# Chapter 5
# Volatile Organic Compounds from Endophytic Fungi

**Sudipta Roy and Debdulal Banerjee**

## 5.1 Introduction

Volatile organics are ubiquitous in nature. Their distinction lies in their unique physical property of readily diffusing in the atmosphere. Volatile compounds have a low molecular weight and a lower boiling point that facilitate their rapid evaporation or sublimation and create a higher vapor density. Most of these compounds do not readily dissolve in aqueous systems. Volatile compounds include any carbon compound (excepting carbon monoxide, carbon dioxide, carbonic acid, metallic carbides, carbonates, and ammonium carbonate) that may be conjugated with other elements such as hydrogen, oxygen, fluorine, chlorine, or nitrogen. VOCs are released from a range of anthropogenic activities such as burning of fuel (gasoline), wood, coal, or natural gas and may also be emitted from oil and gas fields and as diesel exhaust. Volatiles are released as fumes from solvents, paints, glues, and other products in our daily use.

Interestingly, there are significant biogenic sources for volatile organic compounds (VOCs) also. Most of these biogenic volatiles include isoprene, monoterpenes, sesquiterpenes, and oxygenated compounds such as methanol, hexane derivatives, 2-methyl-3-buten-2-ol, and 6-methyl-5-hepten-2-one. Volatiles of animal or plant origin have been extensively studied in the past whereas microbial volatiles (i.e., bacteria and fungi) have not gained serious attention for years. Moreover, although there is a growing literature on VOCs of bacterial origin with their functional aspects (Schulz and Dickschat 2007; Junker and Tholl 2013; Piechulla and Degenhardt 2014), much less attention has paid to the fungal VOCs (FVOCS) (Bennett et al. 2013; Bitas et al. 2013; Schulz and Dickschat 2007; Piechulla and Degenhardt 2014; Kanchiswamy et al. 2015).

---

S. Roy · D. Banerjee (✉)
Microbiology and Microbial Biotechnology Laboratory, Department of Botany and Forestry,
Vidyasagar University, Midnapore, West Bengal, India
e-mail: db@mail.vidyasagar.ac.in

A. N. Yadav et al. (eds.), *Recent Advancement in White Biotechnology Through Fungi*, Fungal Biology, https://doi.org/10.1007/978-3-030-14846-1_5

Approximately 1000 volatile organic compounds (mVOCs) are cited in different reports as produced by 400 different bacteria and fungi so far, among which only 300 VOCs have been characterized from fungi (Chiron and Michelot 2005; Korpi et al. 2009; Lemfack et al. 2014). Interestingly, in the laboratory it has been found that individual fungal species produce a typical pattern of VOCs that may vary depending on growth conditions. The specific profile of volatiles produced by each fungal species is strongly dependent on such environmental factors as temperature, pH, moisture level, nutrients, and age of the culture (Wilkins and Larsen 1995; Bennett et al. 2013; Morath et al. 2012). However, during the past two decades, research on microbial volatile metabolites has significantly intensified. The recent findings about the importance of such volatile metabolites in microbial interactions within fungi or fungi and bacteria and even in the communication between fungi and plants or animals are no doubt very interesting in basic and applied research. Presently, the potential biotechnological applications of such mVOCs are also well illustrated. *Aspergillus*, *Penicillium*, *Alternaria*, *Cladosporium*, *Mucor*, and *Ulocladium* are among the most common VOC-producing fungal genera found in our environment (Bennett and Inamadar 2015). Fungi are capable of producing a plethora of volatile organic compounds (VOCs) belonging to diverse chemical classes, such as terpenoids, straight-chain and branched hydrocarbons, benzene derivatives, naphthalene derivatives, cycloalkanes, alcohols, organic acids, ketones, and aldehydes (Mends et al. 2012; Riyaz-Ul-Hassan et al. 2012; Strobel et al. 2008; Tomsheck et al. 2010), with some special structures.

Molecular approaches such as metagenomics indicate that less than 5% of all fungal species expected to exist on our planet have been characterized thus far (Riyaz-Ul-Hassan et al. 2013). Furthermore, strain variations among the same fungal species suppose them to be highly diverse and suitable for the discovery of new chemical entities, enzymes, and useful volatile organic compounds. The search is now on for novel strains of microorganisms from variegated environments including extreme ecological niches such as geothermal vents, hot springs, the ocean bed, and cold deserts (Foissner 1999). Human beings have been dependent on plants from the very initiation of our civilization, but later gradually we became habituated to synthetic compounds. Surprisingly, after receiving unsatisfying results from synthetic chemical compounds, now we are again in search of natural compounds and here also plants are our savior.

Every plant in this earth harbors a suite of microorganisms which are not harmful to them but on the contrary help their host in many ways. Such classes of microbes are called endophytes. Endophytic microbes are highly diverse and metabolically very sound! Because very little research has been done on endophytic fungi and their volatile organic metabolites, there is a high prospect of finding untold numbers of novel fungal genera existing as plant-associated microbes as well as many novel volatile compounds with significant bioactivity (Strobel and Daisy 2003; Rana et al. 2018a; Rana et al. 2018b; Rana et al. 2016; Suman et al. 2016). Endophytic fungal VOCs are found to induce positive changes in plant growth and vigor, which might be a blessing for agriculture as the demand on agricultural production for fiber, food, and fuel increases exponentially for our ever-growing human population.

Also, at present fungal VOCs (FVOCs) are increasingly being applied in controlling plant pathogens (mycofumigation), in mycodiesel or fuel production and in biosensor production.

Fungal VOCs influence plant growth and defense, interspecies interactions among plants, bacteria, fungi, and nematodes, as attractants of natural enemies, as bio-control agents, and are finding suitable application as pest/insect/herbivore management (Kanchiswamy et al. 2015; Davis et al. 2013; Weise et al. 2014; D'Alessandro et al. 2014). One unique endophytic fungus, *Muscodor vitigenus*, produces sufficient concentration of naphthalene to alter insect behavior (Daisy et al. 2002). This progressive study on endophytic fungal volatile organic compounds (eFVOCs) demonstrates their critical roles in multitrophic interactions and their importance in both the ecosystem and sustainable agriculture systems.

## 5.2 VOC-Mediated Interaction in Fungal Endophytes

Plant roots are thought to be an important entry point for endophytic fungi: it is assumed that soil fungi enter the plant root tissues through any mechanical incision or abrasion. Roots release a diverse mixture of low and high molecular weight organic compounds that make the root tissues and surrounding environment nutrient rich for a diverse community of microbes (Badri and Vivanco 2009). Soil-borne fungi first colonize at the rhizosphere, then invade the intercellular space, and act as commensals or mutualistic endophytes (Yadav 2018; Yadav et al. 2017; Hardoim et al. 2008; Reinhold-Hurek and Hurek 2011), or dwell within the root tissues as intracellular endosymbionts (Bonfante and Genre 2010; Desbrosses and Stougaard 2011).Volatile organic compounds (VOCs) typically occur as a complex mixture of low molecular weight lipophilic compounds generated at different steps of various metabolic pathways.

The term "volatilome" recently has been proposed to illustrate their structural and functional importance (Maffei et al. 2011). VOCs are responsible for interspecies and intraspecies communication, with involvement in innumerable interactions among plants, antagonists, or mutualistic symbionts in the environment both below and above the ground (Maffei et al. 2010; Wang and Maffei 2011; Garbeva et al. 2014; Lemfack et al. 2014; Kanchiswamy et al. 2015). With their comprehensive inter- and intraspecific interactions, VOCs cause genetic and phenotypic variation in the interacting organisms (Effmert et al. 2012; Piechulla and Degenhardt 2014; Penuelas et al. 2014).

Fungal endophytics are able to detect host plants through a composite array of molecular signaling and initiate their colonization in the very rhizoplane by producing specific plant growth regulating volatile organics (Ortiz-Castro et al. 2009). Additional signals from microbes have a role in plant root morphogenesis. Very recently the role of *N*-acyl homoserine lactone (AHL) has been recognized as a signal molecule in plants altering gene expression in roots; shoots thus modulate defense and cell growth responses (Ortiz-Castro et al. 2009; von Rad et al. 2008).

The endophytic fungus *Gilmaniella* sp. AL12 induced ethylene production in *Atractylodes lancea*, as found in a recent study by Yuan et al. (2016). Pre-treatment of this plantlet with amino oxyacetic acid, that is, an ethylene inhibiter, also suppressed endophytic fungi-induced accumulation of ethylene and sesquiterpenoids. Table 5.1 lists the VOCs produced by various endophytic fungi. The hypotheses generated from this study imply VOCs released by endophytic fungi can provide an important signal mediating the biosynthesis of sesquiterpenoids in *Atractylodes lancea*.

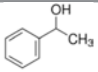
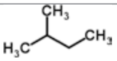
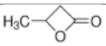
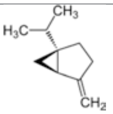
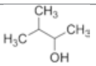
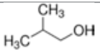
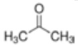
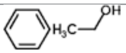
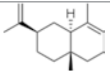
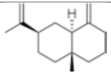
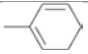
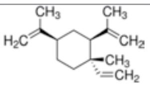
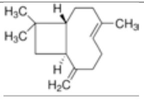
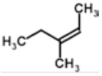
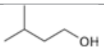
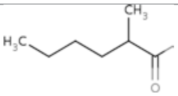
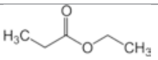
## 5.3 Bioactivity of Endophytic Fungal Volatiles (eFVOCs)

Fungi are an extraordinarily diverse group of microorganisms that are found in many habitats, even competing with other microorganisms. Endophytes spend a long time in mutual relationships with their host plants. Endophytic association often seems confused with plant pathogens or surface dwellers. So, to verify that a microorganism has an endophytic lifestyle in the true sense, it must should be successfully reintroduced into disinfected seedlings and judged by microscopy, thereby also fulfilling Koch's postulates (Hyde and Soytong 2008). Currently, endophytic fungi have received much recognition for interesting metabolic potential and useful secondary metabolites. From the biotechnological point of view, volatile-producing endophytic fungi exert a broad spectrum of odorous compounds with different physicochemical and biological properties that make them useful in both industry and agriculture (Yuan et al. 2012) (Fig. 5.1).

