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MINI-REVIEW

Bacterial volatiles and their action potential

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Abstract During the past few years, an increasing awareness concerning the emission of an unexpected high number of bacterial volatiles has been registered. Humans sense, intensively and continuously, microbial volatiles that are released during food transformation and fermentation, e.g., the aroma of wine and cheese. Recent investigations have clearly demonstrated that bacteria also employ their volatiles during interactions with other organisms in order to influence populations and communities. This review summarizes the presently known bioactive compounds and lists the wide panoply of effects possessed by organisms such as fungi, plants, animals, and bacteria. Because bacteria often emit highly complex volatile mixtures, the determination of biologically relevant volatiles remains in its infancy. Part of the future goal is to unravel the structure of these volatiles and their biosynthesis. Nevertheless, bacterial volatiles represent a source for new natural compounds that are interesting for man, since they can be used, for example, to improve human health or to increase the productivity of agricultural products.

Keywords Bacterial volatiles · Volatile antibiotics · Fungistasis · Quorum sensing · *Serratia odorifera* · *Stenotrophomonas*

The wealth of bacterial volatiles

Microbiologist have recognized for a long time that bacteria emit characteristic scents, e.g., the typical odor of indole from *Escherichia coli* or butyric acid and acetone from *Clostridium acetobutylicum*. Many more odors are known, and humans have exploited microbial volatiles as aroma components of cheese, sauerkraut, yoghurt, wine, etc. Moreover, the repugnant smell of rotting organic matter often results from the release of bacterial volatiles. Although the human nose can distinguish many volatiles, there is a given limitation in the detection and proper description of the relevant smell. Recent investigations using gas chromatography (GC) and mass spectrometry (MS) illustrate the splendid capacity of bacteria to produce a wealth of volatile compounds (Kai et al. 2006; Schulz and Dickschat 2007; Bunge et al. 2008). Whereas the odors and aromas released from bacteria during food transformations and fermentations or from building material have been intensively investigated, only little is presently known about the general ability and efficiency of volatile emissions of bacteria (Dainty et al. 1985, 1989; Korpi et al. 1998).

One of the earliest papers that described the production of volatiles by bacteria (dysentery group) demonstrated the release of formic and butyric acid (Zoller and Clark 1921). Stotzky and Schenck (1976) summarized the volatile organic compounds released from microorganisms and showed that fungi produce a wider variety of volatiles than bacteria, although this might have been attributable to the larger number of studies performed with fungi at that time. Stotzky and Schenck (1976) described bacteria such as *Pseudomonas* spp. and *Streptomyces* spp. as being ethylene and hydrogen cyanide producers, *Clostridium* spp. as emitters of dimethyl disulfide, various short chain acids, 2,3-butanediol, isopentanol, and acetoin, and *Agrobacterium radiobacter*, *A. rhizogenes*, *Bacillus cereus*, *Enterobacter aerogenes*, *E. coli*, *Micrococcus luteus*, *Nocardia corallina*, *Proteus vulgaris*, *Sarcina lutea*, and *Serratia marcescens* as releasers of unidentified volatiles. Furthermore, mixed bacterial cultures and microbes in soil under aerobic and

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anaerobic conditions have the potential to produce organic volatiles (volatile organic compound, VOC). In recent years, the technology has developed further, and the identification and quantification of volatile compounds has been mostly successful. More than 120 different compounds are emitted from actinomycetes (Schöller et al. 2002), comprising alkanes, alkenes, alcohols, esters, ketones, and isoprenoids. *Myxococcus xanthus* has also turned out to be a rich source of volatile compounds, 42 compounds having been collected in a closed-loop stripping apparatus (Dickschat et al. 2004). Two new natural products, (S)-9-methyl-decan-3-ol and 9-methyldecan-3-one, have been identified. With an enormous effort and critical evaluation of the published data, Schulz and Dickschat (2007) have summarized all known bacterial compounds so far detected by using the state-of-the-art methodologies, with 346 different compounds released from various bacteria being described. The classification of bacterial volatiles has revealed 75 fatty acid derivatives, 50 aromatic compounds, 74 nitrogen-containing compounds, 30 sulfur compounds, 96 terpenoids, and 18 halogenated, selenium, tellurium, or other metalloid compounds. Several groups of compounds seem to be especially widespread among bacteria, for example, pyrazines, volatile sulfur components, geosmin, and 2-methylisoborneol. Our investigations with bacterial isolates of *Stenotrophomonas*, *Serratia*, *Pseudomonas*, *Bacillus*, *Burkholderia*, *Erwinia*, *Agrobacterium*, *Staphylococcus*, and *Xanthomonas* species have also indicated the emission of complex bacterial blends of odors, comprising in some cases up to 60 compounds per strain (Kai et al. 2006; Kai/Piechulla, unpublished results). Interestingly, the majority of volatiles cannot unequivocally be identified using the NIST-GC-MS library or others, suggesting that for many of them, their structures remain to be elucidated. Most emitted compounds are species-specific, but overlapping volatile patterns have been found for *Serratia* spp. and *Pseudomonas* spp., indicating that at least in some cases, these volatile profiles or a typical individual compound (characterized by the retention index until the structure is elucidated) can be used as a biomarker (Kai et al. 2006; Henis et al. 1966). Bacterial volatiles are compiled in a publically available "Super Scent" database (Dunkel et al. 2009).

Volatile detection and identification and methodical constraints

State-of-the-art detection and determination of volatiles are generally performed with the headspace technique and gas chromatography and mass spectrometry (GC-MS). Some limitations of the method should be considered here. GC-MS is a sensitive method that allows the determination of the number of different compounds, their relative quantities in the volatile mixture, and also compound identification. However, compounds can only be considered as identified if they demonstrate identical Kovats indices on two columns of different polarity and display mass spectra coincident with the library. We and others have often observed that the available MS libraries [e.g., National Institute of Science and Technology (NIST), Whileys] do not contain compounds that were emitted by bacteria. Therefore, new compounds have to be structurally elucidated by chemists using, e.g., nuclear magnetic resonance analysis. It also should be kept in mind that headspace collections with absorbance material such as tenax, super Q, or charcoal, etc. favor the binding of compounds with specific chemical features and involve extraction with organic solvents (e.g., dichlormethane, pentane, hexane). The huge bulk of solvent leads to insufficient resolution of highly volatile, early eluting compounds. The use of the solid phase microextraction method is a well-accepted alternative, which can also be used to resolve small inorganic or organic volatiles such as CO, CO_2, NH_3, HCN, and ethylene.

