chapter ten

Microbial volatiles and their biotechnological applications

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10.1   Introduction

The capability of the emission of volatile organic compounds (VOCs) is well known for plants and animals. Such volatile compounds are characterized by their molecular weights of less than 300 Da, high vapor pressures, low boiling points, and low polarities. Due to these features, evaporation and distribution into the atmosphere as well as into the air- and water-filled pores below ground are facilitated. These airborne signals exhibit the potential to act as infochemicals for inter- and intra-specific communication in different habitats even over long distances (Kai et al. 2009; Wenke et al. 2010). A comprehensive source of such volatiles is constituted by the microbial world, which has been overlooked in the past. The microbial VOCs (mVOCs) affecting other organisms play a role in plant/fungi-microbe and animal/human-microbe interactions

including microbial pathogens ("the bad") as well as microbes with protecting potential ("the good") (Bailly and Weisskopf 2012; Schenkel et al. 2015; Wenke et al. 2012a). Central questions are: Which volatiles are emitted by microorganisms? Which impact on fitness (health), development and growth do they have on the receiving organism? Are these mVOCs useful for any applications?

At present, headspace analyses of around 490 microbial species resulted in the identification of around 1200 volatiles (for review, see Effmert et al. 2012; Lemfack et al. 2014; Schulz and Dickschat 2007), which are divided into 48 chemical classes dominated by alcohols, alkenes, ketones, and terpenoids (Wenke et al. 2012b). Due to the incredible microbial diversity (10,000 species are known, more than 1 million are expected on Earth) it is foreseen that the actual number of known microbial volatiles represents just the "tip of the iceberg." Furthermore, microorganisms have not been systematically investigated regarding their capabilities of volatile emissions but the present available results indicate that microbes are a good source for novel and unusual volatiles (Lemfack et al. 2014; Von Reuss et al. 2010). The biological and ecological functions of mVOCs are diverse, for example, (i) they play a role in the food chain of the microbial loop since they are assimilated and incorporated into organic matter (bioconversion), (ii) they influence physiological processes in various target organisms (e.g., laccase activity, nitrification, and nitrogen mineralization), (iii) they function as electron acceptors or donors to support metabolic reactions, (iv) they play a role in quorum sensing/quenching, (v) they act as defense compounds against fungi, nematodes/animals and bacteria, (vi) they act as communication signals, or (vii) their functions remain so far elusive (summarized in Effmert et al. 2012; Kai et al. 2009; Wenke et al. 2010 and 2012). Nevertheless, the detailed reactions and adaptations at the physiological, transcriptional, protein and metabolic levels of the target organisms were only recently investigated (Bailly and Weisskopf 2012; Wenke et al. 2012a).

10.2   *Most frequently emitted mVOCs*

The emission of microbial volatiles is commonly—often unconsciously—recognized; for example, the typical smell of cheese varieties, the aroma of wine, and the characteristic odor of mushrooms derive from microorganisms. Furthermore, the earthy, muddy smell in a wet forest is due to the production of geosmin and other volatiles released by *Streptomyces* species. Microbiologists are trained to recognize indole characteristically emitted by *Escherichia coli* and butyric acid released by *Clostridium* spp. These examples indicate that microorganisms contribute significantly to the odors present in our environment. The most comprehensive summary

Table 10.1 Twenty most cited bacterial volatiles in the mVOC database

	mVOC name	Chemical classification	Number of bacteria emitting the compound
1	2-Phenylethanol	Alcohol	100
2	3-Methylbutan-1-ol	Alcohol	90
3	Dimethyl disulfide	Sulfide	88
4	Dimethyl trisulfide	Sulfide	79
5	Undecan-2-one	Ketone	54
6	Benzyl alcohol	Alcohol	53
7	Geosmin	Terpenoid	52
8	Tetradecanoic acid	Carboxylic acid	52
9	Acetic acid	Carboxylic acid	47
10	2-Aminoacetophenone	Ketone	43
11	Benzaldehyde	Aldehyde	43
12	Nonan-2-one	Ketone	43
13	Acetoin	Ketone	42
14	1-Undecene	Alkene	41
15	2-Methylpropan-1-ol	Alcohol	41
16	3-Methylbutanoic acid	Carboxylic acid	41
17	Dodecanoic acid	Carboxylic acid	41
18	Acetone	Ketone	39
19	1-Heptanol	Alcohol	36

