (Marco Kai unpublished). On the other hand, the emission of ammonia was detected in both strains (Teresa Weise unpublished), similar to the NH_3 concentrations released by *S. odorifera* 4Rx13 (<1 μmol, Kai et al. 2010). It is known that ammonia causes decoupling of electron transport (Losada and Arnon 1963), which leads to chlorosis and complete inhibition of plant growth (Britto and Kronzucker 2002). Since at least 2.5 μmol of ammonia is required to cause a distinct inhibition of *A. thaliana* in dual culture (Kai et al. 2010), it can be assumed that ammonia is not solely responsible for volatile-dependent effects on *A. thaliana* in coculture with *S. plymuthica* HRO-C48 and *S. maltophilia* R3089, but has the potential to act synergistically. Based on previous findings, it is concluded that ammonia, dimethyl disulfide, and 2-phenylethanol have a potentially additive or synergistic effect on plants. Additional testing of single compounds or mixtures of these in varying proportions is required to gain more precise information.

16.7 Ecological Aspects of Volatile-Induced Effects

The importance of these effects for the ecosystem should be discussed briefly at this point. In fact, it should be resolved to what extent these effects are applicable to natural conditions, despite the simplicity of the testing system. The conditions chosen proved effective in complete killing the seedlings. The cultivation parameters, in particular the supply of nutrients available to the bacteria, corresponded closely to ideal conditions and resulted in bacterial cell counts of up to 10^{11} cfu. A quantification of *S. plymuthica* HRO-C48 as well as *S. maltophilia* R3089 cells in dual cultures in the presence or absence of *A. thaliana* showed that the seedlings had no significant effect on the rhizobacteria via the air space (unpublished). However, there have been several reports that root exudates are effective against microorganisms, so plants are capable of defending themselves directly (summarized in Bais et al. 2006). This type of interaction via soluble compounds was prevented by spatial separation in Petri dish assay used here. Under these artificial conditions, interaction is not only unilateral but also limited to only two interaction partners. In natural surroundings, a balance within the rhizosphere community would be achieved by intraspecies and interspecies competition. New experiments with various combinations of bacteria in plant cell cultures

have verified that the greater the species diversity, the more stable the microbial community under stress situations (Chatzinotas et al. 2011).

Another aspect for consideration is the type of stress application. The effective volatiles are wafted toward the plants via the airspace above the surface, which does not resemble the natural situation. As for the mechanism of stress recognition, it remains uncertain the involvement of receptors and which plant organs actually detect the volatile signals in dual cultures. This indicates that the simplified experiments carried out here must be followed by performing similar experiment in natural surroundings (spatial separation in soil) in order to obtain ecologically relevant information. Volatile metabolites play an ecological role in the transmission of information under natural conditions. For example, field trials have demonstrated indirect resistance in maize, which was capable of recruiting entomopathogenic nematodes by β-caryophyllene emission (Rasman et al. 2005). In conclusion, the present study provides a sound basis for further studies to shed light on the obvious potential of volatile substances as elicitors of specific responses of plants from an ecological viewpoint.

In addition to artificially induced genetic changes, the occurrence of natural genotypic and phenotypic variants within a species provides an invaluable source for studying complex responses to ever-changing conditions. More than 750 accessions have been described for *A. thaliana*. A comparison of transcriptomes altered by volatiles with the expression profiles of a wide variety of *A. thaliana* accessions under normal conditions revealed the ecotype-specific expression of a large portion of the volatile-regulated genes. This means that the accessions bring along different initial conditions for responding to volatile metabolites at the transcript level. Based on these insights, a number of accessions were tested in dual cultures with *S. plymuthica* HRO-C48 and *S. maltophilia* R3089. Aboveground fresh biomass and root development of 21 natural variants of *A. thaliana* under microarray conditions with *S. plymuthica* HRO-C48 were determined (Wenke et al. 2012b). With regard to the aerial parts of all *Arabidopsis* variants selected, there were no significant differences in relative growth inhibition by the volatiles (Wenke et al. 2012a). Relative inhibition was around 90 % in all accessions in comparison to untreated controls. Such a high percentage of inhibition implies that the seedlings were killed quickly. According to previous findings, chlorosis and killing of plants may be a nonspecific late effect of volatiles, which should be considered separately from specific immediate responses. With regard to the roots, accession-specific responses to volatiles of *S. plymuthica* HRO-C48 were observed. The greatest variation was found between C24 (82 % inhibition) and Ler (42 % inhibition). An adaptation to L-glutamate was confirmed by similar values for relative inhibition of primary root growth of C24 and Ler (approximately 80 % and 40 %, respectively) (Walch-Liu et al. 2006). The natural surroundings of C24 are unknown. It is assumed that this variant originated in the laboratory and has, therefore, not undergone natural adaptation to rhizobacterial volatiles. Ler is an ecotype from Landsberg, Germany, which may be adapted to the effects of volatile elicitors. This initial data supports the notion that the discussed specificity of volatile-induced changes in plant processes has an ecological background.

16.8 Concluding Remarks and Future Perspectives

Volatile signals allow an intra- and interspecies exchange of information between organisms without direct contact, also beneath the soil surface. The rhizobacteria *S. plymuthica* and *S. maltophilia* emit quite different mixtures of volatiles that cause enormous transcriptional, physiological, and morphological changes in *A. thaliana*. These in turn lead to seedling death within 5 days. Research on bacterial volatiles is still in its infancy. It will remain an exciting topic in the coming years: identification of yet unknown infochemicals and in-depth elucidation of their potential as important pharmaceutical, ecological, and agricultural effectors. This includes not only elucidation of the biosynthesis of volatile metabolites but also decryption of volatile-induced signaling pathways in interaction partners.

References

Alström S, Burns RG (1989) Cyanide production by rhizobacteria as a possible mechanism of plant growth inhibition. Biol Fertil Soils 7:232–238

Arthur CL, Pawliszyn J (1990) Solid phase microextraction with thermal desorption using fused silica optical fibers. Anal Chem 62:2145–2148

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266

Baldwin IT, Halitschke R, Paschold A, von Dahl CC, Preston CA (2006) Volatile signaling in plant-plant interactions: "Talking Trees" in the genomics era. Science 311:812–815

Banchio E, Xie X, Zhang H, Pare PW (2009) Soil bacteria elevate essential oil accumulation and emissions in sweet basil. J Agric Food Chem 57:653–657

Barber DA, Martin JK (1976) The release of organic substances by cereal roots into soil. New Phytol 76:69–80

Berardini TZ, Mundodi S, Reiser R, Huala E, Garcia-Hernandez M, Zhang P, Mueller LM, Yoon J, Doyle A, Lander G, Moseyko N, Yoo D, Xu I, Zoeckler B, Montoya M, Miller N, Weems D, Rhee SY (2004) Functional annotation of the *Arabidopsis* genome using controlled vocabularies. Plant Physiol 135:1–11

Berg G, Marten P, Ballin G (1996) *Stenotrophomonas maltophilia* in the rhizosphere of oilseed rape – occurrence, characterization and interaction with phytopathogenic fungi. Microbiol Res 151:1–9

Berg G, Roskot N, Steidle A, Eberl L, Zock A, Smalla K (2002) Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from different *Verticillium* host plants. Appl Environ Microbiol 68:3328–3338

Bernier SP, Létoffé S, Delepierre M, Ghigo JM (2011) Biogenic ammonia modifies antibiotic resistance in physically separated bacteria. Mol Microbiol 81(3):705–716

Bloemberg GV, Wijfjes AHM, Lamers GEM, Stuurman N, Lugtenberg BJJ (2000) Simultaneous imaging of *Pseudomonas fluorescens* WCS365 populations expressing three different autofluorescent proteins in the rhizosphere: new perspectives for studying microbial communities. Mol Plant Microbe Interact 13:1170–1176

Blom D, Fabbri C, Eberl L, Weisskopf L (2011) Volatile-mediated killing of *Arabidopsis thaliana* by bacteria is mainly mediated due to hydrogen cyanide. Appl Environ Microbiol 77 (3):1000–1008

Blumer C, Haas D (2000) Mechanism, regulation, and ecological role of bacterial cyanide biosynthesis. Arch Microbiol 173:170–177

Boland W, Ney P, Jaenicke L, Gassmann G (1984) A "closed-loop-stripping" technique as a versatile tool for metabolic studies of volatiles. In: Scheuer P (ed) Analysis of volatiles. De Gruyter, Berlin, pp 371–380

Boller T, Felix G (2009) A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu Rev Plant Biol 60:379–406

Britto DT, Kronzucker HJ (2002) NH_4 – toxicity in higher plants: a critical review. J Plant Physiol 159:567–584

Bunge M, Araghipour N, Mikoviny T, Dunkl J, Schnitzhofer R, Hansel A, Schinner F, Wisthaler A, Margesin R, Märk TD (2008) On-line monitoring of microbial volatile metabolites by proton transfer reaction-mass spectrometry. Appl Environ Microbiol 74:2179–2186

Chatzinotas A, Schäwe R, Saleem M, Fetzer J, Harms H (2011) Microbial model systems and ecological theory: how does increasing environmental stress affect microbial interactions and ecosystems services. VAAM-Tagung in Karlsruhe, 3–6 April 2011

Chen H, Lai Z, Shi J, Xiao Y, Chen Z, Xu X (2010) Roles of *Arabidopsis* WRKY18, WRKY40 and WRKY60 transcription factors in plant responses to abscisic acid and abiotic stress. BMC Plant Biol 10:281. doi:10.1186/1471-2229-10-281

Chisholm ST, Coaker G, Day B, Staskawicz BJ (2006) Host-microbe interactions: shaping the evolution of the plant immune response. Cell 124:803–814

Cho SM, Kang BR, Han SH, Anderson AJ, Park JY, Lee YH, Cho BH, Yang KY, Ryu CM, Kim YC (2008) 2R,3R-butanediol, a bacterial volatile produced by *Pseudomonas chlororaphis* O6, is involved in induction of systemic tolerance to drought in *Arabidopsis thaliana*. Mol Plant Microbe Interact 21:1067–1075

Dangl JL, Jones JDG (2001) Plant pathogens and integrated defence responses to infection. Nature 411:826–833

Desikan R, Reynolds A, Hancock JT, Neil SJ (1998) Hairpin and hydrogen peroxide both initiate programmed cell death but have differential effects on gene expression in *Arabidopsis* suspension cultures. Biochem J 330:115–120

Dickschat JS, Martens R, Brinkhoff T, Simon M, Schulz S (2005) Volatiles released by a *Streptomyces* species isolated from the North Sea. Chem Biodivers 2:837–865

Dugravot S, Grolleau F, Macherel D, Rochetaing A, Hue B, Stankiewicz M, Huignard J, Lapied B (2003) Dimethyl disulfide exerts insecticidal neurotoxicity through mitochondrial dysfunction and activation of insect K_{ATP} channels. J Neurophysiol 90:259–270

Ercolini D, Russo F, Nasi A, Ferranti P, Villani F (2009) Mesophilic and psychrotrophic bacteria from meat and their spoilage potential in vitro and in beef. Appl Environ Microbiol 75:1990–2001

Etschmann MMW, Bluemke W, Sell D, Schrader J (2002) Biotechnological production of 2-phenylethanol. Appl Microbiol Biotechnol 59:1–8

Ezquer I, Li J, Ovecka M, Baroja-Fernandez E, Munoz FJ, Montero M, Diaz de Cerio J, Hildago M, Sesma MT, Bahaji A, Etxeberria E, Pozueta-Romero J (2010) Microbial volatile emissions promote accumulation of exceptionally high levels of starch in leaves of mono- and dicotyledonous plants. Plant Cell Physiol 51:1674–1693

Fiddaman PJ, Rossall S (1994) Effect of substrate on the production of antifungal volatiles from *Bacillus subtilis*. J Appl Bacteriol 76:395–405

Gautier H, Auger J, Legros C, Lapied B (2008) Calcium-activated potassium channels in insect pacemaker neurons as unexpected target site for the novel fumigant dimethyl disulfide. J Pharmacol Exp Ther 324:149–159

Gerber NN, Lechevalier HA (1965) Geosmin, an earthy-smelling substance isolated from actinomycetes. Appl Microbiol 13:935–938

Goda H, Sasaki E, Akiyama K, Maruyama-Nakashita A, Nakabayashi K, Li W, Ogawa M, Yamauchi Y, Preston J, Aoki K, Kiba T, Takatsuto S, Fujioka S, Asami T, Nakano T, Kato H, Mizuno T, Sakakibara H, Yamaguchi S, Nambara E, Kamiya Y, Takahashi H, Hirai MY, Sakurai T, Shinozaki K, Saito K, Yoshida S, Shimada Y (2008) The AtGenExpress hormone and chemical treatment data set: experimental design, data evaluation, model data analysis and data access. Plant J 55:526–542

Hématy K, Cherk C, Somerville S (2009) Host–pathogen warfare at the plant cell wall. Curr Opin Plant Biol 12:406–413

Hennig L, Menges M, Murray JA, Gruissem W (2003) *Arabidopsis* transcript profiling on Affymetrix GeneChip arrays. Plant Mol Biol 53:457–465

Kai M, Piechulla B (2009) Plant growth promotions due to rhizobacterial volatiles – an effect of CO_2? FEBS Lett 583:3473–3477

Kai M, Effmert U, Berg G, Piechulla B (2007) Volatiles of bacterial antagonists inhibit mycelial growth of the plant pathogen *Rhizoctonia solani*. Arch Microbiol 187:351–360

Kai M, Haustein M, Molina F, Petri A, Scholz B, Piechulla B (2009a) Bacterial volatiles and their action potential. Appl Microbiol Biotechnol 81:1001–1012

Kai M, Wenke K, Piechulla B (2009b) Flüchtige metabolite als infochemikalien: duftstoffe im erdreich. BIUZ 39:3–9

Kai M, Crespo E, Cristescu SM, Harren FJM, Piechulla B (2010) *Serratia odorifera*: analysis of volatile emission and biological impact of volatile compounds on *Arabidopsis thaliana*. Appl Microbiol Biotechnol 88:965–976

Kilian J, Whitehead D, Horak J, Wanke D, Weinl S, Batistic O, D'Angelo C, Bornberg-Bauer E, Kudla J, Harter K (2007) The AtGenExpress global stress expression data set: protocols, evaluation and model data analysis of UV-B light, drought and cold stress responses. Plant J 50:347–363

Kim M, Ahn JW, Jin UH, Choi D, Paek KH, Pai HS (2003) Activation of the programmed cell death pathway by inhibition of proteasome function in plants. J Biol Chem 278:19406–19415

Kirsch C, Logemann E, Lippok B, Schmelzer E, Hahlbrock K (2001) A highly specific pathogen-responsive promoter element from the immediate-early activated CMPG1 gene in *Petroselinum crispum*. Plant J 26:1–12

Kurze S, Dahl R, Bahl H, Berg G (2001) Biological control of soil-borne pathogens in strawberry by *Serratia plymuthica* HRO-C48. Plant Dis 85:529–534

Kwon YS, Ryu CM, Lee S, Park HB, Han KS, Lee JH, Lee K, Chung WS, Jeong MJ, Kim HK, Bae DW (2010) Proteome analysis of *Arabidopsis* seedlings exposed to bacterial volatiles. Planta 232:1355–1370

Lamp C, Dixon RA (1997) The oxidative burst in plant disease resistance. Annu Rev Plant Physiol Plant Mol Biol 48:251–275

Losada M, Arnon DJ (1963) Selective inhibitors of photosynthesis. In: Hochster RM, Quastel JH (eds) Metabolic inhibitors, vol 2. Academic, New York, pp 503–611

Lotze MT, Zeh HJ, Rubartelli A, Sparvero LJ, Amoscato AA, Washburn NR, Devera ME, Liang X, Tör M, Billiar T (2007) The grateful dead: damage associated molecular pattern molecules and reduction/oxidation regulate immunity. Immunol Rev 220:60–81

Mangelsen E, Kilian J, Berendzen KW, Kolukisaoglu UH, Harter K, Jansson C, Wanke D (2008) Phylogenetic and comparative gene expression analysis of barley (*Hordeum vulgare*) WRKY transcription factor family reveals putatively retained functions between monocots and dicots. BMC Genomics 9:194

Mayr D, Margesin R, Klingsbichel E, Hartungen E, Jenewein D, Märk TD, Schinner F (2003) Rapid detection of meat spoilage by measuring volatile organic compounds by using proton transfer reaction mass spectrometry. Appl Environ Microbiol 69:4697–4705

Meyers BC, Morgante M, Michelmore RW (2002) TIR-X and TIR-NBS proteins: two new families related to disease resistance TIR-NBS-LRR proteins encoded in *Arabidopsis* and other plant genomes. Plant J 32:77–92

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. Trends Plant Sci 9:490–498

Müller C, Hilker M (2000) The effect of a green leaf volatile on host plant finding by larvae of a herbivorous insect. Naturwissenschaften 87:216–219

Neill SJ, Desikan R, Clarke A, Hurst RD, Hancock JT (2002) Hydrogen peroxide and nitric oxide as signalling molecules in plants. J Exp Bot 53:1237–1242

O'Connor TR, Dyreson C, Wyrick JJ (2005) Athena: a resource for rapid visualization and systematic analysis of *Arabidopsis* promoter sequences. Bioinformatics 21:4411–4413

Pacioni G (1991) Effects of *Tuber* metabolites on the rhizospheric environment. Mycol Res 95:1355–1358

Pandey SP, Roccaro M, Schön M, Logemann E, Somssich IE (2010) Transcriptional reprogramming regulated by WRKY18 and WRKY40 facilitates powdery mildew infection of *Arabidopsis*. Plant J 64:912–923

Piechulla B, Pott MB (2003) Plant scents-mediators of inter- and intra-organismic communication. Planta 217:687–689

Rasman S, Köllner TG, Degenhardt J, Hiltpold I, Toepfer S, Kuhlmann U, Gershenzon J, Turlings TCJ (2005) Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature 434:732–737

Robert HS, Quint A, Brand D, Vivian-Smith A, Offringa R (2009) BTB AND TAZ DOMAIN scaffold proteins perform a crucial function in *Arabidopsis* development. Plant J 58:109–121

Rossel JB, Wilson PB, Hussain D, Woo NS, Gordon MJ, Mewett OP, Howell KA, Whelan J, Kazan K, Pogson BJ (2007) Systemic and intracellular response to photooxidative stress in *Arabidopsis*. Plant Cell 19:4091–4110

Rudrappa T, Biedrzycki ML, Kunjeti SG, Donofrio NM, Czymmek KJ, Paré PW, Bais HP (2010) The rhizobacterial elicitor acetoin induces systemic resistance in *Arabidopsis thaliana*. Commun Integr Biol 3:130–138

Rushton PJ, Reinstadler A, Lipka V, Lippok B, Somssich IE (2002) Synthetic plant promoters containing defined regulatory elements provide novel insights into pathogen- and wound-induced signaling. Plant Cell 14:749–762

Rushton PJ, Somssich IE, Ringler P, Shen QJ (2010) WRKY transcription factors. Trends Plant Sci 15:247–258

Ryu CM, Farag MA, Hu CH, Reddy MS, Wie HX, Pare PW, Kloepper JW (2003) Bacterial volatiles promote growth in *Arabidopsis*. Proc Natl Acad Sci USA 100:4927–4932

Ryu CM, Farag MA, Hu CH, Reddy MS, Kloepper JW, Pare PW (2004) Bacterial volatiles induce systemic resistance in *Arabidopsis*. Plant Physiol 134:1017–1026

Sakamoto H, Araki T, Meshi T, Iwabuchi M (2000) Expression of a subset of the *Arabidopsis* Cys (2)/His(2)-type zinc-finger protein gene family under water stress. Gene 248:23–32

Schreier P (1980) Wine aroma composition: identification of additional volatile constituents in red wine. J Agric Food Chem 28:926–928

Schulz S, Fuhlendorff J, Reichenbach H (2004) Identification and synthesis of volatiles released by the myxobacterium *Chondromyces crocatus*. Tetrahedron 60:3863–3872

Shang Y, Yan L, Liu ZQ, Cao Z, Mei C, Xin Q, Wu FQ, Wang XF, Du SY, Jiang T, Zhang XF, Zhao R, Sun HL, Liu R, Yu YT, Zhang DP (2010) The Mg-chelatase H subunit of *Arabidopsis* antagonizes a group of WRKY transcription repressors to relieve ABA-responsive genes of inhibition. Plant Cell 22:1909–1935

Shen QH, Saijo Y, Mauch S, Biskup C, Bieri S, Keller B, Seki H, Ulker B, Somssich IE, Schulze-Lefert P (2007) Nuclear activity of MLA immune receptors links isolate-specific and basal disease-resistance responses. Science 315:1098–1103

Stall RE, Hall CB, Cook AA (1972) Relationship of ammonia to necrosis of pepper leaf tissue during colonization by *Xanthomonas vesicatoria*. Phytopathology 62:882–886

Stotzky G, Schenck S (1976) Volatile organic compounds and microorganisms. CRC Crit Rev Microbiol 4:333–382

Strittmatter G, Gheysen G, Gianinazzi-Pearson V, Hahn K, Niebel A, Rohde W, Tacke E (1996) Infections with various types of organisms stimulate transcription from a short promoter fragment of the potato *gst1* gene. Mol Plant Microbe Interact 9:68–73

Swarbreck D, Wilks C, Lamesch P, Berardini TZ, Garcia-Hernandez M, Foerster H, Li D, Meyer T, Muller R, Ploetz L, Radenbaugh A, Singh S, Swing V, Tissier C, Zhang P, Huala E (2008) The Arabidopsis Information Resource (TAIR): gene structure and function annotation. Nucleic Acids Res 36:D1009–D1014

Thimm O, Bläsing O, Gibon Y, Nagel A, Meyer S, Krüger P, Selbig J, Müller LA, Rhee SY, Stitt M (2004) MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. Plant J 37:914–939

Thordal-Christensen H, Zhang Z, Wei Y, Collinge DB (1997) Subcellular localization of H_2O_2 in plants, H_2O_2 accumulation in papillae and hypersensitive response during barley-powdery mildew interaction. Plant J 11:1187–1194

Urbach G (1997) The flavour of milk and dairy products: II. Cheese: contribution of volatile compounds. Int J Dairy Technol 50:79–89

van Dam NM, Qiu BL, Hordijk CA, Vet LEM, Jansen JJ (2010) Identification of biologically relevant compounds in aboveground and belowground induced volatile blends. J Chem Ecol 36 (9):1006–1016

Vespermann A, Kai M, Piechulla B (2007) Rhizobacterial volatiles affect the growth of fungi and Arabidopsis thaliana. Appl Environ Microbiol 73:5639–5641

Walch-Liu P, Liu LH, Remans T, Tester M, Forde BG (2006) Evidence that L-Glutamate can act as endogenous signal to modulate root growth and branching in *Arabidopsis thaliana*. Plant Cell Physiol 47(8):1045–1057

Walker TS, Bais HP, Deziel E, Schweitzer HP, Rahme LG, Fall R, Vivanco JM (2004) *Pseudomonas aeruginosa*-plant root interactions. Pathogenicity, biofilm formations, and root exudation. Plant Physiol 134:3210–3331

Wang Z, Cao G, Wang X, Miao J, Liu X, Chen Z, Qu LJ, Gu H (2008) Identification and characterization of COI1-dependent transcription factor genes involved in JA-mediated response to wounding in Arabidopsis plants. Plant Cell Rep 27:125–135

Wanke D, Berendzen K, Kilian J, Harter K (2009) Insights into the *Arabidopsis* abiotic stress response from the AtGenExpress expression profile dataset. In: Hirt H (ed) Plant stress biology. Wiley-VCH, Weinheim, pp 199–225

Weissteiner S, Schütz S (2006) Are different volatile pattern influencing host plant choice of belowground living insects. Mitt Dtsch Ges Allg Angew Ent 15:51–55

Wenke K, Kai M, Piechulla B (2010) Belowground volatiles facilitate interactions between plant roots and soil organisms. Planta 231:499–506

Wenke K, Wanke D, Kilian J, Berendzen K, Harter K, Piechulla B (2012a) Transcriptional response of Arabidopsis seedlings to rhizobacterial, growth inhibiting volatiles. Plant J 70 (3):445–459

Wenke K, Weise T, Warnke R, Valverde C, Wanke D, Kai M, Piechulla B (2012b) Bacterial volatiles mediating information between bacteria and plants. In: Witzany G, Baluska F (eds) Bio-communication of plants. Springer, Heidelberg

Xie X, Zhang H, Pare PW (2009) Sustained growth promotion in Arabidopsis with long-term exposure to the beneficial soil bacterium *Bacillus subtilis* (GB03). Plant Signal Behav 4:948–953

Xu X, Chen C, Fan B, Chen Z (2006) Physical and functional interactions between pathogen-induced Arabidopsis WRKY18, WRKY40, and WRKY60 transcription factors. Plant Cell 18:1310–1326

Zangerl AR, Berenbaum MR (2009) Effects of florivory on floral volatile emissions and pollination success in the wild parsnip. Arthropod Plant Interact 3:181–191

Zhang H, Kim MS, Krishnamachari V, Payton P, Sun Y, Grimson M, Farag MA, Ryu CM, Allen R, Melo IS, Pare PW (2007) Rhizobacterial volatile emissions regulate auxin homeostasis and cell expansion in Arabidopsis. Planta 226:839–851

Zhang H, Kim MS, Sun Y, Dowd SE, Shi H, Pare PW (2008a) Soil bacteria confer plant salt tolerance by tissue-specific regulation of the sodium transporter HKT1. Mol Plant Microbe Interact 21:737–744

Zhang H, Xie X, Kim MS, Kornyeyev DA, Holaday S, Pare RW (2008b) Soil bacterium augment Arabidopsis photosynthesis by decreasing glucose sensing and abscisic acid levels in planta. Plant J 56:264–273

Zhang H, Sun Y, Xie X, Kim MS, Dowd SE, Pare RW (2009) A soil bacterium regulates plant acquisition of iron via deficiency-inducible mechanisms. Plant J 58:568–577

Zhang H, Murzello C, Sun Y, Kim MS, Xie X, Jeter RM, Zak JC, Dowd SE, Pare PW (2010) Choline and osmotic-stress tolerance induced by the soil microbe *Bacillus subtilis* (GB03). Mol Plant Microbe Interact 23:1097–1104

Chapter 17
Exopolysaccharides of *Paenibacillus polymyxa* Rhizobacteria in Plant–Bacterial Interactions

Irina V. Yegorenkova

17.1 Introduction

Among the myriads of bacteria thriving in the plant rhizosphere, some spore-forming plant-growth-promoting rhizobacteria (PGPR), in particular gram-positive bacilli and streptomycetes, have attracted special attention because of their advantages over non-spore formers in product formulation and stable maintenance in soil (Emmert and Handelsman 1999). Among these, the genus *Paenibacillus* (species of a genus previously included in the genus *Bacillus*, Ash et al. 1993; Trüper 2005) comprises more than 130 species with the type species *Paenibacillus polymyxa*.

The nitrogen-fixing soil rhizobacteria *P. polymyxa* promote the growth and development of a wide range of plants through the establishment of effective associative relationships. This has been associated with the capacities of these microorganisms for nitrogen fixation, phosphate mobilization, and production of phytohormones, antibiotics (Mannanov and Sattarova 2001), and a wide range of lytic enzymes, as well as with their high adaptability to living conditions (Lebuhn et al. 1997; Da Mota et al. 2002; Lal and Tabacchioni 2009). It has been proven experimentally that in association with plants, *P. polymyxa* can increase plant resistance to biotic and abiotic stresses (Timmusk and Wagner 1999; Khan et al. 2008; McSpadden Gardener 2004; Selim et al. 2005; Timmusk et al. 2005). Some investigators believe that in this process, a major role is played by *P. polymyxa*'s capacities for effective colonization and biofilm formation (Haggag and Timmusk 2008; Timmusk et al. 2009b; Haggag 2010). Certain strains not only colonize the surface of roots (Bent et al. 2002) but also penetrate the root interior (Shishido et al. 1999).

I.V. Yegorenkova (✉)
Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences (IBPPM RAS), 13 Prospekt Entuziastov, Saratov 410049, Russian Federation
e-mail: room406@ibppm.sgu.ru

D.K. Maheshwari et al. (eds.), *Bacteria in Agrobiology: Crop Productivity*,
DOI 10.1007/978-3-642-37241-4_17,

P. polymyxa is capable of producing acidic and neutral exopolysaccharides (EPSs; Matora et al. 1992; Hebbar et al. 1992; Lee et al. 1997; Jung et al. 2007), which have unparalleled properties. This explains the diversity of spheres of possible application of these polymers. In addition, *P. polymyxa* exoglycans have been assigned an important role in the formation of plant–microbe associations (Hebbar et al. 1992; Bezzate et al. 2000; Timmusk et al. 2005; Haggag 2007). *P. polymyxa* is widely used as the major component of complex bacterial fertilizers, enriching the environment with excreted polysaccharides (PSs), whose effect on humans and animals is not quite known yet.

The surface localization of the extracellular PSs confers on them the properties of mediators in the interaction of *P. polymyxa* with other micro- and macroorganisms. In addition, by forming a dense layer on the bacterial surface, EPSs may shield other cellular structures underneath them and may also determine bacterial immunological properties. Several investigators (Jung et al. 2007; Chang et al. 2009, 2010) have shown that the EPSs of *P. polymyxa* are biologically active substances (BASs) with an immunomodulatory action.

Despite the intensity of research on these bacterial PSs and the considerable progress made toward elucidating their physiological role, the properties and the chemical structure of a wide range of EPSs remain to be fully clarified. A thorough study of these biopolymers will allow uncovering the functional linkages between the structure of exoglycans and their biological role, which may facilitate a deeper understanding of the molecular foundations of intercellular, interspecies, and interorganismal interactions.

17.2 Ecology and Biotechnological Potential of *P. polymyxa*

P. polymyxa has attracted considerable interest because of its great biotechnological potential in different industrial processes and in sustainable agriculture.

17.2.1 Morphological and Physiological Peculiarities

The genus *Paenibacillus* was created by Ash et al. (1993) to accommodate the former "group 3" of the genus *Bacillus*. *Paenibacillus* species are facultatively anaerobic, endospore-forming, neutrophilic, periflagellated heterotrophic, and low G+C gram-positive bacilli (Euzéby 2011). In Latin, *paene* means *almost*, and therefore the *Paenibacillus* is almost a *Bacillus*. Comparative 16S rRNA sequence analyses revealed that rRNA group 3 bacilli represent a phylogenetically distinct group, exhibit high intragroup sequence relatedness, and are only remotely related to *B. subtilis*—the type species of the genus *Bacillus*. The taxon contains various species such as *B. alvei*, *B. amylolyticus*, *B. azotofixans*, *B. gordonae*, *B. larvae*,

B. macerans, *B. macquariensis*, *B. pabuli*, *B. polymyxa*, *B. pulvifaciens*, and *B. validus* (Ash et al. 1991).

Phenotypically, species of this group react weakly with Gram's stain and even young cultures appear gram negative. They differentiate into ellipsoidal spores, which distinctly swell the mother cell. The combination of morphology and physiology is sufficient to distinguish rRNA group 3 bacilli from all other mesophilic species of *Bacillus* with the exception of *B. circulans*, *B. lautus*, *B. lentimorbus*, and *B. popilliae*. The latter four species are, however, phylogenetically only remotely related to *B. polymyxa*; and its relatives and the described rRNA group 3 specific gene probe provide an unequivocal method for distinguishing these taxa (Ash et al. 1993). Among the 51,713 Firmicutes sequences listed in Ribosomal Database Project II, the family *Paenibacillaceae* comprises 1,057 16S rRNA sequences with 74 as *P. polymyxa* (as on January 2008) (Lal and Tabacchioni 2009). Strains of *P. polymyxa* (the type species of the genus) were found to be capable of suppressing several plant diseases and promoting plant growth (Benedict and Langlykke 1947).

Kim et al. (2010) presented the complete genome sequence of *P. polymyxa* E681. Its 5.4-Mb genome encodes functions specialized to the plant-associated lifestyle and characteristics that are beneficial to plants, such as the production of a plant growth hormone, antibiotics, and hydrolytic enzymes. The complete genome sequence of an important plant-growth-promoting rhizobacterium, *P. polymyxa* SC2, was reported by Ma et al. (2011), who found multiple sets of functional genes in the genome.

P. polymyxa occurs widely in water, soil, and the rhizosphere (Von der Weid et al. 2000; Guemouri-Athmani et al. 2000; Cheong et al. 2005). *P. polymyxa* spores cause sporangium deformation and have thick walls with a star-shaped section. These can remain in a dormant state for long periods, being resistant to heat, drying, radiation, and toxic chemicals (Comas-Riu and Vives-Rego 2002).

17.2.2 Practical Use

Ever since it became known that *P. polymyxa* can elaborate certain antibiotics, this microorganism has continued to generate increased interest owing to the promise for use that it shows. The high activity of N_2 fixation and phosphate mobilization in *Paenibacillus* was a prerequisite to the use of these bacteria as a biofertilizer component (Kozyrovskaya et al. 2005). By now, technologies have been developed for the manufacture and use of *P. polymyxa*-based biopreparations [biopolitsid (BSP) and polimiksobakterin], which have found wide application in Ukrainian agriculture. BSP is based on *B. polymyxa* strain P, which is antagonistic to a wide range of phytopathogenic fungi, including such widespread crop pathogens as *Bipolaris sorokiniana*, *Fusarium avenaceum* Sacc., *F. graminearum*, *Trichothecium roseum*, *Ascochyta pisi* Lib., *Cercosporella herpotrichoides* Fron., *Colletotrichum gloeosporioides* Penz., *Phomopsis leptostromiformis* Bubak,

Rhizoctonia violaceae Tul., and *Sclerotinia sclerotiorum* Lib. De Bar (http://www.ecobiology.com.ua). These preparations are polyfunctional in that they promote transformation of plant-unavailable P-containing mineral and organic compounds, defense against plant pathogens (through the formation of antibiotics), effective use of biological N, and enhancement of soil fertility. Similarly, in India, a consortium, named an Indian Agricultural Research Institute (IARI) microphos culture, was developed that contains two very efficient phosphate-solubilizing bacteria (*Pseudomonas striata* and *B. polymyxa)* and three phosphate-solubilizing fungi (*Aspergillus awamori*, *A. niger*, and *Penicillium digitatum*) (Gaur 1990).

Some investigators believe that *Paenibacillus* bacteria stimulate the growth and development of a wide range of plants (cereals, conifers, legumes, etc.) (Bent et al. 2002; Timmusk et al. 2005), improve the germinability of cultivated plants (Gupta et al. 2000), and are able to degrade pesticides and insecticides. *P. polymyxa* exhibits clear antagonistic activity against soilborne fungal and oomycetic pathogens (Dijksterhuis et al. 1999; Timmusk 2003; Ryu et al. 2006; Choi et al. 2008; Haggag 2007) (Table 17.1).

In recent years, investigators' attention has also been turned to nonagriculture-related possibilities of using *P. polymyxa*. These include biosorption of metals (copper) from polluted soils (Piuri et al. 1998; Philip et al. 2000; Acosta et al. 2005; Chu and Kim 2006); degradation of toxic substances, based on *P. polymyxa*'s ability to degrade phenanthrene and chlorobenzene (Daane et al. 2001; Vogt et al. 2004); wastewater purification, owing to the ability of these bacteria to decompose organic waste (Chockalingam et al. 2003); biosynthesis of a range of BASs (Mavingui and Heulin 1994; Jung et al. 2007); production of enzymes (Budi et al. 2000; Alvarez et al. 2006), e.g., inulinase, which is used in the manufacture of glucose–fructose syrups (Zherebtsov et al. 2003); cellulose degradation (in combination with cellulolytic bacteria) (Gorska et al. 2001); and large-scale production of medicinal antibiotics (Nakajima et al. 1972; Girardin et al. 2002; Zengguo et al. 2007; Tupinambá et al. 2008). The bacterium displays inhibitory activity against human and animal pathogenic microorganisms (Rosado and Seldin 1993; Seldin et al. 1999; Alvarez et al. 2006; Ravi et al. 2007) (Table 17.1).

There are data on the utility of *P. polymyxa* for the building industry and for mining operations, owing to the ability of *Paenibacillus* to adhere to minerals and degrade them (Patra and Natarajan 2004, 2006). Furthermore, predictions have been made that the use of the latest achievements of biotechnology may lead to some fermentation processes becoming competitive with the preparation of the same products (ethanol, butanol, butanediol) from petroleum. Because of its nonpathogenicity, genetic stability, and ability to ferment variously composed polysaccharides of plant raw material, *P. polymyxa* has been assigned to the group of potentially industrial microorganisms, 2,3-butanediol producers (Ui et al. 1983; Nakashimada et al. 1998; Syu 2001) (Table 17.1).

Thus, despite the existing limited information on the genomes of *P. polymyxa*, the past few decades have seen a growing interest in these bacteria owing to their great biotechnological potential in various industrial processes and in agriculture (Lal and Tabacchioni 2009).