### *5.3.1 Fungal Volatiles as Antimicrobial to Plant Pathogens*

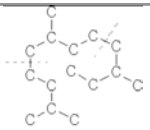
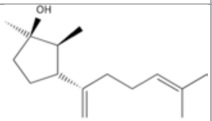
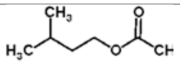
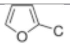
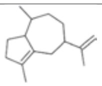
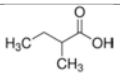
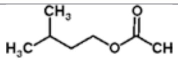
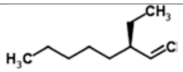
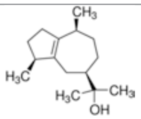
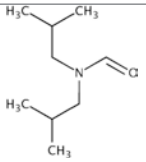
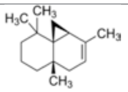
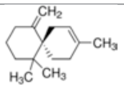
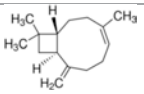
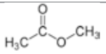
Fungal endophytes somehow manage habitat adaptation within the plant inner tissues, resulting in improved performance concerning plant protection from various biotic and abiotic stresses. Fungi are considered as a large cell factory for volatiles that can harness organic natural products with possibilities for the development of biocontrol agents. Certain endophytes produce antimicrobial VOCs that directly contribute to plant defense against pathogenic microorganisms. The endophytic fungus *Muscodor albus* can be considered as the most active candidate strain in this context. This strain was first reported as an endophyte from *Cinnamomum zeylanicum* (Worapong et al. 2001). It efficiently inhibits and executes selected other plant pathogenic fungi and bacteria by producing a suite of different volatiles (Strobel et al. 2001) that accounts for at least 28 different volatile organic compounds. Surprisingly, although few individual compounds were antagonistic to certain pathogens, a strong synergistic effect was detected, even to lethality, for a broad range of plant and human pathogenic fungi and bacteria. Derivatives of alcohols, esters, ketones, acids, and fatty acids were found as principal components in the

**Table 5.1** List of different volatile organics isolated from endophytic fungi

| Endophytes | Host plant | eFVOCs | Structure | References |
|---|---|---|---|---|
| *Aspergillus niger* | *Rosa damascena* | 2-Phenylethanol |  | Wani et al. (2010) |
| *Botrytis* sp. BFT21 | *Musa* sp. | 2-Methylbutane |  | Ting et al. (2010) |
|  |  | β-Butyrolactone |  |  |
|  |  | 2-Butene dinitrile |  |  |
| *Phomopsis* sp. | *Odontoglossum* sp. | Sabinene |  | Singh et al. (2011) |
|  |  | 3-Methylbutan-1-ol |  |  |
|  |  | 2-Methylpropan-1-ol |  |  |
|  |  | Acetone |  |  |
|  |  | Benzene ethanol |  |  |
| *Nodulisporium* sp. | *Cinnamomum loureirii* | α-Selinene |  | Park et al. (2010) |
|  |  | β-Selinene |  |  |
|  |  | 2,5-Dihydrotoluene |  |  |
|  |  | β-Elemene |  |  |
| *Phialocephala fortinii* | Tree root endophyte | β-Caryophyllene |  | Kramer and Abraham (2012) |
| *Clonostachys rosea* (*Gliocaldium roseum*) strain C-13 = NRRL 50072 | *Eucryphia cordifolia* | 2-Pentene |  | Griffin et al. (2010) |
|  |  | 3-Methylbutan-1-ol |  |  |
|  |  | 2-Methylhexanoate |  |  |
|  |  | Heptane |  |  |
|  |  | Octane |  |  |
|  |  | 2-Butyl propionate |  |  |

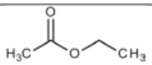
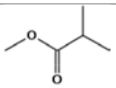
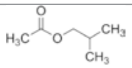
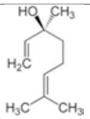
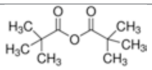
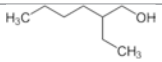
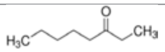
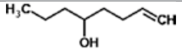
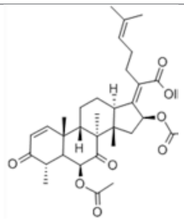
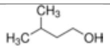
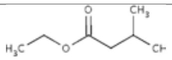
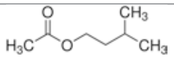
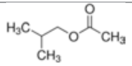
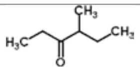
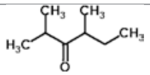
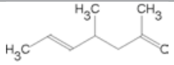
(continued)

**Table 5.1** (continued)

| Endophytes | Host plant | eFVOCs | Structure | References |
|---|---|---|---|---|
| *Epichloe typhina* | *Phleum pratense* | Sesquiterpenes |  | Steinebrunner et al. (2008) |
| | | Chokol K |  | |
| *Muscodor albus* | *Cinnamomum zeylanicum* | 1-Butanol 3-methyl acetate |  | Strobel et al. (2001) |
| *Muscodor albus* I, 41.3 s | Unidentified tree species | 2-Methyl furan |  | Atmosukarto et al. (2005) |
| | | Aciphyllene |  | |
| *Muscodor albus* E-6 | *Guazuma ulmifolia* | 2-Methyl-butanoic acid |  | Strobel et al. (2007) |
| | | 3-Methyl butyl ester |  | |
| | | 3-Ethyl 1-octene |  | |
| | | Guaiol |  | |
| | | *N*-(1-Methylpropyl) formamide |  | |
| *Muscodor sutura* | *Prestonia trifidi* | Thujopsene |  | Kudalkar et al. (2012) |
| | | Chamigrene |  | |
| | | Isocaryophyllene |  | |
| *Muscodor albus* GBA | *Ginkgo biloba* | 3-Methyl acetate |  | Banerjee et al. (2010) |
| | | 1-Butanol |  | |

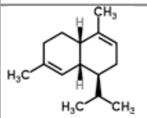
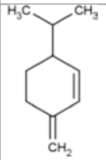
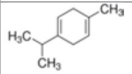
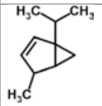
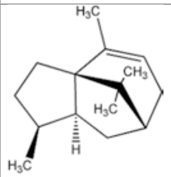
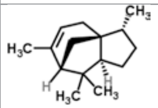
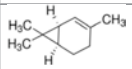
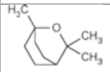
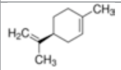
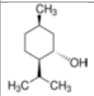
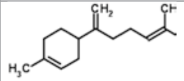
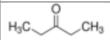
(continued)

**Table 5.1** (continued)

| Endophytes | Host plant | eFVOCs | Structure | References |
|---|---|---|---|---|
| *Muscodor albus* MOW12 | *Piper nigrum* | Acetic acid ethyl ester |  | Banerjee et al. (2014) |
| | | Propanoic acid 2-methyl-methyl ester |  | |
| | | Acetic acid 2-Methylpropyl ester |  | |
| *Daldinia bambusicola* | *Camellia caduca* | Linalool |  | Pandey and Banerjee (2014) |
| | | Pivelic acid anhydride |  | |
| | | 2-Ethylhexanol |  | |
| *Hypoxylon* sp. | *Persea indica* | 3-Octanone |  | Tomsheck et al. (2010) |
| | | 7-Octene-4-ol |  | |
| *Pichia guilliermondii* | *Paris polyphylla* var. *yunnanensis* | Helvolic acid |  | Zhao et al. (2010) |
| *Geotrichum candidum* | *Solanum melongena* | 3-Methyl-1-butanol |  | Mookherjee et al. (2018) |
| | | Ethyl 3-methyl butanoate |  | |
| | | Isopentyl acetate |  | |
| | | Isobutyl acetate |  | |
| *Nodulisporium* sp. (*Hypoxylon* sp.) | *Thelypteris angustifolia* | 4-Methyl-3-hexanone |  | Riyaz-Ul-Hassan et al. (2013) |
| | | 2,4-Dimethyl-3-hexanone |  | |
| | | 4-Methyl 5-hepten 2-one |  | |

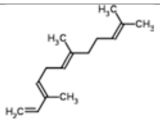
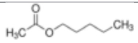
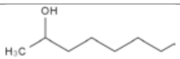
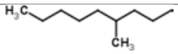
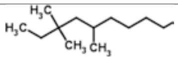
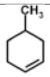
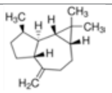
(continued)

**Table 5.1** (continued)

| Endophytes | Host plant | eFVOCs | Structure | References |
|---|---|---|---|---|
| *Diaporthe* spp. | *Catharanthus roseus* | Muurolene |  | Yan et al. (2018) |
| | | Phellandrene |  | |
| | | Terpinene |  | |
| | | Thujene |  | |
| | | Patchoulene |  | |
| | | Cedrene |  | |
| | | 2-Carene |  | |
| *Nodulisporium* sp. GS4d2II1a | *Gliricidia sepium* | Eucaliptol |  | Sánchez-Fernández et al. (2016) |
| | | Limonene |  | |
| *Diaporthephaseolorum* | *Picrorhiza kurroa* | Isomenthol |  | Qadri et al. (2015) |
| | | β-Bisabolene |  | |
| | | 3-Pentanone |  | |

(continued)

**Table 5.1** (continued)

| Endophytes | Host plant | eFVOCs | Structure | References |
|---|---|---|---|---|
| *Gliocladium roseum* (NRRL 50072) | *Dicksonia antarctica* | 4-Decene |  | Strobel et al. (2017) |
| | | α-Farnesene |  | |
| | | Pentyl ester |  | |
| *Gliocladium roseum* (NRRL 50072) | *Eucryphia cordifolia* | Pentyl alcohol |  | Strobel et al. (2008) |
| | | 2-Octyl acohol |  | |
| | | Undecane 2,6-di-methyl |  | |
| | | Decane 3,3,5-trimethyl |  | |
| | | Cyclohexene, 4-methyl |  | |
| *Muscodor yucatanensis* | *Bursera simaruba* | 2-Pentyl furan |  | Macias-Rubalcava et al. (2010) |
| | | Aromadendrene |  | |

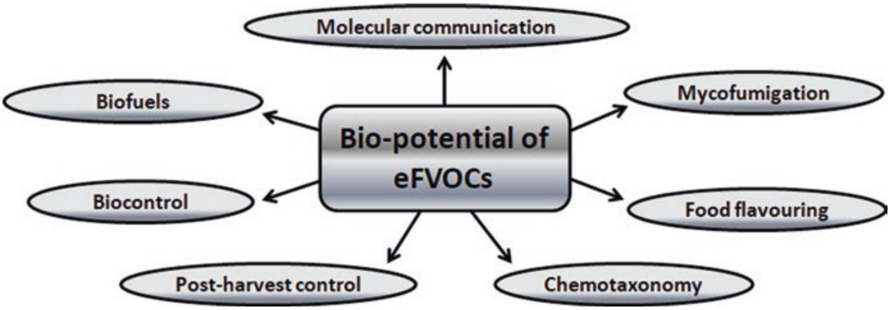


**Fig. 5.1** Bioactive potential of volatile organic compounds from endophytic fungi

metabolite mixture of this endophytic strain, among which isoamyl acetate was the most promising biologically active compound (Strobel et al. 2001).