of microbial volatiles is found in the mVOC database (Lemfack et al. 2014). By data mining, the 20 most frequently emitted mVOCs of bacteria (Table 10.1) and fungi (Table 10.2) were determined. The following compounds are significantly more often released by bacteria: (1) 2-phenylethanol, (2) 3-methylbutan-1-ol, (3) dimethyl disulfide, and (4) dimethyl trisulfide; and 1-octen-3-ol is most frequently emitted by fungi. These most abundant mVOCs derive from three different metabolic pathways: (1) the shikimate pathway synthesizing the amino acid phenylalanine (phenylpropanoid biosynthesis), (2) reduction product of isovaleric acid or caprylic acid (fatty acid biosynthesis), and (3) sulfur metabolism. It is interesting to note that alcohols, ketones, and carboxylic acids are the most frequently emitted mVOCs, while terpenoids and pyrazines appear seldom in the VOC spectra of microorganisms.

10.3   *Habitats of mVOC producers*

Microorganisms appear ubiquitously, in various—even extreme—habitats and ecological niches (Horikoshi et al. 2011). The mVOC database was used to extract the habitats of which volatile emitting microorganisms

Table 10.2 Twenty most cited fungal volatiles in the mVOC database

	mVOC name	Chemical classification	Number of fungi emitting the compound
1	1-Octen-3-ol	Alcohol	62
2	2-Pentylfuran	Furan	44
3	Hexan-1-ol	Alcohol	43
4	2-Pentanol	alcohol	42
5	3-Methylbutanal	Aldehyde	41
6	2-Methylbutanal	Aldehyde	39
7	Hexanal	Aldehyde	38
8	1-Heptanol	Alcohol	37
9	2-Pentanone	Ketone	36
10	2-Ethyl-1-hexanol	Alcohol	34
11	Benzaldehyde	Aldehyde	33
12	Nonanal	Aldehyde	33
13	Decanal	Aldehyde	31
14	Heptanal	Aldehyde	31
15	Styrene	Benzenoid	30
16	1,2,4-Trimethylbenzene	Alkane	29
17	1-Nonanol	Alcohol	29
18	Butan-4-olide	Lactone	29
19	Naphtalene	Alkene	29
20	Pentanal	Aldehyde	29

have been isolated thus far. The microorganisms listed in the mVOC database were obtained from 10 distinct habitats: (1) animals, (2) human/clinical sources, (3) food products, (4) fresh water, (5) humans, (6) marine environment, (7) plants, (8) plant waste, (9) rhizosphere, and (10) soil. Most species listed in the database are of plant sources (70), aquatic environment (66), and from the soil (61). We correlated the VOC profiles of the microorganismal species with the habitat where they originated from (multivariate analysis) (Figure 10.1). No habitat-specific VOC spectra became apparent and it is suspected that it is not the location of isolation, but rather the nutritional supply (growth media) and metabolic capabilities that are relevant for the emission profiles (Fiddaman and Rossall 1993).