Table 17.1 Characteristics of *P. polymyxa*

Strain	Origin	Activity	References
P. polymyxa strains B1 and B2	Wheat rhizosphere	Nitrogen fixation	Lindberg et al. (1985)
P. polymyxa CF43	Wheat rhizosphere	Enhancement of soil porosity	Gouzou et al. (1993)
P. polymyxa PMD216 and PMD230	Wheat rhizoplane	Production of auxin and other indolic and phenolic compounds	Lebuhn et al. (1997)
P. polymyxa PMD112 and PMD128	Wheat rhizosphere		
P. polymyxa PMD66	Soil		
P. polymyxa strain B2	Wheat rhizosphere	Cytokinin production	Timmusk et al. (1999)
P. polymyxa strains B5 and B6	Soil around peanut roots	Production of EPSs, biocontrol of *Aspergillus niger* in the roots and seeds of peanut plants	Haggag (2007), Haggag and Timmusk (2008)
P. polymyxa SCE2	Soil (Brazil)	Protease production, production of antimicrobial compounds active against human pathogenic microorganisms	Rosado and Seldin (1993), Seldin et al. 1999; Alvarez et al. (2006)
P. polymyxa strains CM5-5 and CM5-6	Barley rhizosphere	Production of hydrolytic enzymes, multitarget and medium-independent type of fungal antagonism	Nielsen and Sorensen (1997)
P. polymyxa	Soil, wheat rhizosphere and rhizoplane	Production of chitinase	Mavingui and Heulin (1994)
B. polymyxa ATCC842T	–	Production of xylanase	Budi et al. (2000)
P. polymyxa EJS-3	Root tissue of *Stemona japonica*	Production of fibrinolytic enzymes	Lu et al. (2007)
P. polymyxa ATCC 12321	Spoiled starch	2,3-Butanediol (BDL) production	Ui et al. (1983), Syu (2001)
P. polymyxa T129	Soil	Biocontrol against *Fusarium oxysporum*	Dijksterhuis et al. (1999)
P. polymyxa strains B5 and B6	Wheat rhizosphere	Biocontrol of the oomycete plant pathogens *Phytophthora palmivora* and *Pythium aphanidermatum*	Timmusk et al. (2003)
P. polymyxa strain GBR-1	–	Suppresses root-knot nematodes	Khan et al. (2008)
P. polymyxa strains B2, B3, and B4	Wheat rhizosphere	Increased resistance to plant pathogens (biotic stress) and drought resistance (abiotic stress)	Timmusk and Wagner (1999)

(continued)

Table 17.1 (continued)

Strain	Origin	Activity	References
P. polymyxa JB115	Soil	Production of β-glucan	Jung et al. (2007)
P. polymyxa JB115	Soil	β-Glucan as an immunostimulant or adjuvant for certain animal vaccines	Chang et al. (2009, 2010)
P. polymyxa 1460	Soil	Production of lectin	Karpunina et al. (2003)
P. polymyxa E681	Winter barley roots	Fusaricidin biosynthesis, biocontrol of fungal pathogens on sesame plants	Choi et al. (2008), Ryu et al. (2006)
P. polymyxa OSY-DF	Fermented foods	Coproduction of polymyxin E1 and lantibiotic	He et al. (2007)
P. polymyxa strain M	Marine sediment	Antagonistic activity against *Vibrio* species	Ravi et al. (2007)
P. polymyxa P13	Fermented sausages	Polyxin production and biosorption of heavy metals	Piuri et al. (1998), Acosta et al. (2005)
P. polymyxa BY-28	Soil	Flocculant production	Gong et al. (2003)
P. polymyxa strains B1 and B2	Wheat rhizosphere	Biofilm formation	Timmusk et al. (2005)
P. polymyxa strains B2, B5, and B6	Wheat rhizosphere, peanut rhizosphere	Biofilm formation, antagonistic activity against the oomycete plant pathogens *Phytophthora palmivora* and *Pythium aphanidermatum*	Timmusk et al. (2009b)
P. polymyxa 1465	Soil	Production of EPSs, colonization of wheat-seedling roots	Yegorenkova et al. (2008, 2010)
P. polymyxa 1465, *P. polymyxa* 92	Soil, wheat roots	Production of EPSs, biofilm formation	Yegorenkova et al. (2011)
P. polymyxa SC2	–	Broad-spectrum antimicrobial activity	Ma et al. (2011)
P. polymyxa MB02-1007	Mycorrhizal or nonmycorrhizal systems	Biocontrol of *Ralstonia solanacearum* in tomato	Algam et al. (2010)

Adapted from Lal and Tabacchioni (2009)

17.2.3 Plant Growth Promotion

P. polymyxa occurs widely in various climatic zones and is found in chernozemic, brownearth, serozemic (gray), krasnozemic (red), and sod-podzolic soils. *P. polymyxa* strains have been isolated from the rhizosphere of a variety of crops such as wheat (*Triticum aestivum*), barley (*Hordeum gramineae*; Lindberg et al. 1985), white clover (*Trifolium repens*), perennial ryegrass (*Lolium perenne*), crested wheatgrass (*Agropyron cristatum*; Holl et al. 1988), lodgepole pine *(Pinus contorta latifolia*;

Holl and Chanway 1992), douglas fir (*Pseudotsuga menziesii*; Shishido et al. 1996), green bean (*Phaseolus vulgaris*; Petersen et al. 1996), and garlic (*Allium sativum*; Kajimura and Kaneda 1996). In the wheat root zone, these bacteria may predominate over other N_2-fixing anaerobes, and they have a leading role in the accumulation of N in soils (Döbereiner 1977).

P. polymyxa is grouped with PGPR (Timmusk and Wagner 1999; Haggag 2007). Extensive results are available on the effect of *P. polymyxa* inoculation on the yields of major cereal crops, including wheat, barley, rice, sorghum, millet, and maize (Chanway 1995; Maes and Baeyen 2003). Data from growth chamber experiments have been published concerning yields and N assimilation in winter wheat inoculated with various rhizobacteria. It was shown that *P. polymyxa* inoculation promotes an increase in grain yield (De Freitas 2000). A considerable effect on the growth and yield of wheat and maize was found with a certain plant–bacterial combination and was absent with another combination, demonstrating the existence of an interrelation between the plant genotype and the bacterial strain (Renni and Thomas 1987; Chanway et al. 1988; Da Mota et al. 2002). The most N accumulation was observed when a wheat cultivar was inoculated with *P. polymyxa* isolated from its rhizosphere (Renni and Thomas 1987). Several authors have reported a large positive effect from the introduction of *P. polymyxa* strains into the plant rhizosphere, considering such parameters as plant viability and weight, the concentration of chlorophyll in the leaf mesophyll, the state of the root, and the formation of root hairs. Seed treatment with *P. polymyxa* resulted in better seed germinability and in faster seedling growth (Maes and Baeyen 2003).

Despite the numerous studies of plant interactions with associative N_2-fixing and growth-promoting bacteria, there have so far been no reliable predictions of plant response to inoculation. This response, however, may vary from positive or neutral to negative; and *P. polymyxa* may have adverse effects on plants. For example, when roots of *Arabidopsis thaliana* were soaked for 24 h in cultures of *P. polymyxa* strains B2, B3, and B4 in L medium, plants responded with 30 % growth reduction and a stunted root system, compared to uninoculated plants. These effects were observed in a gnotobiotic system and in soil, pointing to a mild pathogenic effect (Timmusk and Wagner 1999; Timmusk 2003). Consequently, under these conditions, *P. polymyxa* can be considered a deleterious rhizobacterium (DRB). Furthermore, inoculation with *P. polymyxa* strain L6-16R promoted growth of lodgepole pine in one location, inhibited it in a second site, and had no discernible effect in a third site (Chanway and Holl 1994).

The inconsistency of results from *P. polymyxa* inoculations gave impetus to new research on the use of combined inoculation of bacilli and other microorganisms. Combined inoculation of plants with associative bacteria of different genera is one of the most advanced technologies in agriculture. Skvortsova et al. (1998), using cereal grasses, studied the effect of inoculation with two-component cultures composed of *B. polymyxa* and various *Pseudomonas* strains on N_2 fixation, denitrification, and heterotrophic nitrification. Inoculation with such cultures not only produced substantial increases in yields but also significantly enhanced crop N

content. More specifically, yields increased up to 25–77 %. Combined inoculation with *Azospirillum brasilense*, *Azotobacter chroococcum*, *B. polymyxa*, and *Enterobacter cloacae* was found to have positive effects on the yield, dry weight, and total nitrogen of winter wheat (De Freitas 2000); and *P. polymyxa* inoculation, alone or in combination with *Rhizobium*, enhanced the growth of lentil and protected the plant against *Meloidogyne javanica* nematodes (Siddiqui et al. 2007).

For a long time, the beneficial effect of associative rhizosphere bacteria has been attributed largely to fixation of molecular N (a parallel drawn with symbiotic N_2 fixation). In *P. polymyxa*, however, N_2 fixation makes only a partial contribution to the stimulation of plant growth (Chanway and Holl 1991). Apart from the improvement of N nutrition, these bacteria have other mechanisms responsible for the positive effect on plants (Costacurta and Vanderleyden 1995). Several authors have reported that *P. polymyxa* produces hormones of the cytokinin group (Timmusk et al. 1999) and auxins, specifically indole-3-acetic acid (IAA) (Holl et al. 1988; Lebuhn et al. 1997). Treatment with auxins accelerated bacterial colonization of roots and promoted the formation of paranodules (Narula et al. 2006). Lebuhn et al. (1997) examined the actual and potential abilities to form indolic and phenolic compounds on different media in *P. polymyxa* isolated at different distances from the roots of wheat. They observed a gradual decrease in the potential for IAA production by the strains isolated from nonrhizosphere soil, as compared with those from the rhizosphere and the rhizoplane. These metabolic differences indicate that near plant roots, *P. polymyxa* subpopulations undergo selection for genetic and physiological parameters.

Most studies of biological variability within the *P. polymyxa* species have pointed out the influence of various factors on the degree of bacterial genetic polymorphism. Specifically, a study of the effect of plant development stages on a population of *P. polymyxa* in the maize rhizosphere demonstrated that the population observed in the middle stage of plant growth (30–60 days after planting) was more homogeneous than that in the initial stage (10 days) or after 90 days of maize growth (Von der Weid et al. 2000). Long-term cultivation of wheat on Algerian soils (>70 years) was reported to change the rhizosphere population of *P. polymyxa*, increasing its size, decreasing bacterial diversity, choosing the dominant genotype, and enhancing N_2 fixation (Guemouri-Athmani et al. 2000).

The mechanism of *P. polymyxa*'s stimulatory effect on plants is not quite clear yet. It is believed that the effectiveness of plant–*P. polymyxa* associations is determined by such bacterial characteristics as N_2-fixation ability (Lindberg et al. 1985); production of phytohormones (Holl et al. 1988; Lebuhn et al. 1997; Timmusk et al. 1999), antibiotics (Rosado and Seldin 1993), hydrolytic enzymes (Nielsen and Sorensen 1997), and exopolysaccharides (Hebbar et al. 1992; Bezzate et al. 2000; Timmusk et al. 2005; Haggag 2007; Yegorenkova et al. 2010); and improvement of the mineral nutrition and aquatic balance of inoculated plants through phosphate mobilization (Singh and Singh 1993) and soil structure amelioration (Bezzate et al. 2000; Czames et al. 2000).

The significance of the above mechanisms of *P. polymyxa*'s effect on plants is different under different conditions. Apart from climatic factors, a large role is

played by the species and strain characteristics of the bacteria used and by the species and cultivar peculiarities of the plants used. It is believed that growth-promoting factors should be considered collectively, because trying to emphasize the role of any one may lead to a substantial understatement of the effect of each of them (Bashan and Holguin 1997).

17.3 Role of EPSs in the Formation of Plant–Bacterial Associations

Microbial EPSs are the primary or secondary metabolites produced by a variety of microorganisms. These EPSs have been widely used within bioindustries, because the production cost of microbial EPSs is lower than that of algal or plant polysaccharides (Kumar et al. 2007). Additionally, microbial EPSs are nontoxic, biodegradable, and environmentally benign (Shoda and Sugano 2005). *P. polymyxa* elaborates a broad range of neutral and acidic exopolysaccharides (Matora et al. 1992; Hebbar et al. 1992; Lee et al. 1997; Haggag 2007; Jung et al. 2007), which have diverse structures and physical–chemical properties. Most of them are of low or no toxicity. The EPSs of *P. polymyxa* are BASs with immunotropic activity (Jung et al. 2007). They have been assumed to be essential for the development of plant–microbial associations (Bezzate et al. 2000; Timmusk et al. 2005; Haggag 2007).

17.3.1 Physical–Chemical Characterization, Properties, and Use of P. polymyxa *EPSs*

P. polymyxa can synthesize neutral polysaccharides [levan (Iman and Abd-Allah 1974; Han 1989), mannan (Ball and Adams 1959), and glucan (Jung et al. 2007)], acidic polysaccharides, or heteropolysaccharides (Ninomiya et al. 1968a; Glukhova et al. 1986; Matora et al. 1992; Yegorenkova et al. 2008).

Among other bacteria, *P. polymyxa* stands out as one of the most active levan producers. The levans are 2,6-bonded, sometimes branched, regular polymers with a repeating unit (Han 1989, 1990). The levan of *P. polymyxa* can be used to suppress allotransplant rejection; to prolong the action of pharmaceutics; to act as an immunomodulator or a plasma substitute; to increase soil water capacity; to improve plant seed germinability (Iman and Abd-Allah 1974); to prepare pure fructose (Tkachenko and Sevryugina 1989); and to encapsulate substances in the manufacture of cosmetics and in paper and fabric printing (Han 1990). *P. polymyxa* JB115 was isolated from Korean soil as a glucan producer for the development of animal feed additives. As shown by IR, ^{1}H NMR, and ^{13}C NMR spectroscopy, the JB115 glucan is a linear glucan that has β-(1→3) and β-(1→6) structure.

High-molecular-weight glucan (above 100 kDa) can be used as an animal feed additive for immune enhancement and as a potential antitumor agent for livestock (Jung et al. 2007; Chang et al. 2009).

The heteropolysaccharides of *P. polymyxa* have diverse compositions, structures, and properties. The bacteria *B. polymyxa* 458, isolated in Japan, synthesize a highly viscous, nontoxic EPS that is composed of residues of glucose (Glc), mannose (Man), and glucuronic acid (GlcA) at a 7:7:2 molar ratio (Mitsuda et al. 1981). Heating and cooling EPS solutions (>0.7 %) gives rise to agar-like gels, but their strength is slightly inferior to that of gels of the same concentration. *P. polymyxa* strain S-4 produces an acidic EPS that is composed of β-D-Glc, D-Man, D-galactose (Gal), D-GlcA, and D-ManA at a 3:3:1:2:1 ratio. In the main chain of this polymer, Glc and Gal are (1→3)-bonded, Man and Gal are (1→4)-bonded, and GlcA and Man are (1→3)-bonded; the side chains contain Glc, Man, and ManA, which are mostly (1→4)-bonded. The EPS has an "antisclerotic" action by decreasing the concentration of cholesterol in blood and the liver (Fukui et al. 1985).

When grown on saccharose, *P. polymyxa* 271 (FERM P-1824), isolated from Japanese soil, synthesizes two EPSs—an acidic one and a neutral one (Ninomiya et al. 1968a). When grown on glucose, it produces only the acidic polymer, which is composed of D-Glc, D-Man, D-Gal, and D-GlcA at a 3:3:1:2 ratio (Ninomiya et al. 1968b). The neutral EPS is made up of Glc, Man, Gal, and fructose (Fru). The molecular mass (M_m) of the acidic EPS is greater than 1 MDa, and the EPS forms highly viscous aqueous solutions (Ninomiya and Kizaki 1969). An important peculiarity of this EPS is that it forms a stable viscoelastic gel with 40 % ethanol, with the polymer concentration being 2 %. This polymer has both a technical and a pharmacological action. The EPS and its cationic forms lower the concentrations of lipids and cholesterol in blood and the liver, and they reduce the atherogenic index, reducing the probability of atherosclerosis and myocardial infarction (Tanaka et al. 1982).

P. polymyxa mutant strain 1459B excretes two EPSs, one being acidic, viscous, and of a high M_m and the other being weakly viscous, neutral, and of a lower M_m (Glukhova et al. 1986). The ratio between the EPSs depends on the source of carbon in the medium. The neutral EPS is a levan, and the acidic EPS is made up of Glc, Gal, Man, and GlcA residues and trace amounts of arabinose and xylose (Xyl) (Glukhova et al. 1986). Solutions of 1459B EPS are compatible with high concentrations of mono- and bivalent cations and Al^{3+} at pH 3–11. The acidic EPS forms thermolabile elastic gels. It was suggested that the levan of *P. polymyxa* 1459B be used for the preparation of pure fructose, as an immunomodulator, and as a blood substitute, and that the acidic EPS be used for increasing oil reservoir recovery, preparing drill fluids, and regulating the rheological properties of freshwater and mineral water solutions.

A new highly viscous EPS, named polymyxan, was described by Matora et al. (1992). It is synthesized by the producer strain *P. polymyxa* 88A, obtained by short-term treatment with intense microwave radiation at a frequency of 2,375 MHz. Polymyxan consists of an acidic, highly viscous PS (M_m of 1–10 MDa, composition

of 35 % Glc, 36 % Man, 7 % Gal, and 21 % GlcA) and a neutral, low-viscous PS (M_m of 100–300 kDa), which is a glucomannan with equal contents of both monosaccharides and with trace amounts of uronic acids. The structure of this EPS is believed by the authors to be irregular. Data were presented on the use of polymyxan in bread making and also as a polymeric agent for the preparation of drill fluids and the conservation of wells (Matora et al. 1992).

Hebbar et al. (1992) established that *P. polymyxa* ATCC 842 and ATCC 21551 synthesize EPSs composed of Glc, Gal, Man, uronic acids, pyruvate, acetate, and succinate. The EPSs of batch-cultivated *P. polymyxa* 1465 were found to contain neutral and acidic fractions and to be heterogeneous PSs represented by a complex of macromolecules with M_ms ranging from 7×10^4 to 2×10^6 Da (Yegorenkova et al. 2008). When the bacteria were grown on glucose, the acidic component predominated, which correlated with the higher viscosity of aqueous solutions of the EPSs. The exoglycans were found to contain Glc, Man, Gal, and uronic acids. Rabbit polyclonal antibodies were developed to an isolated EPS of *P. polymyxa* 1465, and the presence of common EPS antigenic determinants within the species *P. polymyxa* was shown (Yegorenkova et al. 2008).

P. polymyxa strain P13 was described as an EPS producer by Acosta et al. (2005). Those authors found that 100 ml of a stationary-phase P13 culture formed 27 (±4) mg (±SD) and 15 (±4) mg (±SD) EPS in BHI medium containing 1 M NaCl and in control BHI medium, respectively. This strain exhibited a significant capacity for biosorption of Cu(II) originating from several industries. EPS production was associated with hyperosmotic stress caused by high salt content (1 M NaCl), which led to a significant increase in the biosorption capacity of whole cells (Acosta et al. 2005) (Table 17.1). The adsorption of *P. polymyxa* cells or their EPSs on the surface of several minerals has been reported as a method to selectively separate metal ions from a binary mixture such as sphalerite and galena, galena and pyrite, suggesting their use in biomineral processing by means of microbial flotation and flocculation (Deo and Natarajan 1998; Patra and Natarajan 2004, 2006).

Analysis of the literature data shows that the process of exoglycan biosynthesis and their monosaccharide composition are highly labile—the yield of EPSs, their composition, and their physical–chemical properties depend on several factors (Matora et al. 1992; Lee et al. 1997; Yegorenkova et al. 2008). Sutherland (1972, 1994) examined the interrelation between the structure of polysaccharides and their physical characteristics and functions. There is need for accumulation of data on the chemical structure of these important macromolecules before any justified inferences about the functions of concrete glycopolymers can be made.

The richness of the microbial world determines the diversity of the structures and physical–chemical and biological properties of EPSs, which dictates the possibility of their wide use. Microbial EPSs can be used as an alternative to the traditionally applied synthetic or natural polymers and can also be considered to be new polymers (Sutherland 1986). PSs have already found application in several fields, including environmental management (soil cleanup from petroleum residues), the petroleum industry (enhancement of the effectiveness of petroleum production), metallurgy (involvement in the extraction, processing, and

beneficiation of ore) (Santhiya et al. 2002; Acosta et al. 2005), agriculture (enhancement of crop capacity and soil fertilization), food production (emulsifiers, biofilms, and thickeners) (Matora et al. 1992; Moon et al. 2006), the cosmetic industry (emulsions), and medicine (blood plasma substitutes, drug carriers, and drug components) (Zanchetta et al. 2003). This series will be extended as polysaccharides and their active producers come to be better understood.

Here, I present briefly a selection of data on the EPSs of *P. polymyxa*, because a more detailed consideration would fall outside the scope of the problem being dealt with.

17.3.2 The Capacity of P. polymyxa *for Plant Root Colonization and Root Hair Deformation*

Effective colonization of plant roots by associative bacteria and the maintenance of population size at an ecologically significant level play an important role in plant growth promotion, regardless of the mechanism of action (production of metabolites and of antibiotics against phytopathogens, stimulation with nutrients, or induction of plant resistance) (Timmusk et al. 2005).

17.3.2.1 Attachment to Roots

Notwithstanding the fact that biological control has been used for decades, its use has not been consistent, possibly because its nature and action have not been understood fully (Gamalero et al. 2003). The plant root is not a passive target for soil organisms (Timmusk et al. 2005); therefore, it became necessary to accumulate experimental data concerning the mechanisms responsible for the formation of plant–bacterial associations. The methods used for the study of root colonization by growth-promoting bacteria have been covered in sufficient detail in a review by Gamalero et al. (2003). The endophytic colonization of seedling roots by *P. polymyxa* has been studied with fir (Shishido et al. 1999), pine (Bent et al. 2002), and *Arabidopsis* (Timmusk et al. 2005). Visualization of *P. polymyxa* through FITC-labeled antibodies has demonstrated that this bacterium can colonize the surface of roots (Bent et al. 2002) and can penetrate the root interior (Shishido et al. 1999). *P. polymyxa* was found accumulating in the intercellular spaces outside the vascular cylinder. According to the data of several authors, there was no dispersal at a systemic level, because the bacteria were found to be absent from aerial tissue (Timmusk et al. 2005).

Timmusk et al. (2005), using fluorescence stereomicroscopy, examined the localization of *P. polymyxa* strains B1 and B2 and the formation of biofilms on plant roots in model experiments and in soil systems. They found that colonization begins at the root tip, where the bacteria form microcolonies composed of cells and

a semitransparent matrix. Subsequently (within 2 h), the microcolonies spread over the surface and aggregate, forming biofilms. Root invasion was observed after 5 h of contact, and in a longer period of time, the differentiation zone of the root was colonized. It seems logical that the rhizobacteria predominated at the sites at which the amount of nutrients was greatest and at which nutrient inflow was associated with young plant tissues. Root exudates, secretions, and/or lysates accumulate in such root regions as the tip, the root hairs, and the epithelial cracks at the sites of recent lateral-root formation (Timmusk et al. 2005). The greatest density of the bacteria occurred on the surface of young root tissues, which possibly has to do with the greater intensity of the physiological processes occurring in them (Bent et al. 2002). Timmusk et al. (2005) demonstrated that *P. polymyxa* colonizes the root regions targeted by phytopathogens, thereby keeping these bacteria from accessing the plant and fulfilling a protective function. The polysaccharides produced by *P. polymyxa* are highly complex, and only few organisms may possess the specific enzymatic machinery for their degradation, e.g., *P. polymyxa* itself (Bezzate et al. 1994). For investigating bacterial interactions in natural systems, real-time PCR for the rapid detection of biofilm-forming bacteria was also developed (Timmusk et al. 2009a).

According to the data of many investigators, the complex process of plant–bacterial interaction begins in mucigel, which covers the plant root hairs in large quantities. The interaction of *Paenibacillus* lectins with the carbohydrate moiety of the wheat-root exocomponent fraction changed the enzyme activity of the lectins, as did the interaction with carbohydrate preparations (Karpunina et al. 2003). The authors believe that in the contact of bacteria with plants, a large role is initially played by the adhesive properties of bacterial lectins, which are realized through lectin interactions with the specific sugars present in mucigel.

The formation of N_2-fixing systems calls for a physical and functional interaction between bacterial and plant cells, in which, along with adhesion, a great role is played by enzymatic processes. *P. polymyxa* has complex specific relationships with its plant host at a molecular–genetic level, altering the expression profile for the host's genes (Timmusk and Wagner 1999). There have been reports of stimulation of the activities of chitinase and β-1,3-D-glucanase (Haggag 2007; Algam et al. 2010) and of glucose-6-phosphate dehydrogenase, glutathione reductase, and glutathione S-transferase (Cakmakci et al. 2007) in *P. polymyxa*-inoculated plants. It is known that increased chitinase and β-1,3-D-glucanase activities in plants correlate with resistance to phytopathogens (Timmusk and Wagner 1999). Numerous publications attest that besides plant hydrolytic enzymes, the degradation (hydrolysis) of the plant cell wall involves the work of the hydrolytic enzymes of certain soil bacteria (Ljunggren and Fåhraeus 1961; Hubbell et al. 1978; Tien et al. 1981). From the totality of experimental data obtained, some authors speculate that the penetration of N_2-fixing bacteria into plant root tissues is facilitated by *Rhizobium* agglutinins and *Paenibacillus* lectins, as well as by the enzymatic activity of rhizobial and bacillar cells (Karpunina et al. 2003).

Cell-associated extracellular rhizobial PSs, including lipopolysaccharides (LPSs), the acidic capsular polysaccharides (CPSs), and EPSs, have also been

considered as potential symbiotic factors. York et al. (1996) proposed that EPSs may be involved in cell attachment to plants and in plant infection, ensure protection against plant defense responses, act as signal molecules, and function similarly to flavonoids and lipochitooligosaccharides in the formation of symbiosis. A good example is the oversaturation of the genome of the alfalfa rhizobium *Sinorhizobium meliloti* with genes of EPS synthesis (Kahn et al. 2004). Owing to this, deletion mutants in the *exo* clusters can preserve their symbiotic properties, which are very important, as normal synthesis of EPSs is necessary for the development of nodules (Provorov et al. 2008). Many investigators (Michiels et al. 1991; Yegorenkova et al. 2001) believe that the EPSs of associative bacteria of the genus *Azospirillum* are also involved in the realization of contact between bacterial and plant cells. It has repeatedly been shown that azospirilla on plant roots or root hairs were observed as aggregates surrounded by mucigel or fibril-like material (Bashan et al. 1986; Okon and Kapulnik 1986).

In the root environment, i.e., the rhizosphere, bacterial EPSs contribute to soil aggregation by cementing particles together (Chenu 1995). Inoculation of plants with EPS-producing rhizobacteria, such as *Pantoea agglomerans* (Amellal et al. 1998), *Rhizobium* sp. YAS34 (Alami et al. 2000; Santaella et al. 2008), and *Rhizobium* sp. KYGT207 (Kaci et al. 2005), modifies the aggregation of root-adhering soil and eventually improves plant growth.

As said earlier, *P. polymyxa* can synthesize various EPSs, which are believed to play a large role in cell adhesion to diverse substrates (Deo et al. 2001; Vijayalakshmi and Raichur 2002; Sharma and Rao 2003) and in the formation of plant–microbe associations (Hebbar et al. 1992; Bezzate et al. 2000; Timmusk et al. 2005).

Gouzou et al. (1993) showed that inoculation of wheat with a rhizosphere strain of *P. polymyxa* increased the mass of soil adhering to the roots by 57 %. Comparison of aggregate size distributions suggested a more porous structure for the inoculated rhizosphere soil than for the uninoculated soil. Bezzate et al. (2000) tested the role of levan, a fructosyl polymer produced by strain CF43, in the aggregation of soil adhering to wheat roots. Inoculation of wheat roots with *P. polymyxa* CF43 increased the mass of root-adhering soil. The *P. polymyxa* gene homologous to the *B. subtilis sacB* gene encoding levansucrase was cloned and sequenced. The corresponding gene product synthesized a high-molecular-weight levan. In contrast, inoculation with *P. polymyxa* mutant strain SB03 had no effect on the mass of root-adhering soil, compared with the noninoculated treatment. *P. polymyxa* SB03 is a mutant whose gene encoding the enzyme for levan synthesis, *sacB*, was inactivated. Thus, the results strongly suggest that levan synthesis by strain CF43 is the main mechanism involved in the improvement of the structure of root-adhering soil (Bezzate et al. 2000). Soil structure determines the total volume of soil pores and their size distribution, geometry, and connectivity. The resulting properties of the soil and rhizosphere, such as aeration, resistance to root penetration, water reserves, and therefore water and solute movement, are essential parameters that control plant growth. The stability of soil structure is, therefore, one of the basic determinants of the quality of soil and the rhizosphere,

if not of ecosystem stability (Santaella et al. 2008). Consequently, inoculation by *P. polymyxa* can play an important role in water retention and nutrient transfer in the rhizosphere by increasing porosity.

The effect of two EPS-producing strains of *P. polymyxa* (B5 and B6) on the control of crown rot disease caused by *Aspergillus niger* on peanut was investigated (Haggag 2007; Haggag and Timmusk 2008). Both strains were inhibitory to *A. niger,* but strain B5 proved to be more active. Bacterial growth and protein and biopolymer production were evaluated. Carbohydrate analysis using various color reactions, infrared spectroscopy, and high-performance liquid chromatography revealed that the biopolymer is a homopolysaccharide consisting of various sugars, including Glc, Gal, Man, and Xyl. It was found that *P. polymyxa* B5 produces high levels of sugars compared to the other strain used (Haggag 2007). The ability of *P. polymyxa* to colonize the peanut rhizosphere was evaluated for 60 days (greenhouse) and 140 days (field experiment). The colonization efficiency of B5 was significantly higher than was that of B6 during the first 30 and 60 days (in the greenhouse and in the field experiment, respectively). In both experiments, the author observed a substantial increase in the number of peanut nodules and in plant growth and performance when seeds were treated with B5, as compared to treatment with B6 or to the control (untreated plants) (Haggag 2007).

Enzyme-linked immunosorbent assay (ELISA) with rabbit polyclonal antibodies developed to an isolated EPS of *P. polymyxa* 1465 was used to evaluate the colonization of wheat-seedling roots by this bacterium (Yegorenkova et al. 2010). The assay conditions were optimized for detection of the *P. polymyxa* EPS determinants forming part of the samples used (homogenates of inoculated roots). The dynamics of the immunoenzymatic revealing of specific polysaccharidic antigenic determinants in the samples' composition correlated with an increase in *P. polymyxa* numbers on the roots found by estimation of colony-forming units (Fig. 17.1). The dynamics of *P. polymyxa* attachment was similar to that found for other rhizosphere bacteria: the number of attached cells increased with an extension of the incubation time, and the cell number on the roots stabilized by 18–24 h of contact (Michiels et al. 1991; Zamudio and Bastarrachea 1994; Yegorenkova et al. 2001).

17.3.2.2 Morphological Changes in Root Hairs

The deformation of root hairs is one of the earliest responses of plants to the presence of bacteria in their environment. Some investigators (Gaskins and Hubbell 1979; Baldani et al. 1983) believe that deformation may serve as a quantitative measure of plant responsiveness to inoculation, i.e., it characterizes the activity of a given strain toward the plant. The morphological changes in roots that are induced by soil bacteria have been studied in sufficient detail for the legume–*Rhizobium* symbiosis (Halverson and Stacey 1986) and the plant–*Azospirillum* association (Okon and Kapulnik 1986). Several types of root hair deformations have been recorded, including branches of equal lengths, branches of different lengths, and

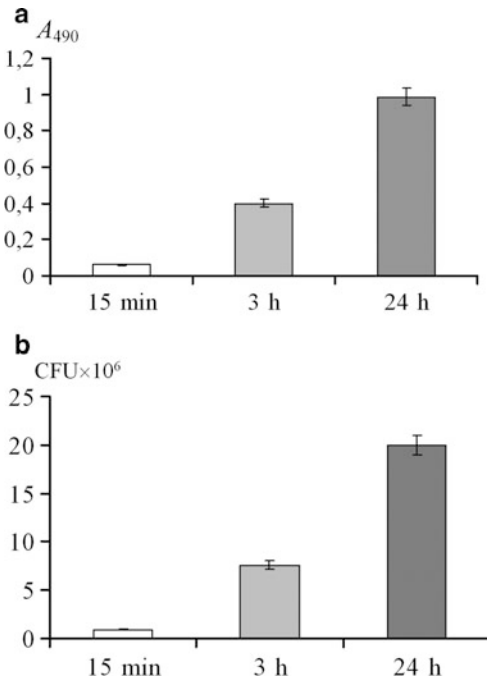


Fig. 17.1 ELISA determination of the number of specific bacterial antigenic determinants in homogenates of wheat roots inoculated with *P. polymyxa* 1465 (**a**), as compared with the results of CFU counting (**b**) (taken from Yegorenkova et al. 2010)

other deformations (curlings, swellings, wavy hairs, etc.). Symmetrical "tuning fork" branches have been observed mostly when homologous strains were used.

The compounds inducing such changes in roots are different in nature, including both high- and low-molecular-weight components. These compounds have been best studied for legume–rhizobial systems. It is known that *Rhizobium* EPSs can stimulate morphological changes in legume root hairs that are similar to the changes produced by whole bacterial cells (Halverson and Stacey 1986). The effect of low-molecular-weight compounds on the deformation of root hairs has been well documented (Patriquin et al. 1983). For *Azospirillum* bacteria, the inducers of morphological changes in root hairs have been found to include both low- and high-molecular-weight compounds. Patriquin et al. (1983) reported the induction of deformations by the supernatant liquid of *A. brasilense* Sp245. Several authors have described the influence on root morphology of the phytohormones produced by azospirilla (Tien et al. 1979) and also of the PS-containing complexes localized in the capsular material and excreted into the environment during bacterial growth (Konnova et al. 1995). It was established that the LPSs of azospirilla also induced deformations and that the changes in the LPS composition of the *A. brasilense* Sp245 outer membrane as a result of omegon insertion into the 120 MDa plasmid decreased the biological activity of the mutant strains toward wheat-seedling roots (Fedonenko et al. 2001). Boyko et al. (2011) concluded that the activity of the LPSs of serogroup 1 azospirilla toward wheat root hair morphology is determined by the fatty acid ratio and the length of the O chains and that in azospirilla whose

O-specific PSs are branched heteropolysaccharides, LPS activity also depends on the character of the side chain substituents.

The molecular mechanism of root hair deformation is fairly complex and almost unknown. Possibly, it includes a chain of reactions, one of which may be the interaction of the root surface receptors with the PS-containing complexes (or their components) on the bacterial surface (Konnova et al. 1995). In the opinion of Patriquin et al. (1983), the deformations may result from altered synthesis in the root hair walls or from stabilization of the cell walls during their growth.

There have been very few publications addressed to the study of the morphology of plant roots inoculated with *P. polymyxa*. When legume plants were inoculated with the cocultures *Rhizobium etli* and *P. polymyxa*, the latter indirectly (through the plant) promoted an increase in the population size of *R. etli* (Petersen et al. 1996). The authors observed an increase in the length of lateral roots and in the number of nodules in the plants. A similar effect was found upon dual inoculation of legumes with *Azospirillum* and *Rhizobium* (Andreyeva et al. 1993). This was explained by *Azospirillum* stimulation of nodule formation, nodule functioning, and possibly plant metabolism. The phytohormones produced by *Azospirillum* facilitate epidermal–cellular differentiation in root hairs, increasing the number of potential sites for rhizobial infection (Yahalom et al. 1991).

The ability of EPS preparations from several *P. polymyxa* strains to induce root hair deformations was studied with seedlings of wheat (cv. Saratovskaya 29). Fåhraeus's glass-slide technique was used to test the EPSs of several *P. polymyxa* strains, including 1465, 1460, 1459, 88A, and 92. It was demonstrated that the isolated EPSs can induce deformations with different intensities, which may count in favor of the assumption that the exoglycans of *P. polymyxa* have an active role in the formation of plant–microbe associations (Yegorenkova et al. 2013).

Among aerobic spore-forming bacteria of the genus *Bacillus*, a *B. subtilis* strain, IB-22, was revealed that excels at cytokinin production. In the culture liquid of this strain, a novel form of biologically active cytokinins was found for the first time: a complex formed between hormones and PSs (Arkhipova 1999). It was suggested that slow dissociation of cytokinins from this complex could have ensured the prolonged and nontoxic action on plants.

17.3.3 EPSs in Biofilms

In natural ecosystems and at industrial and healthcare facilities, microorganisms exist not as free-living cells suspended in their environment (plankton) but mainly as an organized community attached to various biotic and abiotic surfaces. Such communities are called biofilms (Davey and O'Toole 2000). A biofilm is characterized by cells that are attached to a surface or to one another, are enclosed in a matrix of extracellular polymeric substances synthesized by them, and demonstrate a phenotypic change manifested as a change in the growth parameters and in the expression of specific genes (Donlan and Costerton 2002). The development of

biofilm communities is a major strategy used by bacteria for survival in the external milieu. In biofilms, bacteria are stuck to each other by complex intercellular linkages and are functionally similar to multicellular organisms (Ilyina et al. 2004). The EPSs of the contributing microbial flora provide a major part of the dry matter of biofilms, flocs, and related structures. These polymers also play major roles in determining the physical properties and structures of the microbial agglomerations (Sutherland 2001).

17.3.3.1 Biofilm Formation

A bacterial biofilm is formed as a result of complex coordinated interactions of microorganisms with a surface. There are complete reviews in the literature covering biofilm biology and genetics (Watnick and Kolter 2000; Ilyina et al. 2004; Branda et al. 2005; Costerton 2007; Lloyd et al. 2007; Moons et al. 2009; Plakunov et al. 2010; Smirnova et al. 2010). Several investigators have considered the ecological implications of biofilm formation by associative bacteria (Morris and Monier 2003; Danhorn and Fuqua 2007; Eberl et al. 2007; Haggag 2010). Most generally, the sequence of events is as follows.

Biofilm formation begins with an initial, reversible attachment, when planktonic bacteria make contact with the substrate and become temporarily fixed, with some cells being able to detach. In this process, an important role is played by flagella. This stage involves the work of nonspecific physicochemical forces of interaction between the molecules and structures on the surfaces of the microorganism and the solid substrate (Van der Waals, hydrophobic, electrostatic, and London dispersion forces) (Van Loosdrecht 1988). The next developmental stages are the irreversible attachment to the surface; the formation of microcolonies, with aggregation of already attached cells; the formation of macrocolonies; and, finally, the aging of the macrocolonies with the formation of biofilms. The biofilm development cycle is completed when the bacteria resume their planktonic lifestyle (Ilyina et al. 2004). The start of biofilm formation may be signaled by osmolarity, the pH of the medium, soil content of metals, oxygen supply, temperature, and other factors (Davey and O'Toole 2000; Karatan and Watnick 2009).

There is evidence that in biofilms, cells differentiate according to function—the motile, matrix-producing, sporulating cells are located at a distance away from one another and are present in different parts of a biofilm (Vlamakis et al. 2008). Biofilm formation is a complex process that requires the activity of a multitude of genes responsible for both general functions (such as motility, metabolism, and maintenance of cell structure) and special functions, which ensure biofilm formation. In the process of biofilm aging, a multitude of genes are differentially expressed, the work of which requires a regulatory system that would ensure control of expression (Voloshin et al. 2005). In the past decade, there has been an explosion of studies that have led to the discovery of a wide variety of "communication molecules," which are secreted to the medium and induce specific changes in bacterial metabolism when a definite critical concentration of producer cells is

reached. This principle, named quorum sensing (QS), is effected at the cost of various chemical compounds, including low-molecular-weight substances [secondary metabolic products, peptides, lipids, and secreted proteins (Voloshin and Kaprelyants 2004)]. For broader coverage, readers are referred to Faure et al. (2009), Dickschat (2010), and Thoendel and Horswill (2010).

17.3.3.2 Methods of Biofilm Visualization

In recent years, methods have been developed to prepare biofilms in artificial systems, thereby creating controlled conditions for biofilm study. Biofilms are estimated in microtitration plates by spectrophotometric counting of a specific stain bound by the cells in the biofilm (Ferrieres and Clarke 2003). Biofilm formation is also modeled in flow-through chambers and test tubes and on the surface of cover slips and other objects. For studies of living cells and for observations of cells in motion, a phase-contrast microscope and an interference microscope are employed (Smirnova et al. 2010). Stained preparations are examined by using stains specific for the matrix as the major biofilm component. These include the vital dye Congo red, which binds to cellulose and the *curli* pili in the process of staining *Salmonella* biofilm (Römling et al. 1998); fuchsin (Yi et al. 2004); and another cellulose indicator, the fluorescent vital dye calcofluor (Solano et al. 2002). For light-optical observations, investigators use ruthenium red (RR) and alcian blue (AB), which interact with acidic mucopolysaccharides (Smirnova et al. 2009).

