
Bacterial Volatile Organic Compounds: A New Insight for Sustainable Agriculture

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Abstract

Plant growth-promoting rhizobacteria (PGPR) improve plant growth by improved nutrient acquisition and guarding plants from biotic and abiotic stress. PGPR stimulate plant defense system by induction of systemic resistance or tolerance (ISR/IST). A large number of elicitors are known to stimulate plant defense system, and VOCs are one of the most studied elicitors for ISR/IST response which excites plant defense system without direct physical contact. In this chapter review about the current development regarding interactions of PGPR volatiles and plants is discussed. The mechanisms of action of volatile compounds for plant growth promotion as well as stimulation of plant defense to withstand abiotic and biotic stress are also being elaborated to explain elicitation of plant's self-immunity against various stresses.

Keywords

PGPR • ISR • IST • Volatile organic compounds • Self-immunity • Stress

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8.1 Introduction

Living organisms like plants, animals, and microorganisms comprise of a large number of natural chemicals like enzymes, hormones, proteins, and volatile compounds that empower them to survive in nature and play significant roles in organism's metabolism, nutrition, establishment, and conservation in definite ecological location. Volatiles are the compounds having high vapor pressure, which falls into two categories, viz., organic and inorganic. Among inorganic and organic volatiles, volatile organic compounds can travel far from the point of production. Microbial volatile organic compounds (VOC) are found to play key role in antagonism and mutualism. Moreover, various intra- and interspecies cellular and developmental processes are governed by microbial volatiles. Till date the exact mechanism of action of microbial volatiles is to be explained. Since the diversity of volatile-producing microorganisms is huge in nature, if mechanism of action of microbial volatile as an interphase between plant health and microbes can be revealed, then it is likely to disclose unique mechanisms for governing various biological processes critical to plant fitness and will also propose concrete benefits while addressing agricultural and environmental problems.

8.2 Sources of Volatiles in Nature

Biologically produced volatiles comprise of the compounds originating from plants, animals, and microbes. As per general belief, volatile compounds seem to be characteristically linked to the atmosphere, but soil is also considered as a large reservoir of biogenic volatile organic compounds. Volatile organic compounds of biological origin belong to chemical classes such as alcohols, thiols, aldehydes, esters, terpenoids, and fatty acid derivatives which are lipophilic in nature, having low molecular weight and high vapor pressure (Schulz and Dickschat 2007). Usually inside the soil, all the organisms use linkage of signaling pathways to feel the environmental stimuli. This signaling pathway confirms cellular homeostasis which facilitates systematic growth and development as well as controls performance.

8.3 Bacterial Volatile Organic Compounds

In soil, microorganisms like bacteria, fungi, and actinomycetes emit large amount of volatile compounds, among which bacteria are found in higher amount (10^{11} cells/g of soil). Soil bacteria colonize roots, organic residues, and soil particles (Burmolle et al. 2007) as well as the rhizosphere (Mendes et al. 2013). Humans have exploited the potential of microbial volatiles for providing aroma to food and beverages like cheese, sauerkraut, yogurt, wine, etc. The inoculated bacteria release specific odor during fermentation of foodstuffs which is dependent on environmental conditions (Kai et al. 2009). Scientists have discovered more than 1000 different bacterial volatile compounds (Lemfack et al. 2014, <http://bioinformatics>).

charite.de/mvoc) which are employed by bacteria as communication signals with other organisms which in turn decide positive or negative influence on both the interacting communities (Kai et al. 2009; Romoli et al. 2011). Such volatiles enable the organisms to overcome competitive pressure within the same niche. For example, albaflavone and dimethyl disulfide are having negative effect on fungal pathogens, whereas geosmin, 2,3-butanediol, acetoin, and tridecane are having positive effect on plant growth. Stress and antibiotic resistance phenotypes of some of the bacteria are attributed to production of volatile compounds. Recently the role of bacterial volatiles in biofilm formation has also been elucidated. Such volatiles attract the nearby bacterial cells to link together to formulate biofilm. Moreover, some of the bacterial volatiles, viz., ammonia and trimethylamine, can alter gut cell physiology in humans and thereby confer immunization against pathogens. Besides these beneficial effects, some of the volatiles of pathogenic microorganisms are responsible for pathogenesis of the strain. In general bacterial volatiles are having tremendous effect on growth, differentiation, and stress resistance in living organisms (Kai et al. 2009; Kai and Piechulla 2009; Effmert et al. 2012; Wenke et al. 2012; Davis et al. 2013).

