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Fumigant Activity of Volatiles from *Streptomyces alboflavus* TD-1 against *Fusarium moniliforme* Sheldon

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The fumigant activity of volatiles generated by *Streptomyces alboflavus* TD-1 against *Fusarium moniliforme* Sheldon was investigated. The results showed that the mycelial growth, sporulation, and spore germination of *F. moniliforme* were significantly suppressed, and that membrane permeability was disrupted in the presence of the volatiles. Gas chromatography-mass Spectrometry analysis revealed 31 kinds of volatile organic compound from the volatiles. Among them, two earthy-smelling substances, namely, 2-methylisoborneol (50.97%) and trans-1,10-dimethyl-trans-9-decalinol (3.10%) were found. The most abundant compound, 2-methylisoborneol, exhibited inhibitory activity against *F. moniliforme* by fumigation. All these results suggested that *S. alboflavus* TD-1 can be a promising starter for the inhibition of *F. moniliforme* through fumigant action.

Keywords: fumigant, *Fusarium moniliforme* Sheldon, *Streptomyces alboflavus* TD-1, volatile organic compound

Introduction

Fusarium moniliforme (*Fusarium verticillioides*) is a facultative and non-host necrotrophic pathogen that broadly contaminates corn, sorghum, wheat, tomatoes, cotton, peanuts, bananas, soybeans, and other feeds. *F. moniliforme* can cause root rot, stem rot, and ear (grain) rot in plants, which leads to various devastating diseases such as corn ear rot, wheat scab, and rice bakanae disease (Logrieco *et al*., 2002). Biocontrol of *F. moniliforme* is effective alternative method of decreasing crop yield loss.

Volatile organic compounds (VOCs) have low molecular weights and polarities. Antifungal volatiles can be used to control pathogens in plants because VOCs are small molecules that can easily diffuse through the porous structure of soil and over great distances in the atmosphere. The antifungal activity of volatiles produced by *Muscodor albus* can inhibit or kill many plant pathogens, such as *Botrytis cinerea*, *Colletotrichum acutatum*, *Geotrichum* spp., *Monilinia fructicola*, *Penicllium* spp., *Rhizopus* spp., *Rhizoctonia solani*, *Pythium ultimum*, *Aphanomyces cochlioides*, *Verticillium dahliae*, *F. oxysporum* f. sp. *betae*, *Sclerotinia sclerotiorum*, and *Phytophthora capsici* (Strobel *et al*., 2001; Stinson *et al*., 2003; Mercier and Jiménez, 2004; Mercier and Manker, 2005; Mercier and Smilanick, 2005; Gabler *et al*., 2006; Schnabel and Mercier, 2006). The antifungal volatiles of *M. albus* used in agriculture have been patented and are being developed as a commercial antimicrobial biofumigant product (Mercier and Smilanick, 2005).

Actinomycetes, especially genus *Streptomyces*, are known for their ability to produce secondary metabolites such as antibiotics (Manivasagan *et al*., 2009; Thumar *et al*., 2010; Cho *et al*., 2011) and antifungal VOCs (Wan *et al*., 2008; Li *et al*., 2010). Antifungal VOCs from actinomycetes can induce alterations in the morphology of conidiophores and hyphae in several fungi (Moore-Landecker and Stotzky, 1973). Volatile metabolites from *Streptomyces griseoruber* can also inhibit the spore germination of *Cladosporium cladosporioides* (Herrington *et al*., 1985). The VOCs produced by *Streptomyces platensis* F-1 affect several plant diseases such as seedling blight of rice, leaf blight of oilseed rape, and fruit rot of strawberry (Wan *et al*., 2008). Moreover, the volatiles of *Streptomyces globisporus* JK-1 exert antifungal activity against *Penicillium italicum* in *Citrus* microcarpa (Li *et al*., 2010). Nevertheless, information on the fumigant activity of volatiles generated by Streptomyces against *F. moniliforme* is limited.