Biofilms on nontransparent materials are visualized by epifluorescence microscopy. In the Luft method of EPS visualization by transmission electron microscopy (TEM) of biofilms, RR interacts with osmium tetroxide, which forms part of the fixative (Luft 1971). The external PSs of a range of bacteria have been demonstrated with RR and AB (Karlyshev et al. 2001; Hunter and Beveridge 2005). Beveridge (2006) suggested the use of cryoTEM to explore the native hydrated structures of the biofilm.

With accumulation of data on the occurrence and role of biofilms in natural processes, industry, and medicine, the need arose to look for new research methods. With the help of confocal laser scanning microscopy (CLSM), it became possible to directly observe native films. More specifically, the use of CLSM and luminescent dyes allows one to distinguish, within a biofilm, the bacteria selectively stained with propidium iodide and matrix PSs that bind to the FITC-conjugated lectin ConA (Kania et al. 2007). For analysis of biofilm composition, investigators employ fluorochrome-conjugated lectins of different specificities. For example, visualization of *P. polymyxa* through FITC-labeled antibodies has demonstrated that this bacterium can colonize the surface of roots (Bent et al. 2002) and can penetrate the root interior (Shishido et al. 1999). Timmusk et al. (2005), using fluorescence stereomicroscopy, examined the localization of *P. polymyxa* and the formation of biofilms on plant roots in a model and a soil system.

For cell visualization, a labeling method is currently used in which a DNA sequence is inserted into the bacterial chromosome through the agency of a plasmid vector. The DNA sequence codes for a fluorescent label, e.g., green fluorescence protein (GFP). It is possible to conduct a direct real-time observation in vivo of GFR expression in individual cells of cell populations (Zogaj et al. 2001; Santaella et al. 2008).

Recently, atomic force microscopy (AFM) has been applied to studying the components of the metabolites of biofilm-forming bacteria (Hinterdorfer and Dufrêne 2006). By AFM, Jonas et al. (2007) examined the colonies and biofilms of *Salmonella* strains that synthesize cellulose and the surface protein BapA and that form *curli* pili. For example, AFM imaging and force measurement studies have been performed on surface PSs of *Lactobacillus* sp. Lecithin-modified tips were used to examine individual PS molecules on the surface of biofilms (Francius et al. 2008). For understanding their function in biofilms, PSs were characterized by single-molecule force spectroscopy (Sletmoen et al. 2003). Glucans of *Streptococcus mutans* biofilms were characterized, and their possible role in biofilm formation was explored (Cross et al. 2007). The study was conducted with various mutants with an impaired ability to synthesize glucans. The technique also provides the possibility for microbial surface molecular recognition by using specific binding such as antibody–antigen interaction.

17.3.3.3 Extracellular Matrix

Usually, cells in biofilms are embedded in an extrapolymeric matrix that ensures biofilm stability and safety from external stresses (Costerton et al. 1995; Smirnova et al. 2010). The matrix is formed from a mixture of components, including EPSs, proteins, nucleic acids (Voloshin et al. 2005), glycosyl phosphate-containing biopolymers (e.g., teichoic acids), glycoproteins, and (in certain bacteria, e.g., bacilli) polyglutamic acid and other biopolymers (Branda et al. 2006; Safronova and Botvinko 1998). A key structural component of biofilms, which has received close attention in the past decade, is the extracellular polymeric substance called the exopolysaccharide matrix (Sutherland 2001; Verhoef et al. 2005; Smirnova et al. 2010). In different bacterial species, this matrix differs in physical properties and chemical composition; as a rule, however, it is an anionic polymer. The EPS of the matrix consists mostly of homo- and heteropolysaccharides. The EPS is composed of uronic (mainly glucuronic) acids and amino sugars. By now, the EPS composition of several bacteria has been identified (Cunha et al. 2004; Da Re and Ghigo 2006; Hentzer et al. 2001; Ledeboer and Jones 2005).

For example, it has been shown that *Pseudomonas aeruginosa* forms alginate, a copolymer of mannuronic and glucuronic acids (Hentzer et al. 2001). It is an unbranched PS, a property distinguishing it from polymers such as xanthan and dextran. However, several recent reports have shown that other PSs contribute to biofilms formed by nonmucoid *P. aeruginosa* strains, which are believed to be the first to colonize cystic fibrosis patients. A recent example is the expression of the *psl*

operon, which is required in order to maintain the biofilm structure after attachment. Overproduction of the Psl PS led to enhanced cell surface and intercellular adhesion of *P. aeruginosa*, which translated into significant changes in the architecture of the biofilm (Ma et al. 2006).

Branda et al. (2006) discussed the role of PS, proteins, and the extracellular polymer polyglutamic acid as components of the *B. subtilis* matrix. Using microscopical methods, they showed that mutants in the *eps* and *tasA* genes form a weak unstructured film and that double mutants in these genes do not form a film at all. This attests to the need for the presence of both PS and protein in the matrix.

More specifically, Smirnova et al. (2009) found by cytochemical studies that the matrix of biofilms developed by *Salmonella typhimurium* includes acidic mucopolysaccharides, revealed by alcian blue staining, and cellulose, stained with Congo red. Sheludko et al. (2008) compared the thickness and antigenic properties of biofilms produced by *A. brasilense* Sp245 and its mutants deficient in the synthesis of LPSs and calcofluor-binding PSs (CBPSs) at the interface between water and hydrophilic or hydrophobic solid surfaces. They found that the mutants deficient in acidic LpsI synthesis produce thicker biofilms on hydrophilic surfaces. Biofilms produced on hydrophobic surfaces by bacteria that are unable to synthesize CBPSs are less pronounced. Defects in CBPS production in *Azospirillum* mutants with impaired flagellar motility can cause adverse effects on the cell ability to attach to hydrophobic and hydrophilic surfaces. The loss of the neutral LpsII antigen by the mutants capable of producing CBPSs does not affect their behavior on hydrophobic surfaces, which is probably due to the compensatory increase in the total PS production. The fundamental change in the Lps structure correlates with the activation of biofilm formation by the relevant mutants on hydrophilic and hydrophobic surfaces. The effectiveness of biofilm formation by *A. brasilense* Sp7 and its variants was analyzed by Petrova et al. (2010). Those authors reported that spontaneous changes in plasmid composition had a negative effect on biofilm formation by *A. brasilense* on hydrophobic and (more rarely) hydrophilic abiotic surfaces. The derivatives of Sp7 that had lost p115 and harbored an altered pRhico were less active in colonizing plant roots during the first hours of interaction.

Yegorenkova et al. (2010) evaluated the ability of several strains of the rhizobacterium *P. polymyxa*, differing in the yield and rheological properties of their EPSs, to form biofilms on abiotic surfaces. Of these strains, *P. polymyxa* 1465, giving the highest yield of EPSs and the highest kinematic viscosity of the culture liquid and of aqueous PS solutions, proved to be the most active in forming biofilms on hydrophobic and hydrophilic surfaces (Fig. 17.2). Enzyme-linked immunosorbent assay (ELISA) with rabbit polyclonal antibodies developed to isolated EPSs of *P. polymyxa* 1465 and 92 was used to detect *P. polymyxa*'s polysaccharidic determinants in the composition of the biofilm materials. According to the data of Timmusk et al. (2005), the EPSs of *P. polymyxa* participate in biofilm formation on the roots of *Arabidopsis thaliana*.

Santaella et al. (2008) focused on the function of an EPS produced by *Rhizobium* sp. YAS34 in the colonization and biofilm formation on nonlegume plant roots

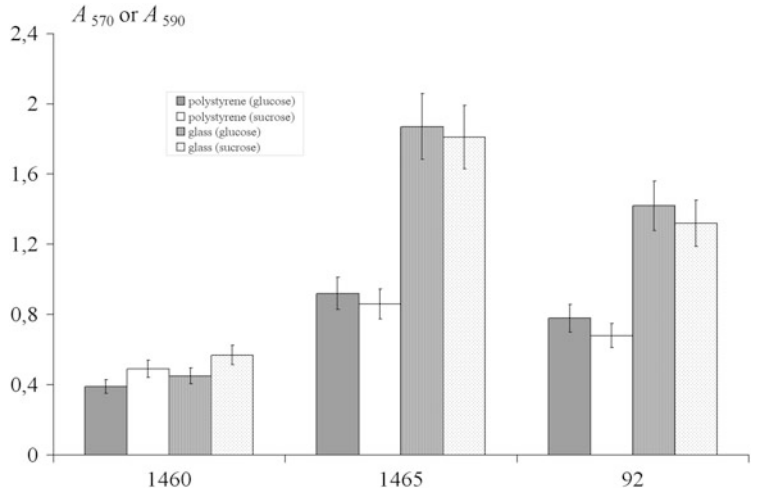


Fig. 17.2 Evaluation of the ability of *P. polymyxa* 92, 1460, and 1465 to form biofilms on hydrophilic and hydrophobic surfaces by using crystal violet staining. A_{570} is the absorbance of samples in polystyrene plates, and A_{590} is the absorbance of samples in glass test tubes (taken from Yegorenkova et al. 2011)

(*Arabidopsis thaliana* and *Brassica napus*). Using random transposon mutagenesis, they isolated an EPS-deficient mutant of strain YAS34 impaired in a glycosyltransferase gene (*gta*). The wild-type and mutant strains were tagged with a plasmid-borne GFP, and for the first time, the EPS produced by the wild-type strain was seen in the rhizosphere by using selective carbohydrate probing with a fluorescent lectin and confocal laser scanning microscopy. The authors observed for the first time that *Rhizobium* forms biofilms on the roots of nonlegumes, independently of the EPS synthesis. When produced by wild-type strain YAS34, EPS is targeted at specific parts of the plant root system. Nutrient fluctuations, root exudates, and the bacterial growth phase can account for such a production pattern. The EPS synthesis in *Rhizobium* sp. YAS34 is not essential for biofilm formation on roots, but it is critical to colonization of the basal part of the root system and to increasing of the stability of root-adhering soil. The authors believe that in the interactions of *Rhizobium* sp. YAS34 with the nonlegume plants, microbial EPS is implicated in the root–soil interface, root colonization, but not in biofilm formation.

Growth and EPS production may be more prolific under attached conditions for some bacteria (Hughes 1997), and attachment to solid surfaces may stimulate PS synthesis, as suggested by Vandevivere and Kirchman (1993). Also, Allison and Sutherland (1987) demonstrated that two strains of freshwater bacteria synthesized significant amounts of EPS only after attachment, indicating that the polymers were not needed for initial adhesion to inert surfaces. These results may again all stem from stress responses. It is possible that even relatively small quantities of preformed EPS from the planktonic cells assisted in adhesion either to the solid surface or to the conditioning film on it. A recent report on colanic acid synthesis in

K12 confirmed these results and also indicated that the PS is required for the formation of the biofilm structure rather than for initial attachment (Danese et al. 2000).

When biofilms or flocs are established, the PS components of microbial origin may exhibit phenotypic differences from planktonic bacteria of the same species. However, it is more likely that the microorganisms secrete EPSs identical in composition and probably also in physical properties with those formed by the same bacteria when grown in planktonic culture. Another possibility is that the polymers formed may be of identical composition to those formed by the free-living bacteria, but, owing to minor structural differences such as the degree of acylation or the molecular mass, they differ in their physical properties. These differences may result in altered viscosity or gel-forming capacity (Sutherland 2001). Costerton et al. (1981) used antibodies against polymers synthesized planktonically to reveal interaction with material in a biofilm matrix. This indicated that at least some of the biofilm EPSs had the same or very similar composition as the planktonic products. Further confirmation of the close similarity or identity of biofilm and planktonic PSs was obtained with highly specific, phage-induced polysaccharases (Hughes et al. 1998). On the other hand, if only very small amounts of one polymer are produced and are impossible to separate from large quantities of a second PS, this might explain the apparent complexity of composition reported for some materials obtained from biofilm isolates (Sutherland 2001).

Possibly, EPSs play various roles (depending on the environmental conditions) in the structure and functions of biofilm communities (Ilyina et al. 2004). Production of EPSs is generally important in biofilm formation, and likewise, it can affect the interaction of microbes with roots and root appendages (Bianciotto et al. 2001). EPSs protect the biofilm against a range of unfavorable environmental effects (UV radiation, changes in the pH of the medium, osmotic shock, and drying), adsorb xenobiotics, promote the mechanisms of nutrient accumulation, and ensure tolerance for antimicrobial agents by limiting the penetration of these agents from the surrounding milieu (Smirnova et al. 2010). For example, water retention varies with the type of PSs, but EPS water retention capacity may exceed 70 g of water per g of PS (Zhang et al. 1998; Vu et al. 2009). Further detail on the EPSs of biofilms and on their structure and functions is available in a review by Sutherland (2001).

The role of the matrix in the formation of polymicrobial biofilms was established (Smirnova et al. 2010). In mixed bacterial populations, the formation of coaggregates is commonly observed, which occurs owing to the cells being stuck together through EPS. On the one hand, coaggregation is conducive to the formation of mixed biofilms, bringing together various microorganisms on the basis of synergism, and on the other hand, it may "cleanse" the surroundings of pathogenic bacteria during their interaction with antagonistic bacteria (Smirnova et al. 2010).

17.3.3.4 Role of Biofilm in the Biocontrol of Plant Diseases

The use of microorganisms for plant disease control is an attractive alternative to the use of synthetic chemical substances. There is a vast literature describing various mechanisms of the biocontrol ability of bacteria, e.g., siderophore production, secretion of hydrolytic enzymes, antibiosis, ISR, and certain others (Timmusk et al. 1999, 2005; Weller et al. 2002; Timmusk 2003; Perneel et al. 2007; Rezzonico et al. 2007; Tran et al. 2007). The biocontrol ability of bacilli has been adequately covered by Kumar et al. (2011). In this section, I will only touch on the possible role of biofilm in the biocontrol of plant diseases.

Bacterial biofilms formed on the roots of plants can protect colonization sites and act as scavengers of nutrients in the rhizosphere, thereby decreasing the availability of root exudate nutrients for stimulation of plant pathogens and subsequent root colonization by them. The mechanism initially reported by Thomashow's group (Weller and Thomashow 1994) has gained less attention, most likely because of difficulties in studying natural systems. However, biofilms can have the potential to be successful in fighting similar root-colonizing pathogens under natural conditions (Timmusk et al. 2009b).

One beneficial rhizobacterium is *Bacillus subtilis*, which is ubiquitous in soil. It can promote plant growth, protect plants against fungal pathogen attack, and play a role in the degradation of organic polymers in soil (Emmert and Handelsman 1999). Recently, it has been reported that *B. subtilis* forms adhering biofilms on inert surfaces under the control of a variety of transcription factors (Stanley et al. 2003). Bais et al. (2004), using an infection model, demonstrated the biocontrol ability of a wild-type *B. subtilis* strain, 6051, against *P. syringae*. *Arabidopsis* root surfaces treated with *B. subtilis* were analyzed by CLSM to reveal a three-dimensional *B. subtilis* biofilm.

Owing to its broad host range and its ability to form endospores and synthesize various types of antibiotics, *P. polymyxa* has the potential of being a commercially useful biocontrol agent. *P. polymyxa* was found to be successful in controlling *Botrytis cinerea*, the causal agent of gray mold, in strawberries (Helbig 2001); *Fusarium oxysporum* and *Pythium spp*., the causal agents of seedling blight, wilt, and root rot of cucumber and watermelon (Dijksterhuis et al. 1999; Yang et al. 2004); sesame damping-off (Ryu et al. 2006); and diseases of *Arabidopsis* caused by *Phytophthora palmivora* and *Pythium aphanidermatum* (Timmusk and Wagner 1999). This can occur by direct antagonism, when protective bacteria and attacking organisms are in close proximity, in which case disease suppression is expected to be restricted to soilborne pathogens. On the other hand, PGPR may stimulate systemic defenses, inducing sustained changes in the plant, which increase its tolerance to further infection by foliar or root pathogens (Timmusk 2003).

So far, most research on the biocontrol activity of *P. polymyxa* has centered on the elaboration of antibiotics by this bacterium. Haggag and Timmusk (2008) investigated the role of biofilm-forming *P. polymyxa* strains in controlling crown root rot disease (*A. niger*) and highlighted the importance of efficient rhizosphere

colonization and biofilm formation in biocontrol. Two plant-growth-promoting *P. polymyxa* strains were isolated from the peanut rhizosphere (from *A. niger*-suppressive soils). The strains were tested under greenhouse and field conditions for inhibition of the crown root rot pathogen of the peanut, as well as for biofilm formation in the peanut rhizosphere. The strains' colonization and biofilm formation were further studied on roots of the model plant *A. thaliana* and with solid surface assays. Their crown root rot inhibition performance was studied in field and pot experiments. The strains' ability to form biofilms in gnotobiotic and soil systems was studied by SEM. It was noted that both strains produced similar amounts of antagonistic substances and were able to suppress the pathogen but that the superior biofilm former offered significantly better protection against crown rot.

Oomycetic pathogens are responsible for one of the most destructive groups of diseases. They are present in almost all cultivated soils and attack the root system, particularly in warm and humid environments. Despite the decades of biological control research, no commercially successful methods for combating diseases caused by *Pythium* and *Phytophthora* have yet appeared. Timmusk (2003) observed a significant yet inconsistent reduction in *Pythium* root rot under natural conditions, when the plants were preinoculated by *P. polymyxa* biocontrol strains. Subsequently, Timmusk et al. (2009b) presented experiments with an *A. thaliana* model system, in which they studied the antagonistic properties of *P. polymyxa* strains toward the oomycete plant pathogens *P. palmivora* and *P. aphanidermatum*. The experiments were conducted on agar plates, in liquid media, and in soil. It was shown that *P. polymyxa* strains significantly reduced *P. aphanidermatum* and *P. palmivora* colonization in liquid assays. Most plants that had been treated with *P. polymyxa* survived the *P. aphanidermatum* inoculations in soil assays. In the authors' opinion, the antagonistic abilities of both systems correlated well with mycoidal substance production and not with the production of antagonistic substances from the biocontrol bacteria. Possibly, the *P. polymyxa* biofilm formed on the roots coincides with the colonization sites of several pathogens and thereby functions as a protective layer to prevent access by the pathogens (Timmusk et al. 2005). The protective layer might also contribute to plant-enhanced drought tolerance (Timmusk and Wagner 1999).

Thus, given the information available to date, one can confidently speak of the substantial role of the biofilm in plant defense against pathogens.

Once the pathways to biofilm development are more fully understood, the management of *P. polymyxa* biofilm formation in resident populations of cropping systems could become possible (Battin et al. 2007). This will be a step to ensuring their reproducible performance in natural environments.

17.4 Concluding Remarks

In this chapter, I have analyzed what is currently known about the EPSs of *P. polymyxa* rhizobacteria, with an emphasis on their role in plant–bacterial interactions. In the past few years, great strides have been made toward elucidating the physiological role of microbial EPSs, and the ecological significance of these polymers has been proven. The involvement of exoglycans in the formation of symbiotic communities and their large role in preserving population viability under extreme conditions are beyond question. PSs constitute an extremely important category of biopolymers with a wide range of biological functions, first and foremost receptor functions, which ensure the interaction of cells with one another and with members of other species. However, for a fuller understanding of the physiological significance of these polymers, it is necessary to elucidate the possibility of a change in their functions that is adequate to external influence.

One of the keys to studying complex biological systems is the development of accurate and realistic models for natural communities in laboratory settings and the application of state-of-the-art research methods that adequately reflect the processes occurring under natural conditions. In the rhizosphere, *P. polymyxa* bacteria operate together with plant roots as communities with increased levels of complexity and plasticity, allowing this system to adapt to the environmental conditions. Within the framework of the concept of symbiogenetics, being developed currently (Tikhonovich and Provorov 2003), symbioses are regarded as biological complexes arising from the functioning (and sometimes the structural integration) of the gene systems of unallied organisms. Owing to the work of the newly formed "supraorganismal" genome, the partners implement new programs of development and adaptation, which are unavailable to free-living organisms (Provorov et al. 2008).

The negative effects of some *P. polymyxa* strains (e.g., root invasion) appear to be mostly strain specific and negligible, as compared with beneficial effects (e.g., growth promotion and pathogen control). Interaction of a bacterial strain with a host plant as a PGPR or a DRB is strongly dependent on the prevalent rhizosphere environment conditions. In any case, the role of *P. polymyxa* in the rhizosphere microbial community requires further studies, also because there is every reason to believe that gaining a greater understanding of these processes will facilitate in the long run the efforts to wean off the dependence on agricultural chemicals (Raza et al. 2008).

Acknowledgments I express my sincere gratitude to my colleagues who have been involved in different stages of the *P. polymyxa* research described in this chapter. Thanks are due to Mr. Dmitry N. Tychinin (this institute) for translating the original manuscript into English.

References

Acosta MP, Valdman E, Leite SGF, Battaglini F, Ruzal SM (2005) Biosorption of copper by *Paenibacillus polymyxa* cells and their exopolysaccharide. World J Microbiol Biotechnol 21:1157–1163

Alami Y, Achouak W, Marol C, Heulin T (2000) Rhizosphere soil aggregation and plant growth promotion of sunflowers by an exopolysaccharide-producing *Rhizobium* sp. strain isolated from sunflower roots. Appl Environ Microbiol 66:3393–3398

Algam SAE, Xie G, Li B, Yu S, Su T, Larsen J (2010) Effects of *Paenibacillus* strains and chitosan on plant growth promotion and control of *Ralstonia* wilt in tomato. J Plant Pathol 92:593–600

Allison DG, Sutherland IW (1987) The role of exopolysaccharides in adhesion of freshwater bacteria. J Gen Microbiol 133:1319–1327

Alvarez VM, von der Weid I, Seldin L, Santos ALS (2006) Influence of growth conditions on the production of extracellular proteolytic enzymes in *Paenibacillus peoriae* NRRL BD-62 and *Paenibacillus polymyxa* SCE2. Lett Appl Microbiol 43:625–630

Amellal N, Burtin G, Bartoli F, Heulin T (1998) Colonization of wheat roots by an exopolysaccharide-producing *Pantoea agglomerans* strain and its effect on rhizosphere soil aggregation. Appl Environ Microbiol 64:3740–3747

Andreyeva IN, Red'kina TV, Izmailov SF (1993) Role of indole-3-acetic acid in the stimulatory effect of *Azospirillum brasilense* on the legume–*Rhizobium* symbiosis. Fiziol Rast (Moscow) 40:902–907 (in Russian)

Arkhipova TN (1999) Study of the cytokinins produced by rhizosphere microorganisms. Extended abstract of Cand. Sci. dissertation, Ufa (in Russian)

Ash C, Farrow JAE, Wallbanks S, Collins MD (1991) Phylogenetic heterogeneity of the genus *Bacillus* revealed by comparative analysis of small subunit – ribosomal RNA sequences. Lett Appl Microbiol 13:202–206

Ash C, Priest FG, Collins MD (1993) Molecular identification of rRNA group 3 bacilli (Ash, Farrow, Wallbanks and Collins) using a PCR probe test. Proposal for the creation of a new genus *Paenibacillus*. Antonie Van Leeuwenhoek 64:253–260

Bais HP, Fall R, Vivanco JM (2004) Biocontrol of *Bacillus subtilis* against infection of *Arabidopsis* roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. Plant Physiol 134:307–319

Baldani VLD, Baldani JI, Dobereiner J (1983) Effects of *Azospirillum* inoculation on root infection and nitrogen incorporation in wheat. Can J Microbiol 29:924–929

Ball DH, Adams GA (1959) A mannan produced by *Bacillus polymyxa*. Can J Chem 37:1012–1017

Bashan Y, Holguin G (1997) *Azospirillum*-plant relationships: environmental and physiological advances (1990–1996). Can J Microbiol 43:103–121

Bashan Y, Levanony H, Klein E (1986) Evidence for a weak active external adsorption of *Azospirillum brasilense* Cd to wheat roots. J Gen Microbiol 132:3069–3073

Battin TJ, Sloan WT, Kjelleberg S, Daims H, Head IM, Curtis TP, Eberl L (2007) Microbial landscapes: new paths to biofilm research. Nat Rev Microbiol 5:76–81

Benedict RG, Langlykke AF (1947) Antibiotic activity of *Bacillus polymyxa*. J Bacteriol 54:24–25

Bent E, Breuil C, Enebak S, Chanway CP (2002) Surface colonization of lodgepole pine (*Pinus contorta* var. *latifolia*) roots by *Pseudomonas fluorescens* and *Paenibacillus polymyxa* under gnotobiotic conditions. Plant Soil 241:187–196

Beveridge TJ (2006) Visualizing bacterial cell walls and biofilms. Microbe 1:279–284

Bezzate S, Steinmetz M, Aymerich S (1994) Cloning, sequencing, and disruption of a levanase gene of *Bacillus polymyxa* CF43. J Bacteriol 176:2177–2183

Bezzate S, Aymerich S, Chambert R, Czarnes S, Berge O, Heulin T (2000) Disruption of the *Paenibacillus polymyxa* levansucrase gene impairs its ability to aggregate soil in the wheat rhizosphere. Environ Microbiol 2:333–342

Bianciotto V, Andreotti S, Balestrini R, Bonfante P, Perotto S (2001) Mucoid mutants of the biocontrol strain *Pseudomonas fluorescens* CHA0 show increased ability in biofilm formation on mycorrhizal and nonmycorrhizal carrot roots. Mol Plant Microbe Interact 14:255–260

Boyko AS, Konnova SA, Fedonenko YP, Zdorovenko EL, Smol'kina ON, Kachala VV, Ignatov VV (2011) Structural and functional peculiarities of the lipopolysaccharide of *Azospirillum brasilense* SR55 isolated from the roots of *Triticum durum*. Microbiol Res 166:585–593

Branda SS, Vik S, Friedman L, Kolter R (2005) Biofilms: the matrix revisited. Trends Microbiol 13:20–26

Branda SS, Chu F, Kearns DB, Losick R, Kolter R (2006) A major protein component of the *Bacillus subtilis* biofilm matrix. Mol Microbiol 59:1229–1238

Budi SW, van Tuinen D, Arnould C, Dumas-Gaudot E, Gianinazzi-Pearson V, Gianinazzi S (2000) Hydrolytic enzyme activity of *Paenibacillus* sp. strain B2 and effects of the antagonistic bacterium on cell integrity of two soil-borne pathogenic fungi. Appl Soil Ecol 15:191–199

Cakmakci R, Erat M, Erdogan U, Donmez MF (2007) The influence of plant growth–promoting rhizobacteria on growth and enzyme activities in wheat and spinach plants. J Plant Nutr Soil Sci 170:288–295

Chang ZQ, Lee JS, Hwang MH, Hong JH, Jung HK, Lee SP, Park SC (2009) A novel β-glucan produced by *Paenibacillus polymyxa* JB115 induces nitric oxide production in RAW264.7 macrophages. J Vet Sci 10:165–167

Chang ZQ, Lee JS, Gebru E, Hong JH, Jung HK, Jo WS, Park SC (2010) Mechanism of macrophage activation induced by β-glucan produced from *Paenibacillus polymyxa* JB115. Biochem Biophys Res Commun 391:1358–1362

Chanway CP (1995) Differential response of western hemlock from low and high elevations to inoculation with plant growth-promoting *Bacillus polymyxa*. Soil Biol Biochem 27:767–775

Chanway CP, Holl FB (1991) Biomass increase and associative nitrogen fixation of mycorrhizal *Pinus contorta* Dougl. seedlings inoculated with a plant growth promoting *Bacillus* strain. Can J Bot 69:507–511

Chanway CP, Holl FB (1994) Growth of outplanted lodgepole pine seedlings, one year after inoculation with plant growth promoting rhizobacteria. For Sci 40:238–246

Chanway CP, Holl FB, Turkington R (1988) Genotypic coadaptation in plant growth promotion of forage species by *Bacillus polymyxa*. Plant Soil 106:281–284

Chenu C (1995) Extracellular polysaccharides: an interface between microorganisms and soil constituents. In: Huang PM, Berthelin J, Bollag JM, McGill WB, Page AL (eds) Environmental impact of soil components interactions. CRC Lewis, Boca Raton, NY, pp 217–233

Cheong H, Park S-Y, Ryu C-M, Kim JF, Park S-H, Chang SP (2005) Diversity of root-associated *Paenibacillus* spp in winter crops from the southern part of Korea. J Microbiol Biotechnol 15:1286–1298

Chockalingam E, Subramanian S, Natarajan KA (2003) Studies on biodegradation of organic flotation collectors using *Bacillus polymyxa*. Hydrometallurgy 71:249–256

Choi SK, Park SY, Kim R, Lee CH, Kim JF, Park SH (2008) Identification and functional analysis of the fusaricidin biosynthetic gene of *Paenibacillus polymyxa* E681. Biochem Biophys Res Commun 365:89–95

Chu KH, Kim EY (2006) Predictive modelling of competitive biosorption equilibrium data. Biotechnol Bioprocess Eng 11:67–71

Comas-Riu J, Vives-Rego J (2002) Cytometric monitoring of growth, sporogenesis and spore cell sorting in *Paenibacillus polymyxa* (formerly *Bacillus polymyxa*). J Appl Microbiol 92:475–481

Costacurta A, Vanderleyden J (1995) Synthesis of phytohormones by plant-associated bacteria. Crit Rev Microbiol 21:1–18

Costerton JW (2007) The biofilm primer. Springer, Berlin

Costerton JW, Irvin RT, Cheng KJ (1981) The bacterial glycocalyx in nature and disease. Annu Rev Microbiol 35:399–424

Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM (1995) Microbial biofilms. Annu Rev Microbiol 49:711–745

Cross SE, Kreth J, Zhu L, Sullivan R, Shi W, Qi F, Gimzewski JK (2007) Nanomechanical properties of glucans and associated cell-surface adhesion of *Streptococcus* mutans probed by atomic force microscopy under in situ conditions. Microbiology 153:3124–3132

Cunha MV, Sousa SA, Leitão JH, Moreira LM, Videira PA, Sá-Correia I (2004) Studies on the involvement of the exopolysaccharide produced by cystic fibrosis-associated isolates of the *Burkholderia cepacia* complex in biofilm formation and in persistence of respiratory infections. J Clin Microbiol 42:3052–3058

Czames S, Hallett PD, Bengough AG, Young IM (2000) Root- and microbial-derived mucilages affect soil structure and water transport. Eur J Soil Sci 51:435–443

Da Mota F, Nobrega A, Marriel IE, Paiva E, Seldin L (2002) Genetic diversity of *Paenibacillus polymyxa* populations isolated from rhizosphere of four cultivars of maize (*Zea mays*) planted in Cerrado soil. Appl Soil Ecol 20:119–132

Da Re S, Ghigo J-M (2006) A CsgD-independent pathway for cellulose production and biofilm formation in *Escherichia coli*. J Bacteriol 188:3073–3087

Daane LL, Harjono I, Zylstra GJ, Haggblom MM (2001) Isolation and characterization of polycyclic aromatic hydrocarbon-degrading bacteria associated with the rhizosphere of salt marsh plants. Appl Environ Microbiol 67:2683–2691

Danese PN, Pratt LA, Kolter R (2000) Exopolysaccharide production is required for development of *Escherichia coli* K12 biofilm architecture. J Bacteriol 182:3593–3596

Danhorn T, Fuqua C (2007) Biofilm formation by plant-associated bacteria. Annu Rev Microbiol 61:401–422

Davey ME, O'Toole GA (2000) Microbial biofilm: from ecology to molecular genetics. Microbiol Mol Biol Rev 64:847–867

De Freitas JR (2000) Yield and assimilation of winter wheat (*Triticum aestivum* L., var. Norstar) inoculated with rhizobacteria. Pedobiologia 44:97–104

Deo N, Natarajan KA (1998) Studies on interaction of *Paenibacillus polymyxa* with iron ore minerals in relation to beneficiation. Int J Miner Process 55:41–60

Deo N, Natarajan KA, Somasundaran P (2001) Mechanisms of adhesion of *Paenibacillus polymyxa* onto hematite, corundum and quartz. Int J Miner Process 62:27–39

Dickschat JS (2010) Quorum sensing and bacterial biofilms. Nat Prod Rep 27:343–369

Dijksterhuis J, Sanders M, Gorris LGM, Smid EJ (1999) Antibiosis plays a role in the context of direct interaction during antagonism of *Paenibacillus polymyxa* towards *Fusarium oxysporum*. J Appl Microbiol 86:13–21

Döbereiner J (1977) Physiological aspects of N_2 fixation in grass-bacteria association. In: Newton W, Postgate JR, Rodriguez-Barrueco C (eds) Recent developments in nitrogen fixation. Academic, London, pp 518–538

Donlan RM, Costerton JW (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 15:167–193

Eberl L, von Bodman SB, Fuqua C (2007) Biofilms on plant surfaces. In: Kjelleberg S, Givskov M (eds) The Biofilm mode of life: mechanisms and adaptations, vol 12. Horizon Bioscience, Norfolk, pp 215–234

Emmert EAB, Handelsman J (1999) Biocontrol of plant disease: a Gram-positive perspective. FEMS Microbiol Lett 171:1–9

Euzéby JP (2011) List of prokaryotic names with standing in nomenclature – genus *Paenibacillus*. http://www.bacterio.cict.fr/p/paenibacillus. Accessed 20 Dec 2011

Faure D, Vereecke D, Leveau JH (2009) Molecular communication in the rhizosphere. Plant Soil 321:279–303

Fedonenko YP, Yegorenkova IV, Konnova SA, Ignatov VV (2001) Involvement of the lipopolysaccharides of Azospirilla in the interaction with wheat seedling roots. Microbiology (Moscow, Russ Fed) 70:329–334

Ferrieres L, Clarke DJ (2003) The RcsC sensor kinase is required for normal biofilm formation in *Escherichia coli* K12 and controls the expression of a regulon in response to growth on a solid surface. Mol Microbiol 50:1665–1682

Francius G, Lebeer S, Alsteens D, Wildling L, Gruber HJ, Hols P, De Keersmaecker S, Vanderleyden J, Dufrene YF (2008) Detection, localization, and conformational analysis of single polysaccharide molecules on live bacteria. ACS Nano 2:1921–1929

Fukui H, Tanaka M, Misaki A (1985) Structure of a physiologically active polysaccharide produced by *Bacillus polymyxa* S-4. Agric Biol Chem 49:2343–2349

Gamalero E, Lingua G, Berta G, Lemanceau P (2003) Methods for studying root colonization by introduced beneficial bacteria. Agronomie 23:407–418

Gaskins MH, Hubbell DH (1979) Response of non-leguminous plants to root inoculation with free-living diazotrophic bacteria. In: Harley JL, Russel RS (eds) The soil–root interface. Academic, New York, pp 175–182

Gaur AC (1990) Phosphate solubilizing microorganisms as biofertilizers. Omega Scientific, New Delhi, p 176

Girardin H, Albagnas C, Dargaignaratz C, Nguyen-The C, Carlin F (2002) Antimicrobial activity of foodborne *Paenibacillus* and *Bacillus* sp against *Clostridium botulinum*. J Food Prot 65:806–813

Glukhova EV, Yarotsky SV, Deryabin VV, Shenderov BA, Ignatov VV (1986) Extracellular polysaccharides of *Bacillus polymyxa* and its mutant strain. Antibiot Med Biotek 31:669–674 (in Russian)

Gong X-Y, Luan Z-K, Pei Y-S, Wang S-G (2003) Culture conditions for flocculant production by *Paenibacillus polymyxa* BY-28. J Environ Sci Health A 38:657–669

Gorska E, Tudek B, Russel S (2001) Degradation of cellulose by nitrogen-fixing strain of *Bacillus polymyxa*. Acta Microbiol Pol 50:129–137

Gouzou L, Burtin G, Philippy R, Bartoli F, Heulin T (1993) Effect of inoculation with *Bacillus polymyxa* on soil aggregation in the wheat rhizosphere: preliminary examination. Geoderma 56:479–491

Guemouri-Athmani S, Berge O, Bourrain M, Mavingui P, Thiery JM, Bhatnagar T, Heulin T (2000) Diversity of *Paenibacillus polymyxa* populations in the rhizosphere of wheat (*Triticum durum*) in Algerian soils. Eur J Soil Biol 36:149–159

Gupta A, Gopal M, Tilak KV (2000) Mechanism of plant growth promotion by rhizobacteria. Indian J Exp Biol 38:856–862

Haggag WM (2007) Colonization of exopolysaccharide-producing *Paenibacillus polymyxa* on peanut roots for enhancing resistance against crown rot disease. Afr J Biotechnol 6:1568–1577

Haggag WM (2010) The role of biofilm exopolysaccharides on biocontrol of plant disease. In: Elnashar M (ed) Biopolymers, vol 14. Sciyo, Rijeka, pp 271–284

Haggag WM, Timmusk S (2008) Colonization of peanut roots by biofilm forming *Paenibacillus polymyxa* initiates biocontrol against crown rot disease. J Appl Microbiol 104:961–969

Halverson LJ, Stacey G (1986) Signal exchange in plant-microbe interactions. Microbiol Rev 50:193–225

Han YW (1989) Levan production by *Bacillus polymyxa*. J Indian Microbiol 4:447–452

Han YW (1990) Microbial levan. Adv Appl Microbiol 35:171–194

He Z, Kisla D, Zhang L, Yuan C, Green-Church KB, Yousef AE (2007) Isolation and identification of a *Paenibacillus polymyxa* strain that coproduces a novel lantibiotic and polymyxin. Appl Environ Microbiol 73:168–178

Hebbar KP, Gueniot B, Heyraud A, Colin-Morel P, Heulin T, Balandreau J, Rinaudo M (1992) Characterization of exopolysaccharides produced by rhizobacteria. Appl Microbiol Biotechnol 38:248–253

Helbig J (2001) Biological control of *Botrytis cinerea* Pers. ex Fr. in strawberry by *Paenibacillus polymyxa* (Isolate 18191). J Phytopathol 149:265–273

Hentzer M, Teitzel GM, Balzer GJ, Heydorn A, Molin S, Givskov M, Parsek MR (2001) Alginate overproduction affects *Pseudomonas aeruginosa* biofilm structure and function. J Bacteriol 183:5395–5401

Hinterdorfer P, Dufrêne YF (2006) Detection and localization of single molecular recognition events using atomic force microscopy. Nat Methods 3:347–355

Holl FB, Chanway CP (1992) Rhizosphere colonization and seedling growth promotion of lodgepole pine by *Bacillus polymyxa*. Can J Microbiol 38:303–308

Holl FB, Chanway CP, Turkington R, Radley RA (1988) Response of crested wheatgrass (*Agropyron cristatum* L.), perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) to inoculation with *Bacillus polymyxa*. Soil Biol Biochem 20:19–24

http://www.ecobiology.com.ua. Cited 20 Dec 2011

Hubbell DH, Morales VM, Umali-Garcia M (1978) Proteolytic enzymes in *Rhizobium*. Appl Environ Microbiol 31:210–213

Hughes KA (1997) Bacterial biofilms and their exopolysaccharides. PhD thesis, Edinburgh University, Edinburgh

Hughes KA, Sutherland IW, Clark J, Jones MV (1998) Bacteriophage and associated polysaccharide depolymerases – novel tools for study of bacterial biofilms. J Appl Microbiol 85:583–590