The appearance of a characteristic volatile profile or compound is attributable to the specific metabolism or metabolic pathway(s) that are active in the bacteria. Depending on the growth media and growth conditions, the bouquet of released compounds can vary, e.g., the growth of *Stenotrophomonas rhizophila* P69 on nutrient broth with and without glucose results in qualitatively and quantitatively different GC profiles, e.g., dimethyl pyrazine and beta-phenylethanol are emitted under both growth conditions, whereas trimethyl pyrazine, tetramethyl pyrazine, and beta-phenylethyl acetate appear when glucose is not present in the medium (Fig. 1). Another example is the addition of L-glucose to the media, which leads to significant less volatile emission compared with growth on D-glucose (Fiddaman and Rossall 1993, 1994). Addition of trehalose to the media resulted in the emission of volatiles from *Pseudomonas monteilii* which stimulate mycelium growth of *Pisollithus albus* (Duponnois and Kisa 2006). Furthermore, bacterial metabolism varies in the lag, log, and stationary phases of *S. rhizophila* P69 batch cultures resulting in different emission patterns of, e.g., 2-piperidone, beta-phenylethanol, dimethyl pyrazine, trimethyl pyrazine, compound retention index 818 during the course of 144 h (Fig. 2). To assess temporal variations of volatile emission, variations that can contain important information for the detection and differentiation of microorganism. Online monitoring of characteristic emission patterns were recently performed with distinct volatiles released from *Salmonella enterica* and *E. coli* (Bunge et al. 2008).

Although temporal patterns and retention index assignments are helpful, a future task will be to elucidate the chemical structures of unidentified volatile compounds, since structural information will allow insights into the

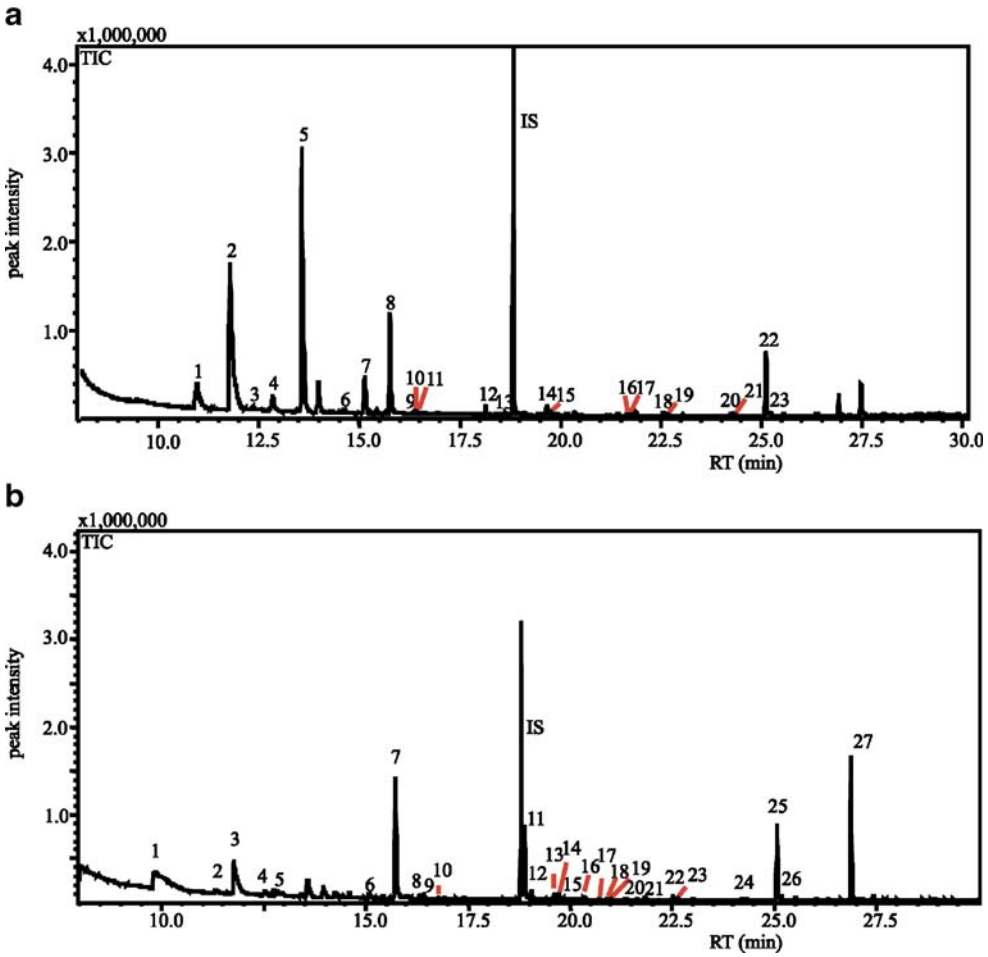

Fig. 1 VOC emission profiles of *S. rhizophila* P69. Headspace volatiles of bacteria grown on nutrient broth with 2% glucose (**a**) and without (**b**) were collected on super Q for 14 h beginning 58 h after inoculation. Volatiles were eluted from the absorbance material and analyzed by GC-MS. Individual compounds were identified: A2 = B3 (compound 2 of **a** = compound 3 of **b**): dimethyl pyrazine (93%), A5: trimethyl pyrazine (94%), A7: tetramethyl pyrazine (92%), A8 = B7: beta-phenylethanol (98%), A12: beta-phenylethyl acetate (90%). (%): identity to compound of the reference data library (NIST140)

underlying metabolic pathway(s) that are active during bacterial growth. A goal for future research is to unravel the identity of the respective synthesizing enzymes and their specific regulation(s) in metabolically active bacteria. To date, only the biosynthesis of geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) responsible for the "earthy" and "musty" odor of many *Streptomyces* species is known (Bentley and Meganathan 1981; Cane and Watt 2003; Gust et al. 2003). Geosmin has been widely investigated because of its major contribution to the "off flavor" of contaminated drinking water, wines, and other foodstuffs, but it also contributes to the palatability of red beet and whisky aroma. *Streptomyces* species utilize both the mevalonate and non-mevalonate pathway, of which most of the enzymes had been discovered, to synthesize the terpenoid intermediate isopentenyl pyrophosphate (Takagi et al. 2000; Kuzuyama et al. 2000). Furthermore, sesquiterpene synthase genes have been isolated, which catalyze the cyclization reaction of farnesyl pyrophosphate (Gust et al. 2003). The increasing numbers of sequenced bacterial genomes, including bacteria that produce large amounts or a large diversity of volatiles, will support the elucidation of the biosynthetic pathways. Interest is presently focused on bacteria that emit volatiles with biological relevance, since the isolation of new bioactive natural compounds remains interesting for man because such compounds can be used, for example, to improve human health or to increase the productivity of agricultural products.

Functions of bacterial volatiles

The diversity of bacterial volatiles has a comparable complexity to that known for plants and fungi, and bacterial volatiles have turned out to be a rich source for new natural compounds. Presently, the biological functions of many bacterial volatiles are not understood in detail. Bacterial volatiles can be assumed to be similar to other volatiles and probably serve as (1) infochemicals for inter- and intra-organismic communication, (2) cell-to-cell communication signals, (3) a possible carbon release valve, or (4) growth-promoting or inhibiting agents. They are important for the sustainment of bacterial populations in ecological niches and for the cooperative development of a community of