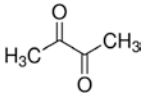
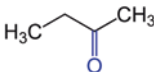
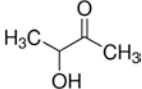
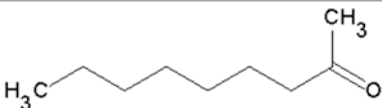
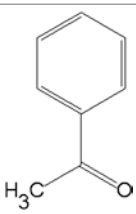
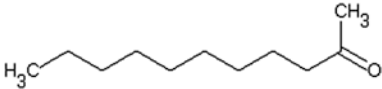
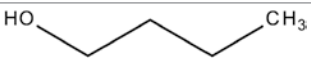
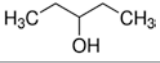
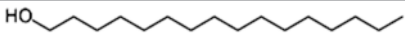
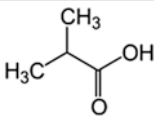
8.3.1 Types of Bacterial Volatile Organic Compounds

Bacterial volatile compounds belonging to different chemical classes are generally produced through catabolic pathways such as glycolysis, protein, and lipid degradation pathways (Schulz and Dickschat 2007; Penuelas et al. 2014). Bacterial volatile compounds derived from organic molecules include numerous chemical classes such as fatty acid derivatives (hydrocarbons, ketones, alcohols), acids, sulfur- and nitrogen-containing compounds, and terpenes (Table 8.1).

8.4 Biological Role of Bacterial Volatiles

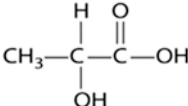
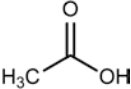
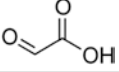
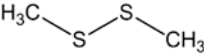

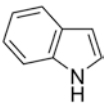
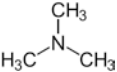
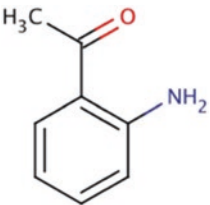
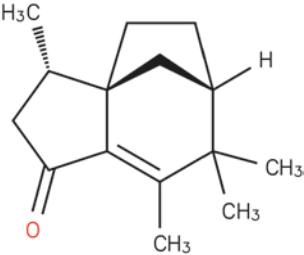
Bacterial volatiles are diverse and complex as compared to that of plants and fungi. Bacterial volatiles are expected to be analogous to other volatiles and possibly assist as communication signals during inter- and intra-organismic communication as well as cell-to-cell communication. It may also act as possible carbon release valve and growth-promoting or growth-inhibiting agents. Volatiles also play an important role in establishment and survival of bacterial populations in ecological niche and for development of different communities. Volatiles can diffuse through aqueous solutions and also travel for far distance in the atmosphere and thereby not only act above ground but also act below ground.

Table 8.1 Types of bacterial volatile organic compounds

Sr. No.	Name of bacterial volatile organic compounds	Structure	Molecular weight
Hydrocarbon			
5	Hexadecane	$\text{H}_3\text{C}-(\text{CH}_2)_{14}-\text{CH}_3$	226.45 g/mol
6	Tridecane	$\text{CH}_3(\text{CH}_2)_{11}\text{CH}_3$	184.36 g/mol
Ketones			
7	2,3-Butanedione		86.0892 g/mol
8	2-Butanone		72.11 g/mol
9	Acetoin		88.11 g/mol
10	2-Nonanone		142.23862 g/mol
11	Phenylethanone		120.151 g/mol
12	2-Undecanone		170.30 g/mol
Alcohols			
13	1-Butanol		74.12 g/mol
14	3-Pentanol		88.148 g/mol
15	Hexadecanol		242.4406 g/mol
Acids			
16	Isobutyric acid		88.11 g/mol