Hunter RC, Beveridge TJ (2005) High-resolution visualization of *Pseudomonas aeruginosa* PAO1 biofilms by freeze-substitution transmission electron microscopy. J Bacteriol 187:7619–7630

Ilyina TS, Romanova YM, Gintsburg AL (2004) Biofilms as a mode of existence of bacteria in external environment and host body: the phenomenon, genetic control, and regulation systems of development. Russ J Genet 40:1189–1198

Iman GM, Abd-Allah NM (1974) Fructosan, a new soil conditishing polysaccharide isolated from the metabolites of *Bacillus polymyxa* AS-1 and its clinical application. Egypt J Bot 17:19–26

Jonas K, Tomenius H, Kader A, Staffan N, Römling U, Belova LM, Melefore O (2007) Roles of curli, cellulose and Bap A in *Salmonella* biofilm morphology studied by atomic force microscopy. BMC Microbiol 7:1–13

Jung H-K, Hong J-H, Park S-C, Park B-K, Nam D-H, Kim S-D (2007) Production and physicochemical characterization of β-glucan produced by *Paenibacillus polymyxa* JB115. Biotechnol Bioprocess Eng 12:713–719

Kaci Y, Heyraud A, Barakat M, Heulin T (2005) Isolation and identification of an EPS-producing *Rhizobium* strain from arid soil (Algeria): characterization of its EPS and the effect of inoculation on wheat rhizosphere soil structure. Res Microbiol 156:522–531

Kahn ML, Schroeder BK, House BL, Mortimer MM, Yurgel SN, Maloney SC, Warren KL, Fisher RF, Barnett MJ, Toman C, Long SR (2004) Foraging for meaning: postgenome approaches to *Sinorhizobium meliloti*. In: Lugtenberg B, Tikhonovich I, Provorov N (eds) Biology of molecular plant–microbe interactions, vol 4. IS-MPMI, St. Paul, MN, pp 416–422

Kajimura Y, Kaneda M (1996) Fusaricidin A, a new depsipeptide antibiotic produced by *Bacillus polymyxa* KT-8. Taxonomy, fermentation, isolation, structure elucidation, and biological activity. J Antibiot 49:129–135

Kania RE, Lamers GEM, Vonk MJ, Huy PTB, Hiemstra PS, Bloemberg GV, Grote JJ (2007) Demonstration of bacterial cells and glycocalyx in biofilms on human tonsils. Arch Otolaryngol Head Neck Surg 133:115–121

Karatan E, Watnick P (2009) Signals, regulatory networks, and materials that build and break bacterial biofilms. Microbiol Mol Biol Rev 73:310–347

Karlyshev AV, McCrossan MV, Wren BW (2001) Demonstration of polysaccharide capsule in *Campylobacter jejuni* using electron microscopy. Infect Immun 69:5921–5924

Karpunina LV, Melnikova UY, Konnova SA (2003) Biological role of lectins from the nitrogen-fixing *Paenibacillus polymyxa* strains 1460 during bacterial–plant-root interactions. Curr Microbiol 47:376–378

Khan Z, Kim SG, Jeon YH, Khan HU, Son SH, Kim YH (2008) A plant growth promoting rhizobacterium, *Paenibacillus polymyxa* strain GBR-1, suppresses root-knot nematode. Bioresour Technol 99:3016–3023

Kim JF, Jeong H, Park SY, Kim SB, Park YK, Choi SK, Ryu CM, Hur CG, Ghim SY, Oh TK, Kim JJ, Park CS, Park SH (2010) Genome sequence of the polymyxin-producing plant-probiotic rhizobacterium *Paenibacillus polymyxa* E681. J Bacteriol 192:6103–6104

Konnova SA, Skvortsov IM, Makarov OE, Prokhorova RN, Rogova TA, Ignatov VV (1995) Polysaccharide complexes secreted by *Azospirillum brasilense* and their possible role in the interaction of bacteria with wheat roots. Mikrobiologiya 64:762–768 (in Russian)

Kozyrovskaya NA, Negrutskaya VV, Koval'chuk MV, Voznyuk TN (2005) *Paenibacillus* sp. is a promising bacterium for the creation of a technology for manufacturing bacteria-based preparations for plants. Biopolim Kletka 21:312–318 (in Russian)

Kumar AS, Mody K, Jha B (2007) Bacterial exopolysaccharides – a perception. J Basic Microbiol 47:103–117

Kumar A, Prakash A, Johri BN (2011) *Bacillus* as PGPR in crop ecosystem. In: Maheshwari DK (ed) Bacteria in agrobiology: crop ecosystems, vol 2. Springer, Berlin, pp 37–59

Lal S, Tabacchioni S (2009) Ecology and biotechnological potential of *Paenibacillus polymyxa*: a minireview. Indian J Microbiol 49:2–10

Lebuhn M, Heulin T, Hartmann A (1997) Production of auxin and other indolic and phenolic compounds by *Paenibacillus polymyxa* strains isolated from different proximity to plant roots. FEMS Microbiol Ecol 22:325–334

Ledeboer NA, Jones BD (2005) Exopolysaccharide sugars contribute to biofilm formation by *Salmonella enterica* serovar typhimurium on Hep-2 cells and chicken intestinal epithelium. J Bacteriol 187:3214–3226

Lee IY, Seo WT, Kim GJ, Kim MK, Ahn SG, Kwon GS, Park YH (1997) Optimization of fermentation conditions for production of exopolysaccharide by *Bacillus polymyxa*. Bioprocess Eng 16:71–75

Lindberg T, Granhall U, Tomenius K (1985) Infectivity and acetylene reduction of diazotrophic rhizosphere bacteria in wheat (*Triticum aestivum*) seedlings under gnotobiotic conditions. Biol Fertil Soils 1:123–129

Ljunggren H, Fåhraeus G (1961) The role of polygalacturonase in root-hair invasion by nodule bacteria. J Gen Microbiol 26:521–528

Lloyd DH, Viac J, Werling D, Reme CA, Gatto H (2007) Role of sugars in surface microbe-host interactions and immune reaction modulation. Vet Dermatol 18:197–204

Lu F, Sun L, Lu Z, Bie X, Fang Y, Liu S (2007) Isolation and identification of an endophytic strain EJS-3 producing novel fibrinolytic enzymes. Curr Microbiol 54:435–439

Luft JH (1971) Ruthenium red and violet. 1. Chemistry, purification, methods of use for electron microscopy and mechanism of action. Anat Rec 171:347–368

Ma L, Jackson KD, Landry RM, Parsek MR, Wozniak DJ (2006) Analysis of *Pseudomonas aeruginosa* conditional Psl variant reveals roles for the Psl polysaccharide in adhesion and maintaining biofilm structure postattachment. J Bacteriol 188:8213–8221

Ma M, Wang C, Ding Y, Li L, Shen D, Jiang X, Guan D, Cao F, Chen H, Feng R, Wang X, Ge Y, Yao L, Bing X, Yang X, Li J, Du B (2011) Complete genome sequence of *Paenibacillus polymyxa* SC2, a strain of plant growth-promoting rhizobacterium with broad-spectrum anti-microbial activity. J Bacteriol 193:311–312

Maes M, Baeyen S (2003) Experiences and perspectives for the use of a *Paenibacillus* strain as a plant protectant. Commun Agric Appl Biol Sci 68:457–462

Mannanov RN, Sattarova RK (2001) Antibiotics produced by *Bacillus* bacteria. Chem Nat Compd 37:117–123

Matora AV, Ignatova EN, Zhemerichkin DA, Yegorenkova IV, Shipin OV, Panasenko VI, Arsenieva LY, Barkovsky AL (1992) Bacterial polysaccharide polymyxan 88A. Main characteristics and possible applications. Prikl Biokhim Mikrobiol 28:731–737 (in Russian)

Mavingui P, Heulin T (1994) *In vitro* chitinase and antifungal activity of a soil, rhizosphere and rhizoplane population of *Bacillus polymyxa*. Soil Biol Biochem 26:801–803

McSpadden Gardener BB (2004) Ecology of *Bacillus* and *Paenibacillus* species in agricultural systems. Phytopathology 94:1252–1258

Michiels KW, Croes CL, Vanderleyden J (1991) Two different modes of attachment of *Azospirillum brasilense* Sp7 to wheat roots. J Gen Microbiol 137:2241–2246

Mitsuda S, Miyata N, Hirota T, Kikuchi T (1981) High-viscosity polysaccharide produced by *Bacillus polymyxa*. Hakkokogaku 59:303–309

Moon SH, Park JM, Chun HY, Kim SJ (2006) Comparisons of physical properties of bacterial cellulose produced in different culture conditions using saccharified food wastes. Biotechnol Bioprocess Eng 11:26–31

Moons P, Michiels CW, Aertsen A (2009) Bacterial interactions in biofilms. Crit Rev Microbiol 35:157–168

Morris CE, Monier J-M (2003) The ecological significance of biofilm formation by plant-associated bacteria. Annu Rev Phytopathol 41:429–453

Nakajima N, Chihara S, Koyama V (1972) A new antibiotic, gatavalin. J Antibiot 25:243–252

Nakashimada Y, Kanai K, Nishio N (1998) Optimization of dilution rate, pH and oxygen supply on optical purity of 2, 3-butanediol produced by *Paenibacillus polymyxa* in chemostat culture. Biotechnol Lett 20:1133–1138

Narula N, Deubel A, Gans W, Behl RK, Merbach W (2006) Paranodules and colonization of wheat roots by phytohormone producing bacteria in soil. Plant Soil Environ 52:119–129

Nielsen P, Sorensen J (1997) Multi-target and medium-independent fungal antagonism by hydrolytic enzymes in *Paenibacillus polymyxa* and *Bacillus pumilus* strains from barley rhizosphere. FEMS Microbiol Ecol 22:183–192

Ninomiya E, Kizaki T (1969) Bacterial polysaccharide produced from *Bacillus polymyxa* № 271. Angew Makromol Chem 6:179–185

Ninomiya E, Kizaki T, Harada K (1968a) High viscous polysaccharide produced by spore-forming bacterium. Part I. Identification of product. J Agric Chem Soc Jpn 42:178–184

Ninomiya E, Kizaki T, Harada K (1968b) High viscous polysaccharide produced by spore-forming bacterium. Part II. Physical properties and consistent sugar ratio. J Agric Chem Soc Jpn 42:431–434

Okon Y, Kapulnik Y (1986) Development and function of *Azospirillum*-inoculated roots. Plant Soil 90:3–16

Patra P, Natarajan KA (2004) Microbially induced flotation and flocculation of pyrite and sphalerite. Coll Surf B 36:91–99

Patra P, Natarajan KA (2006) Surface chemical studies on selective separation of pyrite and galena in the presence of bacterial cells and metabolic products of *Paenibacillus polymyxa*. J Colloid Interface Sci 298:720–729

Patriquin DG, Döbereiner J, Jain DK (1983) Sites and processes of association between diazotrophs and grasses. Can J Microbiol 29:900–915

Perneel M, Heyrman J, Adiobo A, De Maeyer K, Raaijmakers JM, De Vos P, Hofte M (2007) Characterization of CMR5c and CMR12a, novel fluorescent *Pseudomonas* strains from the cocoyam rhizosphere with biocontrol activity. J Appl Microbiol 103:1007–1020

Petersen DJ, Srinivasan M, Chanway CP (1996) *Bacillus polymyxa* stimulates increased *Rhizobium etli* populations and nodulation when co-resident in the rhizosphere of *Phaseolus vulgaris*. FEMS Microbiol Lett 142:271–276

Petrova LP, Shelud'ko AV, Katsy EI (2010) Plasmid rearrangements and alterations in *Azospirillum brasilense* biofilm formation. Microbiology (Moscow, Russ Fed) 79:120–123

Philip L, Iyengar L, Venkobachar C (2000) Site of interaction of copper on *Bacillus polymyxa*. Water Air Soil Pollut 119:11–21

Piuri M, Sanchez-Rivas C, Ruzal SM (1998) A novel antimicrobial activity of a *Paenibacillus polymyxa* strain isolated from regional fermented sausages. Lett Appl Microbiol 27:9–13

Plakunov VK, Strelkova EA, Zhurina MV (2010) Persistence and adaptive mutagenesis in biofilms. Microbiology 79:424–434

Provorov NA, Vorobyov NI, Andronov EE (2008) Macro- and microevolution of bacteria in symbiotic systems. Russ J Genet 44:6–20

Ravi AV, Musthafa KS, Jegathammbal G, Kathiresan K, Pandian SK (2007) Screening and evaluation of probiotics as a biocontrol agent against pathogenic Vibrios in marine aquaculture. Lett Appl Microbiol 45:219–223

Raza W, Yang W, Shen Q-R (2008) *Paenibacillus polymyxa:* antibiotics, hydrolytic enzymes and hazard assessment. J Plant Pathol 90:419–430

Renni RJ, Thomas JB (1987) ^{15}N-determined effect of inoculation with N_2-fixing bacteria on nitrogen assimilation in Western Canadian wheats. Plant Soil 100:213–223

Rezzonico F, Zala M, Keel C, Duffy B, Moënne-Loccoz Y, Défago G (2007) Is the ability of biocontrol fluorescent pseudomonads to produce the antifungal metabolite 2,4-diacetylphloroglucinol really synonymous with higher plant protection? New Phytol 173:861–872

Römling U, Sierralta WD, Eriksson K, Normark S (1998) Multicellular and aggregative behaviour of *Salmonella typhimurium* strains is controlled by mutations in the *agf*D promoter. Mol Microbiol 28:249–264

Rosado AS, Seldin L (1993) Production of a potentially novel anti-microbial substance by *Bacillus polymyxa*. World J Microbiol Biotechnol 9:521–528

Ryu CM, Kim J, Choi O, Kim SH, Park CS (2006) Improvement of biological control capacity of *Paenibacillus polymyxa* E681 by seed pelleting on sesame. Biol Control 39:282–289

Safronova IY, Botvinko IV (1998) Intercellular matrix of *Bacillus subtilis* 271: composition and functions. Microbiology (Moscow, Russ Fed) 67:45–50

Santaella C, Schue M, Berge O, Heulin T, Achouak W (2008) The exopolysaccharide of *Rhizobium* sp. YAS34 is not necessary for biofilm formation on *Arabidopsis thaliana* and *Brassica napus* roots but contributes to root colonization. Environ Microbiol 10:2150–2163

Santhiya D, Subramanian S, Natarajan KA (2002) Surface chemical studies on sphalerite and galena using extracellular polysaccharides isolated from *Bacillus polymyxa*. J Colloid Interface Sci 256:237–248

Seldin L, de Azevedo SF, Alviano DS, de Freire Bastos MC (1999) Inhibitory activity of *Paenibacillus polymyxa* SCE2 against human pathogenic microorganisms. Lett Appl Microbiol 28:423–427

Selim S, Negrel J, Goveraets C, Gianinazzi S, van Tuinen D (2005) Isolation and partial characterization of antagonistic peptides produced by *Paenibacillus* sp. strains B2 isolated from the sorghum mycorrhizosphere. Appl Environ Microbiol 71:6501–6507

Sharma PK, Rao KH (2003) Adhesion of *Paenibacillus polymyxa* on chalcopyrite and pyrite: surface thermodynamics and extended DLVO theory. Coll Surf B 29:21–38

Sheludko AV, Kulibyakina OV, Shirokov AA, Petrova LP, Matora LY, Katsy EI (2008) The effect of mutations affecting synthesis of lipopolysaccharides and calcofluor-binding polysaccharides on biofilm formation by *Azospirillum brasilense*. Microbiology (Moscow, Russ Fed) 77:313–317

Shishido M, Massicotte HB, Chanway CP (1996) Effect of plant growth promoting *Bacillus* strains on pine and spruce seedling growth and mycorrhizal infection. Ann Bot 77:433–441

Shishido M, Breuil C, Chanway CP (1999) Endophytic colonization of spruce by plant growth-promoting rhizobacteria. FEMS Microbiol Ecol 29:191–196

Shoda M, Sugano Y (2005) Recent advances in bacterial cellulose production. Biotechnol Bioprocess Eng 10:1–8

Siddiqui ZA, Baghel G, Akhtar MS (2007) Biocontrol of *Meloidogyne javanica* by *Rhizobium* and plant growth-promoting rhizobacteria on lentil. World J Microbiol Biotechnol 23:435–441

Singh HP, Singh TA (1993) The interaction of a rock phosphate, *Bradyrhizobium,* vesicular-arbuscular mycorrhizae and phosphate-solubilizing microbes on soybean grown in a sub-Himalayan mollisol. Mycorrhiza 4:37–43

Skvortsova NG, Umarov MM, Kostina NV (1998) The effect of nonleguminous plant inoculation with *Bacillus polymyxa – Pseudomonas* mixed cultures on nitrogen transformations in the rhizosphere. Microbiology (Moscow, Russ Fed) 67:201–204

Sletmoen M, Maurstad G, Sikorski P, Paulsen BS, Stokke BT (2003) Characterisation of bacterial polysaccharides: steps towards single-molecular studies. Carbohydr Res 338:2459–2475

Smirnova TA, Didenko LV, Tiganova IG, Andreyevskaya SG, Alexeyeva NV, Stepanova TV, Romanova YM (2009) Study on the structure of biofilm formed by *Salmonella typhimurium* bacteria on abiotic surfaces by the methods of light and transmission electron microscopy. Biotechnology 5:16–23 (in Russian)

Smirnova TA, Didenko LV, Azizbekyan RR, Romanova YM (2010) Structural and functional characteristics of bacterial biofilms. Microbiology (Moscow, Russ Fed) 79:413–423

Solano C, García B, Valle J, Berasain C, Ghigo J-M, Gamazo C, Lasa I (2002) Genetic analysis of *Salmonella enteritidis* biofilm formation: critical role of cellulose. Mol Microbiol 43:793–808

Stanley NR, Britton RA, Grossman AD, Lazazzera BA (2003) Identification of catabolite repression as a physiological regulator of biofilm formation by *Bacillus subtilis* by use of DNA microarrays. J Bacteriol 185:1951–1957

Sutherland IW (1972) Bacterial exopolysaccharides. Adv Microbiol Physiol 8:143–212

Sutherland IW (1986) Industrially useful of microbial polysaccharides. Microbiol Sci 3:5–9

Sutherland IW (1994) Structure-function relationships in microbial exopolysaccharides. Biotechnol Adv 12:393–448

Sutherland IW (2001) Exopolysaccharides in biofilm, flocs and related structures. Water Sci Technol 43:77–86

Syu MJ (2001) Biological production of 2,3-butanediol. Appl Microbiol Biotechnol 55:10–18

Tanaka M, Machida Y, Tanaka S, Okamatsu H, Yatake T (1982) Microbial process for the production of a polysaccharide and its kationic salt and their use in depressing serum and liver cholesterol level and thachogenic index. UK Patent № 2,080,818

Thoendel M, Horswill AR (2010) Biosynthesis of peptide signals in gram-positive bacteria. Adv Appl Microbiol 71:91–112

Tien TM, Gaskins MN, Hubbell DH (1979) Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). Appl Environ Microbiol 37:1016–1024

Tien TM, Diem HG, Gaskins MH, Hubbell DH (1981) Polygalacturonic acid transeliminase production by *Azospirillum* species. Can J Microbiol 27:426–431

Tikhonovich IA, Provorov NA (2003) Symbiotic genetics of plant-microbe interactions. Ekologicheskaya Genetika 1:36–46 (in Russian)

Timmusk S (2003) Mechanism of action of the plant growth promoting bacterium *Paenibacillus polymyxa*. PhD thesis, Uppsala University, Uppsala

Timmusk S, Wagner EGH (1999) The plant-growth-promoting rhizobacterium *Paenibacillus polymyxa* induces changes in *Arabidopsis thaliana* gene expression: a possible connection between biotic and abiotic stress responses. Mol Plant Microbe Interact 12:951–959

Timmusk S, Nicander B, Granhall U, Tillberg E (1999) Cytokinin production by *Paenibacillus polymyxa*. Soil Biol Biochem 31:1847–1852

Timmusk S, van West P, Gow NAR, Wagner EGH (2003) Antagonistic effects of *Paenibacillus polymyxa* towards the oomycete plant pathogens *Phytophthora palmivora* and *Pythium aphanidermatum*. In: Timmusk S (ed) Mechanism of action of the plant growth promoting bacterium *Paenibacillus polymyxa*. Uppsala University, Uppsala, Sweden, pp 1–28

Timmusk S, Grantcharova N, Wagner EGH (2005) *Paenibacillus polymyxa* invades plant roots and forms biofilms. Appl Environ Microbiol 71:7292–7300

Timmusk S, Paalme V, Lagercratz U, Nevo E (2009a) Detection and quantification of *Paenibacillus polymyxa* in the rhizosphere of wild barley (*Hordeum spontaneum*) with real-time PCR. J Appl Microbiol 107:736–745

Timmusk S, van West P, Gow NAR, Huffstutler RP (2009b) *Paenibacillus polymyxa* antagonizes oomycete plant pathogens *Phytophthora palmivora* and *Pythium aphanidermatum*. J Appl Microbiol 106:1473–1481

Tkachenko AA, Sevryugina TV (1989) Levan biosynthesis by *Bacillus polymyxa*. Mikrobiologiya 58:457–461 (in Russian)

Tran H, Ficke A, Asiimwe T, Höfte M, Raaijmakers JM (2007) Role of the cyclic lipopeptide massetolide A in biological control of *Phytophthora* infestans and in colonization of tomato plants by *Pseudomonas fluorescens*. New Phytol 175:731–742

Trüper HG (2005) The type species of the genus *Paenibacillus* Ash *et al*. 1994 is *Paenibacillus polymyxa*. Opinion 77 judicial commission of the international committee on systematics of prokaryotes correspondence. Int J Syst Evol Microbiol 55:513

Tupinambá G, da Silva AJR, Alviano CS, Souto-Padron T, Seldin L, Alviano DS (2008) Antimicrobial activity of *Paenibacillus polymyxa* SCE2 against some mycotoxin-producing fungi. J Appl Microbiol 105:1044–1053

Ui S, Masuda H, Muraki H (1983) Laboratory-scale production of 2,3-butanediol isomers (D(–), L(+), and meso) by bacterial fermentations. J Ferment Technol 61:253–259

Van Loosdrecht MCM (1988) Bacterial adhesion. PhD thesis, Agricultural University of Wageningen, the Netherlands

Vandevivere P, Kirchman DL (1993) Attachment stimulates exopolysaccharide synthesis by a bacterium. Appl Environ Microbiol 59:3280–3286

Verhoef R, Schols HA, Blanco A, Siika-aho M, Ratto M, Buchert J, Lenon G, Voragen AG (2005) Sugar composition and FTIR analysis of exopolysaccharides produced by microbial isolates from paper mill slime deposits. Biotechnol Bioeng 91:91–105

Vijayalakshmi SP, Raichur AM (2002) Bioflocculation of high-ash Indian coals using *Paenibacillus polymyxa*. Int J Miner Process 67:199–210

Vlamakis H, Aguilar C, Losick R, Kolter R (2008) Control of cell fate by the formation of an architecturally complex bacterial community. Genes Dev 22:945–953

Vogt C, Simon D, Alfreider A, Babel W (2004) Microbial degradation of chlorobenzene under oxygen-limited conditions leads to accumulation of 3-chlorocatechol. Environ Toxicol Chem 23:265–270

Voloshin SA, Kaprelyants AS (2004) Cell-cell interactions in bacterial populations. Biochemistry 69:1268–1275

Voloshin SA, Shleeva MO, Kaprelyants AS, Syroeshkin AV (2005) The role of intercellular contacts in the initiation of growth and in the development of a transiently nonculturable state by cultures of *Rhodococcus rhodochrous* grown in poor media. Microbiology (Moscow, Russ Fed) 74:420–427

Von der Weid IA, Paiva E, Nobrega A, van Elsas JD, Seldin L (2000) Diversity of *Paenibacillus polymyxa* strains isolated from the rhizosphere of maize planted in Cerrado soil. Res Microbiol 151:369–381

Vu B, Chen M, Crawford RJ, Ivanova EP (2009) Bacterial extracellular polysaccharides involved in biofilm formation. Molecules 14:2535–2554

Watnick P, Kolter R (2000) Biofilm, city of microbes. J Bacteriol 182:2675–2679

Weller DM, Thomashow LS (1994) Current challenges in introducing beneficial microorganisms into the rhizosphere. In: O'Gara F, Dowling DN, Boesten B (eds) Molecular ecology of rhizosphere biopolymers microorganisms. VCH, Germany, pp 1–18

Weller DM, Raaijmakers JM, McSpadden Gardener BB, Thomashow LS (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu Rev Phytopathol 40:309–348

Yahalom E, Dovrat A, Okon Y, Czosnek H (1991) Effect of inoculation with *Azospirillum brasilense* strain Cd and *Rhizobium* on the root morphology of burr medic (*Medicago polymorpha* L.). Isr J Bot 40:155–164

Yang J, Kharbanda PD, Mirza M (2004) Evaluation of *Paenibacillus polymyxa* pkb1 for biocontrol of *Pythium* disease of cucumber in a hydroponic system. Acta Hortic 635:59–66

Yegorenkova IV, Konnova SA, Sachuk VN, Ignatov VV (2001) *Azospirillum brasilense* colonisation of wheat roots and the role of lectin–carbohydrate interactions in bacterial adsorption and root-hair deformation. Plant Soil 231:275–282

Yegorenkova IV, Tregubova KV, Matora LY, Burygin GL, Ignatov VV (2008) Composition and immunochemical characteristics of exopolysaccharides from the rhizobacterium *Paenibacillus polymyxa* 1465. Microbiology (Moscow, Russ Fed) 77:553–558

Yegorenkova IV, Tregubova KV, Matora LY, Burygin GL, Ignatov VV (2010) Use of ELISA with antiexopolysaccharide antibodies to evaluate wheat-root colonization by the rhizobacterium *Paenibacillus polymyxa*. Curr Microbiol 61:376–380

Yegorenkova IV, Tregubova KV, Matora LY, Burygin GL, Ignatov VV (2011) Biofilm formation by *Paenibacillus polymyxa* strains differing in the production and rheological properties of their exopolysaccharides. Curr Microbiol 62:1554–1559

Yegorenkova IV, Tregubova KV, Ignatov VV (2013) *Paenibacillus polymyxa* rhizobacteria and their synthesized exoglycans in interaction with wheat roots: colonization and root hair deformation. Curr Microbiol 66:481–486

Yi K, Rasmussen AW, Gudlavalleti SK, Stephens DS, Stojiljkovie I (2004) Biofilm formation by *Neisseria meningitidis*. Infect Immun 72:6132–6138

York GM, González JE, Walker GC (1996) Exopolysaccharides and their role in nodule invasion. In: Stacey G, Mullin B, Gresshoff PM (eds) Biology of molecular plant-microbe interactions. International Society for Molecular Plant-Microbe Interactions, St. Paul, MN, pp 325–330

Zamudio M, Bastarrachea F (1994) Adhesiveness and root hair deformation capacity of *Azospirillum* strains for wheat seedlings. Soil Biol Biochem 26:791–797

Zanchetta P, Lagarde N, Guezennec J (2003) A new bone-healing material: a hyaluronic acid-like bacterial exopolysaccharide. Calcif Tissue Int 72:74–79

Zengguo H, Duygu K, Liwen Z, Chunhua Y, Kari BG-C, Ahmed EY (2007) Isolation and identification of a *Paenibacillus polymyxa* strain that coproduces a novel lantibiotic and polymyxin. J Appl Environ Microbiol 73:168–178

Zhang XQ, Bishop PL, Kupferle MJ (1998) Measurement of polysaccharides and proteins in biofilm extracellular polymers. Water Sci Technol 37:345–348

Zherebtsov NA, Abramova IN, Shelamova SA, Popova TN (2003) Identification of catalytically active groups in inulinase from *Bacillus polymyxa* 722. Appl Biochem Microbiol 39:544–548

Zogaj X, Nimtz M, Rohde M, Bokranz W, Römling U (2001) The multicellular morphotypes of *Salmonella typhimurium* and *Escherichia coli* produce cellulose as the second component of the extracellular matrix. Mol Microbiol 39:1452–1463

Chapter 18
Interactions in Rhizosphere for Bioremediation of Heavy Metals

Thounaojam Nevita, Piyush Pandey, Dinesh Kumar Maheshwari, and Anchal Sood

18.1 Introduction

Emphasis on economic growth and industrial activities at the global level has resulted in accumulation of heavy metals in soil. Metals such as lead, copper, cobalt, mercury, cadmium, nickel, selenium and zinc are the most common contaminants of soil. These metals reach the fertile layers of soil through industrial effluents and adversely affect the vegetation. Due to the physiological activities of plants and/or microorganisms associated with root surfaces, the roots of plants growing in soil contaminated with heavy metals have the ability to increase the solubility of metals and may change their speciation, including alteration of the redox potential, exudation of metal chelants and organic ligands and acidification/alkalinization (Glick 1995; Barea et al. 2002; Yang et al. 2009).

Rhizosphere microorganisms, which are closely associated with roots, have been termed plant growth-promoting rhizobacteria (PGPR). These bacteria are capable of promoting plant growth by colonizing the plant root (Moulin et al. 2001). Soil microorganisms play an important role in management of heavy metal contamination because of their ability to increase solubility and change the speciation of metals through the production of organic ligands and release of metabolites like siderophores and organic acids that complex cationic metals or desorbed anionic species by ligand exchange (Hallberg and Johnson 2005). An extension of PGPR technology is the emerging use of these bacteria with plants for environmental

T. Nevita • P. Pandey (✉)
Department of Microbiology, Assam University, Silchar, Assam 788011, India
e-mail: piyushddn@gmail.com

D.K. Maheshwari
Department of Botany and Microbiology, Gurukul Kangri University, Haridwar, Uttarakhand, India

A. Sood
Dolphin P.G. College of Life Sciences, Chunni Kalan, Fatehgarh Sahib, Punjab, India

D.K. Maheshwari et al. (eds.), *Bacteria in Agrobiology: Crop Productivity*,
DOI 10.1007/978-3-642-37241-4_18,

applications. Recent studies in this area include many different uses: growth promotion of soil-stabilizing plants; to counteract flooding stress in plants; aid plant growth in acidic conditions; counter high temperature stress; and the use of PGPR in phytoremediation technologies. Thus, adding PGPR can aid plant growth. This paper focuses on the importance of the rhizosphere for the bioremediation of heavy metals present in the soil and its role in improving plant growth, with discussions on the interaction of microorganisms in the rhizosphere.

18.2 Interactions in the Rhizosphere: Root Exudate and Rhizobacteria

The term "rhizosphere effect" was first described by Hiltner (1904). Due to the nutrients exuded by plant roots, many microorganisms increase in number near the plant roots and provide a carbon-rich environment. The plant roots and microbes initiate colonization by producing and exchanging chemical signals. This process has been described by several authors in detail (Lugtenberg et al. 2001, 2002; de Weert et al. 2002; Bais et al. 2004a, b). The term "rhizosphere" refers to the region of high microbial activity in the soil surrounding the roots, which is influenced by root exudation that affects the microbial communities (Kent and Triplett 2002). Because of the associated microorganisms, the rhizosphere plays a crucial role in the formation and modification of soil (Pate et al. 2001; Pate and Verboom 2009; Taylor et al. 2009) and therefore holds strong potential to overcome heavy metal contamination. In fact, soil microorganisms support bioremediation by using the green or terrestrial plant for cleaning up the hazardous chemicals present in the environment, which is now an emerging technology (Chaney 1983; Baker et al. 1991; Salt et al. 1995).

In addition to its role in mineral acquisition, root exudates have the potential to contribute to the bioremediation of heavy metals. There are several reports that describe the ability of various plant species to accumulate and release heavy metals as volatile root exudates, thus reducing their concentrations in the soil environment (Mench and Martin 1991; Terry et al. 1992; Banuelos et al. 1993; Nanda Kumar et al. 1995; Zayed et al. 1998). Plant roots exude a large number of compounds into the rhizosphere. In the rhizosphere, some complex chemical, physical and biological interactions occur between roots and the soil. The interactions between the roots and soil include root–root, root–insects and root–microbe interactions and therefore various steps have been taken to understand the types of interaction (Hirsch et al. 2003). The rhizosphere is highly dynamic because of the interactions occurring between roots, pathogenic soil microbes and invertebrates (Hirsch et al. 2003).

Symbiotic associations, mycorrhizal fungi and root colonization by bacteria and plant growth-promoting bacteria are all positive interactions. Negative interactions include competition or parasitism in plants, pathogenesis in bacteria or fungi and

invertebrate herbivory (Bais et al. 2006). It is suggested that the root exudates have a major role in determining the outcome of interactions by producing and secreting compounds into the rhizosphere (Gleba et al. 1999; Bais et al. 2001). The secretions include ions, free oxygen and water, enzymes, mucilage and carbon-containing primary and secondary metabolites (Uren 2000; Bertin et al. 2003) in addition to organic ligands (e.g. carbohydrates, organic acids, humic acids, polypeptides, proteins, amino acids, nucleic acids, etc.) and inorganic ligands (e.g. chloride, sulfate, ammonium, phosphate, carbonate etc.). These substances are utilized as the energy source of microorganisms and also function as ligands to be chelated with heavy metal ions. Organic acids released from roots are able to reduce toxicity from heavy metals in soils. Malate, citrate and oxalate have the most dramatic effect due to their implication in the complexation of metals (Hinsinger 2001). Specific organic acids can sequester heavy metals and protect the plants from their toxic effects (Jones et al. 2003; Jung et al. 2003; Liao and Xie 2004; Schwab et al. 2005).

Root exudation is classified into two active processes, excretion and secretion. Excretion involves gradient-dependent output of waste materials with unknown functions and secretion involves exudation of compounds with known functions, such as lubrication and defence (Uren 2000; Bais et al. 2004a, b). The root exudates are also grouped into two classes, low and high molecular weight exudates. Low molecular weight exudates include amino acids, sugars, phenolics and other secondary metabolites; and high molecular weight exudates include mucilage and proteins (Hawes et al. 2000; Vicre et al. 2005). Root exudates have been reported to precipitate heavy metal ions outside the roots by absorbing and binding to them. Cd combined as a complex with oxides of Fe and Mn and with some organic acids was accumulated in rice rhizosphere much more than in non-rhizosphere soil (Lin et al. 1998). Also, root exudates from wheat and rice plants stressed with Pb and Cd heavy metals were found to have a different composition to those from plants not treated with Cd and/or Pb (Lin et al. 2003), as shown by analysis using a capillary electrophoretic method. Equilibrium dialysis demonstrated that root exudates bound metals to an extent that depended on the metal involved; for wheat exudates, the importance of the binding followed the order Pb $>$ Cd. Hence, root exudates can influence Cd and Pb absorption and distribution in plants (Dong et al. 2007). Also, in a different study, it was reported that the Ni-chelating histidine and citrate accumulate in the root exudates of non-hyperaccumulating plants and thus could help to reduce Ni uptake and so play a role in a Ni-detoxification strategy (Salt et al. 2000). Buckwheat secretes oxalic acid from the roots in response Al stress and accumulates nontoxic aluminium oxalate in leaves and, thus, detoxification occurs both externally and internally (Ma et al. 1997).

Plant–microbe interactions positively influence plant growth through various mechanisms such as atmospheric nitrogen fixation, biotic and abiotic stress tolerance and, directly and indirectly, through PGPR (Moulin et al. 2001; Schardl et al. 2004; Gray and Smith 2005). The bacteria can interact positively with plants by secreting biofilms or antibiotics against potential pathogens as a biocontrol (Bais et al. 2004a, b) or by degrading plant–microbe products that might act

allelopathically or autotoxically in soil. However, the rhizosphere bacteria can also affect plant health and survival through pathogen or parasite infection, but the chemical signals secreted both from plants and microbes help to determine whether the interaction is to be stopped or started. Root colonization thus plays a very crucial role in plant and microbe interaction.

18.3 Bioremediation of Heavy Metals

Heavy metals are elements with metallic properties (conductivity, stability as cations, ligand specificity, etc.) and with atomic number >20. The most common heavy metals are Cd, Cr, Cu, Hg, Pb and Ni. The soil contains large amounts of metals, which occur naturally and are required by plants as micronutrients. Heavy metals have a specific gravity five times the specific gravity of water (which is 1 at 4 °C). Small amounts of elements are present in our environment and diet and are essential for good health, but large amounts may cause acute or chronic toxicity (Chabot et al. 1996; Glanze 1996). The heavy metals cannot be degraded easily, not even by biological processes. They can be transformed from an organic state or organic complex to another organic state and because of this degradation of heavy metals in soils is very difficult (Garbisu and Alkorta 2001). Pollution by heavy metals decreases soil microbial activity and crop production and is becoming a severe threat to environmental and human health. When the heavy metals are at an elevated level, they are absorbed by plant roots and transferred to shoots, which results in impaired and reduced growth due to excess accumulation of heavy metals (Foy et al. 1978; Bingham et al. 1986). Different methods have been applied for the degradation of heavy metals (thermal treatment, acid leaching etc.) but they are very costly and also cause destruction of the soil and its fertility. Therefore, the method of phytoremediation for heavy metals is important (Chaney et al. 2000; Cheng et al. 2002; Lasat 2002).

The roots of plants play a very important role because they interact with huge numbers of microorganisms for the degradation of heavy metals, and these interactions lead to their major role in phytoremediation (Glick 1995). The interaction of plants and bacteria with heavy metals affects both the plant and the bacteria. The microbes present in the soil help in recycling plant nutrients, soil and detoxification of noxious chemicals, and also support plant growth (Elsgaard et al. 2001; Filip 2002). The plant and bacteria form both specific and nonspecific kinds of association: first, the plant provides a carbon source to bacteria, which helps the bacteria to decrease the phytotoxicity of the contaminated soil; and second, the plant stimulates the microbial community, which supports the metabolic degradation of contaminated soil.

Rhizobacteria have the ability to alter the bioavailability of heavy metals in the soil (McGrath et al. 2001; Whiting et al. 2001; Lasat 2002) by releasing chelating substances, acidification of the microbial community and changing the redox potential (Smith and Read 1997). Abou-Shanab et al. (2003) have reported that

the uptake of Ni by the plant increases when added with *Sphingomonas macrogoltabidus*, *Microbacterium liquefaciens*, *Microbacterium arabinogalactanolyticum* and *Alyssum murale* as compared with the un-inoculated plant.

18.4 Role of Plants in the Bioremediation of Heavy Metals

The heavy metals are highly reactive at higher oxidation states and therefore highly toxic for plant cells because they react with biomolecules at both the cellular and molecular level. This results in alteration of different plant physiology processes through inactivation and denaturation of enzymes and proteins, blocking of functional groups of metabolically important molecules and functional cellular units, conformational modifications and disruption of membrane integrity (Ramesh 2008; Villiers et al. 2011). The devastating effects of heavy metals may include modified plant metabolism such as inhibition of photosynthesis, respiration and activities of various enzymes (Sharma and Dubey 2007; Hossain et al. 2009, 2010, 2012a; Sharma and Dietz 2009; Hossain and Fujita 2009, 2011; Tan et al. 2010; Dubey 2011). Further, heavy metals are also known to disturb the redox homeostasis by creating free radicals and reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\bullet}OH$) (Sharma and Dietz 2009; Hossain et al. 2010; Dubey 2011; Anjum et al. 2012).