Streptomyces sp. TD-1 is a new strain isolated from soil surrounding a granary in Tianjin, China. The fermentation broth of this strain showed a strong inhibitory effect on *F. moniliforme* (Liu *et al*., 2011). The volatiles generated by the mycelia of fermentation broth (Gause's synthetic medium) can inhibit storage fungi *F. moniliforme* Sheldon, *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus niger*, and *P. citrinum in vitro* (Wang *et al*., 2013). Moreover, the inhibitory rate against *F. moniliforme* of VOCs generated from a wheat seed culture of this strain is higher than the VOCs generated from the mycelium (data not shown). The authors attempted to investigate the antifungal activity of VOCs produced by a wheat seed culture of this strain against *F. moniliforme*. The effects of VOCs on mycelial membrane permeability were evaluated, the VOCs released from a wheat seed culture of *S. alboflavus* TD-1 were identified, and the effects of individual compounds against *F. moniliforme* were analyzed.

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Materials and Methods

Antagonist *S. alboflavus* TD-1

The strain *S. alboflavus* TD-1 was isolated from soil surrounding a granary in Tianjin, China and was identified based on morphological characteristics, physiological characteristics, and 16S rDNA sequences (GenBank accession no.: JX915780) (Wang *et al*., 2013). The strain was deposited in China General Microbiological Culture Collection Center (CGMCC no. 4666). The strain was streaked into test tube slants of Gause's synthetic agar, incubated at 28°C for 5 days, and then stored at 4°C. Working cultures were established by transferring spores of *S. alboflavus* TD-1 onto Gause's synthetic agar plate in Petri dishes before incubation.

F. moniliforme and preparation of spore suspension

F. moniliforme was obtained from the Tianjin Institute of Animal Husbandry and Veterinary Science (Tianjin, China). For conidial production, spores were grown on potato dextrose agar (PDA) in Petri dishes at 28°C. After 7 days of incubation, fresh spores were harvested and filtered through eight layers of sterile cheese clothes. The spores were counted using a haemocytometer and adjusted to 0.5–5×10^6 spores/ml by suspending in sterile distilled water with 0.01% Tween 20.

Preparation of *S. alboflavus* TD-1 cultures for VOC production

About 100 g of autoclaved wheat seeds in conical flasks (250 ml) were inoculated with the spore suspension of *S. alboflavus* TD-1 prepared above at 1 ml per 100 g of wheat seeds. The flasks were incubated at 28°C for 7 days (Wan *et al*., 2008).

Measurement of mycelial growth and sporulation of *F. moniliforme*

An antifungal bioassay was established to study the effects of volatiles from a wheat seed culture of *S. alboflavus* TD-1. A 9 cm-diameter Petri dish containing PDA inoculated with two 6 mm-diameter plugs from the periphery of an actively growing culture of *F. moniliforme* was placed inside a 12 cm-diameter Petri dish containing a wheat seed inoculum of *S. alboflavus* TD-1. In this step, direct contact between *F. moniliforme* plugs and *S. alboflavus* TD-1 was prevented. A lid was placed on the larger dish, which was then sealed with Parafilm™ to enable free gas exchange. Non-inoculated autoclaved wheat seeds were used as controls. After 5 days of incubation at 28°C, the colony diameter of each dish was measured. Each treatment consisted of three replicates, and experiments were performed in triplicate.

Two bottom dishes of a sterilized 9 cm-diameter Petri dish were used to measure the mycelial growth and sporulation of *F. moniliforme*. A dish containing different amounts of wheat seed (0.5 g to 16 g) inocula of *S. alboflavus* TD-1 were prepared and covered with another dish containing PDA inoculated with a 6 mm-diameter plug of *F. moniliforme*. The two dishes were then sealed with Parafilm™ to obtain a double-dish chamber with 200 ml of airspace (Wan *et al*., 2008; Arrebola *et al*., 2010). The non-inoculated autoclaved wheat seeds were used as controls. After 5 days of incubation at 28°C, the diameters of the colonies were measured, and the total number of conidia per square centimeter was assessed. Each treatment consisted of three replicates, and the experiments were performed in triplicate.