10.4   *Applications of mVOCs*

The biological and ecological functions of the microbial volatiles are very diverse and manifold, and often the biological relevances of mVOCs are not known or understood. Beside this lack of knowledge, mVOCs are
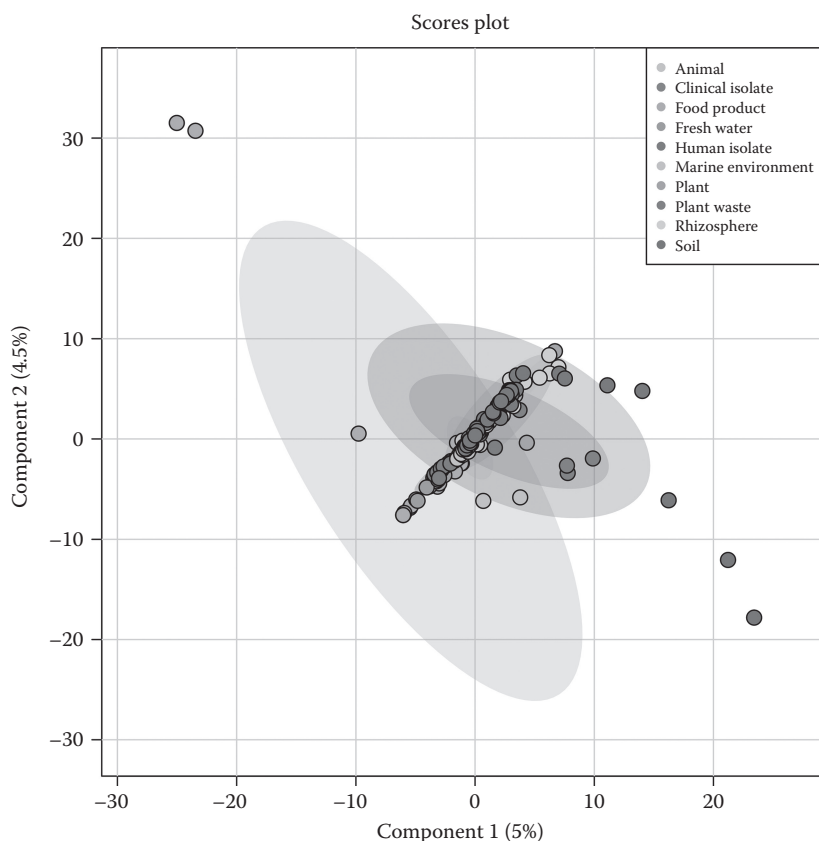
Scores plot

Figure 10.1 (**See color insert.**) Principal component analysis of mVOC-emitting microorganisms of different habitats. Data were extracted from the mVOC database and multivariate analyses were performed using the online comprehensive tool for metabolomic data analysis "MetaboAnalist 3.0." (From Xia, J. et al. 2015. *Nucl. Acid Res.* 43: W251–W257.).

furthermore important cues for applications. Three major areas of implications are addressed: the agricultural, the medical, and the biotechnological application (Figure 10.2).

10.4.1   *mVOCs of foodstuff*

Flavor involves our perception of sugars, organic acids, and of a diverse group of volatile metabolites produced by multiple metabolic pathways. Although the human nose can distinguish many volatiles, there is a given limitation in the detection and proper description of the relevant smell.
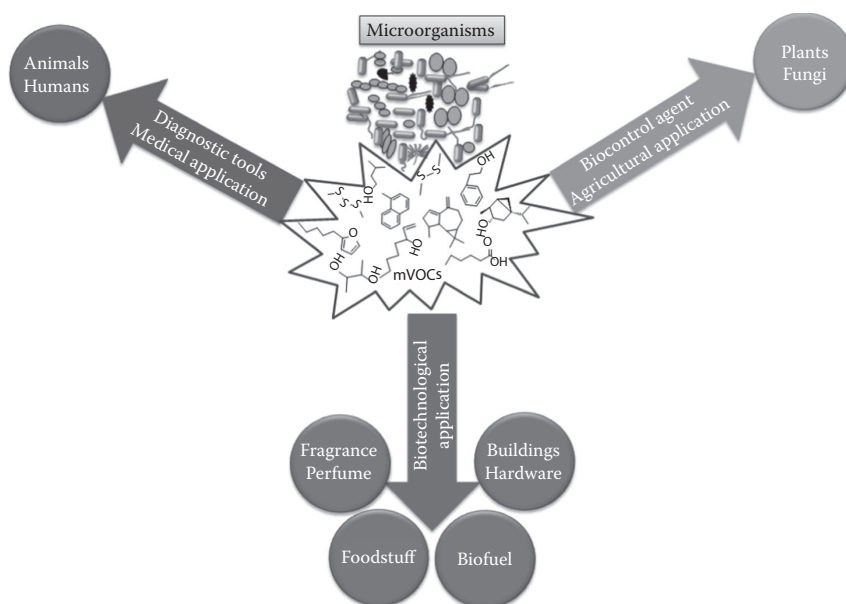
Figure 10.2 Overview of mVOC applications.

Nevertheless, it is still a challenge to improve the flavor of many modern fruit and vegetables. Likewise, the microbial-based volatile aromas of foodstuff such as wine, dairy products, and mushroom (including truffle) are continuously analyzed.