Another endophyte to *Mucor albus* was isolated emitting a number of volatiles, such as tetrohydofuran, aciphyllene, and azulene derivatives (Atmosukarto et al. 2005). The mixture of volatiles effectively inhibited a broad range of plant and human pathogenic bacteria. *Muscodor albus* E-6, an endophyte of *Guazuma ulmifolia*, was isolated by Strobel et al. (2007). This organism produces several unique VOCs that were not previously reported for any other species of *Muscodor*. The volatile metabolites produced by this strain include 2-methyl butanoic acid, 3-methyl butanoic acid, 2-methyl-2-butenal, butanoic acid, 3-methyl butyl ester, 3-methyl-3-buten-1-ol, guaiol, 3-ethyl-1-octene, *N*-(1-methyl propyl) formamide, and certainly azulene with naphthalene derivatives. The mixture is highly effective against an array of plant pathogenic fungi and bacteria.

The endophytic fungus *Muscodor sutura* was observed to produce a mixture of volatile organics including thujopsene, chamigrene, isocaryophyllene, and 2-methyl butanoic acid, which has not been previously reported by any other fungi of the same genus (Kudalkar et al. 2012). This volatile mixture emitted by *M. sutura* inhibited (100% mycelial inhibition) a set of 13 fungal pathogens after only 2 days of exposure. *Muscodor albus* strain GBA was isolated as an endophytic fungus of *Ginkgo biloba*. The strain showed strong inhibitory and killing effects toward test fungal pathogens by its released VOCs. The chemical analysis of VOC revealed derivatives of esters, lipids, alcohols, acids, and ketones with a high concentration of 1-butanol and 3-methyl acetate (Banerjee et al. 2010).

Subsequently, seven new *M. albus* strains were isolated producing various novel mixtures of volatile secondary metabolites. *Muscodor albus* MOW12 (Fig. 5.2), was isolated as an endophytic fungus with antifungal activity from *Piper nigrum* in Mawlong, India. This Xylariaceae-derived endophyte produced low molecular weights of ester, alcohol, and acid derivatives. The major ester components found within a volatile mixture of this isolate are acetic acid ethyl ester, propanoic acid 2-methyl-methyl ester and acetic acid 2-methylpropyl ester (Banerjee et al. 2014). The volatile chemical profile of each *Muscodor* strain significantly varies from one another; even their antagonistic pattern is also remarkable. Some species release large amounts of chemicals as volatile secondary metabolites; all together about 50 different volatiles are documented to be produced by a fungal isolate with endophytic association exerting an impressive antimicrobial spectrum (Ezra et al. 2004). Mycofumigation with *M. albus* against pathogens was already reported in smut-infected barley seeds, and 100% disease control was reported by Strobel et al. (2001). VOC-producing endophytic fungi were also experimentally tested for the treatment of fruits in storage and in transit (Mercer and Jimenez 2004). Soil treatments have also been effectively used in both field and greenhouse situations (Mercier and Manker 2005).

Mitchell and Strobel (2010) isolated *Muscodor crispans*, endophytic to *Ananas ananassoides*. This strain produces a mixture of antifungal and antibacterial volatile organic compounds that strongly inhibits *Pythium ultimum*, a potential plant pathogenic fungus. This strain was also found active against *Alternaria helianthi*, *Botrytis*

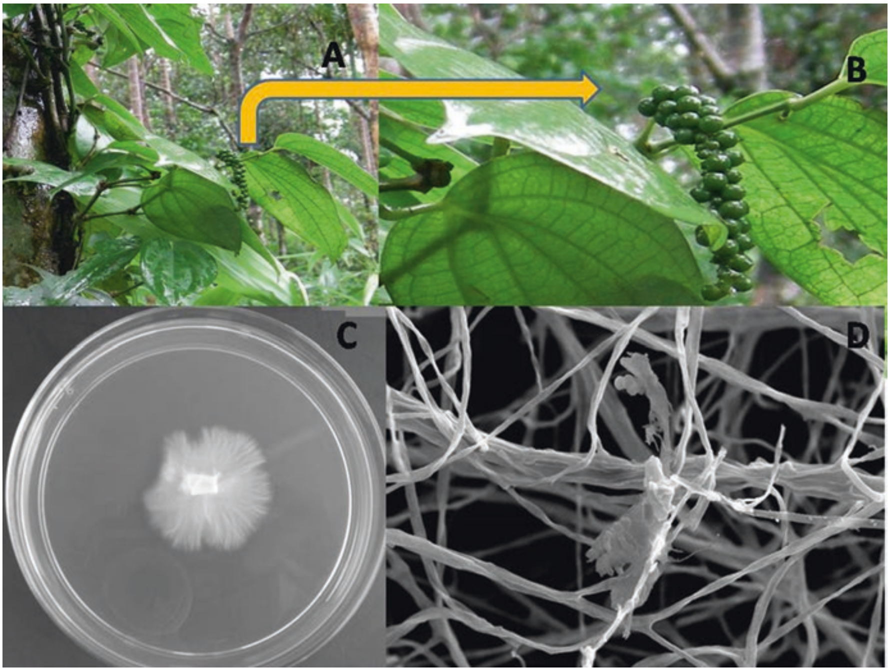


**Fig. 5.2** The endophytic strain of *Muscodor albus* MOW12 isolated from *Piper nigrum*: (**a**) the plant, *Piper nigrum*; (**b**) larger view of leaves and fruits of this plant; (**c**) colony on potato dextrose agar media; (**d**) scanning electron microscopy (SEM) observation of 10-day-old culture of *Muscodor albus* MOW12

*cinerea*, *Fusarium culmorum*, *F. oxysporum*, *Phytophthora cinnamomi*, *P. palmivora*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum,* and *Verticillium dahlia* and even to the plant pathogenic bacterium *Xanthomonas axonopodis* pv. *citri*. The volatile mixture was checked for environmental safety issues and approved as safe by GARS (Generally Regarded as Safe), and no azulene or naphthalene derivatives were detected in the volatile mixture of *Muscodor crispans*, making it a potential candidate strain for commercial application. Thus, this mixture was suggested to have potential utility in applications ranging from food preservation derivatives to agricultural, household, and industrial uses by the US Food and Drug Administration. Stinson et al. (2003) isolated another fungus, *Gliocladium* sp., an endophyte of *Eucryphia cordifolia*, that was discovered to be a volatile antibiotic producer. Many volatile organic compounds were analyzed from the endophytic fungus *Daldinia concentrica*, isolated from olive trees in Israel. This fVOCs mixture was found very promising for post-harvest control. It was effective when used experimentally to protect dried apricots, plums, and raisins from rotting. Moreover, the fVOCs significantly protected peanuts against *Aspergillus niger*, oranges and tomato paste from *Penicillium*, and grapes against *Botrytis*. Another reported endophytic fungus is *Myrothecium inunduatum* from a euphorbeacean herb, *Acalypha indica*, in India (Banerjee et al. 2010).

The authors reported that *M. inundatum* that was cultivated in shake culture flasks produced an abundance of VOCs that showed effective inhibitory effects on the growth of *Phythium ultimum* and *Sclerotinia sclerotiorum*. The volatile mixture contained various hydrocarbons and hydrocarbon derivatives with several terpenes, organic acids, ketones, and alcohols. In another experiment, the volatile antimicrobial metabolites were used to increase the shelf life of fruits and vegetables, as was experimentally shown by Pandey and Banerjee (2014). Endophytic *Daldinia bambusicola* was isolated from *Camellia caduca*. The strain produced linalool, benzene ethanol, and other volatile organics that were able to kill *Phytopthora palmivora* as well as significantly inhibit the growth of *Geotrichium* sp., *Alternaria* sp., *Colletotrichum lagenarium*, *Botrytis cinerea*, and a few others with mild inhibition. The most prevalent compounds were 3-octanone, 3-octanol, and 7-octen-4-ol analyzed, from an endophytic *Hypoxylon* sp. isolated from *Persea indica*, a widespread Laurasian tree of the Mediterranean flora, which produces a plethora of FVOCs with a high effectiveness to treat *Phytophthora cinnamomi*, *P. palmivora*, *Cercospora beticola*, *Aspergillus fumigatus*, and *Sclerotinia sclerotiorum* (Tomsheck et al. 2010). *Pichia guilliermondii* (endophyte to *Paris polyphylla* var. *yunnanensis*) is reported to emit several volatile compounds including helvolic acid. This volatile compound exerts high antifungal activity by inhibiting spore germination of *Magnoporthe oryzae*, one of the most devastating pathogens of rice (Zhao et al. 2010).

Chokol K, another volatile organic compound produced by the grass endophyte *Epichloe* sp. (Clavicipitaceae), effectively inhibited the growth and spore germination of two mycoparasites associated with stomata and two plant pathogenic fungi (Steinebrunner et al. 2008). Endophytic fungi from Orchidaceae were investigated by Singh et al. (2011) for VOCs. *Phomopsis* sp., isolated from *Odontoglosum* sp., produces 3-methyl-1-butanol, 2-methyl-1-propanol, benzene ethanol, and 2-propanone as principal components in the volatile metabolite mixture. Experimental observation indicated that an artificial mixture of these compounds also displayed strong growth inhibition of several fungal pathogens, including *Pythium ultimum*, *Phytophthora palmivora*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Fusarium solani*, *Botrytis cinerea*, *Colletotrichum lagenarium*, and *Verticillium dahlia*. Endophytic *Botrytis* sp. BTF21, which was isolated from *Musa* sp., was found to produce 2-methyl-butane, β-butyrolactone, and 2-butene-dinitrile as volatile secondary metabolites (Ting et al. 2010). The VOC produced by this strain was found to have biocontrol potential to *Fusarium oxysporum*, which is considered to be a serious plant pathogen. *Nodulisporium* sp. and a few more endophytic fungi were isolated from *Cinnamomum loureirii*. As volatile organics, β-elemene, α-selinene, β-selinene, and 2,5-dihydrotoluene were obtained after chemical analysis of the volatile mixture of this *Nodulisporium*. The volatile mixture was successfully applied as a post-harvest disease control of apples (Park et al. 2010).