Measurement of conidial germination of *F. moniliforme*

A double-dish chamber was used as described above. A bottom dish containing different amounts of wheat seed culture of *S. alboflavus* TD-1 and a top dish containing PDA were laid over sterilized cellophane membranes (1.5 × 1.5 cm^2) onto which 0.5 ml aliquots of a conidial suspension of *F. moniliforme* (10^6 conidia/ml) were spread. Non-inoculated autoclaved wheat seeds were used as controls. After 6, 9, and 12 h of incubation at 28°C, cellophane membrane with germinated conidial was taken out, 100 spores per replicate were microscopically observed, and the rate of germination was determined. The spores were considered germinated if the length of the germ tube was twice as long as the spore. Each treatment consisted of three replicates, and the experiments were performed in triplicate.

Measurement of the mycelial membrane permeability of *F. moniliforme*

Many antibacterial agents can interact with bacterial membranes and influence membrane permeability. Once bacterial membranes become compromised, low-molecular-weight species such as K^+ and PO_4^{3-} tend to leak out followed by DNA, RNA, and other materials. These intracellular components are easily detected by ultraviolet (UV) at 260 nm and can be used as an indication of membrane permeability (Chen and Cooper, 2002).

The OD_{260} of the supernatant of *F. moniliforme* was determined using a UV-visible spectrophotometer (8453, Agilent, USA), which determined the mycelial membrane permeability (Virto *et al*., 2005; Oonmetta-aree *et al*., 2006). Two covered bottom dishes were used as described above, with the bottom dish containing the wheat seed cultures of *S. alboflavus* TD-1 and the top dish containing PDA. These dishes were laid over sterilized cellophane membranes (1.5 × 1.5 cm^2) onto which 0.5 ml aliquots of a conidial suspension of *F. moniliforme* were spread. Non-inoculated autoclaved wheat seeds were used as the control group. After 12, 24, 36, and 48 h, the cellophane membrane with inhibited and normal mycelia were taken out to measure the OD_{260}. Each treatment consisted of three replicates, and the experiments were performed in triplicate.

Collection and analysis of VOCs produced by *S. alboflavus* TD-1

Volatiles from wheat seed cultures of *S. alboflavus* TD-1 incubated for 7 days were collected using the headspace solid-phase microextraction (HS-SPME) technique (Gu *et al*., 2007). The sample was placed in a water bath at 45°C for 30 min. The HS-SPME syringe, equipped with 50/30 divinylbenzene/carburen on polydimethylsiloxane on a 65 μm stable fiber (DVB/CAR/PDMS, Supelco, USA) was then inserted through the hole and exposed to the volatiles in the

headspace for 30 min to entrap the volatiles to the fiber material. The syringe containing the volatiles was then inserted into a gas chromatography–mass spectrometry (GC-MS) system. The gas chromatograph was equipped with an ion-trap detector (Varian-4000 GC-MS, Varian Co., USA). A VF-5ms fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm-thick film; Varian Co.) was used to separate the VOCs. Helium with an ultrahigh purity of 99.999% was used as the carrier gas with the flow velocity adjusted at 1 ml/min. The injector temperature was maintained at 250°C. The working temperature for the volatile-separation column was programmed as follows: an initial 40°C for 3 min increased to 150°C at 4°C/min, held at 150°C for 1 min and further increased to 250°C at 8°C/min, and held at 250°C for 2 min. The temperature was set at 220°C for the electronic bombarding ion source (70 eV) and 260°C for the transfer line. Mass spectra were obtained using the scan mode with total ion counts of 43 m/z to 500 m/z. The baseline of volatile compounds from the autoclaved wheat seeds was also measured and subtracted. The experiments were conducted in triplicate.

Investigation of the effects of volatile synthetic compounds against *F. moniliforme*

Two covered bottom dishes were used as described above. The top dish contained a fungal culture, and the bottom dish contained a piece of autoclaved filter paper to which a selected compound was individually added. Different amounts of individual volatile compounds were used, and equivalent amounts of sterile distilled water were used as the control group. After 5 days of incubation at 28°C, the colony diameter and spore counts were assessed. Each treatment had three replicates, and experiments were performed two times.

Statistical analysis

ANOVA was performed on SPSS 13.0 software for Windows (SPSS Inc., USA). Mean comparisons were performed by Fisher's protected least significant difference (LSD) test (P=0.01).