An increase in ROS and methylglyoxal in cells leads to oxidative stress that leads to lipid peroxidation, membrane dismantling, ion leakage and DNA strand cleavage, which leads to plant death (Navari-Izzo 1998; Romero-Puertas et al. 2002; Hossain et al. 2010; Barconi et al. 2011; Rascio and Navari-Izzo 2011). To survive, the plants need a combination of both physiological and biochemical processes, which also require a change in global gene expression and various strategies to cope with the toxic effects of heavy metals. To survive, plants first need "avoidance" to reduce the heavy metals entering the cells through extracellular precipitation, biosorption, uptake or increased efflux. In addition, heavy metals that do enter the cells are chelated intercellularly within the vacuole compartments and by the production of amino acids, organics acids and metallothioneins, phytochelatins and by regulation of the antioxidant defence and glyoxalase system to removed the deleterious effects caused by ROS and MG (Leyval et al. 1997; Cobbett 2000; Hall 2002; Yang et al. 2005; Clemens 2006; Singla-Pareek et al. 2006; Hossain et al. 2009, 2010; Yadav 2010; Seth et al. 2012). Plant-based bioremediation technologies have been collectively termed "phytoremediation", which can be divided into the following categories (Ghosh and Singh 2005):

(a) Rhizofiltration is defined as the use of plants to absorb, concentrate and precipitate contaminants from polluted aqueous sources, with low contaminant

concentration in their roots. Plants like the sunflower have been reported for their ability to remove lead from effluent. Rhizofiltration has been used for lead, cadmium, copper, nickel, zinc and chromium, which are primarily retained within the roots (Chaudhry et al. 1998).

(b) Phytostabilization includes the sorption, precipitation or reduction of metal valence. The plants decrease the amount of water percolating through the soil. This process is the ability of roots to limit contaminant mobility and bioavailability in the soil and is mostly used for the remediation of soil, sediment and sludge (Mueller et al. 1999).

(c) Phytoextraction or phytoaccumulation is the process whereby plants absorb, concentrate and precipitate toxic metals as biomass from contaminated soils. It is the best approach for removing the contamination from soil without destroying the soil structure and fertility.

(d) Phytovolatilization involves the uptake of contaminants by the plants from soil, transforming them into volatile form and transpiring them into the atmosphere. Removal of mercury by this process had been reported; however, mercury released into the atmosphere is expected to be recycled by precipitation and then redeposited back into ecosystem (Henry 2000). Some plants grown in high selenium media were found to produce volatile selenium in the form of dimethylselenide and dimethyldiselenide (Banuelos 2000).

(e) Phytodegradation is the metabolic breakdown of organics to simple products that are incorporated into the plant tissues (Chaudhry et al. 1998). Plants contain enzymes that can break down and convert ammunition wastes, chlorinated solvents such as trichloroethylene and other herbicides. The enzymes are usually dehalogenases, oxygenases and reductases (Black 1995). Rhizodegradation is the breakdown of organics in the soil through microbial activity of the rhizosphere. Further, the rhizobacteria in association with the plant provide efficiency to the process of phytoremediation (Whiting et al. 2001; Abou-Shanab et al. 2003). Giller et al. (1998) reported that in a metal-polluted soil environment, the microbial diversity and activities are affected. Kumar et al. (1995) concluded that the plants of the *Brassicaceae* family have very high ability to accumulate heavy metals, although such plants have a slow growth rate and produce limited biomass when the soil is highly contaminated. Some workers have recommended a *Brassica juncea*–PGPR association as one of the most effective measures for bioremediation of heavy metals (Wenzel et al. 1999; Glick 2003) because *B. juncea* accumulates less metal yet shows high growth rates.

The toxic effect of heavy metals on plant tissues and the physiological mechanism of heavy metal tolerance in plants is not discussed here in detail, although it has been explained in several excellent reviews (Marques et al. 2009; Manara 2010; Hossain et al. 2012b).

18.5 Response of Bacteria to Heavy Metal Contamination

Microorganisms require some metals ions like iron, zinc, copper and manganese as micronutrients, yet metals such as zinc and copper are toxic at high concentrations. Bacteria utilize a variety of resistance mechanisms to protect themselves from toxic concentrations of metals, including permeability barriers, intra- and extracellular sequestration, efflux pumps, enzymatic detoxification and reduction (Nies 1999). The presence of heavy metals at high concentration has great effects on the microbial communities in soils in several ways: (1) it may lead to a reduction of total microbial biomass (Giller et al. 1998), (2) it lowers numbers of specific populations (Chaudri et al. 1993) and (3) it may change microbial community structure (Gray and Smith 2005). Thus, at high concentrations, metal ions can either completely inhibit the microbial population by inhibiting their various metabolic activities or organisms develop resistance or tolerance to the elevated levels of metals. To have a toxic effect, heavy metal ions must first enter the cell and do so via uptake mechanisms that exist because some heavy metals are necessary for enzymatic functions and bacterial growth. Generally there are two uptake systems, quick and unspecific. The quick system is driven by a chemiosmotic gradient across the cell membrane and thus requires no ATP. The other system is slower and more substrate-specific, driven by energy from ATP hydrolysis. Influx of a wide variety of heavy metals occurs because the mechanism is more efficient. When these metals are present at high concentrations, they are more likely to have toxic effects once they are inside the cell (Nies and Silver 1995).

The bacterial resistance mechanisms are encoded generally on plasmids and transposons, and it is probably by gene transfer or spontaneous mutation that bacteria acquire their resistance to heavy metals. In Gram-negative bacteria (e.g. *Ralstonia eutropha*), the czc system is responsible for the resistance to cadmium, zinc and cobalt. The *czc*-genes encode for a cation-proton antiporter (CzcABC), which exports these metals. A similar mechanism, called the *ncc* system, has been found in *Alcaligenes xylosoxidans* and provides resistance against nickel, cadmium and cobalt. In contrast, the cadmium resistance mechanism in Gram-positive bacteria (e.g. *Staphylococcus*, *Bacillus* or *Listeria*) is through Cd-efflux ATPase. Plasmid-encoded energy-dependent metal efflux systems involving ATPases and chemiosmotic ion/proton pumps are also reported for arsenic, chromium and cadmium resistance in other bacteria. The exploitation of these bacterial properties for the remediation of heavy metal-contaminated sites has been shown to be a promising bioremediation option (Lloyd and Macaskie 2000). Although the threshold limit of metal toxicity to soil microorganisms is not conclusive, the interaction between heavy metals and microbes does occur in nature. Microorganisms can interact with metals through many mechanisms, some of which may be used as the basis of potential bioremediation strategies.

There are several reviews available that address the molecular mechanism of heavy metal resistance in bacteria (Summers 1992; Hobman and Brown 1997; Brown et al. 2003; Barkay et al. 2003; Hobman et al. 2005). Bacterial metal

resistance systems are regulated by transcriptional factors from the MerR family (COG0789), ArsR/SmtB family (Wu and Rosen 1993) and by two-component systems such as CusRS (San Francisco et al. 1990), SilRS (Brown et al. 1995) and PcoRS (Rouch and Brown 1997). The mechanisms of allosteric coupling of various metal-dependent regulators have been reviewed by Penella and Giedroc (2005). *Ralstonia* sp. has the genetic ability to survive heavy metal concentrations encoded on a large plasmid, pMOL30, designated as *czc* for cobalt-cadmium-zinc resistance (Nies et al. 1987). This system detoxifies the cell by cation efflux: the three heavy metal cations, which are taken up into the cell by the fast and unspecified transport system for Mg^{2+}, are actively extruded from the cell by products of the *czc* resistance determinants (Nies et al. 1989a, b). *Ralstonia* also has additional structural genes *cnrCBA* for cobalt-nickel resistance (Liesegang et al. 1993) that are located on another megaplasmid, pMOL28 (Taghavi et al. 1997). Protein families involved in bacterial heavy metal metabolism are given in Fig. 18.1.

One of the limitations to rhizoremediation of heavy metals is that the most of the bacteria are unable to survive in near-starvation conditions in the rhizosphere (Normander et al. 1999). Several methods have been developed to improve the degradation process and tolerance of bacteria for contaminated soil. In fact, it has been proposed that specific enzymes should be produced by using genetically engineered microorganisms for sustainable degradation of toxic organic substances, which may be preferred over wild-type organisms in contaminated sites. In contrast to the conventional attributes of wild-type microorganisms, the specially designed metabolic pathways of genetically engineered bacteria can reduce or eliminate undesired toxic intermediates (Pieper and Reineke 2000; Furukawa 2003).

18.6 Plant Growth-Promoting Rhizobacteria: Role in Heavy Metal Degradation

The rhizosphere is known to contain large microbial communities with high metabolic activity compared to bulk soil (Anderson et al. 1993). The microbial community affects the mobility and availability of heavy metals to plants by the release of chelating agents, acidification, phosphate solubilization and redox changes (Smith and Read 1997; Abou-Shanab et al. 2003). The PGPR in association with plants roots provide beneficial effects on plant growth and also provide nutrition through various mechanisms such as N_2 fixation, phytohormone production and siderophores and also by transformation of nutrients when they are applied to seeds or soil (Kloepper et al. 1989; Glick 1995; Glick et al. 1999). In last two decades, the role of rhizobacteria to enhance phytoremediation of heavy metals has attracted major interest in the scientific community (de Souza et al. 1999; Whiting et al. 2001). Apparently, plant–rhizobacteria interaction has the following advantages over other techniques of heavy metal decontamination in soil: (1) it preserves the natural properties of soil, (2) it receives energy from sunlight, (3) high

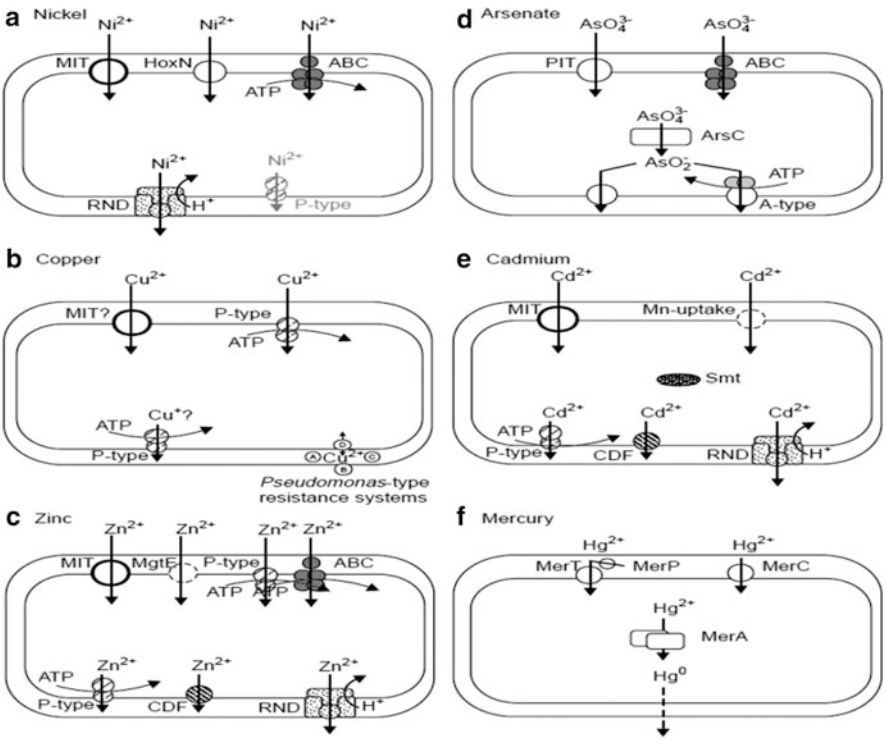


Fig. 18.1 (**a**–**f**) Protein families involved in bacterial heavy metal metabolism. (**a**) Ni^{2+} is accumulated by the fast and unspecific Cor A (metal transport system, *MIT*) Mg^{2+} transport system. Highly specific nickel transporters are either HoxN chemiosmotic transporters or ATP-binding cassette (*ABC*) uptake transporters, which use a periplasmic nickel-binding protein depending on the bacterial species. Characterized nickel resistance systems are based on inducible resistance–nodulation–cell division (*RDN*)-driven transenvelope transporters. Moreover, a nickel-efflux P-type ATPase may exist in *Helicobacter pylori*. (**b**) Cu^{2+} is possibly accumulated by the CorA Mg^{2+} transporter and additionally by P-type ATPases under copper starvation (shown in *Enterococcus hirae*). The mechanism of resistance systems similar to the *Pseudomonas* Cop system is still elusive but, in Gram-positive bacteria, P-type ATPase seems to detoxify copper via efflux. The copper resistance systems of *Pseudomonas* type usually encode four proteins (*circles* with *A*, *B*, *C* or *D*), which bind copper in the periplasm or close to the outer membrane. (**c**) Zn^{2+} is accumulated by the fast and unspecific CorA (MIT) Mg^{2+} system in some bacterial species, and by the fast and unspecific magnesium transporter (*MgtE*) system in others. Inducible, high-affinity ABC transporters supply zinc in times of need. P-type ATPases may transport zinc in both directions, bringing about its uptake as a byproduct of Mg^{2+}-uptake and its efflux as detoxification. Slow efflux is catalyzed by the cation-diffusion facilitator (*CDF*) transporter and the high-efficiency transenvelope efflux by an inducible RND-driven transporter like Czc. (**d**) Arsenate is accumulated by the constitutive, fast and unspecific phosphate inorganic transport (*PIT*) and the phosphate inducible phosphate-specific transport (Pst) systems. Inside the cell arsenate is reduced by ArsC to arsenite, which is removed from the cell by ArsB, either acting alone or together with the A-type ATPase ArsA. (**e**) Magnesium (MIT) and/or manganese uptake systems are responsible for the uptake of Cd^{2+}. Only in cyanobacteria have metallothionein-like proteins (*Smt*) been characterized. Efflux is carried out in Gram-positive bacteria by P-type ATPases; in Gram-negative bacteria it take the form of RND-driven transenvelope transport and

levels of microbial biomass in the rhizosphere can be achieved, (4) it is low in cost and (5) it has the potential to be rapid (Huang 2004c).

Initially, the remediation strategy was confined to the use of some plants and has been used widely in the degradation of heavy metal pollution present in soil. However, the phytoremediation technology also has drawbacks that lead to low rates of seed germination, slow rates of plant development and decreases in plant biomass. These problems can be solved by using selected species of PGPR along with the host plant (Glick 2003). The term rhizoremediation is the combination of phytoremediation and bioaugmentation with PGPR (Kuiper et al. 2004). The addition of PGPR helps in the removal of organic pollutants through an increase in seed germination and stimulates the plant to grow faster (Huang et al. 2004a, b).

One important trait of PGPR is that they can enhance the remediation process by subsequent decrease of ethylene stress in plants (Deikman 1997), which is a major reason for a decrease in plant growth. PGPR consume amino-cyclopropane carboxylic acid (ACC), which is the intermediate to ethylene, through synthesis of 1-amino-cyclopropane-1-carboxylate deaminase (ACC deaminase) that reduces ethylene production in stressed plants (Hall 1996; Reed and Glick 2005; Safronova 2006). The PGPR also have the ability to solubilize phosphates and provide other nutrient for an increase in plant growth (Goldstein 1986). They also help in the suppression of deleterious microorganism growth by the production of siderophores, β-1,3 glucanases, chitinases and antibiotics (Cattelan et al. 1999). Also, as explained earlier, it has been established by several workers that the exudates from roots stimulate bacterial growth by degradation of various contaminants of soil and thus reduce the toxicity to plants, in addition to providing nutrients for the plants and alleviating plant stress by preventing synthesis of stress ethylene (Macek et al. 2000; Hontzeas et al. 2004; Huang et al. 2004b; Chaudhry et al. 2005).

Belimov et al. (2005) isolated cadmium-tolerant plant growth promoting bacteria, namely *Variovorax paradoxus*, *Rhodococcus* sp. and *Flavobacterium* sp., from the roots of Indian mustard. These strains were capable of stimulating root elongation of Indian mustard and were able to tolerate metals including Zn, Ni and Co. The ACC deaminase activity of these bacteria and their stimulating effect on root elongation suggests that the utilization of ACC is helpful in determining the promotion of root growth. In an interesting study, an *Escherichia coli* gene, *ZntA*, which encodes a Pb(II)/Cd(II)/Zn(II) pump, was tested for developing plants with reduced heavy metal content. Yeast cells transformed with this gene had improved resistance to Pb(II) and Cd(II). In Arabidopsis plants transformed with *ZntA*, ZntA

Fig. 18.1 (continued) is possibly carried out by the CDF transporter. (**f**) For mercury, the resistance determinant encodes the transport systems. MerT interacts with a periplasmic mercury-binding protein, MerP. Transport by MerC may be in addition to that by MerT or may substitute for MerT transport, depending on the respective resistance determinant. Inside the cell, Hg^{2+} is reduced to metallic mercury, which diffuses out of the cell and its environment (redrawn from Nies (1999) with permission)

was localized at the plasma membrane and improved the resistance of the plants to Pb(II) and Cd(II) (Lee et al. 2003).

Rajkumar et al. (2006) isolated Cr(VI)-resistant PGPR, *Pseudomonas* sp. Ps 4A and *Bacillus* sp. Ba 32 from contaminated soil and studied their effect on Indian mustard. They reported that the strains protect the plant against the inhibitory effect of Cr due to the production of indoleacetic acid (IAA), siderophores and solubilization of phosphate. In a different study, chromium-resistant bacteria, namely *Ochrobactrium intermedium* and *Bacillus cereus*, were inoculated on the seeds of *Vigna radiate* and it was found that Cr(VI) supplied to the seedling was reduced to Cr(III) in the rhizosphere by the bacterial strain, thus in turn lowering the toxicity of chromium to the seedling (Faisal and Hasnain 2006).

Wu et al. (2006b) demonstrated that the expression of metal-binding peptide (EC_{20}) in a rhizobacterium, *Pseudomonas putida* 06909, and reported that it not only improved cadmium binding but also alleviated the cellular toxicity of cadmium. In addition, inoculation of sunflower roots with the engineered rhizobacteria resulted in marked decrease in cadmium phytotoxicity and a 40 % increase in cadmium accumulation in plant roots. Analysis of the significantly improved growth characteristics of both the rhizobacterium and plant suggested that the use of EC_{20}-expressing *P. putida* endowed with organic degrading capabilities was a promising strategy for remediation of sites contaminated with both organics and metals. *Bacillus subtilis* strain SJ-101 was found to have role in Ni accumulation in Indian mustard, as it exhibited the capability of producing IAA and solubilizing inorganic phosphate. Therefore, owing to its intrinsic ability to promote plant growth and alteration of soil Ni by biosorption or bioaccumulation, it is suggested that the strain SJ-101 is exploited for bacteria-assisted phytoaccumulation of toxic Ni from contaminated sites (Zaidi 2006). The role of PGPR for bioremediation of heavy metals through the plant rhizosphere and their effect on plants is summarized in Table 18.1.

18.7 Role of Mycorrhiza in Management of Heavy Metal Contamination in the Rhizosphere

It is well known that mycorrhizal fungi are a major component of the rhizosphere and form mutualistic associations with most plant species. Mycorrhizal fungi have a great potential for heavy metal remediation in soil. Mycorrhizal fungi form mutualistic associations with plants and contribute to plant growth by improving uptake of minerals from soil and, additionally, protect the plant from heavy metal stress in contaminated soil (Leyval et al. 1997; Perotto and Martino 2001; Ouziad et al. 2005). The fungal symbiont facilitates allocation of heavy metals within the roots and several mechanisms have been suggested for this process, including binding of heavy metals on the cell wall and deposition in vacuoles, siderophore-mediated

Table 18.1 Application of PGPR for bioremediation of heavy metals through the plant rhizosphere and their effect on plants

Plant	Bacteria	Heavy metal	Plant-growth promoting attributes of PGPR	Effect of PGPR on host plant	References
Brassica juncea	*Pseudomonas* sp. PSA 4, *Bacillus* sp. Ba32	Cr(VI)	IAA, phosphate solubilization	Stimulates plant growth and decreases Cr(VI)	Rajkumar et al. (2006)
Vigna radiata	*Ochrobactrum intermedium* and *Bacillus cereus*	Cr(VI)	na	Lowers the toxicity of Cr to seedlings by reducing Cr(VI) to Cr(III)	Faisal and Hasnain (2006)
Brassica napus	*Xanthomas* sp. RJ3, Azomonas sp. RJ4, *Pseudomonas* sp. RJ10, *Bacillus* sp. RJ31	Cd	IAA	Stimulates plant growth and increases cadmium accumulation	Sheng and Xia (2006)
Brassicae juncea	*Azotobacter chroococcum* HKn-5	Pb, Zn	na	Stimulates plant growth	Wu et al. (2006a)
Brassicae juncea	*Bacillus mucilaginosus* HKK-1	Pb, Zn	na	Protects plants from metal toxicity	Wu et al. (2006a)
Brassicae juncea	*Bacillus megatrium* HKPI	Pb, Zn	na	Protects plants from metal toxicity	Wu et al. (2006a)
Brassicae juncea	*Bacillus subtilis* SJ-101	Ni	IAA, phosphate solubilization	Facilitates nickel accumulation	Zaidi et al. (2006)
Trifollium repens	*Brevibacillus*	Zn	na	Enhances plant growth in zinc-contaminated soil	Vivas et al. (2006)
Sunflower	*Ochrobacterium entermedium*	Cr(VI)	na	Increases plant growth and decreases the uptake of Cr(VI)	Faisal and Hasnain (2005)
Tall fescue	*Enterbacter cloacae* CAL 2/*Enterbacter cloacae* UW4	Total petroleum hydrocarbon	IAA, siderophore, ACC deaminase	Increases plant tolerance and accelerates plant growth in heavily contaminated soil	Huang et al. (2005b)
Alfalfa	*Pseudomonas fluorescens*	Polychlorinated biphenyls (PCBs)	na	More effectively metabolizes PCBs with *bph* gene cloned	Villacieros et al. (2005)

Soyabean	*Pseudomonas fluorescens*	Hg	IAA, phosphate solubilization and siderophore production	Increases plant growth	Gupta et al. (2005)
Soyabean	*Pseudomonas* sp. NBR 14014	Cd, Ni, Cr	IAA, phosphate solubilization	Root and shoot elongation	Gupta et al. (2002)
Tomato, canola, Indian mustard	*Kluyvera ascorbeta* SUD 165, *Kluyvera ascorbeta* SUD 165/25	Ni, Pb, Zn	Siderophore	Both strains decrease some plant inhibition by heavy metals. No increase in metal uptake occurs with either strain compared with non-inoculated plants	Burd et al. (2000)
Wheat	*Pseudomonas fluorescens* 2-79	Trichloroethylene (TCF)	na	Degrades TCF with toulene *O*-monooxygenase	Yee et al. (1998)
Tomato, canola, perennial grass, Indian mustard	*Kluyvera ascorbeta* SUD 165 and SUD 165/26, *Pseudomonas lolacisii* RP-23 and *Pseudomonas fluorescens* RS9, *Variovorax paradoxus*, *Rhodococci* sp. and *Flavobacterium* sp.	Cd, Zn, Cu, Ni, Co, Cr, Pb	ACC, siderophore	Resistant to Cd, Zn, Cu, Ni, Co, Cr and Pb; stimulation of root elongation of plant seedlings	Burd et al. (1998, 2000), Dell'Amico et al. (2005), Belimov et al. (2005)

ACC amino-cyclopropane carboxylic acid, *IAA* indoleacetic acid, *na* not available

uptake and the presence of transporters (Galli et al. 1994; Guo et al. 1996; Leyval et al. 1997; Schutzendubel and Polle 2002).

Huang et al. (2005a) observed that speciation of Cu, Zn and Pb changed significantly in the rhizosphere of arbuscular mycorrhiza (AM)-infected and non-infected maize in comparison to bulk soil. The level of exchangeable Cu increased by 26 % in non-infected maize whereas a 43 % increase was recorded in the AM-infected rhizosphere, as compared to bulk soil. Further, an increase in the level of organic-bound Zn and Pb was also recorded in the rhizosphere in comparison to bulk soil. However, carbonate and Fe-Mn oxides of Zn and Pb did not exhibit significant changes. The authors were able to conclude that mycorrhiza protects its host plants from the phytotoxicity of excessive Cu, Zn and Pb by changing their speciation from the bioavailable to the non-bioavailable form. However, the role of AM fungi in the plant stress response is variable when the host is exposed to metal stress. Some authors have reported reduction in metal concentrations in plants due to mycorrhizal colonization (Heggo et al. 1990; Jentschke et al. 1998). An exclusion strategy, showing lower Zn accumulation by AMF-colonized *Zea mays* has been proposed (Huang et al. 2002). Enhanced growth and metal root-to-stem translocation in *Cannabis sativa* plants inoculated with the AM *Glomus mosseae* has been reported (Citterio et al. 2005). Similarly, Chen et al. (2005) observed that a mixed AM inoculum enhanced Pb uptake and growth of *Kummerowia striata*, *Ixeris denticulate*, and *Echinochloa crusgalli* var *mitis*, even resulting in metal levels toxic to plants (Weissenhorn and Leyval 1995).

However, some reports have indicated enhanced uptake and accumulation of heavy metals in plants due to AM colonization (Ahonen-Jonnarth and Finlay 2001; Joner and Leyval 2001; Jamal et al. 2002; Marques et al. 2007a). Most of the reports indicate the possibility of a species-specific effect of AM associations on plant metal uptake and accumulation. Marques et al. (2007b) have shown that inoculation with *G. intraradices* or *G. claroideum* protected the host plant *Solanum nigrum* at high Zn concentration, which was translated into a decrease in metal accumulation in AM-inoculated plants, whereas there was an increase in the metal accumulation at lower Zn levels in the growing matrix. AM are known to produce small cysteine-rich proteins known as metallothioneins, which are similar to phytochelatins, a group of cysteine-rich, heavy metal-binding proteins that are induced when plants are faced with HM stress (Colbet and Goldsbrough 2002). AM association, *G. mosseae–C. sativa* (var. Carmagnola), enhances the root-to-shoot metal translocation to sequester toxic metals in the shoot cell vacuoles by metallothioneins and phytochelatins. Further, transcription of the phytochelatin synthetase gene and three metallothionein genes was increased in mycorrhizal pea roots in response to heavy metal stress (Citterio et al. 2005).

A transcriptional increase of the glutathione-dependent glutathione *S*-transferase gene was observed in *Glomus intraradices* that colonized *Medicago truncatula* plants when subjected to Zn stress (Hildebrandt et al. 2007). Further, upregulation of ROS metabolic genes suggests that fungal heavy metal tolerance also affects the heavy metal tolerance of plants. However, the exact mechanisms of mycorrhizal

fungi-induced heavy metal tolerance and its effect on plants still needs substantial research.

18.8 The Future of Rhizosphere Technology in Heavy Metal Bioremediation

Despite the findings and observations that promote the use of plant–PGPR interaction as an effective strategy for bioremediation of heavy metals in contaminated soil, acceptance of this strategy is not widespread due to the measurement of its performance, ultimate utilization of by-products and its overall economic viability. Till now, the technologies have been rated more on economic efficiency and time. Therefore, this strategy has been evaluated using commercial constraints, i.e. by the expectation that site remediation should be achieved in a time comparable to other clean-up technologies. Also, the observations and data have been collected under laboratory conditions, where promising bacteria are not in competition with indigenous soil bacteria, which is the situation in real soil. The future of this strategy is still in the research and development phase and there are many technical barriers that need to be addressed. To optimize the application of plants and PGPR in bioremediation, it is imperative that not only the efficiency and tolerance of each partner is studied in isolation, but also that there is a proper understanding of symbiosis in the presence of heavy metals.

18.9 Conclusion

The alternative use of PGPR–plant symbiosis for environmental application is a recent outshoot of biofertilizer biotechnology. The main attraction of this technology lies in fact that it is sustainable and inexpensive, and therefore offers a viable alternative to conventional remediation methods. Pertaining to the requirements of the growing population, excessive use of chemicals in agriculture and industrial development will only increase. Therefore, we have no other option but to develop suitable strategies like those using rhizosphere biology to cope with the challenges of heavy metal contamination of soil. This technology not only assures clean and healthy soil free from heavy metals, but PGPR also improve the fertility of soil for future agronomic use, which is the main benefit. Optimizing the process with fast-growing plants with high biomass and good metal-uptake ability along with suitable bacteria in the rhizosphere will provide new insights.

References

Abou-Shanab RA, Angle JS, Delorme TA, Chaney RL, van Berkum P, Moawad H, Ghanem K, Ghozlan HA (2003) Rhizobacterial effects on nickel extraction from soil and uptake by *Alyssum murale*. New Phytol 158(1):219–224

Ahonen-Jonnarth U, Finlay RD (2001) Effect of elevated nickel and cadmium on growth and nutrient uptake of mycorrhizal and non-mycorrhizal *Pinus silvestris* seedlings. Plant Soil 236:128–138

Anderson TA, Guthrie EA, Walton BT (1993) Bioremediation in the rhizosphere. Environ Sci Technol 27:2630–2636

Anjum NA, Ahmad I, Mohmood I (2012) Modulation of glutathione and its related enzymes in plants' responses to toxic metals and metalloids. A review. Environ Exp Bot 75:307–324

Bais HP, Loyola-Vargas VM, Flores HE, Vivanco JM (2001) Root-specific metabolism: the biology and biochemistry of underground organs. In Vitro Plant 37:730–741

Bais HP, Fall R, Vivanco JM (2004a) Biocontrol of *Bacillus subtilis* against infection of Arabidopsis roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. Plant Physiol 134:307–319

Bais HP, Park SW, Weir TL, Callaway RM, Vivanco JM (2004b) How plants communicate using the underground information superhighway. Trends Plant Sci 9:26–32

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266

Baker AJM, Reeves RD, McGrath SP (1991) In situ decontamination of heavy metal polluted soils. Using crops of metal-accumulating plants: a feasibility study. In: Hinchee RE, Olfenbuttel RF (eds) In situ bioreclamation. Butterworth-Heinemann, Stoneham, MA, p 539

Banuelos GS (2000) Phytoextraction of selenium from soils irrigated with selenium-laden effluent. Plant Soil 224(2):251–258

Banuelos GS, Cardon G, Mackey B, Ben-Asher J, Wu L, Beuselinck P, Akohouse S, Zambrzuski S (1993) Boron and selenium removal in boron laden soils by four sprinkler irrigated plant species. J Environ Qual 22:786–792

Barconi D, Bernardini G, Santucci A (2011) Linking protein oxidation to environmental pollutants: redox proteome approaches. J Proteomics 74(11):2324–2337

Barea JM, Azcón R, Azcón-Aguilar C (2002) Mycorrhizosphere interactions to improve plant fitness and soil quality. Anton Leeuwenhoek 81:343–351

Barkay T, Miller S, Summers A (2003) Bacterial mercury resistance from atoms to ecosystems. FEMS Microbiol Rev 27:355–384

Belimov AA, Hontzeas N, Safronova VI, Demchinskaya SV, Piluzza G, Bullitta S (2005) Cadmium-tolerant plant growth-promoting rhizobacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). Soil Biol Biochem 37:241–250

Bertin C, Yang XH, Weston LA (2003) The role of root exudates and allelochemicals in the rhizosphere. Plant Soil 256:67–83

Bingham FT, Pereyea FJ, Jarrell WM (1986) Metal toxicity to agricultural crops. Metal Ions Biol Syst 20:119–156

Black H (1995) Absorbing possibilities: phytoremediation. Environ Health Prospect 103 (12):1106–1108

Brown NL, Barrett SR, Camakaris J, Lee BT, Rouch DA (1995) Molecular genetics and transport analysis of the copper-resistance determinant (pco) from *Escherichia coli* plasmid pRJ1004. Mol Microbiol 6:1153–1166

Brown NL, Stoyanov JV, Kidd SP, Hobman JL (2003) The MerR family of transcriptional regulators. FEMS Microbiol Rev 27:145–163

Burd GI, Dixon DG, Glick BR (1998) A plant growth promoting bacterium that decreases nickel toxicity in plant seedlings. Appl Environ Microbiol 64:3663–3668

Burd GI, Dixon DG, Glick BR (2000) Plant growth promoting bacteria that decrease heavy metal toxicity in plants. Can J Microbiol 46:237–245

Cattelan AJ, Hartel PG, Fuhrmann JJ (1999) Screening of plant growth promoting rhizobacteria (PGPR) to promote early soybean growth. Soil Sci Soc Am J 63:1670–1680

Chabot R, Antoun H, Cescas MP (1996) Growth promotion of maize and lettuce by phosphate solubilizing Rhizobium leguminosarium biovar phaseoli. Plant Soil 184:311–321

Chaney RL (1983) Plant uptake of organic waste constituents. In: Parr JF et al (eds) Land treatment of hazardous wastes. Noyes Data, Park Ridge, NJ, pp 50–76

Chaney RL, Brown SL, Li YM (2000) Progress in risk assessment for soil metals, and in-situ remediation and phytoextraction of metals from hazardous contaminated soils. In: Proceedings of US-EPA conference on phytoremediation: state of the science, 1–2 May 2000, Boston, MA. US EPA, Cincinnati, OH, pp 164–185

Chaudhry TM, Hayes WJ, Khan AG, Khoo CS (1998) Phytoremediation – focusing on accumulator plants that remediate metalcontaminated soils. Australas J Ecotoxicol 4:37–51

Chaudhry Q, Blom-Zandstra M, Gupta S, Joner EJ (2005) Utilizing the synergy between plants and rhizosphere microorganisms to enhance breakdown of organic pollutants in the environment. Environ Sci Poll Res 12:34–48

Chaudri AM, McGrath SP, Giller KE, Rietz E, Sauerbeck DR (1993) Enumeration of indigenous *Rhizobium leguminosarum* biovar trifolii in soils previously treated with metal-contaminated sewage sludge. Soil Biol Biochem 25:301

Chen X, Wu CH, Tang JJ, Hu SJ (2005) Arbuscular mycorrhizae enhance metal uptake and growth of host plants under a sand culture experiment. Chemosphere 60:665–671

Cheng S, Grosse W, Karrenbrock F, Thoennessen M (2002) Efficiency of constructed wetlands in decontamination of water polluted by heavy metals. Ecol Eng 18(3):317–325

Citterio S, Prato N, Fumagalli P, Aina R, Massa N, Santagostino A, Sgorbati S, Berta G (2005) The arbuscular mycorrhizal fungus *Glomus mosseae* induces growth and metal accumulation changes in *Cannabis sativa* L. Chemosphere 59:21–29

Clemens S (2006) Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie 88(11):1707–1719

Cobbett CS (2000) Phytochelatins and their roles in heavy metal detoxification. Plant Physiol 123 (3):825–832

Colbet C, Goldsbrough P (2002) Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol 53:159–182

De Souza MP, Chu D, Zhao M, Zayed AM, Ruzin SE, Schichnes D, Terry N (1999) Rhizosphere bacteria enhance selenium accumulation and volatilization by Indian mustard. Plant Physiol 119(2):565–573

De Weert S, Vermeiren H, Mulders IH, Kuiper I, Hendrickx N, Bloemberg GV (2002) Flagella-driven chemotaxis towards exudate components is an important trait for tomato root colonization by *Pseudomonas fluorescens*. Mol Plant Microbe Interact 15:1173–1180

Deikman J (1997) Molecular mechanisms of ethylene regulation of gene transcription. Physiol Plant 100:561–566

Dell'Amico E, Cavalca L, Andreoni V (2005) Analysis of rhizobacterial communities in perennial Graminaceae from polluted water meadow soil, and screening of metal-resistant, potentially plant growth-promoting bacteria. FEMS Microbiol Ecol 52:153–162

Dong J, Mao WH, Zhang GP, Wu FB, Cai Y (2007) Root excretion and plant tolerance to cadmium toxicity – a review. Plant Soil Environ 53:193–200

Dubey RS (2011) Metal toxicity, oxidative stress and antioxidative defense system in plants. In: Gupta SD (ed) Reactive oxygen species and antioxidants in higher plants. CRC, Boca Raton, FL, pp 177–203

Elsgaard L, Petersen SO, Debosz K (2001) Effects and risk assessment of linear alkyl benzene sulfonates in agricultural soil. 1. Short-term effects on soil microbiology. Environ Toxicol Chem 20(8):1656–1663

Faisal M, Hasnain S (2005) Bacterial Cr (VI) reduction concurrently improves sunflower (*Helianthus annuus* L.) growth. Biotechnol Lett 27:943–947

Faisal M, Hasnain S (2006) Growth stimulatory effect of *Ochrobactrum intermedium* and *Bacillus cereus* on *Vigna radiata* plants. Lett Appl Microbiol 43:461–466
Filip Z (2002) International approach to assessing soil quality by ecologically-related biological parameters. Agric Ecosyst Environ 88(2):689–712
Foy CD, Chaney RL, White MC (1978) The physiology of metal toxicity in plants. Annu Rev Plant Physiol 29(1):511–566
Furukawa K (2003) Super bugs for bioremediation. Trends Biotechnol 21:187–190
Galli U, Schuepp H, Brunold C (1994) Heavy metal binding by mycorrhizal fungi. Physiol Plant 92(2):364–368
Garbisu C, Alkorta I (2001) Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresour Technol 77(3):229–236
Ghosh M, Singh SP (2005) A review on phytoremediation of heavy metals and utilization of its byproducts. Appl Ecol Environ Res 3(1):1–18
Giller KE, Witter E, McGrath SP (1998) Toxicity of heavy metals to microorganisms and microbial process in agricultural soils: a review. Soil Biol Biochem 30:1389–1414
Glanze WD (1996) Mosby medical encyclopedia, revised edition. St. the phytosiderophore secretion under Fe-deficient conditions, in Gramineae. J Exp Bot 45(12):1903–1906
Gleba D, Borisjuk NV, Borisjuk LG, Kneer R, Poulev A, Skarzhinskaya M (1999) Use of plant roots for phytoremediation and molecular farming. Proc Natl Acad Sci USA 25:5973–5977
Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117
Glick BR (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol Adv 21(5):383–393
Glick BR, Patten CL, Holguin G (1999) Biochemical and genetic mechanisms used by plant growth-promoting bacteria. Imperial College Press, London
Goldstein AH (1986) Bacterial solubilization of mineral phosphates: historical perspectives and future prospects. Am J Altern Agric 1:51–57
Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes. Soil Biol Biochem 37:395–412
Guo Y, George E, Marschner H (1996) Contribution of an arbuscular mycorrhizal fungus to the uptake of cadmium and nickel in bean and maize plants. Plant Soil 184(2):195–205
Gupta A, Meyer JM, Goel R (2002) Development of heavy metal resistant mutants of phosphate solubilizing *Pseudomonas* sp. NBRI4014 and their characterization. Curr Microbiol 45:323–332
Gupta A, Ravi V, Bagdwal N, Goel R (2005) In situ characterization of mercury resistant growth promoting fluorescent *Pseudomonads*. Microbiol Res 160:385–388
Hall JA (1996) Root elongation in various agronomic crops by the plant growth promoting rhizobacterium *Pseudomonas putida* GR 12–2. Isr J Plant Sci 44:37–42
Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53 (366):1–11
Hallberg KB, Johnson DB (2005) Microbiology of a wetland ecosystem constructed to remediate mine drainage from a heavy metal mine. Sci Total Environ 338:53–66
Hawes MC, Gunawardena U, Miyasaka S, Zhao X (2000) The role of root border cells in plant defense. Trends Plant Sci 5:128–133
Heggo A, Angle A, Chaney RL (1990) Effects of vesicular arbuscular mycorrhizal fungi on heavy metal uptake of soybeans. Soil Biol Biochem 22:865–869
Henry JR (2000) An overview of phytoremediation of lead and mercury, NNEMS report. US EPA, Washington, DC, pp 3–9
Hildebrandt U, Regvar M, Bothe H (2007) Arbuscular mycorrhiza and heavy metal tolerance. Phytochemistry 68(1):139–146
Hiltner L (1904) Uber neure Erfahrungen und probleme auf dem gebeit der bodenback-teriologie und unter besonderer berucksichtigung der grundungung und brache. Arb Deut Landwirsch Ges 98:59–78