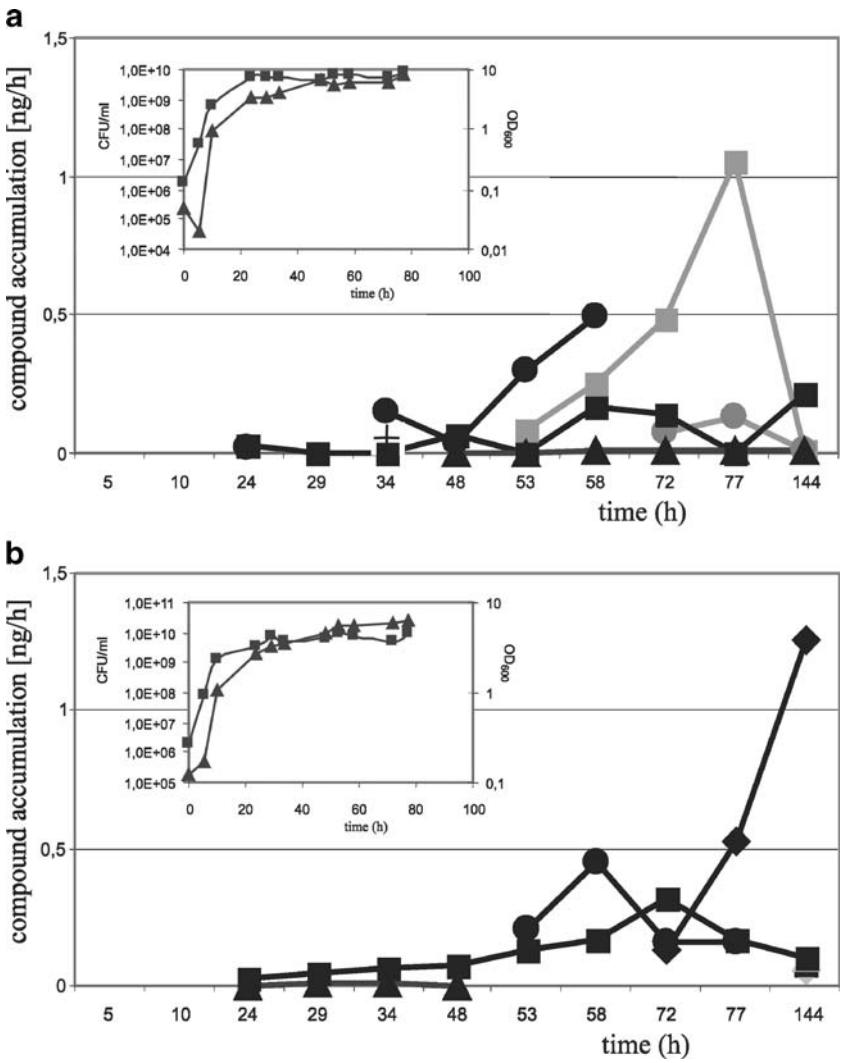

Fig. 2 Emission of volatiles from *S. rhizophila* P69 in various growth intervals of batch culture. *S. rhizophila* was grown on nutrient broth with 2% glucose (**a**) and without (**b**), and volatiles were trapped on super Q in 5 or 14-h intervals. The time course of the unknown compounds with the retention indices (*RI*) 818 (*black rhombus*), 1124 (*black square*), 922 (*black dot*), 1264 (*black triangle*), 1008 (*gray square*), 1090 (*gray dot*), and 748 (**a**) (only 34 h, *cross*), 1189 (**b**) (only 144 h, *black bar*), 1136 (**b**) (only at 144 h, *gray rhombus*) are depicted. *Insert*: growth curves of batch cultures: optical density (*OD*, *triangles*) and living cell number (*squares*)

different organisms, and they can support the selective advantage of some community member(s) and survival during evolution.

Volatiles are important chemicals because they can act over a wide range of scales. Their action profile ranges from the ability to diffuse through aqueous solutions to being able to permeate through the atmosphere. Therefore, volatiles not only play a role above ground but also function below ground.

Bacterial volatiles effecting fungi

An early report on bacterial volatiles affecting fungal growth and development was published by McCain (1966) showing that *Streptomyces griseus* volatiles reduced the sporulation of *Gleosporium aridum* and induced the formation of sclerotia in *Sclerotium cepivorum* and *Rhizoctonia solani*. The effects of bacterial volatiles on fungi known to date range from the stimulation of fruit body formation, to spore germination, to mycelium growth inhibition and promotion, and to the stimulations or reduction of sporulation (Table 1). A large survey by Wheatley (2002) has revealed that 250 bacterial soil isolates cause fungal micelle growth inhibition or promotion, with the two most active bacteria being *Citrobacter freundii* and *Pseudomonas fluorescens*. In a recent investigation of 1,018 randomly selected bacteria, 32% have turned out to produce fungistatic volatiles (Zou et al. 2007). The producers of bioactive volatiles are species of *Alcaligenes*, *Bacillus*, *Ensifer*, *Lysobacter*, *Planomicrobium*, *Sporosarcina*, and *Stenotrophomonas*. Other investigations have named *Burkholderia*, *Pseudomonas*, *Serratia*, *Xanthomonas*, *Pectobacterium*, and *Agrobacterium* species as potent antifungal bacteria (Table 1). The volatiles from any one bacterial strain do not cause the same effects or the same degree of inhibition to all the fungi; rather, the responses depend on the specific fungi–bacteria combination (Tables 1 and 2). The following reasons might be responsible for these differences: (1) different fungi may respond to different component(s) of

Table 1 Effects of bacterial volatiles on fungi

Bacteria	Fungi	Effects	Bioactive compounds	References
Streptomyces griseus	*Gloeosporium aridum*	Reduction of sporulation		McCain 1966
S. griseus	*Sclerotium cepivorum*, *Rhizoctonia solani*	Early formation of sclerotia		McCain 1966
Streptomyces spp.	Various fungi	Antifungal activity		Whaley and Boyle 1967; Hora and Baker 1972; Fries 1973
Pseudomonas	*Agaricus bisporus*	Promotion of fruiting body formation		Lockard and Kneebone 1962; Hayes et al. 1969
Agrobacterium radiobacter, *Bacillus cereus*, *Enterobacter aerogenes*, *Escherichia coli*, *Micrococcus luteus*, *Nocardia corallina*, *Proteus vulgaris*, *Sarcina lutea*, *Serratia marcescens*	*Fusarium oxysporum, Gelasinospora cerealis, Penicillium viridicatum, Trichoderma viridae, Zygorhynchus vuilleminii*	Inhibition of mycelium growth and spore formation, alteration of colony and hyphal morphology		Moore-Landecker and Stotzky 1972, 1973, 1974
	Verticillium dahliae	Mycelium growth inhibition		Alstrom 2001
Unidentified soil bacteria	*Trichoderma viride*, *Phanaerochaete magnoliae*, *Phytophthora cryptogea, Gaeumannomyces graminis*	Growth rate inhibition, growth promotion		Mackie and Wheatley 1998; Wheatley 2002
Unidentified soil bacteria	*Paecilomyces lilacinus, Pochania chlamydospora, Chlonostachys rosea*	Inhibition of spore germination	Trimethylamine, benzaldehyde, *N*,*N*-dimethyloctylamine (no effect: methyl pyrazine, 2,5-dimethyl-pyrazine, nonadecane, 3-methyl-2-pentanone, dimethyl disulfide)	Chuankun et al. 2004
Pseudomonas fluorescens, *P. corrugate, P. chlororaphis, P. aurantiaca*		Inhibition of mycelium growth and spore germination	Cyclohexanol, decanol, 2-ethyl-1-hexanol, nonanal, benzothiazole, dimethyl trisulfide (no effects: dodecane, undecane, nonane, decane, nonadecane, 1-heptadecanol, 4-octylbenzoic acid, phenylenediamine, 2-methyl pyrazine, benzaldehyde, hexadecane, pyrazine, tetradecane, pentadecane, 1-undecene, 2-undecanone, 2-tridecanone)	Fernando et al. 2005
Agrobacterium tumefaciens 7, *Bacillus subtilis B2g*, *Burkholderia cepacia 1S18*, *Pectobacterium carotovorum 436R*, *P. fluorescens L13-6-12*, *Pseudomonas trivialis 3Re2-7*, *Pseudomonas phaseolicola 796*, *Pseudomonas syringae 1142*, *Serratia plymuthica HRO-C48,*	*Fusarium culmorum PR19-12-11*, *Microdochium bolleyi PR5-11-6*, *Neurospora crassa wt 1202A*, *Paecilomyces carneus PR16-10-1, Penicillium waksmanii PR17-11-8, Phoma betae*, *Phoma eupyrena PC17-12-10*, *Rhizoctonia solani AG3*, *Sclerotinia sclerotiorum,*	Inhibition of mycelium growth		Kai et al. 2006; Vespermann et al. 2007; Kai et al. 2008, Table 2, this review