Results

Effects of VOCs on the mycelial growth and sporulation of *F. moniliforme*

The effects of volatiles produced by *S. alboflavus* TD-1 on colonies of *F. moniliforme* are shown in Fig. 1. Radial mycelial growth was markedly suppressed by volatiles from wheat seed cultures of *S. alboflavus* TD-1. The control treatment had no observable effects on growth.

The effects of volatiles produced by *S. alboflavus* TD-1 on the mycelial growth and sporulation of *F. moniliforme* are shown in Table 1. The mycelial growth of *F. moniliforme* declined in the vessel containing autoclaved wheat seeds of *S. alboflavus* TD-1 compared with the vessel without *S. alboflavus* TD-1. After 5 days of incubation, the mycelial growth was 24 mm and the sporulation was 1.4×10^4 spores/cm^2 in the presence of 8 g/plate of wheat seed cultures of *S. alboflavus* TD-1. On the other hand, the mycelial growth was 68 mm and the sporulation was 6.8×10^7 spores/cm^2 in the non-inoculated control groups. No mycelial growth was observed in the presence of volatiles produced by 16 g/plate of *S. alboflavus* TD-1 cultures.

Table 1. Effects of VOCs produced by wheat seed cultures of *S. alboflavus* TD-1 on the mycelial growth and sporulation of *F. moniliforme* after 5 days of incubation

S. alboflavus TD-1[a] (g/plate)	Mycelial growth[b] (mm)	Sporulation[b] ($\times 10^4$ spores/cm^2)
0	68 ± 1.6a	6804 ± 712a
0.5	65 ± 5.7a	6888 ± 452a
1	56 ± 3.1b	609 ± 75b
2	40 ± 3.5c	661 ± 85b
4	33 ± 4.0d	20 ± 2.5c
8	24 ± 1.1e	1.4 ± 0.2c
16	0.0 ± 0.0f	0.0 ± 0.0c

[a] Grams of culture.
[b] Mycelial growth and sporulation were measured after incubation for 5 days at 28°C.
* Means obtained from nine replicates followed by the same letters within the column were not significantly different (P<0.01) according to the LSD test.

Effects of VOCs on the conidial germination of *F. moniliforme*

The effects of volatiles produced by *S. alboflavus* TD-1 on the conidial germination of *F. moniliforme* are shown in Table 2. A delayed effect was observed in conidial germination in the presence of volatiles produced by *S. alboflavus* TD-1. In addition, >50% inhibition of conidial germination was found in 8 g/plate of wheat seed cultures of *S. alboflavus* TD-1 after 6 and 9 h of incubation, whereas conidial germination in the control treatment (autoclaved wheat seed) was as high as 80% and 100% respectively (Table 2). Spore germination was only 12% when the wheat seed cultures of *S. alboflavus* TD-1 was increased to 16 g/ plate at 12 h.

Effects of VOCs on the mycelial membrane permeability of *F. moniliforme*

The effects of volatile compounds produced by *S. alboflavus* TD-1 on the mycelial membrane permeability of *F. moniliforme* are shown in Fig. 2. The OD_{260} of the supernatant

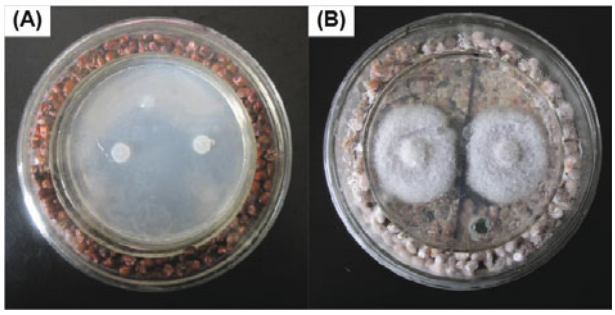

Fig. 1. Antifungal activity of autoclaved wheat seed cultures of *S. alboflavus* TD-1 against *F. moniliforme*.
Notes: a) (A) colonies of *F. moniliforme* in the presence of volatiles (added autoclaved wheat seeds of *S. alboflavus* TD-1); (B) colonies of *F. moniliforme* in the absence of volatiles (added autoclaved wheat seeds). b) *F. moniliforme* and *S. alboflavus* TD-1 were physically separated.