The volatile sesquiterpene β-caryophyllene was identified to be produce by the endophyte *Phialocephala fortinii* with potential anti-fungal activity (Kramer and Abraham 2012). The VOCs of *Oxyporus latemarginatus* EF069, which was isolated as an endophyte from red peppers, also had a negative effect on the mycelial growth of several plant pathogens. Mycofumigation with this endophytic fungus was successfully achieved for control of post-harvest apple decay and root rot by *Rhizoctonia* on moth orchid (Lee et al. 2009). *Aspergillus niger* JUBT 3M, isolated from *Rosa damascena*, is also able to produce VOCs. Chemical analysis reveals the production of 2-phenylethanol as a volatile organic by this isolate. The commercial applications of phenyl ethanol include its use in antiseptics, disinfectants, antimicrobials, and preservatives in pharmaceuticals (Wani et al. 2010).

A *Phoma* sp. was isolated and identified as endophytic of *Larrea tridentata*. This fungus produces a unique mixture of VOCs including a series of sesquiterpenoids, some unusual alcohols, and several reduced naphthalene derivatives. Trans-caryophyllene, considered as a product in the fungal VOCs, was also noted in the VOCs of this plant. The volatile mixture produced by *Phoma* sp. exerts strong antifungal effects on *Verticillium*, *Ceratocystis*, *Cercospora*, and *Sclerotinia*. Here it must be noted that this antifungal profile of endophytic isolates is markedly similar to that of the methanolic extract of the host plant (Strobel et al. 2011). Six volatile organic compounds were obtained from two endophytic fungi (*Nodulisporium* sp. strain GS4d2II1 and *Hypoxylon anthochroum* strain Blaci) that were also determined for their bioactivity by Medina-Romero et al. (2017). Results showed that the VOCs have a significant concentration-dependent antifungal effect individually and also act strongly in a synergic manner in both in vivo and in vitro conditions. They also concluded that the mixture of the six compounds may be used for post-harvest control of *F. oxysporum* against tomato wilt. *Geotrichum candidum* was isolated as an endophytic from the fruit *Solanumm elongena*. The volatile mixture produced by this fungus contains 3-methyl-1-butanol, ethyl-3-methylbutanoate, 2-phenylethanol, isopentyl acetate, naphthalene, and isobutyl acetate in significant proportions. The strain showed significant growth inhibition of *Rhizoctonia solani*, a potent plant pathogen. Mild antifungal activity against a few other fungal pathogens was also recorded by this strain. However, the effectiveness of the antimicrobial property of this volatile mixture was enhanced with the exogenous addition of naphthalene (1.0 mg/plate) by Mookherjee et al. (2018).

The volatile composition produced by endophytic fungi *Alternaria alternata* and *Penicillium canescens* (from the leaves of *Olea europaea* L.) displayed a large antifungal spectrum: the six most abundant volatiles were 3-methyl-1-butanol and phenylethyl alcohol (Malhadas et al. 2017). Another *Nodulisporium* species (*Hypoxylon* sp.) has been isolated as an endophyte to *Thelypteris angustifolia*. The endophyte produces VOCs that produce fuel (mycodiesel) and are also used for biological control of plant disease. The organism was responsible for the unique production of a series of ketones, including acetone, 2-pentanone, 4-methyl-3-hexanone, 2,4-dimethyl-3-hexanone, 2-hexanone, and 4-methyl-5-hepten-2-one, with significant concentration in addition to 1-butanol and phenyl ethanol alcohol. The VOCs produced by this strain were found to be lethal to *Phytophthora palmivora*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Phytophthora cinnamomi* (Riyaz-Ul-Hassan et al. 2013). *Diaporthe* sp. was isolated as an endophytic fungus from *Catharanthus roseus* (Yan et al. 2018). Identification of its volatile metabolites showed terpenes including muurolene, phellandrene, terpinene, and thujene, as well as other minor terpenoids such as caryophyllene, patchoulene, cedrene, 2-carene, and thujone. The isolated VOC mixture exhibited significant antifungal properties against a wide range of plant pathogenic test fungi and oomycetes, including *Alternaria alternata*, *Botrytis cinerea*, *Colletotrichum gloeosporioides*, *Fusarium graminearum*, and *Phytophthora cinnamomi*. A total of 70 VOCs were detected, among which mono- and sesquiterpenes, especially eucalyptol and limonene, were a significant fraction from the endophytic *Nodulisporium* sp. GS4d2II1a (Sanchez-Fernandez et al. 2016).

The antagonism assay indicated strong inhibition to oomycetes plant pathogens, including *Pythium aphanidermatum* of economically important crops. Endophytic *Diaporthe phaseolorum* associated with the rhizome of *Picrorhiza kurroa* was evaluated for antimicrobial properties of its volatile metabolites. The strain was found to produce a unique array of VOCs, particularly menthol, phenylethyl alcohol, (+)-isomenthol, β-phellandrene, β-bisabolene, limonene, 3-pentanone, and 1-pentanol. VOCs produced by this strain are selectively active against the growth of plant pathogenic fungi. The role of this endophyte in endophytic association may be to inhibit the growth of pathogens responsible for root rot of the host (Qadri et al. 2014).

In search of VOC-producing endophytic fungi, Strobel et al. (2017) discovered *Urnula* sp., an endophytic fungus of *Dicksonia antarctica*. About 150 different compounds have been detected and identified from the volatile mixture released by this strain by employing carbotrap methodology. The most notable compounds found in the volatile metabolites produced included 4-decene, tridecene, 2-decene, α-farnesene, butanoic acid, and pentyl ester. In vitro assay showed moderate to strong growth inhibition against some common fungal plant pathogens. Although the antimicrobial potential of eFVOCS is well established, only a very few attempts have made to establish its mechanism and for its commercial application.

A very recent article published by Alpha et al. (2015) nicely described the probable mode of action of VOCs produced by *Muscodor albus* CZ-620 through a series of genetic screening and biochemical assays. This experiment suggests that the VOCs produced by this organism may induce alkylation of DNA and ultimately lead to strand breakage in *E. coli*. Additional cytotoxicity profiling indicated that during VOC exposure, *E. coli* became filamentous, with increased cellular membrane permeability. The volatile nature of the toxic compounds produced by *M. albus* and their broad range of inhibition suggest this fungus as an attractive biological agent.

### *5.3.2 Fungal Volatiles as Diesel Components*

The liquid hydrocarbon fuels have high demand worldwide because of the high volumetric density and relative ease of production, transport, and storage (Santos et al. 2014). Many factors are now forcing us to search for alternative sources of liquid fuels, including issues of the diminishing supplies of these fossil hydrocarbons and serious concerns about climatic changes caused by rising levels of greenhouse gases throughout the world's atmosphere. Plant-derived lipids and bioethanol from the fermentation of sugars and starch are considered an important alternative energy source, but the enormous demand for fossil-based hydrocarbon fuels such as coal, natural gas, and oil cannot be met with the present supply of such alternate energy. Attempts are underway to find still other alternative eco-friendly approaches to increase the production of liquid fuels. Volatile organic compounds (VOCs) are considered to be carbon-based compounds that can readily enter into the gas phase by vaporizing at 0.01 KPa at approximately 20 °C (Pagans et al. 2006).

Most such compounds are lipid soluble and thus have low water solubility. Approximately 250 VOCs have been identified from fungi of diverse ecological niches where they exist as mixtures of simple hydrocarbons, heterocycles, aldehydes, ketones, alcohols, phenols, thioalcohols, thioesters and their derivatives, benzene derivatives, and cyclohexanes (Chiron and Michelot 2005; Korpi et al. 2009; Ortiz-Castro et al. 2009). Recently, endophytic fungi have been extensively studied for production of hydrocarbons and hydrocarbon-like compounds. These compounds have high potential to be used as both "green chemicals" and fuels. *Gliocladium roseum* NRRL 50072, an endophyte isolated from *Eucryphia cordifolia*, produces volatile organics as secondary metabolites (Strobel et al. 2008).

Chemical analysis of the VOC mixture of this fungus revealed an assemblage of alcohols, ketones, and hydrocarbons including pentyl, hexyl, heptyl, octyl, and secondary octyl, decylalcohols, undecane, 2,6,-dimethyl decane, 3,3,5-trimethyl cyclohexene, 4-methyl decane, and 3,3,6-trimethyl undecane. Quantification of the VOCs was determined by proton transfer mass spectrometry (PTR-MS), resulting in organic substances of 80 ppmv (parts per million by volume) in the headspace atmosphere above the media.

The hydrocarbon composition produced by this endophytic fungus contains a number of compounds that are commonly associated with diesel fuel. The mother composition of all types of diesel fuels are the straight-chain hydrocarbons such as hexane, heptane, octane, nonane, and decane along with the branched alkanes, cyclic alkanes, a plethora of benzene derivatives, and some polyaromatic hydrocarbons (Hsu et al. 2000). Currently, all the endophytic fungi producing volatile compounds are being studied for hydrocarbon production or fuel production. An interesting finding by Shaw et al. (2015) states that a nine-carbon polyketide alkene produced by the endophytic fungus *Nigrograna mackinnonii* is likely to be useful for gasoline applications. A great diversity among endophytic fungi is being isolated to date, which implies the occurrence of enormous chemical diversity as invariably moderation of secondary metabolites acts as a weapon in such highly competitive ecosystems (Strobel and Daisy 2003). *Hypoxylon* sp., isolated from *Persea indica*, was found to be an important discovery in this regard: it produces such a volatile organic mixture with fuel properties, that is, mycodiesel.

The VOCs produced by this organism were measured by PTR-MS covering a continuous range of VOC production rate of 7.65 ppmv/h: the VOX mixture consists of 1,8-cineole (a monoterpene), benzene, naphthalene, and 1-methyl-1,4-cyclohexane.