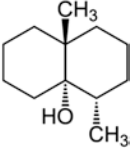
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Table 8.1 (continued)

Sr. No.	Name of bacterial volatile organic compounds	Structure	Molecular weight
17	Lactic acid		90.08 g/mol
18	Acetic acid		60.05 g/mol
19	Glyoxylic acid		74.04 g/mol
Sulfur-containing compound			
20	Dimethyl disulfide		94.2 g/mol
21	1-(Methylthio)-3-pentanone		132.23 g/mol
Nitrogen-containing compound			
22	Indole		117.15 g/mol
23	Trimethylamine		59.11 g/mol
24	2-Aminoacetophenone		135.16 g/mol
Terpenes			
25	Albaflavenone		218.34 g/mol

(continued)

Table 8.1 (continued)

Sr. No.	Name of bacterial volatile organic compounds	Structure	Molecular weight
26	Geosmin		182.31 g/mol

8.5 Role of Bacterial Volatiles in Agriculture

Food safety is adversely affected by climate change and growing pathogens which reduce crop yield. Use of agrochemicals like synthetic pesticides and fertilizers ensures protection against disease and high crop yield, but ultimately, they significantly affect the health of human and environment. In the present era, biological inputs like biopesticides, biofertilizers, and biodegraders are gaining momentum as appropriate alternatives of synthetic agro-inputs. Limiting factors for polarization of such bioinputs include less efficiency, high costs, and inconsistent performance under field conditions (Glare et al. 2012). Researchers have demonstrated that exposure of plants to bacterial volatiles has significant effect on modulation of plant metabolism, physiology, and genetic status which leads to belief that the plants are capable to recognize and react to microbial volatiles. Till date majority of research regarding plant-bacterial volatile interactions are conducted under laboratory conditions, but recently few of the field trials demonstrating efficiency of bacterial volatiles for sustainable crop protection and production have been conducted (Cortes-Barco et al. 2010a, b; Song and Ryu 2013). These studies undoubtedly establish the essentiality for application of bacterial volatiles in open field conditions and emphasize their various roles to escalate pathogen resistance, defense against herbivores, and as biocontrol agents. Operational distribution of bacterial volatiles still remains a major task.

8.5.1 Bacterial Volatile Compounds as Biostimulants

Bacterial volatile compounds are having a major role in promotion of plant growth. Without direct physical contact between plant and microorganism, bacterial volatile compounds can stimulate plant growth (Ryu et al. 2003). Among various PGPR tested, *Bacillus subtilis* GB03 and *B. amyloliquefaciens* IN937a were found to stimulate plant growth by emission of volatile compounds. To reveal a signaling pathway for VOC-mediated plant growth promotion, a series of mutant lines were tested (Ryu et al. 2003). Upon contact with volatile compounds produced by *B. subtilis* GB03, the total leaf surface area was found to increase in mutant lines ethylene insensitive (*etr1*), auxin-transporter-deficient and ethylene insensitive (*eir1*),

gibberellic acid-insensitive (*gai2*), and brassinosteroid-insensitive (*cbb1*). These altogether thereby disprove the necessity of brassinosteroid, gibberellic acid, or ethylene signaling in the plant growth promotion by volatile compounds. Under field condition, *B. subtilis* GB03 is assumed to persist on seeds before planting and then after it uses seed exudates during seed germination and multiply to finally reach up to growing roots where they will conserve a healthy population through plant-microbe interactions (Kloepper et al. 2004). Required bacterial strength to start plant response is recommended to be 10^4 colony-forming units (cfu)/root. *B. subtilis* GB03 was reported to maintain soil populations of 10^5 cfu/root up to 60 days after planting (Kokalis- Burrelle et al. 2006).

8.5.2 Bacterial Volatile Compounds as Bio-protectants Against Abiotic Stress

Bacterial volatile compounds induce systemic tolerance response against abiotic stress such as nutrient deficiency, salinity, and drought (Yang et al. 2009). Induced systemic tolerance is physical and chemical alterations in plants stimulated by PGPR which culminate in improved tolerance to abiotic stresses.