Table 2. **Effect of volatiles produced by wheat seed cultures of *S. alboflavus* TD-1 on the conidial germination of *F. moniliforme***

S. alboflavus TD-1[a] (g/plate)	Conidial germination[b] (%)		
	6 h	9 h	12 h
0	80 ± 10a	100 ± 0a	100 ± 0a
2	77 ± 8ab	99 ± 2a	100 ± 1a
4	68 ± 9b	99 ± 2a	100 ± 0a
8	36 ± 6c	45 ± 6b	59 ± 6b
16	2 ± 1d	8 ± 4c	12 ± 5c

[a] Grams of culture.
[b] Conidial germination was measured after incubation for 6, 9, and 12 h at 28°C.
* Means obtained from nine replicates followed by the same letters within the column were not significantly different (P<0.01) according to the LSD test.

of the mycelium treated with 16 g of wheat cultures of *S. alboflavus* TD-1 was higher than that of the control group during fumigation. After 48 h of fumigation, the OD_{260} of the supernatant in the presence of volatiles produced by *S. alboflavus* TD-1 wheat cultures was 0.87, whereas that of

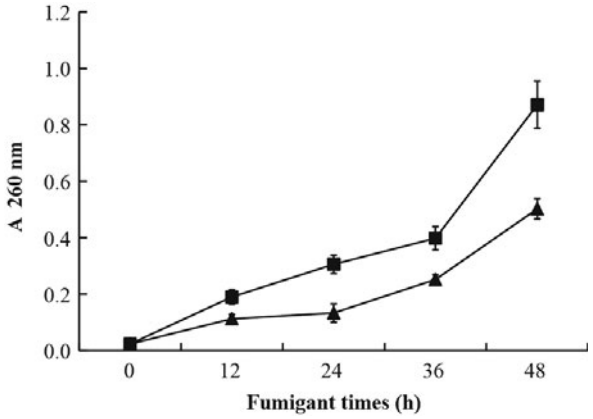

Fig. 2. **Effects of VOCs produced by wheat seed cultures of *S. alboflavus* TD-1 on the mycelial membrane permeability of *F. moniliforme*.** Notes: a) 28°C. b) symbols: (■) *F. moniliform* exposed to volatiles; (▲) normal control group.

Table 3. **VOCs from *S. alboflavus* TD-1 produced on 7-day-old wheat seed cultures detected by GC-MS analysis**

RT[a] (min)	Possible compound	Relative peak area (%)
8.291	(R)-(+)-3-Methylcyclopentanone	0.08
12.78	Benzene,1,2,3-trimethyl	0.02
13.169	1,3-Cycloexadiene-1-carboxadehyde,2,6,6-trimethyl-	4.95
14.745	Bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde,6,6-dimethyl	21.05
21.14	Cyclohexane	0.03
21.365	2-Methylisoborneol*	50.97
21.755	Camphenol,6-*	0.09
22.813	Cis-1,4,dimethyladamantane*	6.04
22.944	1-H-Indene,1-ethylideneoctahydro-7a-methyl-,(1Z,3a,alpha,7a,beta)*	2.66
23.097	1H-Indene,1-ethylideneoctahydro-7a-methyl-,cis-	0.19
23.207	Spiro[2,4]heptane,1,2,4,5-tetramethyl-6-methylene	0.14
23.298	1,4-Dimethyladamantane,[1.alpha.,3.beta.,4.beta.,5.alpha.,7.beta]*	0.07
23.531	Bicyclo[4.3.0]non-3-ene,3,4,7-trimethyl*	0.61
23.676	Cycloehexane,1,1,4,4,-tetramethyl-2,6-bis(methylene)*	3.38
26.735	alpha-Cubebene	0.05
28.152	Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)*	0.18
28.867	4-Isopropenyl-4,7-dimthyl-1-oxspiro[2,5]octane*	0.02
29.076	trans-1,10-Dimethy-trans-9-decalinol*	3.10
29.493	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene	0.09
29.754	Aromadendrene	0.08
29.967	Azulene,1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-	0.12
30.521	Cedran-diol,8S,14*	0.25
30.66	Humulen-(v1)*	0.10
31.136	Isoledene*	0.90
32.151	1H-Cycloprop[e]azulen-4-ol,decahydro-1,1,4,7-tetramethyl*	0.20
32.291	Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)*	0.14
35.354	Cyclohexanemethanol*	0.01
39.949	Hexadecanoic acid,methyl ester	1.29
40.793	Heptadecanoic acid,methyl ester	0.03
41.287	Hexadecanoic acid,14-methyl-methyl ester	0.02
42.505	Octadecanoic acid,methyl ester	0.32