Cineole, known as eucapyptol, can be used in an 8:1 blend with gasoline, whereas this VOC mixture can be used as a diesel fuel additive. The researchers of this group claimed that the ability to produce such rare compounds from a fungal source greatly expands their potential applications in medicine, industry, and energy production. The endophytic *Nodulisporium* sp. also produced some VOC mixtures having both antimicrobial and fuel properties. The presence of cyclohexane, propyl, etc. is considered to be a potential source for a fuel alternative for major diesel components.

The endophytic fungus *Phomopsis* sp. was found to produce 1-butanol, 3-methyl benzene ethanol, 1-propanol, and 2-methyl 2-propanone, which may be used as additives to gasoline. However, extensive basic and applied research is needed to establish hydrocarbon production by these endophytic fungi at the industrial level for additives as liquid biofuel. Such candidate endophytic fungi with fuel potential should be selected for metabolic engineering and scale-up processes for the production of cost-effective alternate fuels that will also be eco-friendly.

### 5.3.3 Fungal Volatiles as Alleochemicals and Communicating Signals

Allelopathy is a biological phenomenon by which metabolites produced by one organism can influence the germination, growth, survival, and reproduction of other organisms. The biochemicals or metabolites having such properties are called allelochemicals. The term allelopathy was first coined by Hans Molisch in 1937. Alleochemicals are in general a subclass of secondary metabolites produced by plants or microorganisms. The negative role of alleochemicals imparts advantages to plant defenses against herbivores. These biochemicals contribute significantly to species distribution and abundance in plant communities and thus give a constant structure of an ecological niche. Applying such allelochemicals in weed management has become an interesting strategy as an environmentally friendly approach. In the agro-ecosystem, weeds compete with valuable crops for nutrient resources and crop handling that results in reduced crop yield with reduced crop quality and ultimately a huge financial loss every year. Although there are several mechanical and chemical strategies for weed control, resistance to chemical herbicides, change in weed composition, and certainly the potential health hazards of such chemicals forces us to find some alternate strategy to control weeds. In this regard, natural products released from plants or microorganisms are considered as promising alternative options. Allelopathic plant growth inhibition has been demonstrated repeatedly in laboratory-scale experiments, but more realistic field studies involving semi-natural or natural soils are often inconclusive (Inderjit et al. 2005; Macias and Galindo 2007; Kaur et al. 2009).

However, an experiment was well conducted by Macias-Rubalcava et al. (2010) in this regard with *Muscodor yucatanensis*, an endophytic fungus, isolated from the leaves of *Bursera simaruba*. The volatile mixture produced by this endophytic

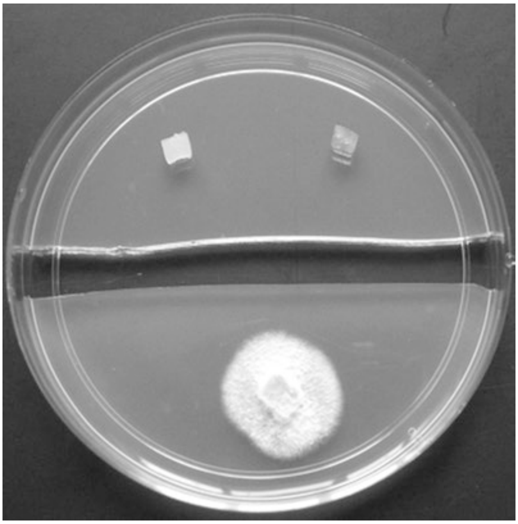


**Fig. 5.3** Split plate assay for determination of antifungal activity of VOCs released by endophytic *Muscodor albus* MOW 12 against test pathogens

fungus was analyzed for allelopathic effect on root elongation in amaranth, tomato, and barnyard grass. Significant phytoinhibition was observed by split plate assay (Fig. 5.3) containing potato dextrose agar (PDA) inoculated with *M. yucatanensis* in one compartment. Maximum root growth inhibition was exhibited by a 10-day-old culture of this strain. .Microbes can colonize the surfaces of plant roots, leaves, and flowers in varied proportions, which is dependent on some chemical signaling. In this context volatile fungal metabolites can be considered important signals in the communication of plant-associated fungi in the rhizosphere and endosphere.

## 5.4 Techniques for VOC Analysis from Endopytic Fungi

Fungi release surplus volatile organic compounds (VOCs) as mixtures of low molecular mass alcohols, aldehydes, esters, terpenoids, thiols, and other small molecules that easily volatilize. Most techniques of VOCs determination including separation and identification now rely on gas chromatography–mass spectrometry (GC-MS). In addition, developments in sensor technology promise to revolutionize this field. Fungal-emitted VOC compositions are complex and highly dynamic. The compounds produced and their abundance also significantly varies with the producing strains, the age of the colony, water availability, substrate utilization type and pattern, incubation temperature, and other environmental parameters.

Endophytic fungi can be isolated from any healthy surface-sterilized plant parts (Strobel and Daisy 2003). There are different authenticated techniques for proper surface sterilization though one can optimize accordingly but authentication of protocol must be done by spreading the last wash water on PDA media to avoid any contamination by surface-dwelling microbes. Different established media are available for isolation of endophytic fungi. Endophytes should be immediately transferred to sterile media to bring in a pure culture. Before VOC analysis of isolates, the age of strains should be synchronized to obtain uniform and optimum results. For this, the strains may be grown first on PDA or other suitable media in individual Petri dishes, and a culture at least 7 days old can be considered as starter material for the next step of VOC production and analysis. An agar block carefully cut with a sterile scalpel from a full-grown fungal plate can be inoculated and immediately sealed with septa of silicone/plastic tape and threaded cap. After proper incubation the culture vials can be placed in an ethylene glycol bath at about 55–65 °C and the VOCs can be extracted by headspace-solid phase microextraction (HS-SPME) using a polydimethylsiloxane/divinylbenzene (PDMS/ DVB) fiber placed at least 1 cm above the surface of the fungal culture. Then, the fiber can be inserted in the GC-MS system for VOC desorption and chemical analysis of the components (Fig. 5.4). Another automated method of adsorbing and desorbing VOCs accumulated in culture headspace is via SPME, where desorption occurs in the GC injector itself. SPME has gained immense popularity recent years as it allows reduced preparation time while increasing sensitivity over other extraction methods (Zhang and Li 2010). Additionally, headspace-SPME coupled with GC-MS can be employed in direct

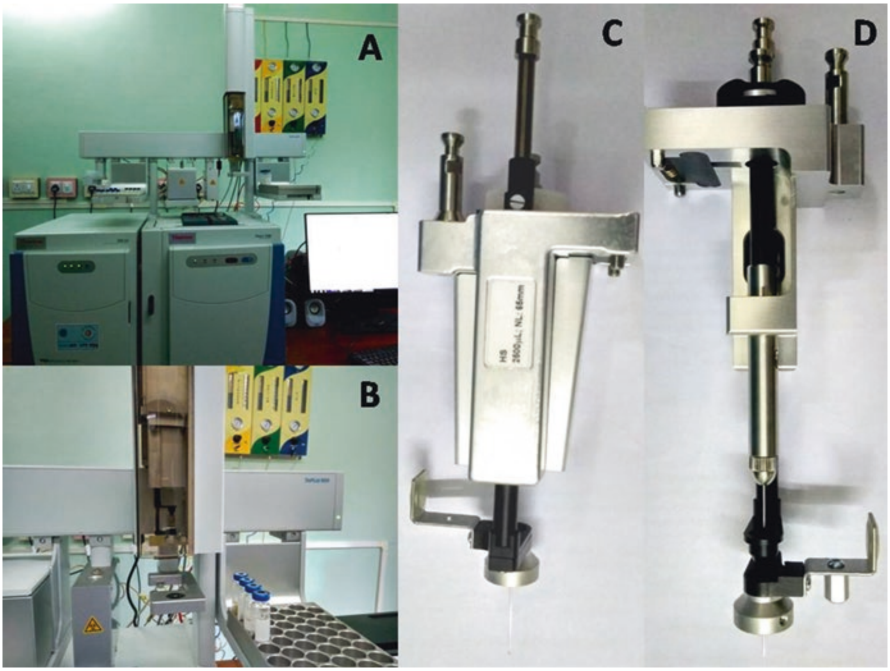


**Fig. 5.4** Gas chromatography-mass spectrometry (GC-MS) system, an important device for VOC analysis. (**a**) The instrument. (**b**) Automated solid-phase microextraction (SPME) and headspace system. (**c**) Headspace sample collection device with syringe. (**d**) Headspace-solid-phase microextraction (HS-SPME) syringe

profiling of living fungal cultures (Stoppacher et al. 2010). In another method, the culture headspace can be concentrated using solid adsorbents such as Tenax, followed by thermal desorption into the GC-MS.

Matysik et al. (2009) demonstrated some technical advantages in adsorbing hydrocarbons, esters, ethers, alcohols, ketones, glycol ethers and halogenated hydrocarbons using activated charcoal filters. The VOCs were then desorbed from the activated charcoal pads with 1.5 ml carbon disulfide and introduced into the GC vials for GC-MS analysis. However, less volatile compounds and reactive compounds such as amines, phenols, aldehydes, and unsaturated hydrocarbons were not recovered efficiently from the charcoal bed because they adsorbed strongly to the adsorbing material. This sampling technique combined with GC-MS was applied for the detection of MVOCs emitted by numbers of fungal species in the genera *Penicillium*, *Aspergillus*, and *Cladosporium*. However, the traditional method of simultaneous distillation extraction (SDE) along with vapor distillation and solvent extraction also can be effective for VOC extraction. SDE has been used to examine the VOCs of *Penicillium roqueforti* and compared with SPME (Jelen 2003). However, in an earlier study involving comparative methods analysis for the VOCs of *Penicillium vulpinum* SDE was inadequate to determine a full volatile profile when compared to headspace sampling methods (Larsen and Frisvad 1995).

Selected ion flow tube–mass spectrometry (SIFT-MS) provides rapid and broad-spectrum detection of even trace VOCs in moderately complex gas mixtures. SIFT-MS quantifies VOCs to low part-per-billion levels even in an unmodified atmosphere (i.e., without pre-concentration) in a real-time manner (Senthilmohan et al. 2001). This technique has been used to study VOCs produced by *Aspergillus*, *Candida*, *Mucor*, *Fusarium*, and *Cryptococcus* sp. (Scotter et al. 2005).