Truffles emit up to 200 volatile metabolites, typical components have a chain length of eight carbon atoms, for example, *trans*-2-octenal, 1-octen-3-ol, and octanol, which are the main components of the mushroom odor (Buzzini et al. 2005; Chitarra et al. 2004) (Table 10.2). The various types of truffles differ in their scent spectra, *Tuber borchii* and *Tuber melanosporum* are distinct to *Tuber indicum* or *Tuber magnatum* (most expensive Piedmont truffle) (Splivallo et al. 2007). New investigations revealed that truffles live in symbiosis with various yeasts, "guest" bacteria, and filamentous fungi, which also contribute to the scent bouquet (Buzzini et al. 2005; Vahdatzadeh et al. 2015). In the case of the white truffle *T. borchii,* it was shown that cyclic sulfur volatiles (thiophene derivatives) actually derive from bacteria inhabiting truffle fruiting bodies (Splivallo et al. 2014). Interestingly, the same thiophene derivatives are also the most important odorants which contribute to human sensed aroma in the latter species (Splivallo and Ebeler 2015). It is believed that truffle fruiting bodies produce their smell to attract mammals and rodents to locate the fungus underground. Actually out of the hundreds of volatiles produced by truffles, only a single one—dimethyl sulfide—has been convincingly

implicated in the attraction of mammals (Talou et al. 1990). The role of other truffle volatiles as a location cue is of great interest for the food industry.

Phenolic content, colour and volatile compounds are the most important *wine* quality attributes. The wine aroma is very much influenced by the bacterial and yeast alcoholic fermentation, when sugars are metabolized into acetic acid as well as ethanol. Acetic esters, ethyl esters and higher alcohols are the dominant aroma compounds, for example, ethyl hexanoate and ethyl octanoate contribute to fruity aromas. Defined bacterial starter cultures are applied to grape juice during wine production. The time point is critical and influences significantly the product outcome. Malolactic fermentation, the decarboxlation of l-malic acid to l-lactic acid is conducted by lactic acid bacteria, for example, *Oenococcus oeni*, results in a slight increase in wine pH and provides microbial stability and positively influences wine aroma and flavour (Abrahamse and Bartowsky 2012). A prolonged or delayed malolactic fermentation increases the risk of spoilage by microorganisms such as *Lactobacillus*, *Pediococcus* and *Brettanomyces* species that may produce, for example, biogenic amines or 4-ethyl-phenol (Curtin et al. 2007; Gerbaux et al. 2009).

Microorganisms play an important role in the development of *dairy product* (cheese, yoghurt, curd, etc.) flavor. For example, more than 100 volatiles, sometimes only present in trace amounts, are found in yoghurt, and most flavor compounds are produced from lipolysis of milkfat and microbial transformation of lactose and citrate (Cheng 2010). Most important for the aroma are acetaldehyde, diacetyl, acetoin, acetone, and 2-butanone, while off-flavor compounds appear during extended shelf-lifes due to lipid oxidation. Since flavor is a very important characteristic from the consumer's point of view, new strains isolated from dairy and nondiary environments are tested for their production of odor-active volatile compounds to modulate dairy product flavors (Pogacic et al. 2015). Multivariate analyses are used to evaluate mVOC profiles of typical milk fermenting bacteria such as *Leuconostoc lactis*, *Lactobacillus* spp., *Brachybacterium* spp., *Brevibacterium* sp., and *Propionibacterium* sp. The survey identified 52 mVOCs and certain VOCs such as ethyl esters, sulfur compounds, branched chain alcohols and acids, and diacetyl as well as related carbonyl compounds turned out to be characteristic for each bacterial species (Pogacic et al. 2015). It was also possible to differentiate mVOC profiles of, for example, the New Zealand and the Italian parmesan cheese based on butanoic acid, phenylacetaldehyde, ethyl butyrate, acetaldehyde and methylbutanals (Langford et al. 2012). Thus, microbial volatile aroma compounds are important tools to assess dairy product quality and flavor although much work is still necessary to understand the complete formation of aromas and flavors. Qualitative and quantitative analyses are surely a first step to achieve this goal, but the volatile compound-matrix

interaction, release mechanism of VOCs and synergistic actions need to be addressed to assess their aroma contributions (Cheng 2010).