Salt Tolerance

Under saline conditions, the plant faces osmotic stresses which results in reduction of crop growth and yield. The basic mechanism underlying induced systemic tolerance in plants against saline condition mediated by bacterial volatiles comprise of decreased sodium uptake in roots and increased discharge of sodium ions from shoots through regulation of various transport proteins including HKT1 and SOS1. Bacterial volatile organic compound (VOC) upregulates HKT1 gene which in turn increases elimination of sodium ions from xylem sap, thereby expediting elimination of sodium ions from plant leaves. Similarly HKT1 is downregulated in the roots. This mechanism was discovered by a thorough study of *B. amyloliquefaciens* GB03 showing VOC-mediated systemic tolerance (Mayak et al. 2004; Barriuso et al. 2008; Zhang et al. 2008a). During the studies it was revealed that *Arabidopsis* plants treated with GB03 VOC showed increased biomass and less sodium ion content as compared to untreated plants (Zhang et al. 2008a). Similar type of induced systemic tolerance was observed in wild-type plants but not in the *hkt1* mutant, proposing a crucial role of HKT1 in facilitating the salinity tolerance activated by GB03 VOCs. Moreover, increasing the shoot-to-root recirculation of sodium ions too can lead to a greater amount of sodium ions in the roots and lower concentration of sodium ions in the shoots. GB03 VOCs concomitantly inhibit and escalate HKT1 expression in roots and shoots, respectively, which assist in VOC-induced salt tolerance (Zhang et al. 2008b). SOS3 (calcium-signaling sensor) may contribute in VOC-mediated salinity tolerance. GB03 VOCs exhibited 50% decrease in sodium ion concentration in whole wild-type plant, whereas *sos3* mutant showed 15% reduction in sodium ion accumulation (Zhang et al. 2008b), proposing that AtSOS3-dependent Na⁺ exudation is also essential for the reduced buildup of sodium ions in

VOC-treated plants. Moreover, VOCs produced by GB3 also cause acidification of the rhizosphere (Zhang et al. 2009), thus generating a proton gradient that could hypothetically aid in SOS1-mediated transfer of Na⁺ from roots. Under saline condition, plants modify their metabolism to survive in osmotic stress triggered by the increased sodium ion concentration. *Pseudomonas simiae* strain AU volatile-induced salt tolerance was observed in soybean plants wherein volatile compounds not only decreased root Na⁺ levels but also increased the buildup of proline, which defend cells from osmotic stress (Vaishnav et al. 2015). Moreover, plants treated with AU volatiles showed higher level of vegetative storage protein (VSP) and numerous other proteins that are known to assist plants to withstand under stress conditions (Vaishnav et al. 2015).

Drought Tolerance

Under dehydrating conditions, raised accumulation of osmoprotectants in plants can increase cellular osmotic pressure to lower the free water potential of cells which thereby avoid water loss and can also stabilize structure of proteins and membrane. Under osmotic stress, *Arabidopsis* plants exposed to GB03 volatiles accumulated greater level of choline and glycine betaine than plants without volatile treatment (Zhang et al. 2010). 2,3-Butanediol is the most common volatile organic compound found in *P. chlororaphis* strain O6. *Arabidopsis* plants inoculated with *P. chlororaphis* O6 or exposed to 2,3-butanediol exhibited increased drought stress tolerance, which clearly leads to increased stomatal closure and reduced water loss (Cho et al. 2008). Upon application of *P. chlororaphis* O6 or 2,3-butanediol, concentration of salicylic acid (SA) was significantly increased which showed dependence of induced systemic tolerance pathway on SA (Cho et al. 2008). Certain bacterial volatiles such as acetic acid are able to induce formation of biofilms containing higher amount of exopolysaccharides (Chen et al. 2015) which indirectly increase plant's drought tolerance by conservation of moisture.