Booth et al. (2011) described a technique that rapidly entrap and collects fungal VOCs having fuel potential. The trapping materials, Carbotrap A and B and bentonite-shale, were placed inside a stainless steel column. The trapped fungal VOCs were then recovered via controlled heating of the column followed by passing the eluted gases through a liquid nitrogen trapper. This method allows significantly higher recovery of compounds normally present in the gas phase for bioassays, further separation, and analyses, and potentially for elucidation of structural basis with nuclear magnetic resonance (NMR) spectroscopy to identify novel compounds produced by fungi.

In a separate method, an analytical system was developed by Schoen et al. (2016) for rapid and accurate estimation of total volatile organic compound production from fungal culture. A platinum catalyst and a sensitive $CO_2$ detector were employed in this system, which determines total VOC production by oxidizing headspace VOCs to $CO_2$ for detection by the integrated $CO_2$ detector. Continuous recording of $CO_2$ data provided a record of respiration and total VOC production throughout the experiments. Respiratory $CO_2$ was satisfactorily bypassed by the catalyst, and the resultant total VOC content could be easily determined from the difference in the two signals. Finally, proton transfer reaction–mass spectrometry (PTR-MS) was used to identify and measure VOCs. After comparing the sum of the individual compounds determined by PTR-MS to the total VOCs established with platinum catalyst, potential differences in detection, identification, and calibration can be identified also.

However, in an earlier study, Strobel et al. (2001) employed simpler techniques for analyzing VOCs produced by endophytic *Muscodor albus* from the cinnamon tree. The method analyzed headspace gases of fungi growing on solid media in a Petri dish. A solid-phase micro-extraction syringe was introduced to conveniently trap fungal volatiles. The syringe was placed through a small hole drilled in the side of the Petri plate, exposed to the vapor phase for 45 min, then removed and inserted into a GC-MS system.

## 5.5 Genetic Engineering with VOC Genes

It is now very clear that microorganisms from diverse ecosystems produce a wide range of volatile organic compounds as secondary metabolites. Compared with other classes of secondary metabolites, volatiles are typically small compounds (up to C-20) with low molecular mass (100–500 daltons), high vapor pressure, low boiling point, and a lipophilic moiety. These properties facilitate their evaporation and diffusion through both water- and gas-filled pores in the rhizosphere and even in the

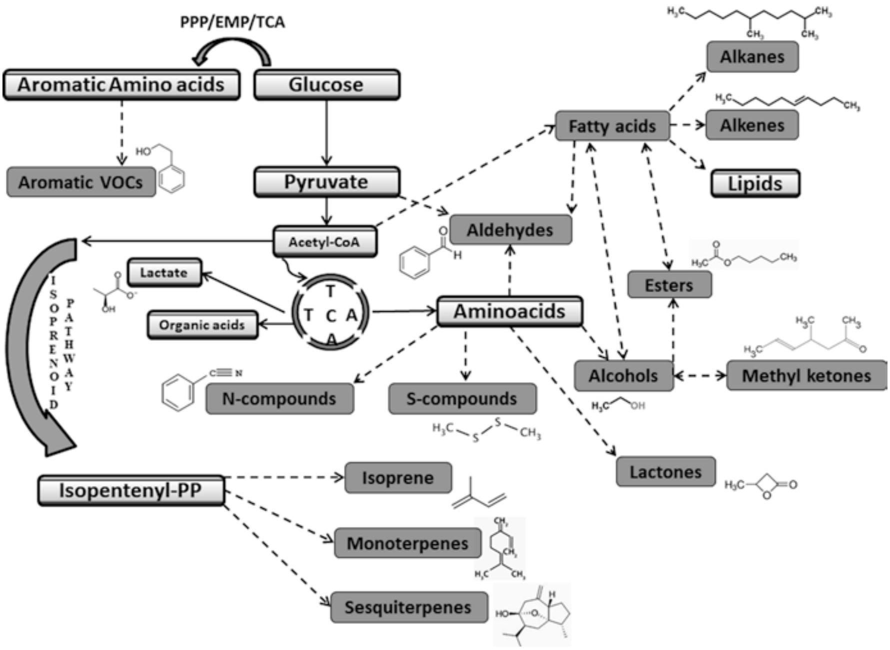


**Fig. 5.5** Biosynthetic pathways for FVOCs. ▢ Represents volatile organic metabolites of different chemical nature; – –► line represent the synthetic path for VOCs

physiological systems of the plant. It is notable that fungal volatiles are dominated by alcohols, benzenoids, aldehydes, alkenes, acids, esters, and ketones (Piechulla and Degenhardt 2014), formed mainly by oxidation of glucose from various intermediates (Korpi et al. 2009). The probable biosynthetic pathways for volatile secondary metabolite production are shown in Fig. 5.5. The fundamental biosynthetic pathways are aerobic and heterotrophic carbon metabolism, fermentation, amino acid catabolism, terpenoid biosynthesis, fatty acid catabolism, and sulfur reduction (Penuelas et al. 2014). Various critical factors in the VOC profile and concentration produced by microorganisms include cultural conditions and the physiological status of the producing microorganism (Insam and Seewald 2010; Romoli et al. 2014). A few genes that are involved in VOC synthesis in endophytic fungi have been characterized, opening a new dimension in volatile research and metabolite engineering. It now seems possible to manipulate the quantity and quality of specific VOC production by editing some metabolic pathways. Terpenes are a chemically diverse class of compounds produced as secondary metabolites by many endophytic fungi. These terpenes not only are biologically active secondary metabolites with great pharmaceutical potential but also have potential as an attractive renewable alternative to fossil fuel. As their energy densities are high, different terpenes such as pinene and bisabolene from endophytic fungi are being actively investigated as potential additive biofuels for replacing diesel and aviation fuel. Wu et al. (2016) have isolated and characterized 26 terpene-producing genes (terpene-synthatase, $tpr_s$) from four mycodiesel-producing endophytic fungi. These $tpr_s$ genes were expressed in an *E. coli* with some modified metabolic pathways to yield an enhanced level of terpene as secondary metabolites. A total of 12 TPR genes among the 26 tested were functional, with most of them exhibiting both monoterpene and sesquiterpene synthase activity.

## 5.6 Conclusion and Future Prospects

Volatile organic compounds of endophytic fungi have drawn much interest to the present day for their novel structure and potential bioactivity. Most studies have focused on the functional role of volatile organics in plant growth and vigor (Bitas et al. 2013; Penuelas et al. 2014). However, the role of volatiles in fungal and host communication and competition in plant physiological systems is still unclear. Even the specific role of each volatile compound in such endophytic associations is still unknown. It has been proposed that volatiles represent waste material or a detox system for the producing microorganisms (Claeson et al. 2007). A few experiments have established the role of VOCs as info-chemicals to communicate among and between species, in gene expression, and as competitive tools directly exerting antimicrobial activity, thus providing an advantage to the host by suppressing or eliminating potential enemies. Moreover, the interesting point lies in the difference between VOC composition produced by the endophytic fungi on laboratory culture media and that in their original *in planta* environment. Compared with diffusible compounds, volatile compounds can travel faster and over longer distances through both liquid and gaseous phase systemically in plant tissues, facilitating VOC-based regulation more promptly and stringently. There are studies proving VOCs as signaling molecules, but the intracellular interactions by VOCs at the cellular macromolecular level are still unclear. Future challenges are therefore to find novel chemicals of fungal volatiles, to discover their biosynthetic and regulatory pathways and the genes involved in the biosynthesis of volatiles in endophytic fungi, to determine biologically relevant concentrations, and to resolve the importance of volatiles in ecosystem interactions.

**Acknowledgments** The authors are thankful to the University Grants Commission (UGC), New Delhi and Department of Biotechnology (DBT), New Delhi for financial support.

## References

Alpha CJ, Campos M, Jacobs-Wagner C, Strobel SA (2015) Mycofumigation by the volatile organic compound-producing fungus *Muscodor albus* induces bacterial cell death through DNA damage. Appl Environ Microbiol 813:147–156

Atmosukarto I, Castillo U, Hess WM, Sears J, Strobel G (2005) Isolation and characterization of *Muscodor albus* I-41.3 s, a volatile antibiotic producing fungus. Plant Sci 169:854–861

Badri DV, Vivanco JM (2009) Regulation and function of root exudates. Plant Cell Environ 32(6):666–681

Banerjee D, Strobel GA, Booth E, Geary B, Sears J, Spakowicz D, Busse S (2010) An endophytic *Myrothecium inundatum* producing volatile organic compounds. Mycosphere 1(3):229–240

Banerjee D, Pandey A, Jana M, Strobel GA (2014) *Muscodor albus* MOW12 an endophyte of *Piper nigrum* L. (Piperaceae) collected from northeast India produces volatile antimicrobials. Indian J Microbiol 54:27–32. https://doi.org/10.1007/s12088-013-0400-5

Bennett JW, Inamadar AA (2015) Are some fungal volatile organic compounds (VOCs) mycotoxins? Toxins 7:3785–3804. https://doi.org/10.3390/toxins7093785

Bennett JW, Hung R, Lee S, Padhi S (2013) Fungal and bacterial volatile organic compounds; an overview and their role as ecological signaling agents. In: Hock B (ed) The Mycota IX fungal interactions. Springer-Verlag, Berlin/Heidelberg, pp 373–393

Bitas V, Kim HS, Bennett JW, Kang S (2013) Sniffing on microbes: diverse roles of microbial volatile organic compounds in plant health. Mol Plant-Microbe Interact 26(8):835–843

Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Nat Commun 1:48. https://doi.org/10.1038/ncomms1046

Booth E, Strobel G, Knighton B, Sears J, Geary B, Avci R (2011) A rapid column technique for trapping and collecting of volatile fungal hydrocarbons and hydrocarbon derivatives. Biotechnol Lett 33:1963–1972

Chiron N, Michelot D (2005) Odeurs de champignons: chimie et role dans les interactions biotiques d' une revue. Cryptogam Mycol 26:299–364

Claeson AS, Sandstrom M, Sunesson AL (2007) Volatile organic compounds (VOCs) emitted from materials collected from buildings affected by microorganisms. J Environ Monit 9:240–245