[a] retention time.
[b] 31 compounds were identified by comparing the mass spectra and retention times from those of available standards in the Library of the National Institute of Standards and Technology (NIST05).
[c] Compounds detected in both the autoclaved wheat seeds of *S. alboflavus* TD-1 and in the control medium are not included in this Table.
[d] Minor compounds detected are not included in this Table.
[e] * the compound has been identified from the volatiles of *S. alboflavus* TD-1 mycelia (Wang *et al.*, 2013).

Table 4. Effects of 2-MIB on the mycelial growth and sporulation of *F. moniliforme* after 5 days of incubation

2-MIB (µl/plate)	Mycelial growth (mm)	Sporulation ($\times 10^4$ spores/cm^2)
0	66 ± 1.4a	6717 ± 1496a
1	66 ± 1.5a	6874 ± 1069a
5	65 ± 2.5a	6529 ± 802a
100	61 ± 3.3b	4118 ± 476b
200	56 ± 2.7c	1032 ± 180c

[a] Mycelial growth and sporulation were measured after incubation for 5 days at 28°C.
[b] 2-MIB, 100 µg/ml in methanol, Sigma-Aldrich, ≥98%, GC.
[c] Means obtained from six replicates followed by the same letters within the column were not significantly different (P<0.01) according to the LSD test.

the non-inoculated control group was 0.52. Longer mycelium exposure to the volatile compounds resulted in higher OD_{260} of mycelial materials.

Composition analysis of volatiles produced by *S. alboflavus* TD-1

The VOCs generated by *S. alboflavus* TD-1 on 7-day-old wheat seed cultures are presented in Table 3. The 31 most frequent VOCs were observed from wheat seed cultures of *S. alboflavus* TD-1 based on the NIST library. These compounds can be classified into several categories: alkenes, aromatic hydrocarbons, terpenoids, esters, and ketones.

Two earthy/muddy-smelling compounds, 2-methylisoborneol (2-MIB) and trans-1,10-dimethyl-trans-9-decalinol (geosmin), were simultaneously detected in the volatile profile of *S. alboflavus* TD-1. The most abundant volatile substance was 2-MIB (50.97%), followed by bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde, 6,6-dimethyl (21.05%).

The compound 2-MIB was detected as the major component in volatiles (>50%) from both mycelium (data not shown) and cultures of *S. alboflavus* TD-1, and was selected for further individual tests of antifungal activity against *F. moniliforme*.

Effects of 2-MIB on the mycelial growth and sporulation of *F. moniliforme*

The effects of 2-MIB on the mycelial growth and sporulation of *F. moniliforme* after 5 days of incubation are shown in Table 4. 2-MIB exerted inhibitory effects on the mycelial growth of *F. moniliforme* at the tested concentrations.

Discussion

F. moniliforme is a phytopathogenic filamentous fungus that can seriously harm crops. Previous studies have shown that *Bacillus subtilis* and its secondary metabolite, fengycin, can inhibit the growth of *F. moniliforme* (Bacon *et al*., 2001; Hu *et al*., 2007). However, information on the volatiles produced by *Streptomyces* for the biocontrol of *F. moniliforme* is limited. In this study, we showed that the mycelial growth and sporulation of *F. moniliforme* were significantly inhibited by VOCs of *S. alboflavus* TD-1 *in vitro*. Mycelial growth was almost fully inhibited when exposed to 16 g/plate wheat seed cultures for 7 days, but grew in length when plated on fresh PDA in the absence of volatiles (data not shown). The spores germinated late when exposed to the same wheat seed culture. This activity against the spores of *F. moniliforme* differed from the fungicidal activity of VOCs from *S. globisporus* JK-1 against *P. italicum* (Li *et al*., 2010).