Inoculation of wheat with *B. thuringiensis* AZP2 under drought stress leads to enhanced plant biomass and fivefold increase in persistence under severe drought due to significant reduction evaporation and maintenance of higher rate of photosynthesis (Timmusk et al. 1999). Detection of volatiles provides promising technique for rapid, noninvasive assay of crop's drought stress and its mitigation (Timmusk et al. 1999). Occupation of roots by *P. chlororaphis* O6 stops water loss by stomatal closure which is mediated by bacterial volatile compound 2R,3R-butanediol, whereas mutant strain deficient in 2R,3R-butanediol production showed no induction of drought tolerance (Cho et al. 2008). Further, *Arabidopsis* mutant lines indicated that induced drought tolerance required the salicylic acid (SA), ethylene, and jasmonic acid-signaling pathways. Both induced drought tolerance and stomatal closure were dependent on *Aba-1* and *OST-1* kinase (Cho et al. 2008). PGPR can also change morphology of plant roots under drought stress. Rhizobacteria affects the physiological processes at plant's cell membrane. Inoculation of wheat seedlings with *Azospirillum brasilense* reduced membrane potentials as well as phospholipid content in the cell membranes of cowpea due to the changes in proton efflux activities (Bashan et al. 1992). Under water stress conditions, there occur an

increase in phosphatidylcholine and a decrease in phosphatidylethanolamine content (Sueldo et al. 1996), but inoculation with *Azospirillum* reverts these changes in wheat seedlings (Pereyra et al. 2006). Rhizobacterial inoculation also stimulates changes in the elasticity of root cell membranes which seems to be the first steps toward enhanced tolerance to drought (Dimkpa et al. 2009). PGPR also strengthens plant cell membranes by activating the antioxidant defense system which in turn enhances drought tolerance in plants (Gusain et al. 2015).

Nutrient Acquisition

Bacterial volatiles generally help in the acquisition of sulfur and iron. Dimethyl disulfide (DMDS) is an S-containing volatile compound commonly produced by many soil bacteria and fungi (Kanchiswamy et al. 2015). Emission of DMDS from *Bacillus* sp. strain B55, a natural symbiont of *Nicotiana attenuata* plants, rescued plant growth retardation caused by S-deprivation (Meldau et al. 2013). The incorporation of bacteria-emitted S into plant proteins was demonstrated by adding radiolabeled ^{35}S to the bacterial growth medium. In addition to detecting DMDS, Meldau et al. (2013) also detected the S-containing compound S-methylpentanethioate in *Bacillus* sp. B55 VOCs. The authors attributed most of the S-nutrition provided by *Bacillus* sp. B55 VOCs to DMDS rather than to S-methylpentanethioate for two reasons. First, DMDS was detected as a major component of the volatile emissions, while S-methylpentanethioate was present in only trace amounts. Second, synthetic DMDS was superior to the natural VOC blends in rescuing S-starvation phenotypes of *N. attenuata* plants (Meldau et al. 2013). Sulfur in SO_4^{2-} is in an oxidative state and thus requires an energy-consuming reduction process for biological assimilation (Takahashi et al. 2011). In contrast, sulfur in DMDS is in a chemically reduced state. Therefore, it appears that DMDS may not only provide S to plants but may also help plants avoid expending energy on sulfate reduction. Consistent with this hypothesis, DMDS supplementation significantly decreased the expression of S-assimilation genes as well as methionine biosynthesis and recycling (Meldau et al. 2013). Like DMDS in *Bacillus* sp. B55 VOCs, other S-containing volatile compounds such as dimethyl sulfide and dimethyl trisulfide have been detected in high concentrations in other microbial VOC blends (Kanchiswamy et al. 2015). Whether these microbial VOCs may also enhance S-assimilation by plants remains to be determined.

The transition between ferrous iron (Fe^{2+}) and ferric iron (Fe^{3+}) generates a redox potential that is important for electron transfer reactions including photosynthesis. Deprivation of Fe severely impairs the photochemical capacity and is accompanied by leaf chlorosis. Gramineous monocots produce siderophores that increase Fe^{3+} mobility in soil and directly uptake Fe^{3+} without reduction, while non-gramineous monocots and dicots not only acidify the rhizosphere to increase Fe^{3+} mobility but also use plasma membrane ferric reductase to reduce Fe^{3+} and subsequently transport Fe^{2+} into the roots (Curie and Briat 2003). Augmented Fe uptake was observed in *Arabidopsis* exposed to GB03 VOCs, which do not contain any known siderophores (Farag et al. 2006; Zhang et al. 2009). Under Fe-sufficient growth conditions, plants treated with GB03 VOCs displayed typical Fe deficiency