Table 1 (continued)

Bacteria	Fungi	Effects	Bioactive compounds	References
Serratia odorifera 4Rx13, *Stenotrophomonas rhizophila P69*, *Stenotrophomonas maltophilia R3089*, *Xanthomonas campestris 2217*, *X. campestris* pv *vesicatoria 85-10*	*Trichoderma strictipile PC26-12-6, V. dahliae V25*			
Pseudomonas monteilii	*Pisolithus albus*	Mycelium growth stimulation		Duponnois and Kisa 2006
Several *Bacillus* species, *S. maltophilia*, *Alcaligenes faecalis*, *Arthrobacter nitroguajacolius*, *Lysobacter gummosus*, *Sporosarcina ginsengisoli*	*Paecilomyces lilacinus, Pochonia chlamydosporia*	Inhibition of Mycelium growth	Acetamide, methanamine, 1-butanamine, benzaldehyde, phenylacetaldehyde, 1-decene, benzothiazole (no effect: methyl pyrazine, dimethyl disulfide, 2,5-dimethyl-pyrazine, dodecane, nonadecane)	Zou et al. 2007
	F. culmorum PR19-12-11, R. solani AG3, S. sclerotiorum, V. dahliaeV25	Inhibition of mycelium growth	Dimethyl disulfide, 1-undecene	Fig. 3, this review

the volatile mixture, or (2) the sites of action may be different, or (3) the fungi might possess different abilities to detoxify the volatile metabolite(s).

Volatiles of rhizobacteria and of phytopathogenic bacteria exert fungistatic effects, for example on the growth of phytopathogenic fungi such as *Verticillium dahliae*, *Sclerotinia sclerotiorum*, and *R. solani*. This adds an additional facet to the antagonistic potential of plant-growth-promoting bacteria and is of agricultural interest (Table 2). Indeed, some bacteria prevent symptom development in field rape plants (Alstrom 2001). Other studies have indicated that the promotion of mycorrhizal formation, e.g., by *Glomus mosseae*, is due to the presence of rhizobacteria or *Streptomyces* species (Azcon-Aguiler and Barea 1985; Azcon-Aguiler et al. 1986; Fitter and Garbaye 1994). Although *Streptomyces* spp. are important positive players in arbuscular mycorrhiza formation, it has to be proven that volatiles are key components (Schrey et al. 2005).

Microscopic observations of the fungal mycelium during *Bacillus subtilis* volatile fumigation have revealed effects on hyphae and conidia (Chaurasia et al. 2005). The longitudinal and traverse septae completely disappeared in *Alternaria alternata*, and conidia became thick-walled and spherical or irregular in shape. Upon volatile exposition, *Cladosporium oxysporum* conidiophores became vegetative and stunted. Lysis of fungal hyphae, vacuolization, and granulation in mycelium structures have been observed in *Fusarium oxysporum* and *Phytium afertile*. Further studies need to be performed in order to understand the underlying mechanisms that lead to these morphological aberrations after exposure to bacterial volatiles.

In addition to the observed morphologic changes, alterations at the molecular level are expected in the fungi.

Table 2 Mycelium growth inhibition of phytopathogenic fungi by volatiles of phytopathogenic bacteria

	Fusarium culmorum PR19-12-11	*Fusarium solani*	*Rhizoctonia solani AG3*	*Sclerotinia sclerotiorum*[a]	*Verticillium dahliae V25*
Agrobacterium tumefaciens 7	55.6±5.1	14.4±7	35.7±8.2	60.4±2.7	43.4±15.1
Pectobacterium carotovorum 436R	40.6±20.8	10.5±3.8	16.7±11.8	64.4±2.8	27.3±7.3
Pseudomonas phaseolicola 796	42.5±13.4	6.9±4.9	9.7±4.6	55.3±3.4	32.9±14
Pseudomonas syringae 1142	48±6.5	11.5±7.6	37.3±13.2	61.5±3.4	52.4±9.3
Xanthomonas campestris pv *vesicatoria* 85-10	57.1±7.5	12.9±10.6	99.5±1	89.2±18	70±16.1

Inhibition measured at day 4 of co-cultivation

[a] Inhibition at day 3 of co-cultivation

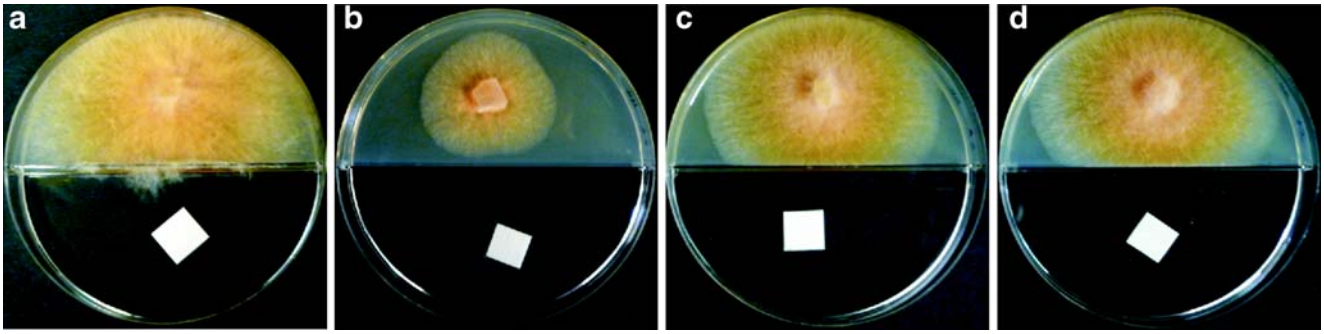

Fig. 3 Mycelium growth of *F. culmorum* exposed to dimethyl disulfide (*DMDS*) or 1-undecene. Bipartite Petri dishes were inoculated with *F. culmorum* on one side. Filter paper with pentane (**a**), 5.2 μg DMDS in pentane (**b**), 0.52 μg DMDS in pentane (**c**), and 3.74 μg 1-undecene in pentane (**d**) was applied on the other side (incubation time, 4 days)

In *Phanaerochaete magnoliae*, enzyme activities change on bacterial volatile exposure, e.g., laccase activity ceases completely, whereas tyrosinase activity increases, both probably as a result of the up- and down-regulation of gene expression rather than of direct enzyme activity changes (Mackie and Wheatley 1998). In several cases, we have observed the dark coloration of the growth media attributable to melanin production of the fumigated fungi (not shown, Kai /Piechulla, unpublished results).