The most well-known "*off-flavor*" volatile geosmin is emitted by soil bacteria such as *Streptomyces* spp., *Anabena* spp., *Oscillatoria* spp., myxo-bacteria, soil-dwelling, aquatic and airborne fungi. Geosmin has the characteristic earthy and moldy odor and is recognized as a volatile of con-taminated food, wine and water (Darriet et al. 2000). The repugnant smell of rotting organic matter results from the release of bacterial and fungal volatiles, consequently mVOCs are good markers for spoiled foodstuff, for example, meat, bread, vegetables and fish. mVOCs used as indicators in food industry will be exemplified here for fish processing. The European sea bass with its white flesh and low fat content is a popular farmed fish. It is very perishable due to microbial spoilage (Gram and Hass 1996). Typical spoilage microorganisms reach high population densities and produce several metabolites (CSIs: chemical spoilage indices), which are respon-sible for the off-odor resulting in their organoleptic rejection (in Parlapani et al. 2015). During fish deterioration, the production of microbial vola-tile compounds such as trimethylamine, dimethylamine, and ammonia is measured with a hydrogel-pH-electrode based near-field passive vola-tile sensor (Bhadra et al. 2015). Other mVOCs have also been studied as potential indicators for CSIs for spoilage/freshness evaluation and became important measures for food quality when the levels vary between the initial and rejection day of seafood. Most of the 40 mVOCs determined on sea bass appeared sporadically or fluctuated, while, for example, 3-methyl-1-butanol, 2-methyl-1-butanol and the ethyl esters acetate, propionate and isobutyrate increased during storage. Various other mVOCs have been reported as metabolites released by *Pseudomonas* spp., *Schewanella* spp., *Enterobacteriaceae*, and *Brochothrix thermosophacta* during fish and/or meat spoilage and specifically ethyl esters were related to *Pseudomonas* activ-ity (Casaburi et al. 2014). Indeed, esters were found only in gutted sea bass stored under air where the aerobic conditions enhance the growth of pseudomonads (Parlapani et al. 2015), while ethyl acetate increased in cod (Olafsdottir et al. 2005) and pangasious filets (Noseda et al. 2012). Acetic acid was mainly attributed to the growth of *B. thermosophacta*. These exam-ples indicate that the characteristic mVOC profiles (= fingerprints) depend on storage conditions (air, vaccum, temperature), fish or meat batches, and on the microorganisms, which preferentially colonize the fish species or the kind of meat. The development of biosensors based on mVOCs is a reachable goal assessing freshness and shelf-life of foodstuff.

10.4.2   *mVOCs as indicators of damp buildings and other hardware*

In the 1990s, mVOCs were used as indicators for indoor air environment (Bayer and Crow 1994; Wessen et al. 1995). With this technique, it was

possible to detect hidden microbial growth behind interior surfaces without destructing the building because it was assumed that gases (mVOCs) may enter the indoor air more easily than fungal or bacterial spores (Lorenz et al. 2002; Wessen et al. 1999). The term "sick building syndrome" (SBS) was coined in the last decade to refer to a set of symptoms that are experienced by the occupants of a building with poor air quality (Polizzi et al. 2012). Buildings with moisture and mold damage were considered risky for health, particular for eyes and the upper respiratory tract (cough and wheeze). Korpi et al. (2009) summarized 96 typical "indoor" mVOCs, of which 15 compounds were toxicologically evaluated (e.g., inhalation studies, lowest administered doses). From the human experimental exposure studies it turned out that symptoms of irritation appeared at mVOC concentrations several orders of magnitude higher than those measured indoors and it was concluded that mVOC concentrations are too low to provoke a nuisance effect on the building occupants. However, a drawback of the present analysis is that the toxicological database is poor, and there may be more potent compounds and endpoints yet not evaluated. Furthermore, in the environment mVOCs may come from various sources, such as building materials, human activities, traffic, foodstuff, smoking and may overlap and act additively in mixtures. The majority of mVOC producers present on buildings are fungi, for example, *Alternaria, Aspergillus, Botrytis, Candida, Fusarium, Penicillium, Trichoderma*, but also bacteria *Streptomyces* and *Pseudomonas* appear frequently (Claeson et al. 2002; Korpi et al. 1998). The main mVOCs produced on building materials were 3-methyl-1-butanol, 1-pentanol, 1-hexanol and 1-octen-3-ol, and Korpi et al. (1998) concluded that no single VOC is a reliable indicator for biocontamination. In contrast Bennett and Inamdar (2015) reported on some mVOCs that have toxic properties. Experimental tests with tissue cultures and *Drosophila melanogaster* have shown that many single mVOCs as well as mixtures emitted by fungi have toxic effects. Subsequently, they are referred to mycotoxins. Inamdar et al. (2010, 2013) tested low vapor concentrations of C-8 compounds, including 1-octen-3-ol, and showed toxicity to larvae and adult flies, selectively affecting dopaminergic neurons in adult *Drosophila* brains and induced Parkinson like behavioral alterations. The toxicity data on 1-octen-3-ol are of particular concern, because this fungal VOC appears ubiquitously (it is the most prominantly released fungal mVOC, Table 10.2) and is largely responsible for the musty odor commonly associated with mold-contaminated damp indoor spaces (Bennet and Inamdar 2015). In accordance, the results of a recent study concerning 6-pentyl-2-pyrone production by *Trichoderma atroviridae* growing on buildings showing that fungi can support SBS symptoms by irritating and damaging mucosal membranes (Polizzi et al. 2011), even when the production of fungal VOCs varies and is suboptimal due to the dependence on temperature and humidity (Polizzi et al. 2012).