responses, including transcriptional upregulation of the root Fe³⁺ reductase gene FRO2 and of the Fe²⁺ transporter gene IRT1, increases in FRO2 enzyme activity, and rhizosphere acidification (Zhang et al. 2009). As a result, Fe levels were elevated in VOC-treated plants, consistent with greater amounts of Fe-rich photosynthetic apparatus (Zhang et al. 2008b). GB03 VOC-triggered gene induction of IRT1 and FRO2 requires the transcription factor FIT1, because VOC failed to induce IRT1 or FRO2 in the fit1 knockout mutant (Zhang et al. 2009). VOC treatment also failed to increase iron uptake or photosynthesis in the fit1 mutant. Still, it remains unknown how VOC-treated plants initiate the inducible iron deficiency responses. One possibility is that a demand for more iron may result from VOC-induced leaf cell expansion (Zhang et al. 2007) and/or photosynthesis augmentation (Zhang et al. 2008b). Also unclear is the identity of the component(s) in GB03 VOCs that induce plant iron deficiency responses. On the other hand, acid component such as diethyl acetic acid possibly accounts for the rhizosphere acidification that is directly caused by VOC exposure (Frag et al. 2006; Zhang et al. 2009).

8.5.3 Bacterial Volatile Organic Compounds to Fight Against Biotic Stress

Phytopathogens are major and chronic threat for agricultural production world over, and losses due to pathogen account for about 13% of the total production losses. Due to increasing production, the producers are becoming more and more dependent on agrochemicals for plant disease management. That's why these agrochemicals dominate the global market of phytosanitary products. But nowadays due to increasing awareness of consumers about pesticide-free safer food, this leads to reduction in the use of these agrochemicals which leads to the development of a new strategy comprising the use of biocontrol agents for plant disease management. Various types of biocontrol agents are presently accessible in the market which differ by the composition of microorganisms within it, namely, bacteria, fungi, viruses, and nematodes. Among which, bacterial biocontrol agents exerts their activity in three ways:

1. Competition: here rhizobacteria due to their fast chemotactic movement toward root exudates outcompete pathogen population in the acquisition of nutrients and specific niche and thereby reduce pathogen population.
2. Antibiosis: the rhizobacteria having capacity to produce antibacterial and antifungal compounds directly inhibit pathogen growth.
3. Plant immunization: here due to plant colonization by rhizobacteria, the plant's innate defense system is activated to respond strongly to the pathogen attack which can be called as induced resistance.

In all these three mechanisms, bacterial volatiles are having major roles. Volatile compounds can travel across membranes unrestrictedly and get released into the atmosphere or soil in the absence of a diffusion barrier (Pichersky et al. 2006).

Moreover mass movement of water through the soil facilitates quick movement of volatile compounds all over the system (Wheatley 2002). Due to its ability to penetrate membranes easily as well as efficient delivery through soil, it improves antagonistic potential of a volatile against target organism.