D'Alessandro M, Erb M, Ton J, Brandenburg A, Karlen D, Zopfi J, Turlings TCJ (2014) Volatiles produced by soil-borne endophytic bacteria increase plant pathogen resistance and affect tritrophic interactions. Plant Cell Environ 37:813–826. https://doi.org/10.1111/pce.12220

Daisy BH, Strobel GA, Castillo U, Ezra D, Sears J, Weaver DK, Runyon JB (2002) Naphthalene, an insect repellent, is produced by *Muscodor vitigenus*, a novel endophytic fungus. Microbiology 148:3737–3741

Davis TS, Crippen TL, Hofstetter RW, Tomberlin JK (2013) Microbial volatile emissions as insect semiochemicals. J Chem Ecol 39:840–859. https://doi.org/10.1007/s10886-013-0306-z

Desbrosses GJ, Stougaard J (2011) Root nodulation: a paradigm for how plant-microbe symbiosis influences host developmental pathways. Cell Host Microbe 20(10):348–358. https://doi.org/10.1016/j.chom.2011.09.005

Effmert U, Kalderas J, Warnke R, Piechulla B (2012) Volatile mediated interactions between bacteria and fungi in the soil. J Chem Ecol 38:665–703. https://doi.org/10.1007/s10886-012-0135-5

Ezra D, Hess WM, Strobel GA (2004) Unique wild type endophytic isolates of *Muscodor albus*, a volatile antibiotic producing fungus. Microbiology 150:4023–4031

Foissner W (1999) Notes on the soil ciliate biota (Protozoa, Ciliophora) from the Shimba hills in Kenya (Africa): diversity and description of three new genera and ten new species. Biodivers Conserv 8:319–389

Garbeva P, Hordijk C, Gerards S, de Boer W (2014) Volatiles produced by the mycophagous soil bacterium *Collimonas*. FEMS Microbiol Ecol 87:639–649

Griffin MA, Spakowicz DJ, Gianoulis TA, Strobel SA (2010) Volatile organic compound production by organisms in the genus Ascocoryne and a re-evaluation of myco-diesel production by NRRL 50072. Microbiology 156(12):3814–3829

Hardoim PR, van Overbeek LS, van Elsas JD (2008) Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol 16:463–471. https://doi.org/10.1016/j.tim.2008.07.008

Hsu CS, Dechert GJ, Abbott DJ, Genowitz MW, Barbour R (2000) Molecular characterization of diesel fuels using modern analytical techniques. In: Song C, Hsu C, Mochida I (eds) Chemistry of diesel fuels. Taylor & Francis, New York

Hyde KD, Soytong K (2008) The fungal endophyte dilemma. Fungal Divers 33:163–173

Inderjit, Weston LA, Duke SO (2005) Challenges, achievements and opportunities in allelopathy research. J Plant Interact 1:69–81

Insam H, Seewald MSA (2010) Volatile organic compounds (VOCs) in soils. Biol Fertil Soils 46:199–213

Jelen HH (2003) Use of solid phase microextraction (SPME) for profiling fungal volatile metabolites. Lett Appl Microbiol 36:263–267

Junker RR, Tholl D (2013) Volatile organic compound mediated interactions at the plant-microbe interface. J Chem Ecol 39(7):810–825. https://doi.org/10.1007/s10886-013-0325-9

Kanchiswamy CN, Mainoy M, Maffie ME (2015) Chemical diversity of microbial volatiles and their potential for plant growth and productivity. Front Plant Sci 6:151

Kaur H, Kaur R, Kaur S, Baldwin IT, Inderjit (2009) Taking ecological function seriously: soil microbial communities can obviate allelopathic effects of released metabolites. PLoS Biol 4:e4700

Korpi A, Jarnberg J, Pasanen AL (2009) Microbial volatile organic compounds. Crit Rev Toxicol 39:139–193

Kramer R, Abraham WR (2012) Volatile sesquiterpenes from fungi: what are they good for? Phytochem Rev 11:15–37. https://doi.org/10.1007/s11101-011-9216-2

Kudalkar P, Strobel G, Hasan SRU, Geary G, Sears J (2012) *Muscodor sutura*, a novel endophytic fungus with volatile antibiotic activities. Mycoscience 53:319–332

Larsen TO, Frisvad JC (1995) Comparison of different methods for collection of volatile chemical markers from fungi. J Microbiol Methods 24:135–144

Lee SO, Kim HY, Choi GJ, Lee HB, Jang KS, Choi YH, Kim JC (2009) Mycofumigation with *Oxyporus latemarginatus* EF069 for control of postharvest apple decay and *Rhizoctonia* root rot on moth orchid. J Appl Microbiol 106:1213–1219. https://doi.org/10.1111/j.1365-2672.2008.04087.x

Lemfack MC, Nickel J, Dunkel M, Preissner R, Piechulla B (2014) mVOC: a database of microbial volatiles. Nucleic Acids Res 42:D744–D748

Macias FA, Galindo JLG (2007) Evolution and current status of ecological phytochemistry. Phytochemistry 68:2917–2936

Macias-Rubalcava ML, Hernandez-Bautista BE, Oropeza F, Duarte G, Gonzalez MC, Glenn AE, Hanlin RT, Anaya AL (2010) Allelochemical effects of volatile compounds and organic extracts from *Muscodor yucatanensis*, a tropical endophytic fungus from *Bursera simaruba*. J Chem Ecol 4:1122–1131

Maffei A, Lambo ME, Turrigiano GG (2010) Developmental regulation of experience-dependent inhibitory plasticity. J Neurosci 30:3304–3309

Maffei ME, Gertsch J, Appendino G (2011) Plant volatiles: production, function and pharmacology. Nat Prod Rep 28(8):1359–1380

Malhadas C, Malheiro R, Pereira JA, de Pinho PG, Baptista P (2017) Antimicrobial activity of endophytic fungi from olive tree leaves. World J Microbiol Biotechnol 33:46. https://doi.org/10.1007/s11274-017-2216-7

Matysik S, Herbarth O, Mueller A (2009) Determination of microbial volatile organic compounds (MVOCs) by passive sampling onto charcoal sorbents. Chemosphere 76:114–119

Medina-Romero YM, Roque-Flores G, Macias-Rubalcava ML (2017) Volatile organic compounds from endophytic fungi as innovative postharvest control of *Fusarium oxysporum* in cherry tomato fruits. Appl Microbiol Biotechnol 101:8209–8222. https://doi.org/10.1007/s00253-017-8542-8

Mends MT, Yu E, Riyaz-Ul-Hassan S, Booth E, Geary B, Sears J, Taatjes CA, Hadi MZ (2012) An endophytic *Nodulisporium* sp. producing volatile organic compounds having bioactivity and fuel potential. J Pet Environ Biotechnol 3:3

Mercer J, Jimenez JI (2004) Control of fungal decay of apples and peaches by the biofumigant fungus *Muscodor albus*. Post Harvest Biol Technol 31:1–8. https://doi.org/10.1016/j.postharvbio.2003.08.004

Mercier J, Manker D (2005) Biocontrol of soil-borne disease and plant growth enhancement in green house soilless mix by the volatile-producing fungus *Muscodor albus*. Crop Prot 24:355–362. https://doi.org/10.1016/j.cropro.2004.09.004

Mitchell AM, Strobel GA, Moore E, Robison R, Sears J (2010) Volatile antimicrobials from *Muscodor crispans*, a novel endophytic fungus. Microbiology 156:270–277. https://doi.org/10.1099/mic.0.032540-0

Mookherjee A, Bera P, Mitra A, Maiti MK (2018) Characterization and synergistic effect of antifungal volatileorganic compounds emitted by the *Geotrichum candidum* PF005, an endophytic fungus from the eggplant. Microb Ecol 75:647–661. https://doi.org/10.1007/s00248-017-1065-0

Morath S, Hung R, Bennett JW (2012) Fungal volatile organic compounds: a review with emphasis on their biotechnological potential. Fungal Biol Rev 30:1–11

Ortiz-Castro R, Contreras-Cornejo HA, ias-Rodriguez L, Lopez-Bucio J (2009) The role of microbial signals in plant growth and development. Plant Signal Behav 4:701–712. https://doi.org/10.4161/psb.4.8.9047

Pagans E, Font X, Sanchez A (2006) Emission of volatile organic compounds from composting of different solid wastes: Abatement by biofiltration. J Hazard Mater 131:179–186

Pandey A, Banerjee D (2014) *Daldinia bambusicola* Ch4/11 an endophytic fungus producing volatile organic compounds having antimicrobial and olio chemical potential. J Adv Microbiol 1:330–337

Park MS, Ahn JY, Choi G-J, Choi YH, Jang KS, Kim JC (2010) Potential of the volatile-producing fungus *Nodulisporium* sp. CF016 for the control of postharvest diseases of apple. Plant Pathol J 26:253–259. https://doi.org/10.5423/PPJ.2010.26.3.253

Penuelas J, Asensio D, Tholl D, Wenke K, Rosenkranz M, Piechulla B, Schnitzler JP (2014) Biogenic volatile emissions from the soil. Plant Cell Environ 37:1866–1891. https://doi.org/10.1111/pce.12340

Piechulla B, Degenhardt J (2014) The emerging importance of microbial volatile organic compounds. Plant Cell Environ 37:811–812. https://doi.org/10.1111/pce.12254

Qadri M, Rajput R, Abdin MZ, Vishwakarma RA, Riyaz–Ul–Hassan S (2014) Diversity, molecular phylogeny, and bioactive potential of fungal endophytes associated with the Himalayan blue pine (*Pinus wallichiana*). Microb Ecol 67:877–887

Qadri M, Deshidi R, Shah BA, Bindu K, Vishwakarma RA, Riyaz-Ul-Hassan S (2015) An endophyte of *Picrorhiza kurroa* Royle ex. Benth, producing menthol, phenylethyl alcohol and 3-hydroxypropionic acid, and other volatile organic compounds. World J Microbiol Biotechnol 31(10):1647–1654. https://doi.org/10.1007/s11274-015-1910-6

Rana KL, Kour D, Yadav AN, Kumar V, Dhaliwal HS (2016) Biotechnological applications of endophytic microbes associated with barley (*Hordeum vulgare* L.) growing in Indian Himalayan regions. In: Proceedings of 86th Annual Session of NASI & Symposium on "Science, Technology and Entrepreneurship for Human Welfare in The Himalayan Region", p 80