Hinsinger P (2001) Bioavailability of soil inorganic P in the rhizosphere as affected by root induced chemical changes: a review. Plant Soil 237:173–195
Hirsch AM, Bauer WD, Bird DM, Cullimore J, Tyler B, Yoder JI (2003) Molecular signals and receptors: controlling rhizosphere interactions between plants and other organisms. Ecology 84:858–868
Hobman JL, Brown NL (1997) Bacterial mercury resistance genes. In: Sigel A, Sigel H (eds) Metal ions in biological systems, vol 34. Dekker, New York, pp 527–568
Hobman JL, Wilkie J, Brown NL (2005) A design for life: prokaryotic metal-binding MerR family regulators. Biometals 2005:429–436
Hontzeas N, Zoidakis J, Glick BR (2004) Expression and characterization of 1-aminocyclopropane-1-carboxylate deaminase from the rhizobacterium *Pseudomonas putida* UW4: a key enzyme in bacterial plant growth promotion. Biochim Biophys Acta 1703:11–19
Hossain MA, Fujita M (2009) Purification of glyoxalase I from onion bulbs and molecular cloning of its cDNA. Biosci Biotechnol Biochem 73(9):2007–2013
Hossain MA, Fujita M (2011) Regulatory role of components of ascorbate-glutathione (AsA-GSH) pathway in plant tolerance to oxidative stress. In: Anjum NA, Umar S, Ahmed A (eds) Oxidative stress in plants: causes, consequences and tolerance. IK International, New Delhi
Hossain MA, Hossain MZ, Fujita M (2009) Stress-induced changes of m ethylglyoxal level and glyoxalaseI activity in pumpkin seedlings and cDNA cloning of glyoxalaseI gene. Aust J Crop Sci 3(2):53–64
Hossain MA, Hasanuzzaman M, Fujita M (2010) Upregulation of antioxidant and glyoxalase systems by exogenous glycinebetaine and proline in mung bean confer tolerance to cadmium stress. Physiol Mol Biol Plants 16(3):259–272
Hossain MA, Piyatida P, Teixeira da Silva JA, Fujita M (2012a) Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. J Bot. doi:10.1155/2012/872875
Hossain MA, Hossain MD, Rohman MM, daSilva JAT, Fujita M (2012b) Onion major compounds (flavonoids, organosulfurs) and highly expressed glutathione-related enzymes: possible physiological interaction, gene cloning and abiotic stress response. In: Aguirre CB, Jaramillo LM (eds) Onion consumption and health. Nova Science, New York, NY
Huang Y, Chen Y, Tao S (2002) Uptake and distribution of Cu, Zn, Pb and Cd in maize related to metals speciation changes in rhizosphere. Chin J Appl Ecol 13:859–862
Huang XD, El-Alawi Y, Penrose DM, Glick BR, Greenberg BM (2004a) A multiprocess phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. Environ Pollut 130:465–476
Huang XD, El-Alawi Y, Penrose DM, Glick BR, Greenberg BM (2004b) Responses of three grass species to creosote during phytoremediation. Environ Pollut 130:453–463
Huang XD (2004c) A multiprocess phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. Environ Pollut 130:465–476
Huang Y, Tao S, Chen YJ (2005a) The role of arbuscular mycorrhiza on change of heavy metal speciation in rhizosphere of maize in wastewater irrigated agriculture soil. J Environ Sci 17 (2):276–280
Huang XD, El-Alawi Y, Gurska J, Glick BR, Greenberg BM (2005b) A multi-process phytoremediation system for decontamination of persistent total petroleum hydrocarbons (TPHs) from soils. Microchem J 81:139–147
Jamal A, Ayub N, Usman M, Khan AG (2002) Arbuscular mycorrhizal fungi enhance Zn and nickel uptake from contaminated soil by soybean and lentil. Int J Phytoremed 4:205–221
Jentschke G, Marschner P, Vodnik D, Marth C, Bredemeier M, Rapp C, Fritz E, Gogala N, Godbold DL (1998) Lead uptake by *Picea abies* seedlings: effects of nitrogen source and mycorrhizaes. J Plant Physiol 153:97–104
Joner EJ, Leyval C (2001) Time-course of heavy metal uptake in maize and clover as affected by root density and different mycorrhizal inoculation regimes. Biol Fertil Soil 33:351–357

Jones DL, Dennis PG, Owen AG, van Hees PAW (2003) Organic acid behavior in soils-misconceptions and knowledge gaps. Plant Soil 248:31–41

Jung C, Maeder V, Funk F, Frey B, Sticher H, Frossard E (2003) Release of phenols from *Lupinus albus* L. roots exposed to Cu and their possible role in Cu detoxification. Plant Soil 252:301–312

Kent AD, Triplett EW (2002) Microbial communities and their interactions in soil and rhizosphere ecosystems. Annu Rev Microbiol 56:211–236

Kloepper JW, Lifshitz R, Zablotowicz RM (1989) Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol 7(2):39–44

Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg BJJ (2004) Rhizoremediation: a beneficial plant–microbe interaction. Mol Plant Microbe Interact 17:6–15

Lasat HA (2002) Phytoextraction of toxic metals: a review of biological mechanisms. J Environ Qual 31(1):109–120

Lee J, Bae H, Jeong J, Lee J-Y, Yang Y-Y, Hwang I, Martinoia E, Lee Y (2003) Functional expression of a bacterial heavy metal transporter in *Arabidopsis* enhances resistance to and decreases uptake of heavy metals. Plant Physiol 133:589–596

Leyval C, Turnau K, Haselwandter K (1997) Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7 (3):139–153

Liao M, Xie XM (2004) Cadmium release in contaminated soils due to organic acids. Pedosphere 14:223–228

Liesegang H, Lemke K, Siddiqui RA, Schlegel H-G (1993) Characterization of the inducible nickel and cobalt resistance determinant *cnr* from pMOL28 of *Alcaligenes eutrophus* CH34. J Bacteriol 175:767–778

Lin Q, Zheng CR, Chen HM, Chen YX (1998) Transformation of cadmium species in rhizosphere. Acta Pedol Sin 35:461–467

Lin Q, Chen YX, Chen HM, Zheng CM (2003) Study on chemical behavior of root exudates with heavy metals. Plant Nutr Fertil Sci 9:425–431

Lloyd JR, Macaskie LE (2000) Bioremediation of radioactive metals. In: Lovley DR (ed) Environmental microbe–metal interactions. ASM, Washington, DC, pp 277–327

Lugtenberg BJ, Dekkers L, Bloemberg GV (2001) Molecular determinants of rhizosphere colonization by *Pseudomonas*. Annu Rev Phytopathol 39:461–490

Lugtenberg BJ, Chin-A-Woeng TF, Bloemberg GV (2002) Microbe-plant interactions: principles and mechanisms. Ant Leeuw 81:373–383

Ma JF, Zheng SJ, Matsumot H (1997) Detoxifying aluminium with buckwheat. Nature 390:569–570

Macek T, Mackova M, Kas J (2000) Exploitation of plants for the removal of organics in environmental remediation. Biotechnol Adv 18:23–34

Manara A (2010) Plant responses to heavy metal toxicity. In: Furini A (ed) Plants and heavy metals. Springer, Berlin, pp 27–53

Marques APGC, Oliveira RS, Samardjieva KA, Pissarra J, Rangel AOSS, Castro PML (2007a) *Solanum nigrum* in contaminated soil: effect of arbuscular mycorrhizal fungi on zinc accumulation and histolocalisation. Environ Pollut 145:691–699

Marques APGC, Oliveira RS, Samardjieva KA, Rangel AOSS, Pissarra J, Castro PML (2007b) EDDS and EDTA-enhanced zinc accumulation by *Solanum nigrum* inoculated with arbuscular mycorrhizal fungi grown in contaminated soil. Chemosphere 70:1002–1014

Marques APGC, Rangel AOSS, Castro PML (2009) Remediation of heavy metal contaminated soils: phytoremediation as a potentially promising clean-up technology. Crit Rev Environ Sci Technol 39:622–654

McGrath SP, Zhao FJ, Lombi E (2001) Plant and rhizosphere processes involved in phytoremediation of metal-contaminated soils. Plant Soil 232(1–2):207–214

Mench M, Martin E (1991) Mobilization of cadmium and other metals from two soils by root exudates of *Zea mays* L., *Nicotiana tabacum* L. and *Nicotiana rustica* L. Plant Soil 132:187–196

Moulin L, Munive A, Dreyfus B, Boivin-Masson C (2001) Nodulation of legumes by members of the beta-subclass of *Proteobacteria*. Nature 411:948–950

Mueller B, Rock S, Gowswami Dib, Ensley D (1999) Phytoremediation decision tree. Interstate Technology and Regulatory Cooperation Workgroup, ITRC, Washington, DC

Nanda Kumar PBA, Dushenkov V, Motto H, Raskin I (1995) Phytoextraction: the use of plants to remove heavy metals from soils. Environ Sci Technol 29:1232–1238

Navari-Izzo F (1998) Thylakoid-bound and stromal antioxidativeenzymes in wheat treated with excess copper. Physiol Planta 104(4):630–638

Nies DH (1999) Microbial heavy metal resistance. Appl Microbiol Biotechnol 51:730–750

Nies DH, Silver S (1995) Ion efflux systems involved in bacterial metal resistances. J Ind Microbiol 14:186–199

Nies D, Mergeay M, Friedrich B, Schelegel HG (1987) Cloning of plasmid genes encoding resistance to cadmium, zinc and cobalt in *Alcaligenes eutrophus* CH34. J Bacteriol 169:4865–4868

Nies A, Nies DH, Silver S (1989a) Cloning and expession of plasmid genes encoding resistance to chromate and cobalt in *Alcaligenes eutrophus*. J Bacteriol 171:5065–5070

Nies DH, Nies A, Chu L, Silver S (1989b) Expression and nucleotide sequence of a plasmid determined divalent cation efflux system from *Alcaligenes eutrophus*. Proc Natl Acad Sci USA 86:7351–7355

Normander B, Hendriksen NB, Nybroe O (1999) Green fluorescent protein-marked *Pseudomonas fluorescens*: localization, viability, and activity in the natural barley rhizosphere. Appl Environ Microbiol 65:4646–4651

Ouziad F, Hildebrandt F, Schmelzer E, Bothe H (2005) Differential gene expressions in arbuscular mycorrhizal colonized tomato grown under heavy metal stress. J Plant Physiol 162(6):634–649

Pate JS, Verboom WH (2009) Contemporary biogenic formation of clay pavements by eucalypts: further support for the phytotarium concept. Ann Bot 103:673–685

Pate JS, Verboom WH, Galloway PD (2001) Co-occurrence of proteaceae, laterite and related oligotrophic soils: coincidental associations or causative inter-relationships? Aust J Bot 49:529–560

Penella MA, Giedroc DP (2005) Structural determinants of metal selectivity in prokaryotic metal-responsive transcriptional regulators. Biometals 18:413–428

Perotto S, Martino E (2001) Molecular and cellular mechanisms of heavy metal tolerance in mycorrhizal fungi: what perspectives for bioremediation? Minerva Biotechnol 13(1):55–63

Pieper DH, Reineke W (2000) Engineering bacteria for bioremediation. Curr Opin Biotechnol 11:262–270

Rajkumar M, Nagendran R, Kui JL, Wang HL, Sung ZK (2006) Influence of plant growth promoting bacteria and Cr (vi) on the growth of Indian mustard. Chemosphere 62:741–748

Ramesh G (2008) Cloning and characterization of metallothione in genes of ectomycorrhizal fungus *Hebeloma cylindrosporum*. PhD Thesis, Thapar University, Punjab, India

Rascio N, Navari-Izzo F (2011) Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant Sci 180(2):169–181

Reed MLE, Glick BR (2005) Growth of canola (*Brassica napus*) in the presence of plant growth-promoting bacteria and either copper or polycyclic aromatic hydrocarbons. Can J Microbiol 51:1061–1069

Romero-Puertas M, Palma JM, Gomez M, Del Rio LA, Sandalio LM (2002) Cadmium causes the oxidative modification of proteins in pea plants. Plant Cell Environ 25(5):677–686

Rouch DA, Brown NL (1997) Copper-inducible transcriptional regulation at two promoters in the *Escherichia coli* copper resistance determinant pco. Microbiology 143(4):1191–1202

Safronova VI (2006) Root associated bacteria containing 1-aminocyclopropane-1-carboxylate deaminase improve growth and nutrient uptake by pea genotypes cultivated in cadmium supplemented soil. Biol Fertil Soil 42:267–272

Salt DE, Blaylock M, Kumar NPBA, Dushenkov V, Ensley BD, Chet I (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology 13:468–474

Salt DE, Kato N, Kramer U, Smith RD, Raskin I (2000) The role of root exudates in nickel hyperaccumulation and tolerance inaccumulator and nonaccumulator species of Thlaspi. In: Terry N, Banuelos G (eds) Phytoremediation of contaminated soil and water. Lewis, Boca Raton, pp 189–200

San Francisco MJD, Hope CL, Owolabi JB, Tisa LS, Rosen BP (1990) Identification of the metalloregulatory element of the plasmidencoded arsenical resistance operon. Nucl Acid Res 18:619–624

Schardl CL, Leuchtmann A, Spiering MJ (2004) Symbioses of grasses with seed-borne fungal endophyte. Annu Rev Plant Biol 55:315–340

Schutzendubel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot 53(372):1351–1365

Schwab AP, He YH, Banks MK (2005) The influence of organic ligands on the retention of lead in soil. Chemosphere 61:856–866

Seth CS, Remans T, Keunen E (2012) Phytoextraction of toxic metals: a central role for glutathione. Plant Cell Environ 35(2):334–346

Sharma SS, Dietz KJ (2009) The relationship between metal toxicity and cellular redox imbalance. Trends Plant Sci 14(1):43–50

Sharma P, Dubey RS (2007) Involvement of oxidative stress and role of antioxidative defense system in growing rice seed-ling s exposed to toxic concentrations of aluminum. Plant Cell Rep 26(11):2027–2038

Sheng XF, Xia JJ (2006) Improvement of rape (*Brassica napus*) plant growth and cadmium uptake by cadmium-resistant bacteria. Chemosphere 64:1036–1042

Singla-Pareek SL, Yadav SK, Pareek A, Reddy MK, Sopory SK (2006) Transgenic tobacco over expressing glyoxalase pathway enzymes g row and set viable seeds in zinc-spiked soils. Plant Physiol 140(2):613–623

Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Plant Growth Regul 25(1):71

Summers AO (1992) Untwist and shout – a heavy metal responsive transcriptional regulator. J Bacteriol 174:3097–3101

Taghavi S, Mergeay M, van der Lelie D (1997) Genetic and physical maps of the *Alcaligenes eutrophus* CH34 megaplasmid pMOL28 and its derivative pMOL50 obtained after temperature induced mutagenesis and mortality. Plasmid 37:22–34

Tan YF, O'Toole N, Taylor NL, Millar AH (2010) Divalent metal ions in plant mitochondria and their role in inter actions with proteins and oxidative stress-induced damage to respiratory function. Plant Physiol 152(2):747–761

Taylor LL, Leake JR, Quirk J, Hardy K, Banwarts SA, Beerling DJ (2009) Biological weathering and the long-term carbon cycle: integrating mycorrhizal evolution and function into the current paradigm. Geobiology 7:171–191

Terry N, Carlson C, Raab TK, Zayed AM (1992) Rates of selenium volatilization among crop species. J Environ Qual 21:341–344

Uren NC (2000) Types, amounts and possible functions of compounds released into the rhizosphere by soil grown plants. In: Pinton R, Varanini Z, Nannipieri P (eds) The rhizosphere: biochemistry and organic substances at the soil interface. Dekker, New York, pp 19–40

Vicre M, Santaella C, Blanchet S, Gateau A, Driouich A (2005) Root border-like cells of *Arabidopsis*. Microscopical characterization and role in the interaction with rhizobacteria. Plant Physiol 138:998–1008

Villacieros M, Whelan C, Mackova M, Molgaard J, Sanchez-Contreras M, Lloret J (2005) Polychlorinated biphenyl rhizoremediation by *Pseudomonas fluorescens* F113 derivatives,

using a *Sinorhizobium meliloti* nod system to drive bph gene expression. Appl Environ Microbiol 71:2687–2694

Villiers F, Ducruix C, Hugouvieux V (2011) Investigating the plant response to cadmium exposure by proteomic and metabolomic approaches. Proteomics 11(9):1650–1663

Vivas A, Biro B, Ruiz-Lozano JM, Barea JM, Azcon R (2006) Two bacterial strains isolated from a Zn-polluted soil enhance plant growth and mycorrhizal efficiency under Zn toxicity. Chemosphere 52:1523–1533

Weissenhorn I, Leyval C (1995) Root colonization of maize by a Cd-sensitive and a Cd-tolerant *Glomus mosseae* and cadmium uptake in sand culture. Plant Soil 167:189–196

Wenzel WW, Lombi E, Adriano DC (1999) Biochemical processes in the rhizosphere: role in phytoremediation of metal-polluted soils. In: Prasad MNV, Hagemeyer J (eds) Heavy metal stress in plants: from molecules to ecosystems. Springer, Berlin, pp 273–303

Whiting SN, de Souza MP, Terry N (2001) Rhizosphere bacteria mobilize Zn for hyperaccumulation by *Thlaspi caerulescens*. Environ Sci Technol 35(15):3144–3150

Wu J, Rosen BP (1993) Metalloregulated expression of the ars operon. J Biol Chem 268:52–58

Wu SC, Cheung KC, Luo YM, Wong MH (2006a) Effects of inoculation of plant growth-promoting rhizobacteria on metal uptake *by Brassica juncea*. Environ Pollut 140:124–135

Wu CH, Wood TK, Mulchandani A, Chen W (2006b) Engineering plant-microbe symbiosis for rhizoremediation of heavy metals. Appl Environ Microbiol 72:1129–1134

Yadav SK (2010) Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. S Afr J Bot 76(2):167–179

Yang XE, Jin XF, Feng Y, Islam E (2005) Molecular mechanisms and genetic basis of heavy metal tolerance/hyperaccumulation in plants. J Integr Plant Biol 47(9):1025–1035

Yang J, Kloepper JW, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14:1–4

Yee DC, Maynard JA, Wood TK (1998) Rhizoremediation of trichloroethylene by a recombinant, root-colonizing *Pseudomonas fluorescens* strain expressing toluene ortho-monooxygenase constitutively. Appl Environ Microbiol 64:112–118

Zaidi S, Usmani S, Singh BR, Musarrat J (2006) Significance of *Bacillus subtillis* strain SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in *Brassica juncea*. Chemosphere 64:991–997

Zayed A, Lytle CM, Terry N (1998) Accumulation and volatilization of different chemical species of selenium by plants. Planta 206:284–292

Chapter 19
The State of the Rhizoinhabitants in Bridging the Gap Between Plant Productivity and Persuasiveness During Remediation

Narayanan Rajendran

19.1 Introduction

The proliferation of contaminants and chemicals in various ecosystems is increasingly posing major environmental and global concerns. Soil and ground water contamination by hazardous chemicals such as arsenic and other contaminants poses major environmental and plant productivity problems (Vithanage et al. 2012), and thus remediation of contaminants for improved plant productivity by plant inhabitants through the means of biotic and abiotic remediation systems is of great interest. A number of eco-friendly technology-related transformations in several bioremediation subsets such as microbial remediation, phytoremediation, phycoremediation and macrophyte remediation (Basile et al. 2012) offer insights into the problems of chemical contamination in agro-ecosystems (Hinsinger et al. 2011). Among these subsets, rhizoinhabitant-based remediation (e.g. use of endophytic microbes) has a foremost benefit for enhanced plant productivity (Qin et al. 2011). The "green look" of plants while they remediate soil contaminants has a high public acceptability over any other method of remediation, besides generating a profitable influence among ecosystem consumers. It has been reported that the feasibility of commercial use of such rhizoinhabitants is favored by policy makers and scientists for environmental clean-up because of better ecosystem functionality (Sanon et al. 2009) in addition to better plant productivity.

Plants have the innate ability to transform some of the complex organic pollutants into simple, nontoxic compounds (Meagher 2000) and/or the inherent capability to transform inorganic pollutants to less toxic substances. Engineered transgenic plants have an improved but acquired ability to do the same, in addition to the amazing stabilization capability for metal contaminants by acting as "filters or traps" (Raskin 1996). The high achiever status of both groups of remediator

N. Rajendran (✉)
Molecular Microbiology, Kentucky State University, Frankfort, KY 40601, USA
e-mail: narayanan.rajendran@kysu.edu

D.K. Maheshwari et al. (eds.), *Bacteria in Agrobiology: Crop Productivity*,
DOI 10.1007/978-3-642-37241-4_19,

plants strongly depends not only on their innate or acquired capacity but also on the correlation between survivability of the plants and the state of their inhabitant partners such as bacteria. Some plant growth-promoting bacteria (PGPR) have a higher influence on plant growth and yield (Hameeda et al. 2006) and because of the "most-favorable status", microorganisms play a key role in ecosystem restoration and functioning (Balser et al. 2006). Microbial associations with plants are versatile and are a well-established combinatorial field of study on interactions. However, the interface between plants and microbes is very intriguing at every level of affiliation, from the rhizosphere to the phyllosphere, as well as at every stage of on-and-off symbiotic or antagonistic relationships. It is fair to articulate that a network of inter-related ecological and biological relationships exists between them. Hence, plant–microbe interactions and their population dynamics are essential (Sanon et al. 2009). Moreover, how the composition of the co-occurring microbial community responds to the plant communities is crucial to an understanding of their innate ability and acquired capability for remediation.

The knowledge of molecular mechanisms of detoxification of chemical contaminants by rhizobial inhabitants has merged with engineering strategies to improve phytoremediation processes (Vance 1996) and has already proved to be a viable and safe alternative for remediation of many contaminants, especially in situ (Compton et al. 1998). A number of green remediation techniques have now been developed and are commercially available in some form or other. As the technology continues to offer new, low-cost green remediation options some of them are intensively applied in practice for better plant productivity in different terms and forms along with some of the synergistic subsets of phytoremediation technology. This article reviews the state of the plant inhabitants, rhizobia in particular, and the factors that influence the balance between plant productivity and plant persuasiveness so that their inhabitants are held and thrive for better productivity in terms of survival and effectiveness. We attempt to address the potential influence of the co-occurring soil bacterial communities, the types of chemical speciation available at the sites and how plant populations deal with plant–plant, plant–microbe and plant–chemical interactions and bridge the gap between all three in order to show productivity while remediating toxic chemicals.

19.2 Norming of Plant Productivity-Based Remediation

Whether in a fertile soil or any other setting, the association of plants and its rhizobial inhabitants must pass through several phases before they fully become "associated". The shared expectation of contact, otherwise called the "norms", is crucial for such association in order to remediate the contaminant sites while enhancing plant productivity. In practice, if the state of the rhizospheric microbes, soil bacteria in particular, remains unmired by their performance, the plant productivity goes down while remediation of toxicants goes up (Madhaiyan et al. 2009). The more association there is towards a common goal, the more they tend to

converge toward the common perspective of plant productivity and remediation. The rhizobacteria depend on the plant roots, acknowledge and abide by the norms set by the plants. Such a norming process has an effect on the level of effort set by plants for their productivity associated with the kind of remediation it follows. The primary role of the host plant in this association with soil bacteria is to gain enhanced productivity. Hence, they make persuasiveness their priority and facilitate the practice of cohesion with soil microbes (Buée et al. 2009). This association can be applied to any type of "green remediation", a collective expression for a combination of several remediation techniques such as plant-based (phytoremediation), algae-based (phycoremediation), microbe-based (microbial remediation) and other form of nature-based (bioremediation) remediation processes, for contaminant clean-up processes since the association of two partners is structurally similar. Due to the synergistic effect of chemicals on ecosystems (Barbosa et al. 1998), which poses a significant threat to life forms, green remediation dictates the need for such alternative approaches to solve such emerging risks. The success of green remediation is closely associated with the plants and its inhabitants such as microbes and the ability of plants (as well as their persuasiveness) to deal with toxic chemicals in spite of barriers such as environmental factors and the functional stability of the inhabited ecosystem itself.

The seasonal dynamics of plant populations in any given ecosystem plays a major role in plant productivity and ecosystem restoration. Similarly, rhizobial inhabitants (especially soil bacteria) have a significant role to play in the functional stability of ecosystems (Cotner and Biddanda 2002). Beyond the state of the rhizobacteria, it is also important to recognize that various chemicals in the form of nutrients at the contaminated sites play a key role in the survival of both plants and the rhizobial inhabitants. The biochemical transformation of these chemicals by plants and microbes, as studied extensively in the laboratory, may vary more than in-situ transformation at the toxic plumes. For example, selenium was accumulated much less in Canola plants when grown in field conditions than in greenhouse conditions (Banuelos et al. 1998). This indicates that beyond the biological factors, other environmental factors affect plant persuasiveness. These factors include, but are not limited to, the diversity of the main microbial community, the co-existence of microbial communities, plant–microbial population dynamics in relation to chemical remediation and the responsible role of "plant norms" in chemical remediation. Moreover, the shape of the co-occurring rhizobacterial communities and their composition respond to the plant communities and also make difference. Since this emerging biologically safe green remediation technology is expanding, not only for toxicant removal on contaminated plumes but also as a land-recovery strategy, especially on mountain top removal and mining sites, it is important to know about the effects of plant persuasiveness on chemical remediation.

Green remediation has been developed from a conceptual methodology to a viable plant productivity-based technology for contaminant clean-up. There are several technical terms used to explain the different remediation processes in this plant productivity-based green remediation technology. For example, many terminologies (Table 19.1) such as the biomechanism of chemical resistance,

Table 19.1 Technical terms commonly used in phytoremediation

Terms	Description
Aquifer	Any sediment with spaces that hold water in sufficient quantities to yield economically valuable amounts of water to wells and springs
Biomining	The process of uptake of minerals by plants or microbial life forms
Flood plain	The nearly level land that borders a stream and is subject to flooding
Hyperaccumulators	Plants that accumulate high level of contaminants, especially heavy metals
Leaching	The removal of soluble matter from soil by percolating water
Phytoaccumulation	Accumulation of contaminants in leaves and stems followed by the uptake
Phytodegradation	Transformation of chemical contaminants into another form by plants during their metabolic activities, instead of accumulation or volatilization
Phytoextraction	After remediation, the plant parts such as leaves, stems, roots, etc. are harvested and crushed to separate the chemical contaminants
Phytostabilization	Use of plants to eliminate the bioavailability of toxic chemicals, especially metals and radionuclides, in soil thus rendering them nontoxic
Phytostimulation	To improve the remediation process, plants and/or its habitat are treated with a microbial consortium or tracer elements or enzymes, etc
Phytovolatilization	Evaporation of remediated chemicals through leaves; e.g. poplar trees volatilizee 90 % of TCE, which they uptake from soil
Recharge	The addition of water to the zone of saturation for better remediation
Rhizofiltration	Tufted plant roots are used to clean up water contaminants to precipitate and concentrate chemicals from polluted effluents
Rhizosecretion	A subset of molecular farming techniques designed to produce and secrete valuable natural products and recombinant proteins from roots
Rhizosphere	A zone directly related to the surface area of the plant roots associated with the growth of microorganisms directly related to the roots
Saturation zone	Typically referred to as the area below the water table where pore spaces in the sediment or rock are filled with water

uptake, translocation, accumulation etc. are unique to phytoremediation. Hence, selection of a suitable synergistic remediation process and its plant species has a profound effect on the enhanced productivity-based decontamination process. Phytoremediation in particular is well known (Macek et al. 2002b) for its ability to take up and concentrate contaminants in biological tissues without destroying the environment. Several plant species have a high innate ability to uptake (Jha et al. 2010) metals, and to accumulate and metabolize toxic chemicals. The higher the plant productivity, the better is the accumulation of such toxic chemicals, beyond providing esthetic value over any other biological life forms. As a result, the incinerated plant ash of the accumulated plants or parts of the plants can be reused similarly to "commercial ore" or can be recycled. The plants can also be decomposed into modified green manure or disposed of as metal-based organic matter through an authorized method of disposal of hazardous items. The association of plants and toxic contaminants therefore offers a viable means of accomplishing the in-situ remediation of contaminated sites.

19.2.1 The Role of Rhizobial Inhabitants

Phytoremediation has its limitation like any other technology. It is a long process and is restricted to deep-depth remediation sites. Moreover, the productivity of remediator plants goes down when accumulation of the toxicants increases in their biological tissues due to intercellular colonization by the rhizobacteria (Murugaiyan et al. 2009). The other significant constraint is the use of non-native or invasive species of phytoremediators (Chaudhry et al. 2002). However, phytoremediation many advantages over physical and chemical means of remediation. It is cost-effective as well as environmentally friendly (Liphadzi and Kirkham 2006). It is safe, biologically feasible and long-lasting to use plants to remove metal contaminants (especially nickel, zinc and copper from explosives and ammunition wastes), to remove organic contaminants from crude oil wastes and oil spillage or to remove a variety of other contaminants such as pesticides, solvents and landfill leachates. Many factors help to achieve a progressive phytoremediation for heavy metal remediation (Kamnev and Lelie 2000). Compared to conventional technologies, which render the soil unusable for several years and destroy the natural components and structure of the soil, plant productivity-based phytoremediation offers the advantages of being truly in situ, has lower capital and labor costs, retains the functionality of the soil structure and ecosystem, involves minimal disturbance to the environment and allows reuse of the remediated site within a short period. At high-risk sites of contaminants, phytoextraction from plants with high accumulation of metals can be used as a supportive method to remove the leftover contaminants. Because of the ability of the deep roots of specific terrestrial plants, which reach a specific depth and clean up the last remains of contaminants, productivity-based phytoremediation is a viable alternative technology for progressive sites, where high concentrations of metallic elements are present in the soil (Baker 1989) and rhizosphere.

Phytoremediation is a passive technique and has a high public acceptance. The green canopy of remediator plants has an esthetic value (Beard and Green 1994) and is useful in urban areas as a reducer of noise pollution. Trees added to contamination sites as forest cover not only increase the percentage of forest but also give a green cover to protect the barren soil, protect direct evaporation of ground water and prevent human settlement. They are very good solar energy-driven potential energy trappers as well as good stimulants for rainfall. Besides, nematode communities and other soil populations can interact and improve the soil structure because of the remediator tree plantations. The plant material with accumulated mine contaminants can be evenly distributed and decomposed in trace metal-deficient soils to enhance the soil fertility. Depending on the mineral content of the incinerate ash, it could also be used as compost or fertilizer with other substrates. Besides, revitalization of contaminated sites, particularly the metaloferrous mining sites (Bradshaw and Johnson 1992) through re-vegetating the land helps to create more public acceptance.

Beyond focusing on unique remediator plants with specific characterization, it is necessary to typify the physiological, biochemical and molecular responses of other phytoinhabitants besides the rhizobacteria. For example, organisms like Hornworm, Thrips, Leafhoppers etc. as well as environmental chemical stimuli (including airborne chemicals) play roles in plant productivity. The most common organisms such as *Thysanoptera* (Thrips) and *Homoptera* (Leafhoppers), *Hymenoptera* (bees and wasps), *Neuroptera* (lacewings), *Collembola* (springtails) and *Psocoptera* (barklice) are found around exotic plants, while *Coleoptera* (beetles), *Homoptera* (aphids, cicadas), *Heteroptera* (true bugs) and *Lepidoptera* (butterflies and moths) are found on native plants (Devinny et al. 2005). In general, observation of those plants could reveal their relevant phytoinhabitants. For example, tomato plants of *Solanaceae* attract Hornworms (*Manduca sexta*, equipped with a red-tipped horn at the end of the abdomen as shown in Fig. 19.1) through volatile emission of its "signature" alkaloid-rich secretions. Similarly, Tobacco Hornworms (*Manduca sexta sexta*) can sense a similar alkaloid secretion and feed on alkaloid-rich tobacco plants. Its special mechanism for selective sequestering of alkaloids (such as nicotine in tobacco plants) basically helps it to feed on nicotine-rich tobacco or alkaloid-rich tomato plants. Furthermore, the nicotine in the tobacco leaf is toxic to many insects but not to the Tobacco Hornworm, therefore it is able to successfully feed on this plant. The evolutionary existence of the same innate mechanisms in two entirely different species has resulted in two diverse functionalities: one that protects the plant remediator from several insect pests, and another that overcomes the same protection barrier by an ability to selectively sequester alkaloids.

19.2.2 The Role of Plants and Their Persuasiveness

In general, success of a phytoremediation process starts with the selection of remediator plants in compliance with the contaminated sites. For the past two decades, more than 1,700 plant species have been used to remove contaminations from polluted sites (Hoseini et al. 2012). Approximately 400 plant species have been used to take up unusually large amounts of contaminants both in terrestrial and aquatic sites. Among them, 47 plant species are considered as hyper-accumulator plants for radioactive elements including cesium and strontium (Singh et al. 2008). Table 19.2 shows some of the prominent plants that have been studied up to now. Phytoremediator cultivation and management strategies such as selection of plant species, planting densities and crop rotation can have profound influence on the its persuasiveness and degree of remediation. Selection of indigenous plants that are capable of degrading the pollutant especially at industrial sites (Kirschner 1995) is not only significant but also vital because plants are required that will grow well in that specific local environment, such as ecologically sensitive islands where only native plants should be allowed to grow. This is not only acceptable by the local community but also considered a biogeographically successful approach, as the indigenous plants are adopted well by the

Fig. 19.1 Chemical sensing of plant inhabitants for survival. Tobacco Hornworms (*Manduca sexta*), equipped with a red-tipped horn at the end of the abdomen, feed on alkaloid-rich tomato plants. Its special alkaloid-sensing ability and mechanism for selectively sequestering alkaloids helps it to feed on the alkaloid-rich tomato plants of *Solanaceae* and on nicotine-rich tobacco leaves. The alkaloid nicotine in the tobacco leaf is toxic to several insects but not to this hornworm. Understanding such metabolic substances of plants could reveal their phytoinhabitants, including macro- or microorganisms

community. Therefore, a comprehensive understanding of phytoremediators and the uptake, tolerance and transport of heavy metals is essential for the development of phytoremediation technologies (Basile et al. 2012).