Nematicidal Activity of Bacterial Volatile Organic Compounds

Meager efforts were done for testing antagonistic potential of bacterial volatile organic compounds against phytopathogenic nematodes. Till date laboratory tests were done to determine influence of bacterial volatile organic compounds on second-stage juvenile (J2) of plant parasitic nematodes. Gu et al. (2007) evaluated the nematicidal activity (NA) of 200 bacterial isolates against *Panagrellus redivivus* in using compartmentalized petri dishes and found more than 20% nematicidal activities by 149 isolates wherein 49 isolates showed more than 80% NA including *B. weihenstephanensis*, *B. simplex*, *B. subtilis*, and *Serratia marcescens*. Same bacterial strains were also tested against *Bursaphelenchus xylophilus* wherein 165 bacterial strains showed more than 20% NA. Six bacterial strains (two of *B. simplex*, three of *weihenstephanensis*, and one of *S. marcescens*) revealed strong NA (80%) against both tested nematode species. Huang et al. (2010) reported that volatile organic compounds produced by *B. megaterium* showed 100% mortality of *Meloidogyne incognita* J2 and strong inhibition of egg hatching. It was observed that same isolates showed significant variation in their nematicidal activity because of their VOC production pattern. Among the 81 different VOCs identified in the 15 bacterial isolates by Gu et al. (2007), 46 VOCs were not having any NA and 18 showed strong NA and 2 VOCs (benzaldehyde and trimethylpyrazine) occurred in all samples at high concentrations. Among all the tested 20 VOCs, 9 VOCs, viz., 2-undecanone, 2-octanol, decanol, benzaldehyde, 2-nonanone, dimethyl disulfide, benzeneacetaldehyde, cyclohexene, and phenol, showed 100% NA against tested nematodes. Huang et al. (2010) identified a total of 17 VOCs from *B. megaterium* which were tested in vitro, against *M. incognita*, by using commercial compounds. Among a total of 17 compounds tested, 2-nonanone, 2-undecanone, decanal, dimethyl disulfide, and benzeneacetaldehyde showed more than 80% nematicidal activities.

Control of Phytopathogenic Fungi by Bacterial Volatiles

Presently many of the researchers have evaluated the role of bacterial volatiles in fungicidal activity. Fernando et al. (2005) isolated various bacterial strains, viz., *Pseudomonas chlororaphis* (five isolates), *P. corrugate* (one isolate), *P. fluorescens* (three isolates), and *P. aurantiaca* (one isolate), from canola and soybean plants, which showed production of antifungal VOCs which inhibited sclerotia and ascospore germination as well as mycelial growth of *Sclerotinia sclerotiorum* in laboratory and soil tests. Similarly, cyanide produced by *P. fluorescens* CHAO inhibits tobacco rot caused by the fungus *Thielaviopsis basicola* (Voisard et al. 1989). Liu et al. (2008) reported the production of volatiles by bacterium species *Paenibacillus polymyxa*, *B. pumilus*, and *B. subtilis* isolated from cucumber rhizosphere. These volatiles showed 20–100% inhibitory effect on phytopathogenic fungi, viz., *S. sclerotiorum*, *B. cinerea*, *A. brassicae*, *A. solani*, *Ascochyta citrullina*, *F.*

oxysporum, *F. graminearum*, *Cercospora kikuchii*, *Rhizoctonia solani*, *Phoma arachnidicola*, and *Verticillium dahliae*. Moreover, Arrebola et al. (2010) reported that *B. subtilis* and *B. amyloliquefaciens* obtained from Valencia and Shamouti oranges produced volatile organic compounds having 25–50% inhibitory effect on *Penicillium crustosum* and *P. italicum*. Wan et al. (2008) reported that VOCs produced by *Streptomyces platensis* F-1 reduce mycelial growth of *R. solani*, *S. sclerotiorum*, and *B. cinerea* and controlled the disease caused by them in rice, oilseed rape, and strawberry, respectively. Huang et al. (2012) reported that application of DMDS produced by *B. cereus* CIL significantly protected tobacco against *Botrytis cinerea* under greenhouse conditions.

Baysal et al. (2013) detected the production of 2,3-butanediol by *B. subtilis* strains, FZB24, QST713, and EU07, which can efficiently control *Fusarium oxysporum* f.sp. *radices-lycopersici*.

Giorgio et al. (2015) reported that six strains of volatile-producing rhizobacteria inhibited the growth of *Sclerotinia sclerotiorum* strain. The presence of 1-undecene, 2-nonanone, 2-undecanone, 2-propanone, 1-tetradecanol, acetic acid, m-cymene, dl-limonene, dimethyl disulfide, and dimethyl trisulfide was detected in bacterial culture filtrate through GC–MS analysis.