Microbial biofilms are also formed on much other hardware, for example, clinical tubings, hulks, and air conditioners of houses and automobiles, which may result in malodors. Alpha-proteobacteria, methylobacteria, Shingomonadales, Burkholderiales, Bacillales, *Alcanovorax* spp., and *Stenotrophomonas* spp. were found on contaminated heat exchanger fins of evaporators from cars, which produced di-, tri-, and multiple sulfides, acetylthiazole, aromatic compounds and diverse pyrazines (Diekmann et al. 2013). Interestingly, a close relationship of the VOC profile and microbial community to the climate and air quality where the car was operating (European, American, Arabic, and Asian) was determined.

10.4.3   *mVOCs for the perfume industry*

Volatile organic compounds are predestinated and prestige flavor, fragrance, and aroma compounds. The perfume industry has an increasing demand for new VOCs. Although traditionally plants were the sources for aromas and fragrances, many other sources including microorganisms are presently tapped (Rutkin 2015).

10.4.4   *mVOCs as the next generation biofuel*

The limitations of fossil fuels are foreseen and contrast steadily increasing fuel requirements (reviewed in Rude and Schirmer 2009). Furthermore to combat climate change and to reach the goal of energy independence, search for alternative and renewable energy sources is ongoing. Three routes to convert such resources into energy-rich, fuel-like molecules or fuel precursors are considered: (i) photosynthesis related production by plants, alga and cyanobacteria, (ii) fermentative and nonfermentative production by heterotrophic microorganisms, and (iii) chemical conversion of biomass. Biofuel platforms based on food and nonfood biomass conversion have several limitations including the energy output per land area, the compatibility with current fuel infrastructures, and insufficient capacity the meet Renewable Fuel Standard (RFS) (Wang et al. 2015). mVOCs are considered as potential alternatives of biofuels from renewable resources. Microbial fuels are fermentative short–chain alcohols (e.g., ethanol and butanol), nonfermentative short-chain alcohols (1-propanol, 1-butanol, isoforms and derivatives of butanol, pentanol, and 1-hexanol), fatty acid-derived hydrocarbons (fatty acid alcohols of C12 to C18 are useful for fuels, while very-long-chain fatty acid alcohols C24–C26 are unsuitable), and isoprenoid-derived hydrocarbons. To overcome the disadvantages of ethanol, fuel research enquired the production of isoprenoid derived biofuels. Sesquiterpenes (e.g., farnesol, farnesene, and farnesane bisabolene) are being developed as precursors for fuel and monoterpenes (e.g., α-pinene, camphene, limonene, and sabinene and terpinene) are discussed