Rana KL, Kour D, Sheikh I, Yadav N, Yadav AN, Kumar V, Singh BP, Dhaliwal HS, Saxena AK (2018a) Biodiversity of endophytic fungi from diverse niches and their biotechnological applications. In: Singh BP (ed) Advances in endophytic fungal research. Springer, Switzerland. https://doi.org/10.1007/978-3-030-03589-1_6

Rana KL, Kour D, Yadav AN (2018b) Endophytic microbiomes: biodiversity, ecological significance and biotechnological applications. Res J Biotechnol 14:1–30

Reinhold-Hurek B, Hurek T (2011) Living inside plants: bacterial endophytes. Curr Opin Plant Biol 14:435–443. https://doi.org/10.1016/j.pbi.2011.04.004

Riyaz-Ul-Hassan S, Strobel GA, Booth E, Knighton B, Sears J (2012) Modulation of volatile organic compound formation in the mycodiesel producing endophyte *Hypoxylon* sp. CI-4. Microbiology 158:465–473

Riyaz-Ul-Hassan S, Strobel G, Geary B, Sears J (2013) An endophytic *Nodulisporium* sp. from Central America producing volatile organic compounds with both biological and fuel potential. J Microbiol Biotechnol 23:29–35

Romoli R, Papaleo MC, De Pascale D, Tutino ML, Michaud L, Lo G, Fani R, Bartolucci G (2014) GC-MS volatolomic approach to study the antimicrobial activity of the Antarctic bacterium *Pseudoalteromonas* sp. TB41. Metabolomics 10:42–51

Sanchez-Fernández RE, Diaz D, Duarte G, Lappe-Oliveras P, Sánchez S, Macias-Rubalcava ML (2016) Antifungal volatile organic compounds from the endophyte *Nodulisporium* sp. strain GS4d2II1a: a qualitative change in the intraspecific and interspecific interactions with *Pythium aphanidermatum*. Microb Ecol 71:347–364. https://doi.org/10.1007/s00248-015-0679-3

Santos RG, Loh W, Bannwart AC, Trevisan OV (2014) An overview of heavy oil properties and its recovery and transportation methods. Braz J Chem Eng 31(3):571–590. https://doi.org/10.1590/0104-6632.20140313s00001853

Schoen HR, Peyton BM, Knighton WB (2016) Rapid total volatile organic carbon quantification from microbial fermentation using a platinum catalyst and proton transfer reaction-mass spectrometry. AMB Express 6:90. https://doi.org/10.1186/s13568-016-0264-2

Schulz S, Dickschat S (2007) Bacterial volatiles: the smell of small organisms. Nat Prod Rep 24(4):814–842. https://doi.org/10.1039/b507392h

Scotter JM, Langford VS, Wilson PF, Mcewan MJ, Chambers ST (2005) Real-time detection of common microbial volatile organic compounds from medically important fungi by Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS). J Microbiol Methods 63:127–134

Senthilmohan ST, Mcewan MJ, Wilson PF, Milligan DB, Freeman CG (2001) Real time analysis of breath volatiles using SIFT-MS in cigarette smoking. Redox Rep 6:185–187

Shaw JJ, Spakowicz D, Dalal RS, Strobel SA (2015) Biosynthesis and genomic analysis of medium-chain hydrocarbon production by the endophytic fungal isolate *Nigrograna mackinnonii* E5202H. Appl Microbiol Biotechnol 99(8). https://doi.org/10.1007/s00253-014-6206-5

Singh SK, Strobel GA, Knighton B, Geary B, Sears J, Ezra D (2011) An endophytic *Phomopsis* sp. possessing bioactivity and fuel potential with its volatile organic compounds. Microb Ecol 61:729–739. https://doi.org/10.1007/s00248-011-9818-7

Steinebrunner F, Twele R, Francke W, Leuchtmann A, Schiestl FP (2008) Role of odour compounds in the attraction of gamete vectors in endophytic *Epichloe* fungi. New Phytol 178:401–411

Stinson M, Ezra D, Hess WM, Sears J, Strobel G (2003) An endophytic *Gliocladium* sp. of *Eucryphia cordifolia* producing selective volatile antimicrobial compounds. Plant Sci 165:913–922

Stoppacher N, Kluger B, Zeilinger S, Krska R, Schuhmacher R (2010) Identification and profiling of volatile metabolites of the biocontrol fungus *Trichoderma atroviride* by HS-SPME-GC-MS. J Microbiol Methods 81:187–193. https://doi.org/10.1016/j.mimet.2010.03.011

Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67(4):491–502

Strobel GA, Dirske E, Sears J, Markworth C (2001) Volatile antimicrobials from *Muscodor albus*, a novel endophytic fungus. Microbiology 147:2943–2950

Strobel GA, Katreena K, Hess WM, Sears J, Ezra D, Vargas PN (2007) *Muscodor albus* E-6, an endophyte of *Guazuma ulmifolia* making volatile antibiotics: isolation, characterization and experimental establishment in host plant. Microbiology 153:2613–2620

Strobel GA, Knighton B, Kluck K, Ren Y, Livinghouse T, Griffen M, Spakowicz D, Sears J (2008) The production of myco-diesel hydrocarbons and their derivatives by the endophytic fungus *Gliocladium roseum* (NRRL 50072). Microbiology 154:3319–3328

Strobel G, Singh SK, Riyaz-Ul-Hassan S, Mitchel AM, Geary B, Sears J (2011) An endophytic/pathogenic *Phoma* sp. from creosote bush producing biologically active volatile compounds having fuel potential. FEMS Microbiol Lett 320:87–94

Strobel G, Ericksen A, Sears J, Xie J, Geary B, Blatt B (2017) Urnula sp., an endophyte of *Dicksonia antarctica*, making a fragrant mixture of biologically active volatile organic compounds. Microb Ecol 74(2):312–321

Suman A, Yadav AN, Verma P (2016) Endophytic microbes in crops: diversity and beneficial impact for sustainable agriculture. In: Singh D, Abhilash P, Prabha R (eds) Microbial inoculants in sustainable agricultural productivity: research perspectives. Springer, New Delhi, pp 117–143. https://doi.org/10.1007/978-81-322-2647-5_7

Ting ASY, Mah SW, Tee CS (2010) Identification of volatile metabolites from fungal endophytes with biocontrol potential towards *Fusarium oxysporum* F. sp. *cubense* Race 4. Am J Agric Biol Sci 5:177–182

Tomsheck A, Strobel GA, Booth E, Geary B, Spakowicz D, Knighton B, Floerchinger C, Sears J, Liarzi O, Ezra D (2010) *Hypoxylon* sp., an endophyte of *Persea indica*, producing 1,8-cineole and other bioactive volatiles with fuel potential. Microb Ecol 60:903–914

von Rad U, Klein I, Dobrev PI, Kottova J, Zazimalova E, Fekete A, Hartmann A, Schmitt-Kopplin P, Durner J (2008) Response of *Arabidopsis thaliana* to *N*-hexanoyl-dl-homoserine-lactone, a bacterial quorum sensing molecule produced in the rhizosphere. Planta 229:73–85. https://doi.org/10.1007/s00425-008-0811-4

Wang A, Maffei A (2011) Inhibition dictates the sign of plasticity at excitatory synapses. In sfn (2010) 415.02, San Diego Minisymposium “Beyond the Connectome”

Wani MA, Kaul S, Kumar DM, Lal DK (2010) GC-MS analysis reveals production of 2-phenylethanol from *Aspergillus niger* endophytic in rose. J Basic Microbiol 50:110–114

Weise T, Thuermer A, Brady S, Kai M, Daniel R, Gottschalk G, Piechulla B (2014) VOC emission of various *Serratia* species and isolates and genome analysis of *Serratia plymuthica* 4Rx13. FEMS Microbiol Lett 352:45–53. https://doi.org/10.1111/1574-6968.12359

Wilkins K, Larsen K (1995) Identification of volatile (micro) biological compounds from household waste and building materials by thermal desorption-capillary gas chromatography-mass spectroscopy. J High Resolut Chromatogr 18:373–377

Worapong J, Strobel GA, Ford EJ, Li JY, Baird G, Hess WM (2001) *Muscodor albus* anam. nov., an endophyte from *Cinnamomum zeylanicum*. Mycotaxon 79:67–79

Wu W, Tran W, Taatjes CA, Alonso-Gutierrez J, Lee TS, Gladden JM (2016) Rapid discovery and functional characterization of terpene synthases from four endophytic Xylariaceae. PLoS One 11(2):e0146983. https://doi.org/10.1371/journal.pone.0146983

Yadav AN (2018) Biodiversity and biotechnological applications of host-specific endophytic fungi for sustainable agriculture and allied sectors. Acta Sci Microbiol 1:1–5

Yadav AN, Kumar R, Kumar S, Kumar V, Sugitha T, Singh B, Chauhan VS, Dhaliwal HS, Saxena AK (2017) Beneficial microbiomes: biodiversity and potential biotechnological applications for sustainable agriculture and human health. J Appl Biol Biotechnol 5:1–13

Yan DH, Song X, Li H, LuoT DG, Strobel G (2018) Antifungal activities of volatile secondary metabolites of four *Diaporthe* strains isolated from *Catharanthus roseus*. J Fungi 4:65. https://doi.org/10.3390/jof4020065

Yuan JR, Shen WQ, Huang Q (2012) Antifungal activity of *Bacillus amyloliquefaciens* NJN-6 volatile compounds against *Fusarium oxysporum* f. sp. *cubense*. Appl Environ Microbiol 78:5942–5944

Yuan J, Sun K, Deng-Wang MY, Dai CC (2016) The mechanism of ethylene signaling induced by endophytic fungus *Gilmaniella* sp. AL12 mediating sesquiterpenoids biosynthesis in *Atractylodes lancea*. Front Plant Sci 23(7):361. https://doi.org/10.3389/fpls.2016.00361

Zhang Z, Li G (2010) A review of advances and new developments in the analysis of biological volatile organic compounds. Microchem J 65:127–139

Zhao CZ, Xia H, Frazier TP, Yao YY, Bi YP, Li AQ, Li MJ, Li CS, Zhang BH, Wang XJ (2010) Deep sequencing identifies novel and conserved micro-RNAs in peanuts (*Arachis hypogaea* L.). BMC Plant Biol 10:3