The inhibition mechanism of VOCs released from microorganisms on *F. moniliforme* is poorly understood. A previous study has reported the feature of hyphal protoplasm retraction of *Botrytis cinerea* in the presence of *B. subtilis* JA volatiles (Chen *et al*., 2008). Moreover, the volatiles of *B. subtilis* induce morphological abnormalities in several fungal structures. The hyphae of *Fusarium oxysporum* become lysed, vacuolar, and granulated. The conidia of *Alternaria alternata* become thick walled and spherical or irregular in shape. *Cladosporium oxysporum* conidiophores become vegetative and stunted (Chaurasia *et al*., 2005). In the present study, the lysis of fungal hyphae was also observed, and the OD_{260} of the supernatant with the treated mycelium increased. The volatile compounds released from *S. alboflavus* TD-1 affected the mycelium, which resulted in low-molecular-weight metabolites (nucleotides, amino acid, and inorganic ions) seeping away from the mycelium of *F. moniliforme*.

Studying volatile metabolites from *Streptomyces* revealed considerable biosynthetic diversity and antifungal activity in culture plates or planta (Jáchymová *et al*., 2002; Schöller *et al*., 2002; Dickschat *et al*., 2005; Nourozian *et al*., 2006; Kai *et al*., 2007). In this study, 31 volatile compounds from *S. alboflavus* TD-1 were identified by GC-MS analysis. Two earthy-smelling substances, 2-MIB and geosmin, were simultaneously found. Most *Streptomyces* strains generate one of these substances (Wilkins and Schöller, 2009). Gerber (1969) found these substances in Actinomycetes, which are perceived to be the major sources of the earthy odor and are proven to affect sea urchin development and the germination of some Brassicaceae seeds (Nakajima *et al*., 1996; Ogura *et al*., 2000). This study is the first to report on the antifungal properties of 2-MIB against *F. moniliforme* as fumigants, and other individual volatile compounds that can efficiently suppress pathogens must be investigated.

Several compounds in this study have been found to possess antifungal activity in previous reports, including aromadendrene, but have not been found in the leaf oils of *Piper caninum*, *Melaleuca alternifolia*, and *S. globisporus* JK-1 (Hammer *et al*., 2003; Li *et al*., 2010; Salleh *et al*., 2011), isoledene produced by *S. globisporus* JK-1, and in essential oils of *Illicium griffithii* (Saraswathy *et al*., 2010). By contrast, aromadendrene and isoledene had no significant effect on the mycelial growth of *P. italicum* as fumigant (Li *et al*., 2010).

Alpha-cubebene is reportedly present in essential oils of *Origanum vulgare*, *Piper lanceaefolium* Kunth, and *Cinnamomum cassia* Presl (Pino Benitez *et al*., 2009; Verma *et al*., 2011). Five of the VOCs detected in the present study [1-H-indene, 1-ethylideneociahydro-7a-methyl (1Z,3a,alpha, 7a,beta), alpha-cubebene, geosmin, aromadendrene, and isoledene] are commonly found as components of volatiles from *S. globisporus* JK-1 (Li *et al*., 2010), but significant differences exist between VOCs from *S. alboflavus* TD-1 and other *Actinomycetes* (Jáchymová *et al*., 2002; Schöller *et al*., 2002; Dickschat *et al*., 2005). Thus, the same types of VOCs may be released from different organisms, and different microorganisms can specifically produce volatile compounds.

In conclusion, the present study showed that volatiles from *S. alboflavus* TD-1 had efficacy against *F. moniliforme in vitro* as fumigants. *F. moniliforme* contaminates crops and their processed products during pre-harvest and post-harvest stages. Thus, *S. alboflavus* TD-1 can be an effective agent for controlling diseases caused by *F. moniliforme*. Whether the volatile substances of *S. alboflavus* TD-1 can inhibit *F. moniliforme* growth in plants remains unknown and requires further investigation. The safety of practically applying these compounds to humans and the environment also needs to be studied.

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