as potential next generation jet fuel components (reviewed in Gupta and Phulara 2015; Rude and Schirmer 2009). Isoprenoides are considered as good diesel alternatives because of their low hygroscopy, high energy content and good fluidity at low temperatures. Limitations are the low yields, toxicity and their stereochemical complexities. Presently attempts are undertaken to use genetically engineered microbes that produce a variety of infrastructure-compatible drop-in fuel molecules and to boost up terpene synthesis. In recent years, particularly *Escherichia coli* and *Saccharomyces cerevisiae* as well as less commonly used microbial strains were used as hosts to tune expression of endogenous enzymes of the isoprenoid pathway or to introduce heterologous enzymes. Several obstacles have been addressed and overcome, and advances in isoprenoid-based biofuels have been made using synthetic biology tools, for example, modulating the MEP pathway because it is stoichiometrically more competent than the MVA pathway, introducing the second terpene pathway into the microorganism, reprogramming metabolic nodes of pathways via mutations of transcription factors, improving terpene synthase catalysis, and installing efficient storage and excretion strategies are essential keys for progress (summarized in Gupta and Phulara 2015). Cyanobacteria, due to their fast growth, high photosynthetic rates, ability to grow in nonarable areas, availability of genome sequences, and production from CO_2 and solar energy make them a useful platform for biofuel production (Gupta and Phulara 2015; Wang et al. 2015). Although isoprenoid-based biofuel titers have been significantly improved in microorganisms in recent years, they still do not match with those of ethanol.

10.4.5   mVOCs as biocontrol agents in agriculture

Microbiota are attracted by suitable microenvironments in the soil to colonize and create microecosystems. Consequently, the "networking" communities are characterized by mutualism, commensalism, cooperation, antagonism, competition, and coexistence. In this arena, interactions between bacteria and fungi could have a positive or negative impact on third parties, which is useful if the weakened party is a pathogen and the strengthened party is a valuable member of the community (plant growth–promoting bacteria or fungi). An example of such a kind was recently shown (Kottb et al. 2015). The dominant volatile 6-pentyl-pyrone (coconut-like aroma, 6PP) of *Trichoderma asperellum* was perceived as a stress compound by *A. thaliana* and subsequently initiated multilayered defence adaptations including morphological and physiological alterations as well as activation of signaling cascades to withstand this environmental influence. Most noticeable is that *A. thaliana* preexposed to 6PP showed significantly reduced symptoms when challenged with *Botrytis cinerea* and *Alternaria brassicicola*, indicating that defense-activated plants

subsequently became more resistant to pathogen attack. Together, these results support that products that are based on *Trichoderma* volatiles have the potential of being a useful biocontrol agent in agriculture.

Another example is a GFP-tagged *Bacillus subtilis* strain which was able to successfully suppress cucumber wilt by confining growth of *Fusarium oxysporum* f. sp. *cucumerinum* by colonizing the root and persisting on the rhizoplane (Cao et al. 2011). The authors proposed antibiosis as one mode of action caused by diffusible agents. Other experiments with a *B. subtilis* strain isolated from the rhizosphere of wheat and soybean showed bacterial volatiles being involved in the biocontrol of *Botrytis mali* and *Phytophthera sojae*, respectively (Jamalizadeh et al. 2010; Sharifi et al. 2002).

Many *Bacillus* species have been reported to synthesize plant growth promoting VOCs. Ryu et al. (2004) reported that 2,3-butandiol and acetoin emitted by *Bacillus subtilis* GB013 and *Bacillus amyloliquefaciens* IN937a triggered induced systemic resistance against *Erwinia carotovora* in *Arabidopsis thaliana.* Volatiles of *Bacillus badius* M12 were documented to possess organogenetic potential on callus cultures of *Sesamum indicum* and introduce antioxidative activity in tobacco cultures (Gopinath et al. 2015).

Microorganisms of the soil and rhizosphere are able to protect plants from infections by specific root pathogens, a phenomenon called disease-suppression of soils (Hornby 1983). Beside other antagonistic modes of actions, only in the last decade it appeared that mVOCs play a key role in this pathogen inhibition (Insam and Seewald 2010; McNeal and Herbert 2009). The biological activity of such mVOCs might be an alternative regarding the usage of the methyl bromide for fumigation of soils infected by soil-borne fungal pathogens; in fact dimethyl disulfide, which is frequently emitted by bacteria (Table 10.1), is already used as a novel soil fumigant PALADIN®. Fungistasis results from the presence of mVOCs, for example, blocking germination of spores and inhibiting mycel growth (summarized in Kai et al. 2009). Minerdi et al. (2009) also showed that virulence genes are repressed in *Fusarium oxysporum*. Only a few studies indentified single compounds being responsible for the antifungal activity. Fernando et al. (2005) and Cordovez et al. (2015) demonstrated that six VOCs (cyclohexanal, decanal, 2ethyl-1-hexanol, nonanal, benzothiazole, and dimethyl trisulfide) of *Pseudomonas* and 1,3,5-trichloro-2-methoxy benzene and methyl 2-methylpentanoate of *Streptomyces* species possessed antifungal activity. However, the concentrations tested were quite high and the ecologically relevant doses remain to be determined.