The fungistatic effect attributable to bacterial volatiles has been substantiated during the past 10 years. Volatile mixtures emitted from bacteria have often been applied, but the bioactive compound(s) remain to be determined. For example, the mycelium growth of *Fusarium culmorum* shows a dose-dependent inhibition by dimethyl disulfide, and small effects have been obtained with 1-undecene (Fig. 3). Additional bacterial compounds that act as bioactive agents on fungi include various amines, benzaldehyde, cyclohexanol, decanal, 2-ethyl-1-hexanol, nonanal, benzothiazole, and dimethyl trisulfide; compounds such as methyl pyrazine, 2,5-dimethyl-pyrazine, phenylenediamine, 4-octylbenzoic acid, several middle and long chain alkans, alkenes, aldehydes, and alcohols have surprisingly low inhibitory effects on various fungi (Table 1). Dimethyl disulfide and benzaldehyde possess inhibitory but sometimes non-inhibitory effects; this might be due to different experimental setups, e.g., the way that the compounds are applied and their concentrations. Since the GC profiles of bacterial volatiles allude to many more compounds, future work has to concentrate on the structural elucidation and on the determination of biological activity.

Bacterial volatiles effecting plants

The first (and so far only) report on the growth promotion of *Arabidopsis thaliana* attributable to two typical VOCs emitted by bacilli, 2,3-butandiol and acetoin, was published by Ryu et al. (2003), whereas the biological functions of the other 38 compounds, comprising short chain alcohols, aldehydes, acids, esters, ketones, hydrocarbons, S-containing compounds, and CO_2 that are emitted from *B. subtilis* and *B. amyloliquefaciens* remain to be elucidated (Farag et al. 2006). In contrast, strong inhibitory effects on *A. thaliana* and the moss *Physcomitrella patens* have been demonstrated by other bacteria, such as *Pseudomonas* spp., *Serratia* spp., and *Stenotrophomonas* spp., although the bioactive compounds are presently unknown (Table 3). To

Table 3 Effects of bacterial volatiles on plants

Bacteria	Plants	Effects	Bioactive compounds	References
B. subtilis GB03, *Bacillus amyloliquefaciens* IN937a	*Arabidopsis thaliana*	Growth promotion (leaf surface area) induced systemic resistance (ISR) regulation of auxin homeostasis	2,3-Butandiol, acetoin	Ryu et al. 2003; Farag et al. 2006; Zhang et al. 2007
B. subtilis B2g, *B. cepacia 1S18*, *P. fluorescens L13-6-12*, *P. trivialis 3Re2-7*, *S. odorifera 4Rx13*, *S. plymuthica HRO-C48*, *S. maltophilia R3089*, *S. rhizophila P69*	*Arabidopsis thaliana*	Growth inhibition (leaf fresh weight)		Vespermann et al. 2007; Tarkka and Piechulla 2007; Kai et al. 2008
S. odorifera 4Rx13	*Physcomitrella patens*	Growth inhibition		Kai/Piechulla, unpublished

Table 4 Effects of bacterial volatiles on animals

Bacteria	Animals	Effects	Bioactive compounds	References
B. subtilis B2g, P. fluorescens L13-6-12, S. odorifera 4Rx13, X. campestris 85-10	*Acanthamoeba castellanii, Paramecium caudatum*	Growth inhibition, death		Fig. 4, this review
B. subtilis B2g, *P. fluorescens L13-6-12*, *S. odorifera 4Rx13*, *X. campestris 85-10*	*Caenorhabditis elegans*	Growth and development not altered, chemo-attraction		This review
Bacillus simplex, *B. subtilis*, *Bacillus weihenstephanensis*, *Microbacterium oxydans*, *S. maltophilia*, *Streptomyces lateritius*, *Serratia marcescens*	*Panagrellus redivivus, Bursaphelenchus xylophilus*	Reduction of movement or death	Phenol, 2-octanol, terpineol, benzenacetaldehde, decanal, 2-nonanone, 2-undecanone, cyclohexene, dimetyl disulfide, benzaldehyde, phenylethanone, nonane (no or little effect: 1-hexadecanol, benzene ethanol, propanone, hexadecane, tetradecane, dodecane, propionic acid, trimethyl pyrazine)	Gu et al. 2007
Alpha and gamma proteobacteria	*Aedes aegypti*	Stimulation of oviposition, directing egg laying to favorable habitat	Nonanoic acid, octadecanoic acid, carboxylic acid methyl esters	Ponnusamy et al. 2008
Porphyromonas gingivalis, *Prevotella loescheii*, *Fusobacterium nucleatum*	Lymphocyte cells	Inhibition of proliferation and cytokine production	Butyric acid, propionic acid, valeric acid, isovaleric acid	Kurita-Ochiai et al. 1995

our knowledge, bacterial volatiles have so far not been tested on agriculturally relevant plant species. The scientific advantage of studying *A. thaliana*, however, is that whole genome microarray analysis can be performed. Out of 26,000 protein-encoding transcripts, 600 differentially expressed genes related to cell wall modifications, primary and secondary metabolism, stress responses, and hormone regulation have been identified in *A. thaliana* exposed to *B. subtilis* volatiles (Zhang et al. 2007). These data implicate the regulation of auxin homeostasis and cell expansion and provide a new paradigm as to the way that bacilli promote plant growth.

Bacterial volatiles effecting animals

Since rhizobacterial volatiles influence the growth of fungi and plants, the question has arisen as to whether soil-living animals are also affected (Table 4). The protozoan *Acanthamoeba castellanii* und *Paramecium caudatum* are dominant soil organisms (1.6×10^5 individuals per gram soil) primarily feeding on bacteria (Bonkowski 2004). Co-cultivations in which organisms have direct contact have shown that *E. coli* and *Stenotrophomonas maltophilia* serve as a good food source for *A. castellanii*, whereas *Staphylococcus epidermidis*, *S. marcescens*, and *Pseudomonas aeruginosa* are not feeder bacteria (Wang and Ahearn 1997). Although not investigated by the authors, it cannot be excluded that the bacterial volatiles have a direct impact on the feeding behavior of *A. castellanii*. We have shown that in bipartite Petri dishes in which the different organisms never come into direct contact, the volatiles of *B. subtilis*, *P. fluorescens*, *S. odorifera*, and *Xanthomonas campestris* pv *vesicatoria* negatively influence the growth of *A. castellanii* and *P. caudatum*. Although the inhibition of *A. castellani* after 4 days of co-cultivation ranges between 60% and 95%, depending on the density of the protozoa, the bacterial volatiles are lethal for *P. caudatum* at all population densities (Fig. 4).

