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Soil volatile fungistasis and volatile fungistatic compounds

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Abstract

Fungistasis is a widespread phenomenon that can be mediated by soil microorganisms and volatile organic compounds (VOCs). The relationship between soil microorganisms and VOCs is still unclear, however, and many fungistatic compounds remain to be identified. We assessed the effects of soils (soil direct fungistasis) and VOCs produced by natural soils (soil volatile fungistasis) on the spore germination of several fungi. Both strong soil direct fungistasis and soil volatile fungistasis were observed in a wide range of soils. Soil fungistasis and VOC fungistasis were significantly correlated ($P<0.001$). The volatile fungistatic activity of soils stopped after autoclaving. Some VOCs were identified by using solid-phase microextraction-gas chromatography/mass spectrum. VOC composition and in vitro antagonism of relatively pure commercial compounds also were measured. Some VOCs, trimethylamine, 3-methyl-2-pentanone, dimethyl disulfide, methyl pyrazine, 2,5-dimethyl-pyrazine, benzaldehyde, *N*,*N*-dimethyloctylamine and nonadecane, were produced by various fungistatic soils. Moreover, antifungal activity test of above VOCs showed that trimethylamine, benzaldehyde, and *N*,*N*-dimethyloctylamine have strong antifungal activity even at low levels (4–12 mg l^{-1}).

Keywords: Fungistasis; Antifungal; Soil volatile compounds; GC/MS; SPME

1. Introduction

Fungal propagules are inhibited by most soils, a phenomenon first described as 'fungistasis' by Dobbs and Hinson (1953). Many factors, that affect the intensity of fungistasis including soil physical and chemical traits, environmental changes, fungal characteristics, the community composition, and the metabolic activities of other soil microbes, have been studied extensively over the last 50 years, and there are many explanations for the mechanism of soil fungistasis. The nutrient-deprivation hypothesis (Lockwood, 1964, 1977) and germination inhibitor hypothesis (Romine and Baker, 1973) are the two most popular. The former hypothesis states that exogenous energy sources, e.g. fixed carbon, is relatively unavailable to germinating spores due to strong competition from other soil microorganisms. The latter hypothesis states that fungistasis can be caused by antifungal compounds produced by other microorganisms. But until now, no definitive answers have emerged.

In recent years, significant data supporting microbial activitiy in soil fungistasis process has become available (Alabouvette, 1999; Mondal and Hyakumachi, 1998; Toyota et al., 1996; Ellis et al., 2000). For example, soil fungistasis can be reduced by soil sterilization or the addition of exogenous antibiotics. De Boer (2003) found that soil microbial community composition was an important factor determining soil fungistasis, especially the pseudomonads, and is consistent with the germination inhibitor hypothesis of fungistasis.

Some volatile organic compounds (VOCs) from soil environments, e.g. ethylene (Hora and Baker, 1970; Smith, 1973), ammonia (Ko et al., 1974; Ko and Hora, 1974), allyl alcohol, and acrylic acid (Balis, 1976), can reduce or inhibit spore germination of a variety of fungi. Most of fungistatic compounds were observed in alkali or neutral soils (Lockwood, 1977; Liebman and Epstein, 1992, 1994). The relatively high volatility and relatively low molecular weights of these compounds means that they could not be

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identified and traced easily during the soil fungistatic process. We know that no reports demonstrating highly volatile fungistatic compounds are produced under natural conditions or that identify activity thresholds for these VOCs.

The objective of this study was to determine the relationship between soil direct fungistasis and soil volatile fungistasis and to find important fungistatic volatile compounds. The fungistatic activity of 146 soils and VOCs produced by the soils were assessed and the correlation between soil direct fungistasis and soil volatile fungistasis was calculated. Furthermore, the specific volatile organic components produced in strong fungistatic soils were identified by the method of solid-phase microextraction-gas chromatography/mass spectrum (SPME-GC/MS) and their role in fungistasis was assayed.

2. Materials and methods

2.1. Fungi and growth conditions

Three fungal strains, which differ in spore sizes and sensitivities to soil fungistasis, *Paecilomyces lilacinus* IPC (CGMCC No. 0241), *Pochonia chlamydospora* ZK7 (CGMCC No. 0418), and *Clonostachys rosea* GR87 (CGMCC No. 0807), were obtained from China General Microbiological Culture Collection Center (CGMCC, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China), and were used as model fungi. Conidia of these fungi are nutrient-independent for germination.

These strains were cultured on peptone glucose agar (1% peptone, 1% glucose, 2% agar) for 7 d at 25 °C. Conidia were then scraped from the media and suspended in sterile distilled water. To remove mycelial fragments and germinated conidia, the sporangium suspension was filtered through four layers of lens wiping paper and washed twice by centrifugation at 5000 rpm at 4 °C for 20 min. The suspension was adjusted to 1×10^4 conidia per ml by adding sterile distilled water.