mVOCs are also nematicidial. Gu et al. (2007) showed that mVOCs of bacilli isolates reduced movement of the nematodes *Panagrellus redivivus* and *Bursaphelenchus xylophilus.* Microbial volatiles also influence the tritrophic interactions comprising bacteria, fungi, and nematodes. *Paenibacillus polymyxa* and *Paenibacillus lentimorbus* exhibited strong

antifungal activities, thereby interfering with the nematode-fungus interaction (*Meloidogyne incognita—Fusarium oxysporum)*, which significantly reduced nematode infestation of tomato plants (Son et al. 2009). Additionally, soil bacteria, including one rhizobacterial strain, enhanced the nematophagous activity of the nematode-trapping fungus *Arthrobotrys oligospora* by increasing trap formation and predaceous activity (Duponnois et al. 1998). There are also numerous instances of mVOCs associated with insect feeding behavior, but some mVOCs are also powerful repellants (summarized in Davis et al. 2013). In some ecosystems, bacterial and fungal volatiles incite insect aggregations, or mVOCs can resemble sexual pheromones that elicit mating and oviposition behaviors from responding insects. An interesting example, which is considered a novel approach to control aphids in the field and greenhouse, is due to the release of volatiles by *Staphylococcus sciuri* allowing to locate the prey in the tritrophic aphid–bacteria hoverfly interaction (Leroy et al. 2010).

Considerable progress has been made in understanding the functions of mVOCs in plants; however, implanting this knowledge under field conditions remains in its infancy (summarized in Kanchiswamy et al. 2015). Field trials are needed to prove the value of mVOCs, but there is a realistic chance to develop new sustainable, cheaper, efficient, effective, and eco-friendly alternatives to pesticides and fertilizers (Kanchiswamy et al. 2015).

10.4.6   *mVOCs for chemotyping and diagnostic tools*

In addition to considering the biological and ecological roles of mVOCs being important for the homeostasis (fitness and health) of humans and plants, these compounds can be used as noninvasive markers. Individual mVOCs as well as clusters of volatiles are useful for phenotyping fungi (Müller et al. 2013) and bacteria (Peñuelas et al. 2014). Even phylogenetically closely related but phenotypically different species of *Streptomyces* isolates or *Clostridum difficile* ribotypes could be differentiated (Cordovez et al. 2015; Kuppusami et al. 2015). Noninvasive markers are particularly demanded and already used as medical diagnostic tools, for example, for the recognition of methyl nicotinate as an indicator for tuberculosis caused by *Mycobacterium tuberculosis* (Syhre and Chambers 2008; Mgode et al. 2012). *Pseudomonas aeruginosa* infections are associated with declining lung function in cystic fibrosis and high mortality rates. 2-aminoacetophenone is a small molecule and an intermediate of the quinazoline biosynthesis and was shown to be significantly higher in *P. aeruginosa* colonized subjects than control patients and is therefore a promising breath biomarker (Scott-Thomas et al. 2010). A noninvasive VOC-based detection was proposed as an alternative technique suitable for surveillance and as diagnostic tool applicable for urological infections by *Proteus* spp. (Aarthi et al.

2014). These examples stand for a number of other cases described in the literature; this chapter was not initiated to present a comprehensive review of this topic.

10.5   Conclusion

Microbial VOCs have been overlooked in the past, but due to the ubiquitous appearance of microorganisms and their broad metabolic capabilities they have to be considered to understand organismal interactions in ecosystems. Besides these semiochemical functions and biological relevances, mVOCs offer chances to provide sustainable and eco-friendly alternatives to pesticides and fertilizers in the agriculture and the environment, and noninvasive diagnostic tools for animal and human health and fitness. mVOCs are already used in biotechnological processes to control, for example, foodstuff, buildings, and other hardware. It is expected that techniques with better sensitivity and wider applications will be used routinely to make our lives safer. Furthermore, we expect to find novel (lead) structures of natural products that are good sources for new applications.

Acknowledgments

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