Other prominent soil creatures are nematodes such as the non-parasitic bacterial feeding *Caenorhabditis elegans*. *C. elegans* lives in the soil at the air–water interface; it therefore can encounter both water-soluble and volatile chemicals that might influence its behavior. *Pseudomonas* and other microbes produce small chain alcohols, ketones, diacetyl, and esters as metabolic byproducts (Zechman and Labows 1985; Dainty et al. 1985) that can act as natural chemoattractants for *C. elegans* (Bargmann et al. 1993). Indeed, *C. elegans* provides two types of chemosensory neurons that can detect volatile attractants (Sengupta et al.

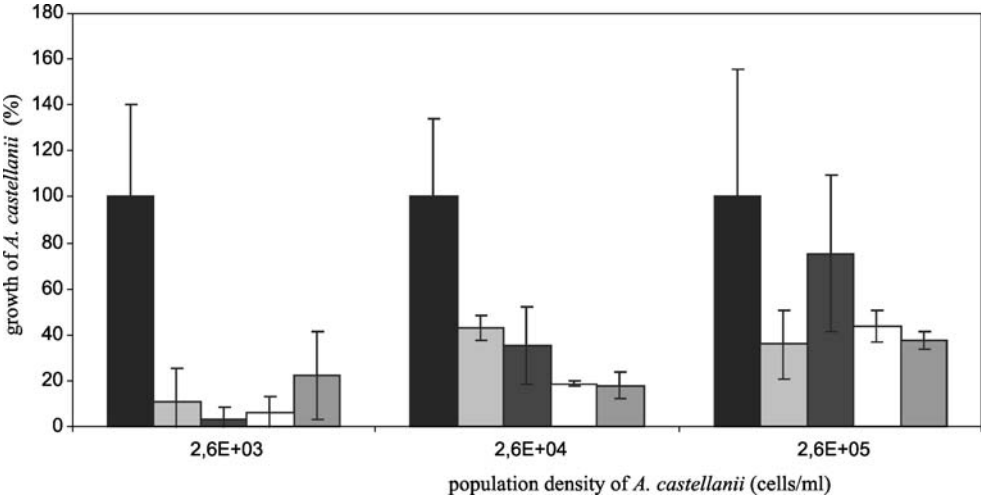

Fig. 4 Co-cultivation of bacteria with *A. castellanii*. *S. odorifera* 4R×13 (*light gray bar*; CFU/ml, 1×10^9), *B. subtilis* B2g (*dark grey bar*; CFU/ml, 8.6×10^7), *P. fluorescens* L13-6-12 (*white bar*; CFU/ml, 6.1×10^8), and *Xanthomonas vesicatoria* pv. *vesicatoria* 85-10 (*middle grey bar*; CFU/ml, 1.9×10^8) were co-cultivated in bipartite Petri dishes with *A. castellanii* at three population densities (2.6×10^3, 2.6×10^4, 2.6×10^5 cells/ml). *Black bar*: control with water

1996). Each pair of neurons detects several different odorants: One set of olfactory neurons senses diacetyl, pyrazine, and thiazols, whereas the other set is activated by benzaldehyde, butanone, isoamyl alcohol, and thiazoles. Our co-cultivation experiments of *C. elegans* with *B. subtilis*, *P. fluorescens*, *S. odorifera*, and *X. campestris* pv *vesicatoria* in bipartite Petri dishes have substantiated previous observations. The volatiles emitted by these bacteria are so attractive for this worm that they manage to crawl over a 3-cm-high barrier, most likely to establish a new feeding source (Molina/Piechulla, unpublished results). In contrast, the free-living nematode *Panagrellus redivivus* and the pinewood nematode *Bursaphelenchus xylophilus* gradually reduce their motility (or die) during exposure to bacterial volatiles such as phenol, octanol, benzaldehyde, benzene acetaldehyde, decanal, 2-nonanone, 2-decanone, cyclohexene, and dimethyl disulfide (Gu et al. 2007). In summary, nematode susceptibility to one or another compound varies, indicating a species-specific reaction to individual compounds; therefore, certain bacterial isolates have the potential to be effective biocontrol agents against nematodes.

Recently, gravid mosquito (*Aedes aegypti*) females have been shown to use bacterial (alpha and gamma proteobacteria) volatiles, e.g., carboxylic acids and methyl esters, as potent oviposition stimulants to direct egg laying in favorable habitats (Ponnusamy et al. 2008). Another example of bacterial odor-dependent oviposition has been reported for gravid *Anopheles gambiae* (Huang et al. 2006). Such knowledge of bioactive bacterial volatiles/volatiles mixtures and their respective stimulative concentrations can

Table 5 Effects of bacterial volatiles on bacteria

Bacteria	Bacteria	Effects	Bioactive compounds	References
Gram-negative bacteria		Cell-to-cell communication, quorum sensing	*N*-acylhomoserine lactone (*N*-AHLs)	Summarized in Ryan and Dow 2008
X. campestris pv *campestris*		Diffusible signal factor (DSF)	*cis*-11-methyl-2-dodecenoic acid	Ryan and Dow 2008
E. coli	*E. coli*, *P. fluorescens*, *Pseudomonas aeruginosa*	Regulation of expression of multi-drug exporter genes, inhibition of biofilm formation	Indole	Ryan and Dow 2008
X. campestris pv *campestris*, *Streptomyces* spp.		Diffusible factor (DF) regulates the production of pigments (xanthomonadins) and extracellular polysaccarides	Butyrolactone	Ryan and Dow 2008
S. odorifera, *Serratia plymuthica*	*B. cepacia*	Reduction of red colony coloration		Fig. 5, this review
Veillonella spp., *Bacteroides fragilis*	*Salmonella typhimurium*, *Salmonella enteritidis*, *E. coli, P. aeroginosa*, *Clostridium perfringenes*, *Clostridium difficile*	Reduction of heat resistant spores, prevention of spore formation	Short chain volatile fatty acids: acetate, isobutyrate, isovalerate, succinate	Hinton and Hume 1995; Wrigley 2004

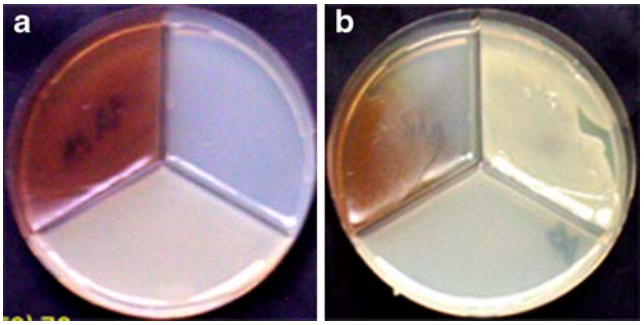

Fig. 5 Co-cultivation of *B. cepacia* and *S. odorifera*. *B. cepacia* 1S18 (*left sector*) cultivated with (**a**) non-inoculated nutrient broth II (*right sector*) or (**b**) with *S. plymuthica* HRO-C48 (*right sector*). Discoloration of *B. cepacia* colonies is visible after 72 h of co-cultivation

be used to study insect epidemiology and might have an impact on mosquito-abatement programs.