2.2. Soils

A total of 146 soils with various characteristics, such as pH, organic, water content, carbon content, vegetation/cultivation history and geographic origin, were obtained in summer from various regions of Yunnan province in southwest China (Table 1). Soil samples were taken from a depth of 2–20 cm. Physical and chemical characteristics, e.g. pH, moisture, organic mater, inorganic N, and inorganic P, were determined and then the soils were sieved through a 0.34 μ-pore screen, air-dried, and then stored in plastic bags at 4 °C for at least 2 weeks. Prior to use, soil moisture was adjusted to 20% (wt wt^{-1}).

2.3. Soil direct fungistasis determination

Soil direct fungistasis of all soils was assayed by measuring the repression of spore germination. Low spore germination meant high fungistasis. About 200 μl of a spore suspension of a model fungus was placed on a patch of nitrocellulose filter (ca. 20 mm×20 mm×0.02 mm). The filter patch was covered with cellophane and buried in a soil block. Compounds in the soils could diffuse through the filter and cellophane freely. After incubation at 25 °C for 24 h, the filter patch was taken out from soil, and spore germination was counted under a microscope. In preliminary experiments, it was observed that the general period for complete spore germination of these model fungi on WA media was 24 h, and the degree of fungistasis on soil plates did not have any changes after 24 h. Therefore we applied 24 h of incubation for our test.

Three replicates were used for each treatment and experiments were done in triplicate.

2.4. Soil volatile fungistasis

Volatile fungistatic activity of the 146 soils was estimated by the following method. Ten grams of soil was added to an empty Petri dish (6.0 cm diameter). The top of the plate was replaced with the bottom portion of another Petri dish with a 5-mm thickness of 1% water agar (WA). The plate bottoms were sealed together with Parafilm (American National Can, Chicago, USA), so that volatile compounds could diffuse into the WA. After incubation at 25 °C for 4 d, the WA was inoculated with 10 μl of a fungal spore suspension (approximately 300 spores), and re-sealed with Parafilm. Plates were incubated at 25 °C for 24 h and the percentage of spores germinated determined.

As a control, all the soils were treated by autoclaving (twice, each time for 30 min at 121 °C) to kill soil microorganisms, and were then assayed volatile fungistasis to model fungi by same method described above.

Three replicates were used for each treatment and experiments were done in triplicate.

Table 1
Soil characteristics

Soil texture	Color	Vegetation/cultivation	pH	Organic carbon (%)	Total $CaCO_3$ (%)	CEC
Clay, sandy loam, loam, clay loam	Red, yellow, black, grey, brown	Rice, corn, upland crops, vegetables, forest, fruit trees, non-cultivated	3.1–8.8	0.2–5.1	2.3–29.6	3.0–25.7

2.5. Volatile organic compounds (VOCs) extraction

The method of SPME (Augusto and Valente, 2002; Wady et al., 2003) was used for VOCs extraction and GC/MS assay. As a relatively new technique, SPME allows simple, solventless sampling of volatiles and semivolatiles from a wide variety of matrices, including drugs and their metabolites in body fluids, VOCs in water, and explosive residues from postblast soil samples. Compared with other volatile compounds extraction methods, SPME can get various kinds of volatile compounds and avoid them losing during treatment process.

In our other previous study, the extractive ability of four kinds of SPME fibers (100 μm PDMS, 65 μm PDMS/DVB, 75 μm CAR/PDMS, 65 μm CAR/PDMS, purchased from Supelco, Bellefonte, PA, USA) had been compared. For its special extractive ability to various kinds of volatile components, 75 μm CAR/PDMS SPME fiber was used in this study.

A new 75 μm CAR/PDMS SPME fiber was conditioned with helium at 250 °C for 2 h prior to use. After each extraction cycle, the fiber was kept back inside the SPME needle to prevent contamination and was conditioned before re-used with helium at 220 °C for 10 min.

Two grams of soil was added into a 15 ml SPME vials and incubated at room temperature for over 10 d to recovery microbial community. The SPME needle was allowed to pierce the septum, and the fiber was exposed to the headspace of the vial. Extraction condition was 30 °C for 10 h. Sterile distilled water was used as blank. A manual holder and a sampling stand/vial puck designated for SPME analysis (Supelco, Bellefonte, PA, USA) were used during the process.

Three replicates were used for each treatment and experiments were done in triplicate.