Plants have the ability to take up metals or solvents, depending on the habitat and habits of the plants (Zenk 1996). The uptake of chemical contaminants by specific remediator plants depends on the specific contaminant sites. Surface contaminations including toxic metals can be remediated by terrestrial herbs such as *Hibiscus cannabinus*, which has gained attention for remediation processes as reported recently (Meera and Agamuthu 2012). These herbs show cost-effective mechanisms in remediating Fe and As from landfill leachate-contaminated soil according to the assessment based on bioconcentration and translocation factors. Results on the sequestration of As and Fe indicate that this Hibiscus variety can tolerate these metals and hence are suitable for phytoextraction of leachate-contaminated sites. Another such terrestrial herb, Sunflower, has significant physiological response to trace elements such as Cd, Zn, Cu and nutrients accumulation in contaminated soil (Rivelli et al. 2012). Contamination with Cd alone did not affect the growth or physiological parameters; however, Zn, Cu and Cd together showed toxic effects on chlorophyll content. In aquatic sites, many surface contaminants have major influences on remediation processes such as the bioaccumulation ability of plants. For example, three aquatic macrophytes *Lemna minor*, *Elodea canadensis* and *Leptodictyum riparium* are considered good bioaccumulators for the heavy metals (Vajpayee et al. 1995). Out of these, *L. riparium* was the most effective in accumulating Cu, Zn and Pb, whereas *L. minor* was the most effective in accumulating Cd (Basile et al. 2012). There are many such aquatic and terrestrial remediator plants that exclusively pertain to their habitat and that are reported to have growth characteristics and adaptability to a wide range of soil, aquatic and climate conditions (Hooper and Vitousek 1998). For example *Spartina alterniflora* and *Juncus roemerianus* are for the salt marsh; *Carex acutiformis* and *C. gracilis* for river and streambanks; *Phragmites australis*, *Typha latifolia*, *T. angustifolia* and *T. dominguensis* for lakeshores; *Juncus effusus*, *Cyperus giganteus*, *Scirpus polyphyllus*, *S. tabernaemontani* and *S. californicus*

Table 19.2 Promising plants for phytoremediation*

Plant species	Target chemicals
Abelmoschus esculentus (Okra)	Pb, crude oil
Achillea millefolium (Yarrow)	Cu, Cd
Acer rubrum (Red maple)	Leachate
Aelonthus biformifolis	Cu
Agrostis tenuis	Cu, Zn
Agrostis castellana (Colonial bent grass)	Metals
Agropyron smithii (Western wheat grass)	Hydrocarbons (TPHs, PAHs)
Aleopecurus pratensis	Hydrocarbons (TPHs, PAHs)
Alpine pennycress	Zn, Ni
Allium schoenoprasum (Chives)	Cd
Amaranthus hybridus (Slim amaranth)	PCBs, Ni
Amorpha fruicosa (Indigo bush)	Pb
Anthurium andraeanum (Foliage herb)	Formaldehyde
Arenaria rubella	Ni
Artemisia frigid (Prairie sagewort)	Crude oil, PCBs
Atriplex hortensis (Garden Orach)	PCBs
Avena sativa (Oat)	Zn, Cd
Bacopa monnieri (Water hyssop)	Metals
Berkheya coddii	Ni
Beta vulgaris L. (Sugar beet)	Zn, Cd
Betula pendula (Euro white birch)	PCBs, PAHs
Bouteloua gracilis (Blue gamma grass)	Hydrocarbons (PAHs), selenium, sulfide complexes, uranium, uranyl cation
Brassica juncea (Indian mustard)	Se, Ar, Cd, sulfide complexes
Brassica napus (Canola)	Cd, Pb, Zn
Brassica oleracea var. *botrytis* (Broccoli)	Fe, Mn, Zn, Ni, Cd
Brassica pekinensis Rupr.	Pb
Brassica rapa (Field mustard)	Zn, Cd
Bromus biebersteinii (Meadow brome)	Mn, Zn
Bromus inermis	Metals, Cd
Buchloe dactyloides (Buffalo grass)	Hydrocarbons
Callitriche stagnalis	Uranium
Cannabis sativa	Sr, Cs
Cerastium arvense	Cd
Chlorophytum bichetii (Foliage herb)	Formaldehyde
Claytonia perfoliata (Miner's lettuce)	Cd
Cucurbita pepo	Mn, Zn, Cd, Fe
Cynodon dactylon (Burmuda grass)	Hydrocarbons
Datura innoxia	2,4,6-Trinitrotoluene
Dichapetalum gelonioides	Ni
Dieffenbachia spp. (Foliage herb)	Formaldehyde
Digitalis purpurea (Common Foxglove)	Cd
Eichhornia crassipes (Water hyacinth)	Heavy metals
Elymus canadensis (Canadian wild rye)	Hydrocarbons
Elymus dauricus (Dahurian wild rye)	Hydrocarbons
Eucalyptus camaldulensis	Arsenic, sodium

(continued)

Table 19.2 (continued)

Plant species	Target chemicals
Festuca arundinacea (Tall fescue)	PAHs, pyrene, hydrocarbons
Festuca rubra (Red fescue)	Hydrocarbons
Ficus pumila L. (Figs tree)	Nitrogen dioxide
Fontinalis antipyretica	Uranium
Gleditsia triacanthos (Honey locust)	Pb
Haumaniastrum spp.	Co, Cu
Heliathus annuus (Sunflower)	Metals, radioactive contaminants
Holcus lanatus L.	Arsenate
Hordeum vulgare (Barley)	Zn, Cu
Hydrilla verticillata	Metals
Juncus accuminatus	Metals
Juniperium virginiana	Cd, metals
Larrea tridentata	Mn
Lavender augustifolia Mill (Lavender)	Cd, Pb, Cu, Mn, Fe
Liquidambar styraciflua (American gum)	Perchlorate
Liriodendron tulipifera (Yellow poplar)	Metals
Lolium perenne (English ryegrass)	Hydrocarbons
Lotus corniculatus (Birds-foot trefoil)	Hydrocarbons
Lupinus albus (White lupin)	Arsenic
Lupinus angustifolius	Metals
Lupinus luteus	As, Cd, Cu, Pb, Zn
Maclura pomifera (Osage orange)	PCBs
Medicago sativa (Alfalfa)	PAHs
Melilotus officinalis (Yellow clover)	Hydrocarbons
Mimulus guttatus	Cu
Morus rubra (Mulberry)	PAHs, PCBs
Myriophyllum spicatum (Water milfoil)	Heavy metals
Myriophyllum aquaticum (Parrot feather)	Heavy metals
Oryza sativa L. (Rice)	TCAB, Sm, sulfide complexes
Panicum virgatum (Switch grass)	PAHs, hydrocarbons
Phacelia seicea	Mn, Zn, Ni
Phaseolus acutifolius (Tepary bean)	Uranium, uranyl cations
Phaseolus vulgaris	Fe, Mn
Phleum pretense (White clover)	PAHs, Ni, Cd
Phragmites australis (Common reed)	Fe
Picea mariana (Black spruce)	Hg
Picea pungens (Blue spruce)	Mn
Pinus ponderosa	Metals
Pisum sativum	Uranium, uranyl cation
Psidium guajava (Guava)	Formaldehyde
Poa alphine (Alpine blue grass)	Organic contaminants
Populus deltoides (Poplar tree)	Volatile organic compounds
Populus hopeiensis	Cd, TCE
Populuseucoides	Cd, Zn
Populus nira var. *thevestina*	Cd
Populus tomentosa	Cd, metals

(continued)

Table 19.2 (continued)

Plant species	Target chemicals
Populus tremula (Aspen)	Pb
Rhapis excels	Formaldehyde
Ricinus communis (Castor)	Crude oil, toxic contaminants
Rosmarinus officinalis	Formaldehyde
Scenedesmus acutus	Ni
Scirpus acutus	Atrazine
Schizachyrium scoparium (Bluestem grass)	PAHs
Sebertia acuminata	Ni
Senecia glaucus	Hydrocarbons
Silene vulgaris (Bladder campion)	Zn, Cd
Sinapis alba	Pb, Cd, Hg, As, Cr
Solidago hispida (Hairy golden rod)	Metals
Sonchus oleraceus L.	Pb
Sorghum bicolor (Guinea corn)	Crude oil
Sorghum sudanense (Sudan grass)	PAHs
Spartina alternifolia	Metals
Spartina patens	PAHs
Spinacia oleracea (Naket Spinach)	Crude oil, hydrocarbons
Spirodela polyrhiza L. Scheid	Cd
Stellaria calycantha (Northern starwort)	Cd
Stenotaphrum secundatum St. A. grass)	Hydrocarbons
Tamarix parviflora (Tamarisk)	As, high sodium
Tamarix aphylla	$Cd(NO_3)_2$
Thlaspi caerulescens (*Alpine pennycress*)	Zn, Cd, Ni
Thlaspi ochroleucum	Heavy metal uptake
Thlaspi rotundifolium	Pb
Thlaspi goesingense	Ni
Tillandsia cyanea (Foliage herb)	Formaldehyde
Trifolium pratense (Red clover)	Hydrocarbons
Trifolium repens (White clover)	PCBs, hydrocarbons
Triticum aestivum (Wheat)	Pb
Typha latifolia	Heavy metals
Typha domingensis	Fe, Mn, Zn, Ni, Cd
Vicia faba (Broad beans)	Metals, hydrocarbons
Vigna unguiculata (Cowpea)	PAHs, metals
Wolffia globosa (Duckweed)	Cr, Cd
Zamia pumila (Woody foliage plant)	Formaldehyde

PAH polyaromatic hydrocarbon, *PCB* polychlorinated biphenyl, *TCAB* tetrachloroazobenzene, *TCE* trichloroethylene, *TPH* total petroleum hydrocarbons
*It will help readers to select a group of promising plants for their comparative phytoremediation works and even compare their phylogenic approach, with targeted chemical speciation.

for shallow wetlands; and *Arundo donax*, *Paspalum sp*. and *Cyperus pseudovegetus* for various moist habitats. It is therefore recognized that the plants and their habitat co-exert an influencing role on remediation, which can be exploited as a cost-effective alternative for the treatment of contaminated soil and polluted aquifers.

19.3 Factors Affecting Plant Productivity-Based Remediation

Every successful plant productivity-based remediation involving multiple parameters requires collective action of plants and its involved rhizoinhabitants; without these, the entire process tends to drift apart and lose efficiency. Environmental factors such as rainfall, soil structure and seasonal cycles, as well as chemical factors such as toxicants, chemical species, distribution pattern, chemo-environmental stimuli, chemically enriched assemblage and dissolved organic/inorganic matters play significant roles in plant productivity and phytoremediation. Biotic factors like selection of remediator species can equally affect the phytoremediation process and the state of the plant community itself. Also playing a major role are biological factors like bacterial populations residing at the rhizosphere, other phytoinhabitants, plant–microbial population dynamics and shifts in the community richness and distribution, as well as plankton populations residing at the aqua sphere, including seasonal algal dynamics ,. The entire process depends on both biotic and abiotic factors, including chemical species that contribute to changes in the remediation process as well as plant community composition (Fig. 19.2). Even though individual plant or microbial population are highly dynamic, they can act strangely in their response to chemical species and their availability when acting together (Sivamani et al. 1992). The state of the plant inhabitants depends, especially in chemically enriched assemblages, on the availability of dissolved organic and inorganic matter (Newman et al. 1998). Although the processes are related to seasonal cycles, their interactions with chemicals and the state of the rhizoinhabitants are related to resource management at the site. This occurs in response to seasonal shifts and is influenced by factors such as seasonal stability, temperature and the state of the plant community itself. Thus, the process can depend on the pattern variability of seasonal shifts and other environmental factors.

19.3.1 Environmental and Biological Factors

Studies on environmental factors including soil structure, water conditions, toxicants, light intensity, temperature, mechanical injury, insect feeding and exposure to pathogens and airborne chemicals have demonstrated that plants can be developed as a reliable biological response to such stimuli. The chemical responses of plants to environmental stimuli have profound implications for the development of reliable phytoremediation processes. These responses are often expressed through physiological pathways that can be readily measured by unique phenotypical observation of phytoinhabitants (Rajendran and Venkatesan 1993) as well as by routine biochemical assays (Consuelo et al. 2004). All levels of their interactions in response to irrigation or flooding of a nearby creek require an in-depth understanding of their symbiotic relationships, especially when they act together in a hostile

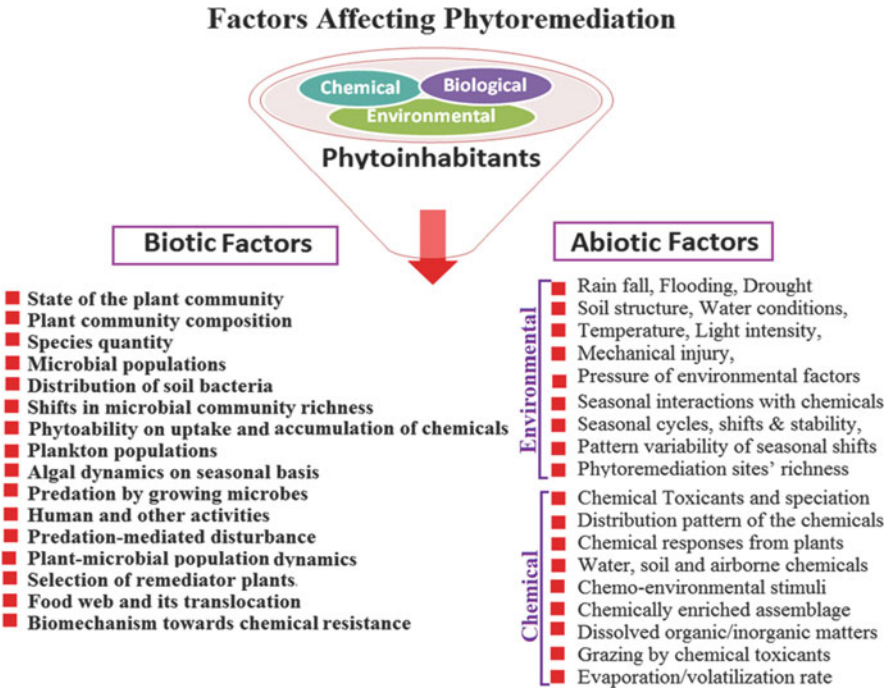


Fig. 19.2 The most common factors affecting the phytoremediation process. Environmental factors such as rainfall, soil structure, seasonal cycles, as well as chemical factors such as toxicants, chemical species, distribution pattern, chemo-environmental stimuli, chemically enriched assemblage and dissolved organic/inorganic matter can all play a significant role in phytoremediation. Biotic factors like selection of remediator species can equally affect the phytoremediation process as can the state of the plant community itself. Microbial populations residing at the rhizosphere as phytoinhabitants, plant–microbial population dynamics, shifts in the community richness, bacterial distribution and plankton populations (including the seasonal dynamics of algae residing at the aqua sphere) can also play a major role in the remediation process

environment, where toxic chemicals threaten the very existence of plants. For example, in an earlier study it was noticed that water quality in Stekoa Creek (a major tributary of the Chattooga River, Georgia, USA) is degraded by sediment that runs off from construction sites near the creek, as well as by bacterial contamination from the wastewater treatment facilities in Clayton (Kent and Bayne 2010). Consequently, the remediation site experienced shifts in the pattern of distribution of the chemicals and other toxicants (Fig. 19.3) that can affect the state of the phytoremediators as well as rhizoinhabitants. For example, remediation of polyaromatic hydrocarbons (PAHs), like naphthalene, in soil plumes can occur at different rates. The evaporation rate of naphthalene for example is slow when the concentration of the organic matter increases in a deep soil environment, whereas the rate is elevated at areas of high concentration, especially the loading sites. The PAHs are produced due to partial combustion of fossil fuels in traffic exhausts, coal fires, heating etc. For example, naphthalene is a low molecular weight PAH widely

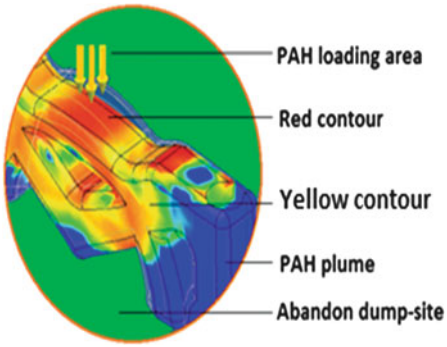


Fig. 19.3 General pattern of dispersal of a PAH toxicant from its highly concentrated state of entry to least diffusion levels. Consequently, the remediation site (shown in *green*) may experience shifts in the pattern of distribution of the chemical toxicants that can affect the state of the phytoinhabitants. For example, remediation of polyaromatic hydrocarbons (PAHs) in soil plumes (as shown in *blue*) can occur at different rates. On the basis of surface sediment scanning, the evaporation rate of naphthalene (a significantly predominant PAH found in most PAH dumping sites), is significantly higher at the loading area (where it is highly concentrated as shown by the *red* contour) than the lower depth of the soil (where the concentration gradually decreases) due to the augmentation of increased organic matter (as shown by the *yellow* contour)

used in manufacturing industries as an additive or intermediate product for manufacture of dyes, plastics, repellents etc. (USEPA 2001). Over 500 tons of naphthalene were released into the US soil in 1998 (US-EPA 2001), and 120 tons were released by Canada in 2005 (Environment Canada 2008). Over 4,133,000 tons of naphthalene were consumed by industries in 1 year throughout Western Europe (Lacson 2000). The National Priorities List on hazardous waste sites compiled by US-EPA indicates that naphthalene (Fig. 19.4) is one of the most common PAHs found in dumping sites. Using novel techniques like the nutrient film technique, it is possible to easily screen multiple clones of phytoremediators for growth in the presence of various toxicants (Migeon et al. 2012).

Plant productivity-based phytoremediation is one of the most promising bioremediation techniques. However, the quality of the plants used in remediation processes generally dictates the successes of the process (Anderson et al. 1993), even though it depends on many other influencing factors. Using native phytoremediator species has more advantages than use of invasive or introduced agricultural species. Such native plants have dense, deep root systems adapted to the local conditions, including seasonal conditions such as rainfall and instant environmental exposures such as local contamination networks. Besides, native species may have more phytoinhabitant associations, such as microbial alliance at their rhizosphere, which improves plant productivity. The availability of root exudates of native phytoremediators to the rhizoinhabitants enhances microbial composition in the rhizosphere (Rambelli 1973), thus the native plant communities may provide a network of chemical signaling that could enhance nutrient cycling (Hooper and Vitousek 1998) and plant productivity. A group of native and quality

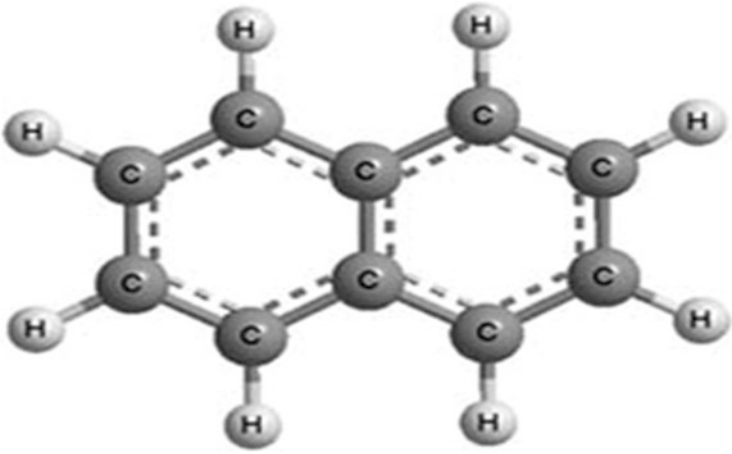


Fig. 19.4 Chemical structure of one the most common PAHs, naphthalene. Naphthalene, a bicyclic aromatic hydrocarbon, is frequently used in manufacturing sectors especially for dyes, additives, plastics, repellents, etc. Large amounts of naphthalene are produced through partial combustion of coal and other fossil fuels (traffic, heating, etc.). It was first registered as a pesticide in the USA in 1948. The National Priorities List on hazardous waste sites compiled by the US-Environmental Protection Agency indicates that naphthalene is one of the most widespread PAHs and is found in most PAH dumping sites

phytoremediators and their assemblages may provide enriched above-ground habitats for phyllosphere microbes and macroorganisms. It is fair to say that the network of such inter-related ecological and biological relationships make the food web a more intricate process, with many linkages to the plant community.

The toxicants and pressure of environmental factors such as temperature, rainfall, drought etc. influences the state of the bioaccumulation process by phytoremediators and their inhabitants. The concentrations of dominant toxicants and related elements such as N and P could play a significant role in the growth of phytoremediators along with other influencing factors such as the plant's physiological factors, the plant's innate bioaccumulation ability and the quantity and quality of the chemical resources available at the phytoremediation sites. Although chemical species affect the bioaccumulation rate of the remediators, the presence of an abundance of microbial populations with consistent patterns of distribution at the remediation sites also has a significant influence on remediation. Heavy metal accumulation in rhizobial tissues always exceeds that in phyllosphere tissues such as in stem and leaves, with a lower translocation from roots to shoots (Rivelli et al. 2012). Hence, native phytoremediators like *Helianthus annuus* have developed an innate ability to compartmentalize toxic wastes to preserve the young biological tissues (Rivelli et al. 2012). This suggests that the state of the phytoremediation depends not only on the toxicant resources and availability in the remediation sites but also on microbial dynamics, which are determined by seasonal factors, biological factors and the abundance of plant–microbe interactions. Dramatic shifts in microbial community richness and abundance due to seasonal factors such as

rainfall could result in the loss of remediation capability, which may be responsible for the failure of a specific remediation process.

19.3.2 Resources for Enhanced Plant Productivity

There is a greater understanding today than ever before of the importance of resources for high plant productivity. Any moderate changes in resource availability could influence overall productivity and the phytoremediation process itself. The pH, for example, greatly affects trace element accumulation in above-ground biomass (Migeon et al. 2012). Such variation affects not only different phytoremediators but also the biomass productivity. A recent study with large range of poplar clones revealed the potential of the poplar clones for use in phytostabilization, where some clones rather than others were suitable for production of less-contaminated above-ground biomass . Plant biomass and trace element accumulation patterns in leaves also showed variation among clones. For example, the highest Cd and Zn concentrations in leaves were detected in *P. trichocarpa* and *P. trichocarpa* hybrids and varied with the clones when exposed to a multipollution context (Migeon et al. 2012). Similarly, altering the quantity and composition of dissolved organic matter over time may play an important role in determining the rate of the phytoremediation. Mercury contamination in aquatics and wetlands is harmful to life on earth. Although the relative importance of these methylation sites may vary seasonally and spatially, several studies suggest that wetlands are the principal source of methylmercury (MeHg) to lakes when wetland runoff dominates the catchment hydrology (Watras et al. 2005). Such toxic contaminants have a major impact on the plant–microbial food web. The increase in soil bacterial population parallel with phytoinhabitant abundance may represent a combination of direct and indirect effects, including release of nutrients from the remediation sites. Such changes can rapidly induce compensatory alterations in plant–microbial community structure and their interactions that result in the formation of a size reduce from multiplication.

19.3.3 Over-grazing and Predatory Influences

Over-grazing of the plant community may strongly influence biomass productivity and phytoinhabitant dynamics in any remediation sites, including terrestrial or aquatic sites. Soil rhizobial inhabitant communities in association with plants are better at scavenging nutrients and for degradation of contaminants. The total quantity of microorganisms degrades more toxic contaminants than the phytoremediator itself. Some bacterial groups have unusual degradative metabolic pathways to degrade soil containments, such as *Pseudomonas stutzerii* against carbon tetrachloride (Sepulveda et al. 1999) and some have particular biochemical

pathways when acting synergistically, but not alone (Lappin et al. 1985). In some cases, plant exudates provided easy access of nutrients to rhizobial inhabitants and promote synergistic remediation. Changes in the proportion of soil bacteria thus makes a difference and could be a mechanism that influences plant dynamics and plant inhabitant community structure in the soil remediation environment. Rapid changes in soil microbial community-abundance have consequences for the plant inhabitant community (Kent and Triplett 2002), which ultimately affects the phytoremediation process. Predation by other populations, including growing microbes, and human activities determine the species composition and quantity at the phytoremediation sites. The distribution and community structure of bacteria play major roles (Yannarell and Kent 2009) and it is essential to study predation by competing bacterial populations. In addition, the absence of biological defensive mechanism such as thorns or poisonous exudates allows large predators to play a dominant role in plant existence. Shifts in plant community dynamics may be an indirect effect of the suppression of microbial populations by chemical toxicants or a direct effect of chemo-grazing of chemical aggregates similar to that of predation-mediated disturbance (Jurgens and Sala 2000). Thus, the consideration of rhizosphere communities, phytoremediator species ecology and diversity of phytoinhabitants suggests that a set of predatory influences will always affect the performance of phytoremediators as well as the native phytoinhabitant assemblages in terms of toxicant degradation. If all elements of the ecosytem are thriving, they will offer a greater biomass increase and better remediation in bridging the gap between plant productivity and chemical remediation.

19.4 Chemical Contaminants and Toxicant Species

Soils polluted with polycyclic aromatic hydrocarbons (PAHs) and other toxicants are of environmental concern. Wetlands are often net sources of methylmercury (MeHg). It accumulates in aquatic food webs and contaminates fish and related aquatic life-forms (Watras et al. 2005). It poses the greatest risk to drinking water and humans. Toxic heavy metals and organic pollutants are major potential targets for phytoremediation. Enhancement of PAH dissipation in vegetated soil is often suggested to be a result of a rhizosphere effect caused by root exudates (Aprill and Sims 1990). One of the methods that can be effectively applied for cleaning up many such toxicants particularly PAH is rhizosphere bioremediation (Frick et al. 1999). Many toxicants persist hundreds of years in smelter and mining sites and in ammunition waste. Major toxic chemicals and their species are mercury (Hg) (Bizily et al. 1999; Eckley et al. 2005), polychlorinated biphenyls, chlorinated benzoic acid, hexachlorobiphenyl, 2,4,6-trinitrotoluene (TNT), naphthalene, pyrene (Liste and Alexander 2000), chloroacetamide herbicides, benzo(a)pyrene, 3,3′,4,4-tetrachloroazobenzene (TCAB), arsenic (As) (Pickering et al. 2000), polycyclic aromatic hydrocarbons (PAHs) (Pradhan et al. 1998), metals such as nickel (Ni), zinc (Zn), copper (Cu), rubidium (Rb), cesium (Cs), manganese (Mn), iron

(Fe), selenium (Se), chromium (Cr), cadmium (Cd) (Nedelkoska and Doran 2000) and lead (Pb) or radioactive isotopes such as uranium (U), Cs-137, strontium (Sr) and cobalt (Co). Beranova et al. (2007) have studied rhizoremediation for decontamination of long-term PCB-contaminated soil with a focus on microbial diversity. Bacteria are able to transform PCBs under aerobic or anaerobic conditions. The anaerobic process, reductive dechlorination, leads to the formation of lower chlorinated PCBs that are aerobically more easily degraded than congeners with a higher level of chlorination (Furukawa 2006). Aerobic bacteria that are able to degrade PCBs have been isolated and identified from, for example, strains of the genera *Pseudomonas*, *Achromobacter*, *Alcaligenes*, *Burkholderia*, *Ralstonia* and *Rhodococcus* among others. These bacteria are able to use biphenyl as a sole source of carbon and energy and PCBs are co-metabolized by the enzymes of biphenyl pathway. These enzymes are encoded by genes included in biphenyl operon (Abramowicz 1990).

Uptake and accumulation of chemical contaminants by plants varies from species to species, based on the chemical contaminant speciation. Some plant species act as powerful remediators of the primary chemical contaminants but others remediate only the chemical species of the primary contaminants. For example, uptake and accumulation of selenium and its chemical species were varied in Indian mustard (De Souza et al. 1998), broccoli, sugarbeet and rice (Zayed et al. 1998). Similarly, remediation of trichloroethylene (TCE), tetrachloroethylene (PCE) (Newman et al. 1998) and species such as trichloroethanol, and trichloroacetic acid as well as nitroaromatic compounds (Rivera et al. 1998) such as aminodinitrotoluene, diaminonitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7,-tetraazocine (HMX) were varied as reported earlier. Among the plant species, dewberry (*Rubus caesius*), vipers-bugloss (*Echium vulgare*), scarlet-pimpernel (*Anagallis arvensis*) and narrow-leaf plantain (*Plantago lanceolata*) can accumulate significant amount of Pb, Cu, Zn and Cd. Besides xenobiotic degradation of 2,4,5-trichlorophenoxyacitic acid (Boyle and Shann 1998) and the high potential for removal of chlorinated compounds such as carbon tetrachloride and PCE, the degradation of brominated compounds such as ethylene dibromide and dibromochloropropane as well as nonhalogenated compounds such as methyl-*t*-butyl ether (Newman et al. 1998) significantly depends on the quality of the plant species used rather than the zone of their accumulation.

19.4.1 Contaminants and Plume Systematics

The concentration of xenobiotics in a contaminated plume may have variable effects on phytoremediator plants. Unless we study the array of toxicants and their level of persistence in the contaminated sites, it is ineffective to use any phytoremediator to achieve the intended remediation. For example, many grasses may be suited for heavy metal remediation (Peterson et al. 1998) but not all; why

does such inequity exist in nature? Because of the multiple degree of toxicity present in a contaminant site, there may be a variable intensity of influences on the biological accumulation of toxic chemicals in grasses while the remediation process in progress. For example, Cd is one the most toxic metals, followed by Pb, Cu and Zn. At the molecular level, sublethal concentrations of the heavy metals cause induced cell plasmolysis and alterations in the chloroplast arrangement (Basile et al. 2012). Therefore it is prudent to pay attention to the contour of the plume before selecting a phytoremediator. The pinpointing of the remediation spot followed by categorization of its spreading outline is the foremost step in the phytoremediation process. Once attained, the remediators can be grouped according to their levels of remediation potential and the ability of the remediator, in association with its rhizosphere, to reach the contaminants. The types of remediators (such as terrestrial or aquatic) as well as their level of remediation are schematically explained in Fig. 19.5.

Terrestrial phytoremediators are divided into five groups based on the ability of the roots in the rhizosphere to reach the contaminants.

1. Surface remediator: The rhizosphere of this group of phytoremediator can reach less than 10 cm from the focal point of the root of origin
2. Sub-surface remediator: The rhizosphere spreads beyond 12 but less than 24 cm depth in soil (example: herbs like *Alpine pennycress*)
3. Deep-surface remediator: The rhizosphere spreads beyond 24 but less than 36 cm depth in the soil (example: *Picea* spp.)
4. Core remediator: It has a moderately deep rhizosphere of more than 3 ft depth in the soil (example: *Juniperium virginiana*)
5. Deep-core remediator: The roots can penetrate beyond 6 ft deep and spread very broadly in the soil. (example: trees like *Ficus pumila* L.).

Aquatic photoremediators can scavenge the toxicants directly from the aqueous surface (Rajendran and Arokiasamy 1990) by floating on the surface area or fully/partially submerge into water. In a recent heavy metal removal experiment, it was revealed that macrophytes showed excellent performance in removing the selected metals, thus suggesting that they are good candidates for wastewater remediation purposes (Basile et al. 2012). These aquatic remediators are divided into five groups based on their remediation ability.

1. Floating remediator: They have tough roots and can move with the water current.
2. Emerged remediator: They can float but most of the green parts are emerged on the water like a water hyacinth.
3. Submerged remediator: They show less surface area to the sun and most of the plant is underwater, for example plants that live under water but protrude out to get a minimum of sunlight for survival.
4. Deep-submerged remediator: They are fully submerged aquatic plants and may move with the water current.
5. Anchored remediator: They are rooted on the floor of the water body in deeper areas.

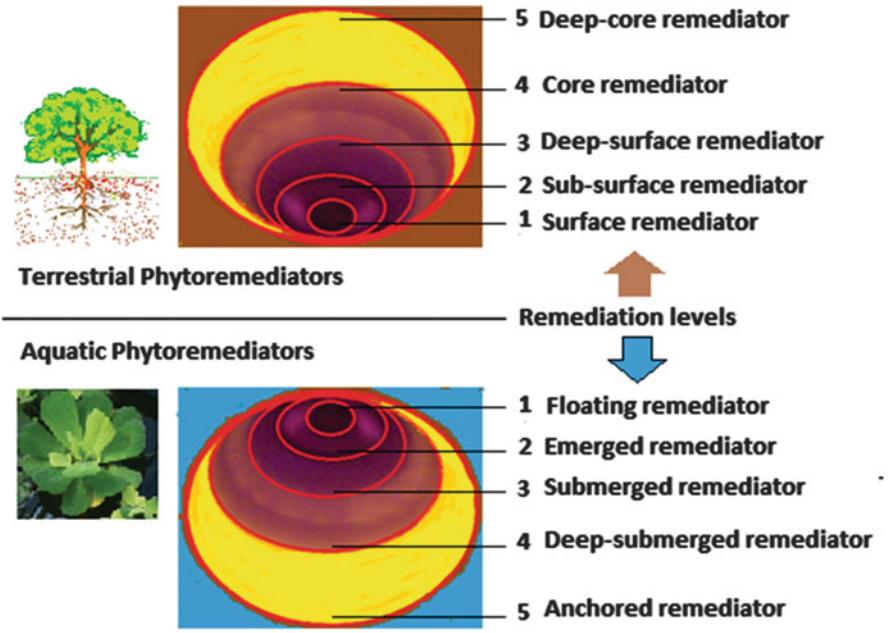


Fig. 19.5 Types of remediator plants and their potential remediation levels. The two states of remediator lifeforms, terrestrial and aquatic, play major roles in our everyday existence by remediating chemical toxicants from the environment. The state of the soil remediator group is divided into five assemblages based on the ability of the roots in the rhizosphere to reach the contaminants: (1) surface remediator, (2) sub-surface remediator, (3) deep-surface remediator, (4) core remediator and (5) deep-core remediator. The state of the aquatic phytoremediator group is divided based on the scavenging of toxicants directly from the aqueous facade by floating on the surface area or fully or partially submerged into water. Their five assemblages are: (1) floating remediator, (2) emerged remediator, (3) submerged remediator, (4) deep-submerged remediator and (5) anchored remediator

19.4.2 Phytoinformatics and Data Analysis

Gathering large amounts of data and interpreting outsized datasets for successful phytoremediation is emerging into a frontier field of study in remediation. Fine-scale phylogenetic analysis may open new possibilities on the patterns and roles of specific phytoinhabitant populations in ecosystem processes. Applying such community datasets to research examining microbial, algal and related populations in different environments (including aquatic and terrestrial systems) helps ecosystem management and analysis. Many techniques are scattered across different fields of study and it is time to collectively apply them together in phytoremediation. For example, molecular biology-related microbial ecological techniques provide an enormous amount of data, but it is not reaching phytoremediation researchers. It is an urgent need to utilize modern techniques from other disciplines, e.g.

"ecosystem process models" that are used for synthesizing biogeochemical cycles and generating data on nutrient cycling and patterns.

The results on specific mechanisms that are the most influential for changing carbon and nitrogen budgets can be used in experimental trials to synthesize prediction data (Davis et al. 2010). A similar approach can be used for phytoremediator plants by applying various agricultural and cultivation practices. This would provide large datasets describing microbial community composition and variation across time and space. It could provide C and N cycling of phytoremediation ecosystems that can be used. The design principles and data model should be used during database development and will help researchers to get bioinformatics data on remediator plants and phytoinhabitants, especially microbial bioinformatics data. Such data-mining processes have enormous potential for the design of environmentally friendly phytoremediation methods for practical use in a broader perspective by utilizing other biological databases. Microbial community datasets must be linked with phytoremediator plants in relation to related environmental factors. This challenging task is how to get reasonable data from the slow-growing phytoremediators and the real-time remediation process. Analytical and molecular techniques available in microbial ecology have the potential to reveal more about the relationship between phytoremediator plants and phytoinhabitants, especially microbes and their role in ecosystem functions (Jacob et al. 2005).

19.5 Synergistic Strategies

Plant-based remediation is an attractive, low-cost, in-situ green technology for the progressive clean-up of toxicants (Sandermann 1992). In specific cases, it offers the possibility of selectively removing only the metal contaminants, leaving the soil unaffected in every other way. However, there are a few disadvantages to phytoremediation technology. It is considered to be a slower process than mechanical or chemical remediation methods (Schnoor et al. 1995). It has limitations such as lower contaminant concentrations as well as site-specificity of contamination in shallow soils, streams and very deep ground water. However, a synergistic approach that takes advantage of the remarkable decontamination ability of plants could enhance the accumulation of contaminants in feasible way. This approach includes integrating the traditional remediation concepts with recent phytoremediation methods to concentrate the contaminants from the environment and to metabolize the various molecules in plant tissues. For example, in aquatic environments, phytoplankton-based algal exudates influence bacterial community structure (Paver and Kent 2010). Thus, similar influences and correlations between phytoremediator aquatic macrophytes and phytoinhabitants such as bacterial and algal communities need to be measured to understand the abundance and activity of the phytoremediation. Physical (abiotic) parameters (Shah 2000) such as irrigation, fertilization, plant rotation, alteration of the physical and chemical condition of soil,

use of chelating agents and control of the transportation of contaminants from deeper levels into the subsurface of roots zones together with biotic parameters such as microbial formulations and symbiotic actions are some of the traditional methods suitable for synergistic phytoremediation process. Biotic and abiotic forces vary in relative importance at different spatial and temporal scales. For example, abiotic factors such as geography, landscape position, hydrology, seasonal events, tropic status and biotic factors such as competition, tropic interactions and effect of plankton may all play a role in shaping the dynamics of lake bacterial communities (Shade et al. 2007).

19.5.1 Biological Adaptation of Native Plants

Plant species used in remediation processes should adapt to the local climate and soils conditions. Native plants as well as plants that are capable of supporting the phytoinhabitants are well suited for such remediation processes, since they are adapted to the area (Hellmers et al. 1955). In order to survive in the chemo-pressured hostile conditions at the contaminated sites, many native hyperaccumulator plants have developed larger root systems and succulent stems and are able to compartmentalize the contaminants. Wetland remediator plants such as *Arundo*, *Phragmites*, *Typha* and *Scirpus* act as pumps for nutrients and ions by remediating from sediments. When plants are stabilized in the soil, roots of plants such as Poplar and Cottonwoods reach down in depth towards the water table and establish a dense mass of secondary roots that absorb large quantities of water along with soluble contaminants. Once the rhizofiltration process is completed with saturated contaminants, the biological pumping mechanism adopted by the plants permits the contaminants to pass through the xylem in a rational way to reach the leaves and other parts of the tree, where they are accumulated, partly metabolized or partially reached volatile status. Based on nature's pumping concept, phytoremediation technologists adopt similar methods of mechanical means to pump the ground water to the surface area to remediate the volatile contaminants. However, a complete remediation cannot be achieved with this process due to the persistence of non-volatile contaminants in the sites. This process can be coupled with ground-remediation process similar to that of carbon tetrachloride remediation at the polluted plume itself (Sepulveda et al. 1999) in order to achieve optimum remediation.

19.5.2 Traditional Remediation Approaches

Harvesting the remediator plants before flowering and re-plantation of the site supports fast uptake of contaminants rather than waiting for the plant's full cycle. This vegetative growth remediation process not only prevents formation of fruits,

which may not be suitable for birds and other fruit-dependents, but also restricts the offspring. Similarly, plant rotation with other kinds of remediator plants can help to re-accumulate the contaminants in different proportions from the same site. This type of re-vegetation of the landscape can be repeated to bring down the contaminant levels in the soil to allowable limits. Synergistic use of phytoremediators along with wetting the site permits the free flow of water-transport to the remediator sites. Certain plants under monoculture absorb unusually large amounts of certain metals in comparison to other plants. The monoculture plantation helps decontamination if the site has a particular contaminant. In other cases, a combination of different plants would help to eliminate to multiple contaminants. In an in-situ testing method, such as the target-neighbor method, it was evaluated how planting density influences the uptake and to manipulate plant density for optimal removal of contaminants (Shann 1995). Similarly, the metal distribution in contaminated sites also influences the uptake, where metals in the sediments in the inlet zone are at greater concentrations than in other areas (Kongroy et al. 2012). Therefore, the high metal-removing potential of plants may need to be significantly supplemented by in-situ remediation operations, especially for biomonitoring studies. This could be a useful phytoremediation technology for restoring water quality by harvesting submerged and floating biomass (Ali et al. 1999).

19.5.3 Rhizobial Association and Plant Productivity

Phytoremediation is attractive (Siciliano and Germida 1998) since it uses renewable resources like plant remediators to remove toxic wastes rather than using chemical compounds. These plants can secrete root exudates such as phytoenzymes, which can strongly support degradation of hydrocarbons and other contaminants (Kathi and Khan 2011). In many cases, the biodegradation rate is dependent on the individual composition of plant exudates (Merkl et al. 2005). During natural attenuation and/or bioaugmentation, some of the indigenous rhizobial microflora survive with the help of such plant exudates, which comes with a plentiful supply of carbon sources and carry out bioremediation process more effectively. This proves that a combinatorial green remediation approach, beyond using plants or microbial communities to treat toxic wastes (Timmis et al. 1994), is more successful if it is used effectively. Recently, the combination of both plant and microbes has emerged as a promising synergistic rhizobacterial remediation process (Nichols et al. 1997). Many studies used bacterial and bacterioplankton communities in various environmental conditions to study their aggregate community pattern as well as their influence on the environment (Kent et al. 2004). Many attempts were made successfully to remediate toxicants, including organochlorine herbicides, by using this bacterially enhanced phytoremediation method (Hogan et al. 2006). The effectiveness of such microbe-associated rhizobial activity depends on specific interactions between plants and their associated root microorganisms (Saraf et al. 2008) in relation to the soil property and the nature of compounds to be degraded

(Reilley et al. 1996). For example, as high as 90 % reduction in the concentration of diesel range organics were observed over a 24-week period when a microbial-enhancing process was used within the rhizosphere of willow trees at an oil-contaminated site (Carman et al. 1998). Similarly, inoculating Dahurian wild rye (*Elymus dauricus*) or meadow brome (*Bromus biebersteinii*) with a combination of *Pseudomonas aeruginosa* strain R75 and *P. savastanoi* strain CB35 increases degradation of 2-chlorobenzoic acid in soil (Siciliano et al. 1998b).