The aforementioned examples indicate that bacterial volatiles affect invertebrate animal growth and behavior. The effects of bacterial volatiles on vertebrate animals or respective cell cultures has been reported by Kurita-Ochiai et al. (1995) who have shown that fatty acids (e.g., butyric acid, propionic acid, valeric acid, and isovaleric acid) produced by periodontophatic bacteria, such as *Porphyromonas gingivalis*, *Prevotella loescheii*, and *Fusobacterium nucleatum*, impair lymphocyte proliferation and cytokine production.

Bacterial volatiles effecting bacteria

Bacteria use a cell-to-cell communication system to monitor their population density (quorum sensing; reviewed by Ryan and Dow 2008). This allows a colony or a group of organisms to behave in a coordinated fashion in order to regulate processes contributing to virulence, antibiotic production, biofilm formation, and other developmental programs (Table 5). Additionally, bacteria can sense signal molecules that they do not synthesize, thereby eavesdropping on signaling by other organisms in their immediate environment. The autoinducer signal molecules produced by bacteria are structurally diverse; however, many are compounds with small molecular masses that might have the potential to act as a volatile. Gram-negative bacteria use *N*-acylhomoserine lactones (*N*-AHLs), fatty acid derivatives (3-hydroxypalmitic acid methyl ester, *cis*-unsaturated fatty acids), and cyclic dipeptides, whereas Gram-positive bacteria use amino acids and modified peptides or gamma-butyrolactones (*Streptomyces* spp.) for communication. Other known signals are methyl-2,3,3,4-tetrahydroxyhydrofuran (S-THMF), indole, quinolones, and S-3-hydroxytridecan-4-one. The production of indole is widespread among soil bacteria and is generated through the degradation of tryptophan by tryptophanase. Indole has been shown to regulate the expression of several multi-drug reporter genes in *E. coli* via two two-component signal transduction pathways; it also inhibits biofilm formation in *E. coli*, *P. fluorescens*, and *P. aeruginosa*. A butyrolactone (diffusible factor) has been shown to regulate the production of the yellow pigments (xanthomonadins) in *X. campestris*. During our investigations, we have observed that the typical red colony coloration of *B. cepacia* diminishes on exposure to volatiles from *S. odorifera* and *Serratia plymuthica* (Fig. 5), showing that volatiles of bacteria can influence the metabolism of other bacteria. Another example is the release of volatile short chain fatty acids from *Veilonella* species and *Bacteroides fragilis*, which thus control the growth of the enteropathogens *Salmonella typhimurium*, *Salmonella enteritidis, E. coli*, and *Pseudomonas aeroginosa* (Hinton and Hume 1995; Wrigley 2004). The acids reduce the pH milieu of the intestine, resulting in a higher sporulation rate of *Clostridium difficile* or *Clostridium perfringenes*. These are conditions under which such anaerobic bacteria produce their toxins that finally cause diarrhea in the patient. This example shows that communication via bacterial volatiles is also of significant clinical relevance.

Conclusions and outlook

This review was intended to summarize the present knowledge of bacterial volatile emission as well as the effects of the volatiles exert on different organisms. The inquest clearly demonstrates that the diversity and complexity of bacterial volatile emanation is similar to other organisms and that it was underestimated in the past. Two major tasks should be addressed in the future. Firstly, structural elucidation of new natural products with specific action potentials would increase the impact of biologically active compounds, and secondly, functional analysis would clarify the role of volatiles in the interactions of organisms, populations, and communities and reveal their importance for the ecological balance.

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References

Alstrom S (2001) Characteristics of bacteria from oil seed rape in relation to their biocontrol of activity against *Verticillium dahliae*. J Phytopathol 149:57–64

Azcon-Aguiler C, Barea JM (1985) Effect of soil microorganisms on formation of vesicular–arbuscular mycorrhizas. Trans Br Mycol Soc 84:536–537

Azcon-Aguiler C, Diaz-Rodriguez RM, Barea JM (1986) Effects of soil microorganisms in spore germination and growth of the vesicular–arbuscular mycorrhizal fungus *Glomus mossae*. Trans Br Mycol Soc 86:337–340

Bargmann CI, Hartwieg E, Horvitz HR (1993) Odorant-selective genes and neurons mediate olfaction in *C. elegans*. Cell 74:515–527

Bentley R, Meganathan R (1981) Geosmin and methylisoborneol biosynthesis in *Streptomycetes*: evidence for an isoprenoid pathway and the absence in non-differentiating isolates. FEBS Lett 125:220–222

Bonkowski M (2004) Protozoa and plant growth: the microbial loop in soil revisited. New Phytol 162:617–631

Bunge M, Araghipour N, Mikoviny T, Dunkl J, Schnitzhofer R, Hansel A, Schinner F, Wisthaler A, Marfesin R, Märk TD (2008) On-line monitoring of microbial volatile metabolites by proton transfer reaction-mass spectrometry. Appl Environ Microbiol 74:2179–2186

Cane DE, Watt RM (2003) Expression and mechanistic analysis of a germacradienol synthase from *Streptomyces coelicolor* implicated in geosmin biosynthesis. PNAS 100:1547–1551

Chaurasia B, Pandey A, Palni LMS, Trivedi P, Kumar B, Colvin N (2005) Diffusible and volatile compounds produced by an antagonistic *Bacillus subtilis* strain cause structural deformation in pathogenic fungi in vitro. Microbiol Res 160:75–81

Chuankun X, Minghe M, Leming Z, Keqin Z (2004) Soil volatile fungistasis and volatile fungistatic compounds. Soil Biol Biochem 36:1997–2004

Dainty RH, Edwards RA, Hibbard C (1985) Time course of volatile compound formation during refrigerated storage of naturally contaminated beef in air. J Appl Bacteriol 59:303–309

Dainty RH, Edwards RA, Hibbard CM, Marewick JJ (1989) Volatile compounds associated with microbial growth on normal and high pH beef stored at chill temperature. J Appl Microbiol 66:281–289

Dickschat JS, Wenzel SC, Bode HB, Müller R, Schulz S (2004) Biosynthesis of volatiles by the Myxobacterium *Myxococcus xanthus*. Chem Biol Chem 5:778–787

Dunkel M, Schmidt U, Struck S, Berger L, Gruening B, Hossbach J, Jaeger IS, Effmert U, Piechulla B, Eriksson R, Knudsen J, Preissner R (2009) Super Scent—a database of flavors and scents. Nucleic Acid Res 37. doi:10.1093/nar/gkn695

Duponnois R, Kisa M (2006) The possible role of trehalose in the mycorrhiza helper bacterium effect. Can J Bot 84:1005–1008

Farag MA, Ryu CM, Summer LW, Pare PW (2006) GC-MS SPME profiling of rhizobacterial volatiles reveals prospective inducers of growth promotion and induced systemic resistance in plants. Phytochemistry 67:2262–2268

Fernando WGD, Ramarathnam R, Krishnamoorthy AS, Savchuk SC (2005) Identification and use of potential bacterial organic antifungal volatiles in biocontrol. Soil Biol Biochem 37:955–964

Fiddaman PJ, Rossall S (1993) The production of antifungal volatiles by *Bacillus subtilis*. J Appl Bacteriol 7:119–126

Fiddaman P, Rossall S (1994) Effect of substrate on the production of antifungal volatiles from *Bacillus subtilis*. J Appl Bacteriol 76:395–405