Mackie and Wheatley (1999) detected that there exist variations in inhibitory effects of single bacterial isolate against various fungal pathogens which may be attributed to the facts that different fungi may respond to different component(s) of the volatile mixture as the site for reaction may be different; some of the fungi have developed mechanism to detoxify the volatile metabolite(s) (Kai et al. 2007). Mechanism of action of bacterial VOCs includes inhibition of fungal mycelial growth or enzyme activity (Wheatley 2002). Exposure to both larger and older bacterial populations greatly increases both the degree and the rate of inhibitory effects on the fungi (Mackie and Wheatley 1999). VOCs can be fungicidal or fungistatic and water soluble. Mackie and Wheatley (1999) found that the inhibitory effects on many fungi by the bacterial VOCs were not fungicidal and the persistence of the effects due to VOC adsorption into agar medium indicated that the active compounds are water soluble. VOCs produced by microorganisms played an important role during their evolution in the context of their interactions, community population, and functional dynamics. Such interactions will result in functional responses by the organisms involved to some community members and coincidental disadvantage to others. The substrate-dependent variation in VOC production will result in variations in microbial, and consequently systemic, response (Wheatley 2002).

Bactericide Activities of VOC Substances Produced by Microorganisms

Gram-positive *Bacillus* sp. strains producing volatile compounds, viz., acetoin and butanediol, induced systemic resistance in tobacco against *Erwinia carotovora* SCC1 and promoted plant growth (Ryu et al. 2003, 2004). Han et al. (2006) reported colonization of cucumber roots by *P. chlororaphis* O6 deliberates defense against *Corynespora cassiicola*. Rudrappa et al. (2010) reported that *Arabidopsis thaliana* (Col-0) plants, inoculated with *B. subtilis* strain FB17, showed lower disease severity against *P. syringae* pv. tomato DC3000 compared to plants without FB17

treatment as *B. subtilis* produced acetoin (3-hydroxy-2-butanone), which triggers induced systemic resistance (ISR) and protects plants against DC3000 pathogenesis. To further confirm the role of acetoin, *B. subtilis* acetoin biosynthetic mutants were created, and it showed reduced emission of acetoin which in turn showed reduction on protection. Further analysis suggested that resistance to DC3000 occurs through NpR, salicylic acid (SA)/ethylene (ET)-mediated pathway. Choi et al. (2014) indicated that *B. amyloliquefaciens* strain IN937a encourages induced systemic resistance (ISR) against bacterial spot disease caused by *Xanthomonas axonopodis* pv. *vesicatoria* in pepper through VOC emission. Among all the volatiles tested, 3-pentanol was tested. Treatment receiving 3-pentanol significantly reduced disease severity in field trials over 2 years. To further elucidate the role of bacterial volatile in stimulation of plant defense, expression of defense genes was studied and revealed that the expression of CaPR1, CaPR2, and CaPIN2 increased in 3-pentanol-treated pepper plants. Dandurishvili et al. (2011) reported that VOCs produced by the *Serratia plymuthica* IC1270, *P. fluorescens* Q8r1-96, and *P. fluorescens* B-4117 inhibited the growth of *Agrobacterium tumefaciens* and *A. vitis* under laboratory conditions. Further analysis revealed presence of dimethyl disulfide (DMDS) as the major volatile produced by antagonistic bacterial strains as well as emitted by tomato plants treated with bacterial strains. Further to rule out possibility of involvement of antibiotics in suppression of pathogen, mutants of *P. fluorescens* Q8r1-96 and *S. plymuthica* IC1270 deficient in 2,4-diacetylphloroglucinol or pyrrolnitrin production, respectively, were tested and revealed that mutants also showed suppression of pathogens and thereby established the role of bacterial volatile in protection of plants against crown gall disease.

8.6 Future Prospects

Exploration, implementation, and adoption of BVOCs for crop production and protection should be emphasized for sustainable crop production. Till date majority of research pertaining to BVOCs is carried out under laboratory conditions and only few species of volatile-producing microorganisms are explored, but still BVOCs showed considerable influence on plant growth, development, and defense. If we want to explore the potential of BVOCs as low-cost, eco-friendly bioinoculant, then more experiments should be conducted under field trial conditions to provide scientific evidence. Generally BVOCs are most attractive as biological pesticides; their use was restricted up to 4% of the global pesticide market. We need to recognize the multidimensional communication of BVOCs with other microorganisms and crops. Research on BVOCs is in its infancy, but in the future, BVOCs will outcompete chemical pesticides and fertilizers as natural products which benefit crops.

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