Many bacteria can be directly used to remediate toxic wastes including PCBs (Roberts 1987). The process called bacterization of seeds (Rajendran et al. 1991) and/or seedlings of remediator plants with a microbial monoculture promotes synergistic biomining. However, the mechanism by which bacterial inoculants such as *Pseudomonas* species promote phytoremediation differs from plant to plant (Siciliano et al. 1998b). Plants can be engineered to carry bacterial enzymes to degrade biphenyls (Francova et al. 2003). Microorganisms and higher plants have been used to clean waste water (Wolverton et al. 1983). In recent years, biologically safe microbial compounds or growth-inducing tracer nutrients or organic stimulants have been used at low concentrations to improve the rhizosphere microbial communities, supports a hyper-growth of plants and help the growth of more hyperaccumulator roots (Weyens et al. 2009). For example, low concentration of ethylnediamine-*N*,*N*′-diacetic acid (EDDA) helps to chelate iron and increase siderophore synthesis in the rhizobacteria in and around the roots of sunflowers. A similar combined effect of biostimulation and phytoremediation was observed in a post oil-spill habitat restoration and enhanced oil degradation in the soil when marsh sods of *Spartina alterniflora* and *Spartina patens* were used. The results suggest that vegetative transplantation can simultaneously restore oil-contaminated wetlands and accelerate oil degradation in the soil (Lin and Mendelssohn 1998). Tank and Saraf (2008) have reported that species of *Pseudomonas* NT1 and C5 show highest Ni decontamination from the soil as well as plant growth promotion in Ni-spiked soil.

19.5.4 Symbiotic and Biocatalytic Approaches

Plant productivity-based restoration of hydrocarbon-contaminated soil, especially in intense deep areas, is an intricate process because it strongly influences the plants and its individual cells (Sadunishvili et al. 2009). In nature, it is proven that plants, in association with other inhabitants, clean up petroleum contamination as a self-sustaining remediation approach. This is the single most important symbiotic approach to successful remediation. The rhizosphere of these plants plays a major role in achieving the recycling process (Knatznelson 1965). Many grasses have a similar innate ability for degradation of chemical species of PAH such as benzo[a] pyrene, benzo[a]anthracene, dibenzo [a, h] anthracene and chrysene. For example, *Andropogon gerardii*, *Schizachyrium scoparium*, *Sorghastrum nutans*, *Panicum virgatum*, *Elymus Canadensis*, *Bouteloua curtipendula*, *Bouteloua gracilis* and

Pascopyrum smithii are considered the best grasses for the phytoremediation of drought and desert areas and to remediate crude oil (Kathi and Khan 2011). Phytoremediation, on the other hand, especially of salt marsh and upland man-made contamination sites is more successful when the plants symbiotically serve as remediator by hosting other organisms. This involves not only efficient phytoremediator plants but also the ecological characteristics of the rhizosphere inhabitant communities they support. Aquatic macrophytes (Rajendran and George 1989) can scavenge more nutrients and contaminants in waterways when applied synergistically. For example, the moss *Ceratodon purpureus*, water ferns like *Azolla* and *Athyrium yokoscense* and fungi like *Phanerochaete chrysoporium* also follow the synergistic biodegradation process, accompanied by plants and phytochelates. Mycorrhizal association with marsh and other rhizospheres makes the process more feasible (Shetty et al. 1995). For example, *Pinus ponderosa* hosts the mycorrhizal fungal *Hebeloma crustuliniforme* to symbiotically degrade atrazine. Root exudates released from plants (such as enzymes, sugars, alcohols or acids containing organic carbon) serve as substrates for soil or aquatic microflora (Rovira 1959). Sometimes they encounter adversity due to the catalytic action of other exudates; however, such catalytic action itself could be effective in improving plant productivity by digesting the organic substances during the biodegradation process.

Another promising approach is to using certain biocatalysts to break down complex chlorinated solvents like TCE or ammunition wastes at the contaminated plume. The primary breakdown process allows remediator plants to scavenge the rest of the contaminants of the plume. For example, cytochrome p450 catalyzes a variety of mono-oxygenation reactions for a wide range of lipophilic compounds, including PAHs and a few other environmentally persistent pollutants. These microbial cytochrome p450 pathways can be applied in the detoxification and biodegradation of recalcitrant pollutants. Aquatic and wetland treatment processes, against such types of TNT and RDX ammunition contaminants and for their removal in ground water, can be employed by using similar approach (Best et al. 1999). For example, the root exudate of Dahurian wild rye (*Elymus dauricus*) degrades 2-chlorobenzoic acid (Siciliano et al. 1998b) and overexpression of the enzyme γ-glutamylcysteine synthetase in *Brassica* spp. enhances Cd tolerance and accumulation in this Indian mustard (Zhu et al. 1999). These symbiotic restorations of land can be used to restore the terrain for wildlife habitat and can be modified in later years for commercial development.

19.6 Challenges in Plant Productivity and Remediation Processes

Plant productivity-based phytoremediation faces several challenges. Optimizing the process itself certainly moves the remediation process forward. For example, instead of introducing invasive species into cleaning sites, using native plants

provides a link between the clean-up and habitat restoration (Shrimp et al. 1993). The rhizosphere of the phytoremediators promotes degradation of toxicants and at the same time provides a habitat for many micro-and macroinhabitants to further sustain the restoration process. Maintaining more efficient cooperation among the partners means that more work is done. Unraveling the mechanisms of mineralization and volatilization of contaminants in plants may lead to ways of increasing the efficiency of the phytoremediation process. In a controlled environment, a 100 % remediation can be obtained by using a "continuous flow phytoreactor", as in the case of TNT remediation (Rivera et al. 1998). However, in field studies, this may not be the case due to the observed environmental influencing factors. For example, approximately 10 % of selenium was accumulated in Canola plants when grown in field conditions, whereas 50 % was noticed in greenhouse studies (Banuelos et al. 1998). To achieve high results, studies of soil characteristics, activities of microbial communities, biomass and mineralization of hazardous chemicals are necessary before and after planting of plant species (Boyle and Shann 1998). In contaminated soil, the success of phytoremediation depends on the structure of the soil. The void space or pore space of the soil dictates the soil phytoremediation process. It not only holds water for plants and microflora but also keeps air needed for roots and aerobic decomposition of organic molecules. Many metals are less soluble and thus less mobile when high oxidation states persist in the contaminated soil. They are maintained by the aerobic conditions and are very favorable for phytoremediation technology (USEPA 1997).

19.6.1 Identification of Chemical Toxicants

Identification of the species of contaminants, including translocated, accumulated or volatilized chemicals, as well as various converted forms in different parts of aqueous and soil plumes is a challenge, and the first step to determine the efficiency of plants in remediation processes. While the plant productivity increases, it is equally important and challenging to detect the chemicals species and the accumulation of toxic compounds in plant tissues and other parts of the high-accumulator plants. The latter can be identified by speciation analysis using X-ray absorption spectroscopy (Zayed et al. 1998). The volatilization of chemical species can be measured by using gas chromatography coupled with electron capture detection (GC-ECD) (Scheidemann et al. 1998). The translocated compounds can be extracted using dichloromethane with acid hydrolysis followed by alkalization, and the major metabolites separated using high-pressure liquid chromatography (HPLC) and identified by nuclear magnetic resonance (NMR). In the soil, the diversity of substrates utilized by the root-associated microbial communities can be assessed using Biolog Gram-negative (GN) and Gram-positive (GP) plates. The communities can be also characterized by extracting fatty acid methyl esters (FAME) from rhizobacteria associated with plant roots (Siciliano et al. 1998a). In the greenhouse, hydraulic control of supplementary water could serve as a

monitoring device to observe remediation in aquatic plants. In fields, besides these instruments, some other monitoring devices can also be used in order to monitor heavy-metal uptake from contaminated soil. For example, ascorbate is used as a marker of herbicide stress in wetland plants (Lytle and Lytle 1997). Measurement of chlorophyll fluorescence-induction kinetics was introduced recently for similar purposes in field phytoremediation. This portable chlorophyll fluorometer can be used to identify the most applicable parameter for monitoring the remediation process (Richter et al. 1998). In some cases, cytochrome p450 enzymes (Nelson et al. 2004) in plants catalyze the high range of chemically divergent substrates. Potential transgenic plants with tailored enzymatic activities could play major roles in the removal of environmentally stable organic pollutants from contaminated fields. Such enzymatic bioassays can be used to identify such toxicants to detect contaminants at specific sites.

19.6.2 Custom-Designed Approaches

The challenges in phytoremediation are use of the right phytoremediator and the handling of its highly productive biomass since it contains accumulated contaminants. For some sites, there is a co-remediation solution available but for other sites custom-designed methods are needed. For example, novel biotechnological approaches that harness recent advances (Abhilash et al. 2012) in our understanding of phytoremediator plants, their chemical interactions and their phytoinhabitants (such as microorganisms in the rhizosphere as well as within plant tissue) can be revisited to optimize in-situ applications. For example, the phytoremediation process can be custom-designed based on the organic matters (Newman et al. 1998) available in the remediator soil. The degree of efficiency of the remediation process in the rhizosphere varies according to the plant species involved as well as the depth of the contaminants present in the plume. Based on the available data, other forms of green remediation processes, including phycoremediation in parallel with phytoremediation especially in aqueous plumes, can be efficiently tailored for application. Adopting combinatorial phytoremediation, such as plant and microbe-based methods especially in terrestrial plumes, are more successful. The techniques such as phytoextraction, phytodegradation, phytostabilization, rhizofiltration, phytovolatilization etc. make green remediation more user-friendly and as efficient as commercial filtration techniques. Custom-designed nanoparticles such as Ag, Au, Cu, Si, and C may have unique accumulation patterns and solution properties that can significantly impact particle fate and effects. A recent study on biomass, transpiration and element content in zucchini plants revealed that plants are unaffected by Au nanoparticles, regardless of particle size or concentration. Ag and Si nanoparticles reduced plant biomass and transpiration to a significant degree. Cu nanoparticles were phytotoxic but much of the effect was alleviated by humic acid. The shoot Ag

and Cu content did not differ based on particle size or concentration (Hawthorne et al. 2012).

19.6.3 Signature Model Using Bioindicator Plants

The so-called hyperaccumulator weeds have been identified as signature remediator plants for the metal ores and radionuclides (Entry et al. 1996). They spread more in alpine areas such as found in central Europe, Japan and the Rocky Mountains of the USA. Based on the signature quality, many of these plants are identified as bioindicator plants. For example, the duckweed (*Wolffia globosa*) acts as an indicator of heavy metal pollutants and is especially sensitive to chromium and cadmium. In combination with some biochemical markers, many plants can be identified as good bioindicators including for radioactive elements (Hoseini et al. 2012). For example, ascorbate can be a biomarker for herbicide stress. Plants in combination with soil pH can also be used as moderate indicators in some phytoremediation studies. For example, the uptake of the chemical species of uranium, the uranyl cation (UO_2^{2+}) was observed in *Pisum sativum* at soil pH 5. The tepary bean (*Phaseolus acutifolius*) and red beet (*Beta vulgaris*) show the highest accumulation of uranium at pH 6 and 8. This indicates that soil pH is an interesting influencing factor in phytoremediation processes, at least in the case of uranium uptake (Ebbs et al. 1998).

19.6.4 Priority-Based Advancement Using a Transgenic Approach

Many plant species and/or phytoremediator species are classified as first-rate phytoremediation candidates for non-commercial sites such as homes, where volatile formaldehyde is a big concern. Manipulation of the genetic outcome of a plant growth-promoting rhizobacterium (PGPR) such as *Pseudomonas fluorescens* through transposon mutagenesis (Rajendran et al. 1994), protoplast fusion (Rajendran and Jayaraman 1994) or electroporation into nitrogen-fixing bacterium such as *Azospirillum lipoferum* (Ramalingam and Rajendran 2001) or screening for mutant clones in carbon tetrachloride-degrading soil bacterium such as *Pseduomonas stuzeri* (Sepulveda et al. 1999) makes such soil bacteria more suitable for association with domesticated plants for remedial applications. Using such modified rhizobial inhabitants with transgenic remediator plants for higher productivity makes the process more viable and successful. The advantage of using engineered plants with chemo-degrading microbial genes is that one can continuously improve plant productivity and elevate them to the next stage. It is also possible to monitor the efficiency of productivity and the remediation process

simultaneously with the pathways involved (Stomp et al. 1994). For example, studies with Cd remediation by transgenic tobacco (Macek et al. 2002a) or the isolation of a Cd^{2+}-sensitive Cad1 mutant of *Arabidopsis thaliana*, which is deficient in a peptide called phytochelatin synthetase, demonstrates conclusively the importance of such engineered approaches for heavy metal tolerance. Similarly, natural variation in cadmium hyperaccumulation can be assayed in *Thlaspi caerulescents* (Roosens et al. 2003). A variety of assays are now available to use with the model plants *Arabidopsis thaliana* and *Nicotiana tabacum* (Heaton et al. 1998) to assesses phytoremediation capability with a modified bacterial mercuric reductase gene, *merA* (Rugh et al. 1996), which is capable of converting ionic mercury and Hg(II) to the less toxic, volatile Hg(O).

19.7 Conclusion

In this chapter, an attempt was made to explain the state of the rhizobial inhabitants of phytoremediator communities and a number of factors that may potentially influence plant productivity and the process of remediation. Problems of chemical contamination and its salvage will continue because of the continuous use of metals, chemicals and their derivatives in our everyday life in one way or other. The continuous use of chemicals today will have a profound effect on the environment and public health tomorrow, which will surpass the current emerging risks. By coalescing different forms of nature's bioremediation processes, plants can clean up a large plume of contaminated soil and/or aquifer sites in association with their inhabitants. Although it has been recognized for more than 20 years that plants can be used as a biological clean-up tool for inorganic contaminants, the technology has not yet been adopted in many counties. This could be due to lack of public awareness of the potential success and/or even of the real existence of such technology. There is an urgent need for knowledge on ecosystem restoration through domesticating a vast group of model hyperaccumulator plants and trees from the wild, herbarium collections and taxonomic literatures. The success of this process demands a multidisciplinary approach, because the precise mechanisms for the removal of contaminants of concern and the complex survival ability of the plants are still not fully understood and necessarily depend on a multidisciplinary approach. Hence, it is appropriate to start with selection of novel remediator plants and combine this with environmental engineering for in-situ remediation studies. It is therefore important to incorporate traditional tissue culture with nanobiological approaches and genetic engineering tools to enable the plant to metabolize a particular toxic pollutant. Currently, many interdisciplinary terms are being introduced and potential applications are being employed in phytoremediation. More understanding is needed on hyperaccumulator plants and incorporation of genes into the plant growth-promoting bacteria (PGPR) for synergistic phytoremediation. Improved methods for recycling the remediator plants themselves are necessary to make phytoremediation more economically and

commercially viable. In the near future, phytoremediation might become an integral part of environmental management.

Acknowledgments The author gratefully acknowledges the US Nuclear Research Council (USNRC) for the faculty research grant and Professor M. Saraf, Department of Microbiology and Biotechnology, Gujarat University, Ahmedabad, Gujarat (India) for helpful discussions during the preparation of this manuscript. This work was supported by the Oak Ridge Association of Universities and Kentucky State University (ORAU-KSU) initiative.

References

Abhilash PC, Powell JR, Singh HB, Singh BK (2012) Plant–microbe interactions: novel applications for exploitation in multipurpose remediation technologies. Trends Biotechnol 30 (8):416–420

Abramowicz DA (1990) Aerobic and anaerobic biodegradation of PCBs – a review. Crit Rev Biotechnol 10:241–249

Ali MB, Tripathi RD, Rai UN, Pal A, Singh SP (1999) Physico-chemical characteristics and pollution level of Lake Nainital: role of macrophytes and phytoplankton in biomonitoring and phytoremediation of toxic metal ions. Chemosphere 39(12):2171–2182

Anderson TA, Guthrie EA, Walton BT (1993) Bioremediation in the rhizosphere. Environ Sci Technol 27:2630–2636

Aprill W, Sims RC (1990) Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. Chemosphere 20:253–265

Baker AJM (1989) Terrestrial higher plants which hyperaccumulate metallic elements: a review of their distribution, ecology and phytochemistry. Biorecovery 1:81–126

Balser T, McMahon KD, Bronson BD, Coyle DR, Craig N, Flores-Mangual M, Forshay K, Jones S, Kent A, Shade A (2006) Bridging the gap between micro- and macro-scale perspectives on the role of microbial communities in global change ecology. Plant Soil 289:59–70

Banuelos GS, Ajwa HA, Wu L, Zambrzuski S (1998) Selenium accumulation by *Brassica napus* grown in Se-laden soil from different depths of Kesterson Reservoir. J Soil Contam 7 (4):481–496

Barbosa AC, Silva SR, Dorea JG (1998) Concentration of mercury in hair of indigenous mothers and infants from the Amazon basin. Arch Environ Contam Toxicol 34:100–105

Basile A, Sorbo S, Conte B, Cobianchi RC, Trinchella F, Capasso C, Carginale V (2012) Toxicity, accumulation, and removal of heavy metals by three aquatic macrophytes. Int J Phytoremed 14 (4):374–387

Beard JB, Green RL (1994) The role of turfgrasses in environmental protection and their benefits to humans. J Environ Qual 23:3

Beranova K, Kochankova L, Macek T, Mackova M (2007) Rhizoremediation for decontamination of long-term PCB contaminated soil with focus on microbial diversity. J Biotechnol 131(2): S243

Best EP, Sprecher SL, Larson SL, Fredrickson HL, Bader DF (1999) Environmental behavior of explosives in groundwater from the Milan Army Ammunition Plant in aquatic and wetland plant treatments. Removal, mass balances and fate in groundwater of TNT and RDX. Chemosphere 38(14):3383–3396

Bizily SP, Rugh CL, Summers AO, Meagher RB (1999) Phytoremediation of methylmercury pollution: merB expression in *Arabidopsis thaliana* confers resistance to organomercurials. Proc Natl Acad Sci USA 96(12):6803–6813

Boyle JJ, Shann JR (1998) The influence of planting and soil characteristics on mineralization of 2,4,5, T in rhizosphere soil. J Environ Qual 27(3):704–709

Bradshaw A, Johnson M (1992) Revegetation of metalloferrous mine waste: the range of practical techniques used in Western Europe. In: Anthony MT (ed) Minerals, metals and environment. Elsevier Science, London, pp 481–491

Buée M, De Boer W, Martin F, van Overbeek L, Jurkevitch E (2009) The rhizosphere zoo: an overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. Plant Soil 321(1–2):189–212

Carman EP, Crossman TL, Gatliff EG (1998) Phytoremediation of No.2 fuel oil-contaminated soil. J Soil Contam 7(4):455–466

Chaudhry Q, Schröder P, Werck-Reichhart D, Grajek W, Marecik R (2002) Prospects and limitations of phytoremediation for the removal of persistent pesticides in the environment. Environ Sci Pollut Res Int 9:4–17

Compton HR, Haroski DM, Hirsh SR, Wrobel JG (1998) Pilot-scale use of trees to address VOC contamination. In: Wickramanayake GB, Hinchee RE (eds) Bioremediation and phytoremediation. Battelle, Columbus, pp 245–250

Consuelo MDM, Jack CS, Mark CM, James HT (2004) Induced plant signaling and its implications for environmental sensing. J Toxicol Environ Health 67(8–9):819–834

Cotner JB, Biddanda BA (2002) Small players, large role: microbial influence on biogeochemical processes in pelagic aquatic ecosystems. Ecosystems 5:105–121

Davis SC, Parton WJ, Dohleman FG, Smith CM, Kent AD, DeLucia EH (2010) Comparative biogeochemical cycles and sustainability of bioenergy crops. Ecosystems 13:144–156

De Souza MP, Pilon-Smits EA, Lytle CM, Hwang S, Tai J, Honma TS, Yeh L, Terry N (1998) Rate-limiting steps in selenium assimilation and volatilization by Indian mustard. Plant Physiol 117(4):1487–1494

Devinny J, Longcore T, Bina A, Kitts C, Osborne KH (2005) Phytoremediation with native plants: final report for Zumberge Fund for Innovation. University of Southern California, CA

Ebbs SD, Brady DJ, Kochian LV (1998) Role of uranium speciation in the uptake and translocation of uranium by plants. J Exp Bot 49(324):1183–1190

Eckley C, Watras C, Hintelmann H, Kent AD, Regnell O (2005) Mercury methylation in the hypolimnetic waters of lakes with and without connection to wetlands in northern Wisconsin. Can J Fish Aquat Sci 62:400–411

Entry JA, Vance NC, Hamilton MA, Zabowski D, Watrud LS, Adriano DC (1996) Phytoremediation of soil contaminated with low concentrations of radionuclides. Water Air Soil Pollut 88:167–176

Environment Canada (2008) Risk management scope for naphthalene. http://www.ec.gc.ca/substances/ese/eng/challenge/batch1/batch1_91-20 3_rmscopes_en.pdf

Francova K, Sura M, Macek T, Szekeres M, Bancos S, Demnerova K, Sylvestre M, Mackova M (2003) Generation of plants carrying bacterial enzyme for degradation of polychlorinated biphenyls. Fresen Environ Bull 12(3):309–313

Frick CM, Farrwll RE, Germida JJ (1999) Assessment of phytoremediation as an in-situ technique for cleaning oil-contaminated Sites. Calgary, Canada, pp 1–82. http://www.clu-in.info/download/remed/phyassess.pdf

Furukawa K (2006) Oxygenases and dehalogenases: molecular approaches to efficient degradation of chlorinated environmental pollutants. Biosci Biotechnol Biochem 70:2335–2348

Hameeda B, Rupela OP, Reddy G, Satyavani K (2006) Application of plant growth-promoting bacteria associated with composts and macrofauna for growth promotion of Pearl millet (*Pennisetum glaucum* L.). Biol Fertil Soil 43(2):221–227

Hawthorne J, Musante C, Sinha SK, White JC (2012) Accumulation and phytotoxicity of engineered nanoparticles to Cucurbita pepo. Int J Phytoremed 14(4):429–442

Heaton ACP, Rugh CL, Wang NJ, Meagher RB (1998) Phytoremediation of mercury and methylmercury-polluted soils using genetically engineered plants. J Soil Contam 7(4):497–509

Hellmers H, Horton JS, Juhren G, O'Keefe J (1955) Root systems of some chaparral plants in southern California. Ecol 36(4):667–678

Hinsinger P, Brauman A, Devau N, Gérard F, Jourdan C (2011) Acquisition of phosphorus and other poorly mobile nutrients by roots. Where do plant nutrition models fail? Plant Soil 348 (1–2):29–61

Hogan D, Ryan D, Dowling N (2006) Bacterial endophyte-enhanced phytoremediation of the organochlorine herbicide 2, 4-dichlorophenoxyacetic acid. FEMS Microbiol Ecol 57 (2):302–310

Hooper DU, Vitousek PM (1998) Effects of plant composition and diversity on nutrient cycling. Ecol Monogr 68(1):121–149

Hoseini PS, Poursafa P, Moattar F, Amin MM, Rezaei AH (2012) Ability of phytoremediation for absorption of strontium and cesium from soils using *Cannabis sativa*. Int J Environ Health Eng 1:17

Jacob C, Kent AD, Benson BJ, Newton RJ, McMahon KD (2005) Biological databases for linking large microbial and environmental datasets. Proceedings of the 9th world multiconference on systematics, cybernetics and informatics VII: Informatics Sys. Dev. II, 14–18 July 2002, Orlando, FL

Jha CK, Dhara P, Rajendran N, Saraf M (2010) Combinatorial assessment on dominance and informative diversity of PGPR from rhizosphere of *Jatropha curcas* L. J Basic Microbiol 50 (3):211–217

Jurgens K, Sala MM (2000) Predation-mediated shifts in size distribution of microbial biomass and activity during detritus decomposition. Oikos 91:29–40

Kamnev AA, Lelie DVD (2000) Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. Biosci Rep 20(4):239–258

Kathi S, Khan AB (2011) Phytoremediation approaches to PAH contaminated soil. Indian J Sci Technol 4(1):56–63

Kent AD, Bayne ZL (2010) Degraded water quality influences microbial community composition and perception of health risks in the Chattooga River. DNA Cell Biol 29:509–517

Kent AD, Triplett EW (2002) Microbial communities and their interactions in soil and rhizosphere ecosystems. Annu Rev Microbiol 56:211–236

Kent AD, Jones SE, Yannarell AC, Graham JM, Lauster GH, Kratz TK, Triplett EW (2004) Annual patterns in bacterioplankton community variability in a humic lake. Microbiol Ecol 48 (4):550–560

Kirschner EM (1995) Botanical plants prove useful in cleaning up industrial sites. Chem Eng News 73(50):22

Knatznelson H (1965) Nature and importance of the rhizosphere. In: Baker KF, Snyder WC (eds) Ecology of soil-borne plant pathogens: prelude to biological control. University of California Press, Berkley, CA, pp 187–207

Kongroy P, Tantemsapya N, Lin YF, Jing SR, Wanpen W (2012) Spatial distribution of metals in the constructed wetlands. Int J Phytoremed 14(2):128–141

Lacson JG (2000) CEH product review – naphthalene. In: Chemical economics handbook (CEH). SRI International, Menlo Park, CA

Lappin HM, Greaves MP, Slater JH (1985) Degradation of the herbicide Mecoprop [2-(2-methyl-4-chlorophenoxy) propionic acid] by a synergistic microbial community. Appl Environ Microbiol 49(2):429–433

Lin QX, Mendelssohn IA (1998) The combined effects of phytoremediation and biostimulation in enhancing habitat restoration and oil degradation of petroleum contaminated wetlands. Ecol Eng 10(3):263–274

Liphadzi M, Kirkham M (2006) Availability and plant uptake of heavy metals in EDTA-assisted phytoremediation of soil and composted biosolids. S Afr J Bot 72:391–397

Liste HH, Alexander M (2000) Phytoremediation of toxic elemental and organic pollutants. Curr Opin Plant Biol 3(2):153–162

Lytle TF, Lytle JS (1997) Ascorbate: a biomarker of herbicide stress in wetland plants. In: Kruger EL, Anderson TA, Coats JR (eds) Phytoremediation of soil and water contaminants, ACS symposium series no. 664. American Chemical Society, Washington, DC, pp 106–113

Macek T, Macková M, Pavlíková D, Truksa M, Singh CA, Kotrba P, Yancey N, Scouten WH (2002a) Accumulation of cadmium by transgenic tobacco. Acta Biotechnol 22(2):101–106

Macek T, Macková M, Chromá L, Burkhard J, Demnerova K (2002b) Phytoremediation. In: Agathos SN, Reineke W (eds) Biotechnology for the environment. Kluwer Academic, Brussels, pp 115–137

Madhaiyan M, Poonguzhali S, Senthilkumar M, Santhanakrishnan P (2009) Responses of agronomically important crops to inoculation with plant-associated benefical bacteria in crop-farming systems–a review. In: Arn TD (ed) Corn crop production – growth fertilization and yield. Nova Science, New York. ISBN 978-1-60741-955-6

Meagher RB (2000) Phytoremediation of toxic elemental and organic pollutants. Curr Opin Plant Biol 3(2):153–162

Meera M, Agamuthu P (2012) Phytoextraction of As and Fe using *Hibiscus cannabinus* L. from soil polluted with landfill leachate. Int J Phytoremed 14(2):186–199

Merkl N, Schultze-Kraft R, Infante C (2005) Phytoremediation of petroleum-contaminated soils in the tropics assessment of tropical grasses and legumes for enhancing oil-degradation. Water Air Soil Pollut 165:195–209

Migeon A, Richaud F, Blaudez D, Chalot M (2012) Hydroponic screening of poplar for trace element tolerance and accumulation. Int J Phytoremed 14(4):350–361

Murugaiyan S, Munusamy M, Subbiah S, Kannaiyan S (2009) Intercellular colonization and growth promoting effects of *Methylobacterium* sp. with plant-growth regulators on rice (*Oryza sativa* L. cv CO-43). Microbiol Res 164:92–104

Nedelkoska TV, Doran PM (2000) Hyperaccumulation of cadmium by hairy roots of *Thlaspi caerulescens*. Biotechnol Bioeng 67:607–615

Nelson DR, Schuler MA, Paquette SM, Werck-Reichhart D, Bak S (2004) Comparative genomics of rice and *Arabidopsis*. Analysis of 727 cytochrome P450 genes and pseudogenes from a monocot and a dicot. Plant Physiol 135:756–772

Newman LA, Doty SL, Grey K, Heilman PE, Muiznieks I, Shang TQ, Siemieniec ST, Strand SE, Wang XP, Wilson AM, Gordon MP (1998) Phytoremediation of organic contaminants: a review of phytoremediation research at the University of Washington. J Soil Contam 7 (4):531–542

Nichols TD, Wolf DC, Rogers HB, Beyrouty CA, Reynolds CM (1997) Rhizosphere microbial populations in contaminated soils. Water Air Soil Poll 95(1–4):165–178

Paver SF, Kent AD (2010) Temporal patterns in glycolate-utilizing bacterial composition correlate with phytoplankton population dynamics in humic lakes. Microb Ecol 60:406–418

Peterson MM, Horst GL, Shea PJ, Comfort SD (1998) Germination and seedling development of switchgrass and smooth bromegrass exposed to 2,4,6-trinitrotoluene. Environ Poll 99(1):53–59

Pickering IJ, Princes RC, George MJ, Smith RD, George GN, Salt DE (2000) Reduction and coordination of arsenic in Indian mustard. Plant Physiol 122(4):1171–1178

Pradhan SP, Conrad JR, Paterek JR, Srivastava VJ (1998) Potential of phytoremediation for treatment of PAHs in soil at MGP sites. J Soil Contam 7(4):467–480

Qin S, Xing K, Jiang JH, Xu LH, Li WJ (2011) Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. Appl Microbiol Biotechnol 89(3):457–473

Rajendran N, Arokiasamy DI (1990) Nitrogen fractions of aquatic growth forms. Bionat 11 (1):21–23

Rajendran N, George VK (1989) Chlorophyll and carotenoid contents in eleven aquatic plants. J Aqua Org 2(2):54–56

Rajendran N, Jayaraman K (1994) Expression of the insecticidal crystal protein genes from *Bacillus thuringiensis* in a Gram-negative *Pseudomonas fluorescens* by protoplast fusion. FEMS Microbiol Lett 122:103–108

Rajendran N, Venkatesan S (1993) Synergistic action of plant defense chemicals and the endotoxin of *Bacillus thuringiensis* in relation to *Heliothis armigera* and *Spodoptera litura* (Lepidoptera). Proc Chem Ecol Phytophag Ins 4:300–311

Rajendran N, Sivamani E, Jayaraman K (1991) Green house trials of *Bacillus thuringiensis* toxin on cotton plants. In: Proceedings of the 31st annual conference of Association of Microbiologist of India, TN Agricultural University, Coimbatore, pp 23–25

Rajendran N, Jahn D, Marahiel MA (1994) Tn5 mutagenesis of *Pseudomonas fluorescens* to isolate mutants deficient in antibacterial activity. FEMS Microbiol Lett 115:191–196

Ramalingam G, Rajendran N (2001) Transformation studies of *Bacillus thuringiensis* cryIC gene into nitrogen fixing *Azospirillum lipoferum*. Zeitschrift für Naturforschung C 56c:245–248

Rambelli A (1973) The rhisozphere of mycorrhizae. In: Marks GC, Kozlowski TT (eds) Ectomycorrhizae. Academic, New York

Raskin I (1996) Plant genetic engineering may help with environmental cleanup. Proc Natl Acad Sci USA 93:3164–3166

Reilley KA, Banks MK, Schwab AP (1996) Dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. J Environ Qual 25:212–219

Richter PI, Barocsi A, Csintalan Z, Kuperberg MJ, Szdzuj J (1998) Monitoring soil phytoremediation by a portable chlorophyll fluorometer. Field Anal Chem Technol 2 (4):241–249

Rivelli AR, De Maria S, Puschenreiter M, Gherbin P (2012) Accumulation of cadmium, zinc, and copper by *Helianthus annuus* L.: impact on plant growth and uptake of nutritional elements. Int J Phytoremed 14(4):320–334

Rivera R, Median VF, Larson SL, Mc Cutcheon SC (1998) Phytotreatment of TNT-contaminated groundwater. J Soil Contam 7(4):511–529

Roberts L (1987) Discovering microbes with a taste for PCBs. Science 237:975–977

Roosens N, Verbruggen N, Meerts P, Ximanez De Emban P, Smith JAC (2003) Natural variation in cadmium hyperaccumulation and its relationship to metal hyperaccumulation for seven populations of *Thlaspi caerulescens* from western Europe. Plant Cell Environ 26:1657–1672

Rovira AD (1959) Root excretions in relation to the rhizosphere effect. IV. Influence of plant species, age of plants, light, temperature, and calcium nutrition on root exudation. Plant Soil 11:53–64

Rugh C, Wilde D, Stack NM, Thompson DM, Summers AO, Meagher RB (1996) Mercuric ion reduction and resistance in transgenic *Arabidopsis thaliana* plants expressing a modified bacterial merA gene. Proc Natl Acad Sci USA 93:3182–3187

Sadunishvili T, Kvesitadze E, Betsiashvili M, Kuprava N, Zaalishvili G, Kvesitadze G (2009) Influence of hydrocarbons on plant cell ultrastructure and main metabolic enzymes. World Acad Sci Eng Technol 57:271–276

Sandermann H Jr (1992) Plant metabolism of xenobiotics. TIBS 17:82–84

Sanon A, Andrianjaka ZN, Prin Y, Bally R, Thioulouse J (2009) Rhizosphere microbiota interfers with plant-plant interactions. Plant Soil 321(1–2):259–278

Saraf M, Tank N, Rajendran N (2008) Rhizomediation in soil for sustainable agriculture. In: Maheshwari DK, Dubey RC (eds) Potential microorganisms for sustainable agriculture, vol 19. I.K. International, New Delhi, pp 364–393

Scheidemann P, Klunk A, Sens C, Werner D (1998) Species dependent uptake and tolerance of nitroaromatic compounds by higher plants. J Plant Physiol 152(2–3):242–247

Schnoor JL, Light LA, McCutcheon SC, Wolfe NL, Carreira LH (1995) Phytoremediation of organic and nutrient contaminants. Environ Sci Technol 29:318–324

Sepulveda T, Lycely DC, Rajendran N, Dybas MJ, Criddle CS (1999) Generation and initial characterization of *Pseudomonas stutzeri* KC mutants with impaired ability to degrade carbon tetrachloride. Arch Microbiol 171:424–429

Shade AL, Kent AD, Jones SE, Newton RJ, Triplett EW, McMahon KD (2007) Inter-annual dynamics and phenology of bacterial communities in an eutrophic lake. Limnol Ocean 52:487–494

Shah KL (2000) Basics of solid and hazardous waste management technology. Prentice Hall, Upper Saddle River, NJ, p 23

Shann JR (1995) The role of plants and plant/microbial systems in the reduction of exposure. Environ Health Perspect 103(5):13–15
Shetty KG, Hetrick BD, Schwab AP (1995) Effects of mycorrhizae and fertilizer amendments on zinc tolerance of plants. Environ Pollut 88(3):307–314
Shrimp KE, Tracy JC, Davis LC, Lee E, Haung W, Erickson LE, Schnoor JL (1993) Beneficial effects of plants in the remediation of soil and groundwater contaminated with organic materials. CRC Crit Rev Environ Sci Technol 23(1):41–77
Siciliano SD, Germida JJ (1998) Mechanisms of phytoremediation: biochemical and ecological interactions between plants and bacteria. Environ Rev 6:65–79
Siciliano SD, Germida JJ, Siciliano SD (1998a) Biolog analysis and fatty acid methyl ester profiles indicate that *Pseudomonas* innoculants that promote phytoremediation alter the root-associated microbial community of *Bromus biebersteinii*. Soil Biol Biochem 30(13):1717–1723
Siciliano SD, Goldie H, Germida JJ (1998b) Enzymatic activity in root exudates of Dahurian wild rye (*Elymus dauricus*) that degrades 2-chlorobenzoic acid. J Agric Food Chem 46(1):5–7
Singh S, Eapen S, Raj K, D'Souza S (2008) Phytoremediation of 137cesium and 90strontium from solutions and low-level nuclear waste by *Cannabis*. Ecotoxicol Environ Saf 69:306–311
Sivamani E, Rajendran N, Senrayan R, Ananthakrishnan TN, Jayaraman K (1992) Influence of some plant phenolics on the activity of delta endotoxin of *Bacillus thuringiensis* var. *galleriae* on *Heliothis armigera*. Ent Exp Appl 63:243–248
Stomp AM, Han KH, Wilbert S, Gordon MP, Cunningham SD (1994) Genetic strategies for enhancing phytoremediation. Ann NY Acad Sci 721:481–491
Tank N, Saraf M (2008) Enhancement of plant growth and decontamination of nickel spiked soil using PGPR. J Basic Microbiol 49(2):195–204
Timmis KN, Steffean RJ, Unterman R (1994) Designing microorganisms for the treatment of toxic wastes. Annu Rev Microbiol 48:528–557
USEPA (1997) Status of in situ phytoremediation technology. In: Recent developments for in situ treatment of metal contaminated soils. Report no. EPA-542-R-97-004. US Environmental Protection Agency, Washington, DC, pp 31–42
USEPA (2001) Contaminant candidate list; preliminary regulatory determination support document for naphthalene. Report no. EPA 815-R-01-008. US Environmental Protection Agency, Washington, DC
Vajpayee P, Rai UN, Sinha S, Tripathi RD, Chandra P (1995) Bioremediation of tannery effluent by aquatic macrophytes. Bull Environ Contam Toxicol 55:546–553
Vance DB (1996) Phytoremediation: enhancing natural attenuation processes. Natl Environ J 6:30–32
Vithanage M, Dabrowska BB, Mukherjee AB, Sandhi A, Bhattacharya P (2012) Arsenic uptake by plants by and possible phytoremediation applications: a brief overview. Environ Chem Lett 10 (3):217–224
Watras CJ, Morrison KA, Kent AD, Price N, Regnell O, Hintelmann CH, Hubacher T (2005) Sources of methylmercury to a wetland-dominated lake in northern Wisconsin. Environ Sci Technol 39:4747–4758
Weyens N, Lelie D, Taghavi S, Vangronsveld J (2009) Phytoremediation: plant endophyte partnerships take the challenge. Curr Opin Biotechnol 20:248–254
Wolverton BC, McDonald RC, Duffer WR (1983) Microorganisms and higher plants for waste water treatment. J Miss Acad Sci 31:79–89
Yannarell AC, Kent AD (2009) Bacteria, distribution and community structure. In: Likens G (ed) Encyclopedia of inland waters. Elsevier, Oxford
Zayed A, Lytle CM, Terry N (1998) Accumulation and volatilization of different chemical species of Selenium by plants. Planta 206(2):284–292
Zenk MH (1996) Heavy metal detoxification in higher plants–a review. Gene 179(1):21–30
Zhu YL, Pilon-Smits ES, Tarun AS, Weber SU, Jouanin L, Terry N (1999) Cadmium tolerance and accumulation in Indian mustard is enhanced by overexpressing gamma-glutamylcysteine synthetase. Plant Physiol 121(4):1169–1178

Index

D.K. Maheshwari et al. (eds.), *Bacteria in Agrobiology: Crop Productivity*,
DOI 10.1007/978-3-642-37241-4,

Q

R