Fitter AH, Garbaye J (1994) Interactions between mycorrhizal fungi and other soil organisms. Plant Soil 159:123–132

Fries N (1973) Effects of volatile organic compounds on the growth and development of fungi. Trans Br Mycol Soc 60:1

Gu Y-Q, Mo M-H, Zhou JP, Zou C-S, Zhang K-Q (2007) Evaluation and identification of potential organic nematicidal volatiles from soil bacteria. Soil Biol Biochem 39:2567–2575

Gust B, Challis GL, Fowler K, Kieser T, Chater KF (2003) PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. Proc Natl Acad Sci U S A 100:1541–1546

Hayes TS, Randle PE, Last FT (1969) The nature of the microbial stimulus affecting sporophore formation in *Agaricus bisporus* (Lange) Sing. Ann Appl Biol 64:177–187

Henis Y, Gould JR, Alexander M (1966) Detection and identification of bacteria by gas chromatography. Appl Microbiol 14:513–524

Hinton A Jr, Hume ME (1995) Antibacterial activity of the metabolic by-prodcuts of a *Veillonella* species and *Bacteroides fragilis*. Anaerobe 1:121–127

Hora TS, Baker R (1972) Soil fungistasis: microflora producing a volatile inhibitor. Trans Br Mycol 59:491–500

Huang J, Miller JR, Chen S, Vulule JM, Walker ED (2006) *Anopheles gambiae* (Diptera: Culicidae) oviposition in response to agarose media and cultured bacterial volatiles. J Med Entomol 43:498–504

Kai M, Effmert U, Berg G, Piechulla B (2006) Volatiles of bacterial antagonists inhibit mycelial growth of the plant pathogen *Rhizoctonia solani*. Arch Microbiol 187:351–360

Kai M, Vespermann A, Piechulla B (2008) The growth of fungi and *Arabidopsis thaliana* is influenced by bacterial volatiles. Plant Signal Behav 3:1–3

Korpi A, Pasanen A-L, Pasanen P (1998) Volatile compounds originating from mixed microbial cultures on building materials under various humidity conditions. Appl Environ Microbiol 64:2914–2919

Kurita-Ochiai T, Fukushima K, Ochiai K (1995) Volatile fatty acids, metabolitic by-products of periodontopathic bacteria, inhibit lymphocyte proliferation and cytokine production. J Dent Res 74:1367–1373

Kuzuyama T, Takagi M, Takahashi S, Seto H (2000) Cloning and characterization of 1-deoxy-D-xylulose-5-phosphate synthase from *Streptomyces* sp strain CL190, which uses both the mevalonate and the non-mevalonate pathways for isopentenyl diphosphate biosynthesis. J Bacteriol 182:891–897

Lockard JD, Kneebone LR (1962) Investigation of the metabolic gases produced by *Agaricus bisporus* (Lange) Sing. Mushroom Sci 5:281–299

Mackie A, Wheatley RE (1998) Effects and incidence of volatile organic compound interactions between soil bacterial and fungal isolates. Soil Biol Biochem 31:375–385

McCain AH (1966) A volatile antibiotic by *Streptomyces griseus*. Phytopathology 56:150

Moore-Landecker E, Stotzky G (1972) Inhibition of fungal growth and sporulation by volatile metabolites from bacteria. Can J Microbiol 18:957–962

Moore-Landecker E, Stotzky G (1973) Morphological abnormalities of fungi induced by volatile microbial metabolites. Mycologia 65:519–530

Moore-Landecker E, Stotzky G (1974) Effects of concentration of volatile metabolites from bacteria and germinating seeds on fungi in the presence of selective absorbents. Can J Microbiol 20:97–103

Ponnusamy L, Yxu N, Nojima S, Wesson DM (2008) Identification of bacteria and bacteria-associated chemical cues that mediate oviposition site preferences by *Aedes aegypti*. Proc Natl Acad Sci U S A 105:9262–9267

Ryan RP, Dow JM (2008) Diffusible signals and interspecies communication in bacteria. Microbiology 154:1845–1858

Ryu C-M, Farag MA, Hu C-H, Reddy MS, Wei HX, Pare PW (2003) Bacterial volatiles promote growth in *Arabidopsis*. Proc Natl Acad Sci U S A 100:4927–4932

Schrey SD, Schellhammer M, Ecke M, Hampp R, Tarkka MT (2005) Mycorrhiza helper bacterium *Streptomyces* AcH505 induces

differential gene expression in the ectomycorrhizal fungus *Amanita muscaria*. New Phytol 168:205–216

Schöller CEG, Gürtler H, Pedersen R, Molin S, Wilkins K (2002) Volatile metabolites from actinomycetes. J Agric Food Chem 50:2615–2621

Schulz S, Dickschat JS (2007) Bacterial volatiles: the smell of small organisms. Nat Prod Rep 24:814–842

Sengupta P, Chou JH, Bargmann CI (1996) odr-10 encodes a seven transmembrane domain olfactory receptor required for responses to the odorant diacetyl. Cell 84:578–587

Stotzky G, Schenk S (1976) Volatile organic compounds and microorganisms. CRC Crit Rev Microbiol 4:333–382

Takagi M, Kuzuyama T, Takahashi S, Seto H (2000) A gene cluster for the mevalonate pathway from *Streptomyces* sp strain CL190. J Bacteriol 182:4153–4157

Tarkka MT, Piechulla B (2007) Aromatic weapons: truffles attack plants by the production of volatiles. New Phytol 175:383–386

Vespermann A, Kai M, Piechulla B (2007) Rhizobacterial volatiles affect the growth of fungi and *Arabidopsis thaliana*. Appl Environ Microbiol 73:5639–5641

Wang X, Ahearn DG (1997) Effect of bacteria on survival and growth of *Acanthamoeba castellanii*. Curr Microbiol 34:212–215

Whaley JW, Boyle AM (1967) Antibiotic production by *Streptomyces* species from the rhizosphere of desert plants. Phytopathology 57:347–351

Wheatley RE (2002) The consequences of volatile organic compounds mediated bacterial and fungal interactions. Antonie van Leeuwenhoek 81:357–364

Wrigley DM (2004) Inhibition of *Clostridium perfringens* sporulation by *Bacteroides fragilis* and short-chain fatty acids. Anaerobe 10:295–300

Zechman JM, Labows JNJ (1985) Volatiles of *Pseudomonas aeruginosa* and related species by automated headspace concentration-gas chromatography. Can J Microbiol 31:232–237

Zhang H, Kim M-S, Krishnamachari V, Payton P, Sun Y, Grimson M, Farag MA, Ryu C-M, Allen R, Melo IS, Pare PW (2007) Rhizobacterial volatile emissions regulate auxin homeostasis and cell expansion in *Arabidopsis*. Planta 226:839–851

Zoller HF, Mansfield Clark W (1921) The production of volatile fatty acids by bacteria of the dysentery group. J Gen Physiol 3:325–330

Zou C-S, Mo M-H, Gu Y-Q, Zhou J-P, Zhang K-Q (2007) Possible contributions of volatile-producing bacteria to soil fungistasis. Soil Biol Biochem 39:2371–2379