2.6. Gas chromatography/mass spectrometry (GC/MS) assay

A Hewlett Packard 6890GC/5973MSD (Agilent Technologies, USA) equipped with a HP-5MS capillary column was used to separate and identify the VOCs. The carrier gas was helium with a flow rate of 1 ml/min in the split mode (10:1). After extraction, the SPME fiber was inserted into the GC injection port directly and exposed at 250 °C for 1.5 min. The temperature of the oven was 50 °C for 2 min, 50–180 °C at a rate of 8 °C/min, 180–240 °C at 10 °C/min, and held at 240 °C for 6 min. The temperature of the transfer line and ion trap were 250 and 300 °C, respectively. The identification of a volatile compound was based on a comparison of the mass spectrum of the substance with a GC/MS system data bank (Wiley 138 and NBS 75 k library).

2.7. Antifungal test of commercial products of VOCs

Commercial products of VOCs which were specifically produced in fungistatic soils, trimethylamine, 3-methyl-2-pentanone, dimethyl disulfide, methyl pyrazine, 2,5-dimethyl-pyrazine, *N*,*N*-dimethyloctylamine and nonadecane were purchased from Sigma, St Louis, MO. Their fungistatic activities were assessed using following methods.

Treatment A. A Petri dish (9.0 cm diameter) with 5 mm thickness of 2% WA was inoculated with 10 μl of the spore suspension. A piece of filter paper (ca. 10 mm × 10 mm) moistened with 10 μl of distilled water was placed into the lid of another Petri dish, and 0.03–50 mg of the commercial volatile compound was added on the filter paper quickly. Distilled water was used as a control. The Petri dish with WA was inverted and placed over the Petri dish lid with filter paper. The two portions were sealed with Parafilm and incubated at 25 °C for 24 h, after which percent spore germination was determined. Concentration of compounds is calculated in mg l^{-1} by reference to the air volume in the Petri dish. Addition of 0.03–50 mg commercial volatile compounds results in 1–1570 mg l^{-1} concentration.

Treatment B. Ten grams of soil (mixture of 10 different kinds of soils selected at random from 146 soils, each soil 1 g) were placed in a Petri dish (6.0 cm diameter) and sterilized by autoclaving (at 120 °C, twice each 30 min). Aliquots (0.03–50 mg) of the volatile compounds were mixed into the soil. The cover of the Petri dish was replaced with the bottom of another Petri dish containing a 5 mm thickness of 2% WA that had been inoculated with 10 μl of the fungal spore suspension. The two portions were sealed together with Parafilm, incubated at 25 °C for 24 h, and the percent spore germination determined.

All the tests were triplicate.

2.8. Data analysis and statistics

Percent spore germination data are the means of triplicate assays, and the significance of each value was verified with the one-tailed *t* test. Percent inhibition of spore germination was calculated as (% inhibition = (germination of control − that of treatment)/control × 100). Here, inhibition $\geq 75\%$ were considered as strong fungistasis, 50–75% as moderate, 25–50% as slight, and $\leq 25\%$ as no fungistasis. Differences between autoclaved and non-autoclaved treatments in the soil volatile fungistasis assay were evaluated with a paired-sample *t* test. The correlation between direct soil fungistasis and soil volatile fungistasis was evaluated by using calculation of correlations (Pearson), linear regression equations, and correlation coefficients (*r*). Differences of the response of the three fungi to soil fungistasis and commercial volatile compounds were inspected with ANOVAs and Fisher's least significant difference if significant differences were found ($P \leq 0.05$).

Table 2
Soil direct fungistasis and volatile fungistasis

Assay	Model fungi	Percentage of soils of different fungistatic activities (%)				Mean inhibition ± SD (%)
		Strong[a]	Moderate	Slight	No	
Direct fungistasis	*Pae. lilacinus*	96.6	3.4	0.0	0.0	92.2 ± 6.0
	Po. Chlamydospora	34.9	42.5	19.9	2.7	64.7 ± 18.6
	C. rosea	69.9	26.0	4.1	0.0	79.9 ± 14.7
Volatile fungistasis	*Pae. lilacinus*	35.1	45.8	16.5	2.6	75.5 ± 17.6
	Po. Chlamydospora	6.7	23.4	44.1	25.8	51.4 ± 22.8
	C. rosea	8.9	35.2	43.4	12.5	58.1 ± 18.0

[a] Degree of fungistasis. Strong, 100–75% inhibition; moderate, 75–50% inhibition; slight, 50–25% inhibition; no: no fungistasis, 25–0% inhibition.

Data statistics were performed using SPSS 11.0 for Windows (SPSS Inc., Chicago, USA).

3. Results

3.1. Soil direct fungistasis and soil volatile fungistasis

A total of 146 soils were estimated for their direct and indirect/volatile fungistatic activity to three model fungi. Most of the tested soils showed more or less fungistasis to the model fungi (Table 2), and the positive soils showed similar pattern of inhibition to three model fungi although the degree of fungistasis to three model fungi was different, and the textures of these soils were various. If a certain soil suppressed one model fungus, it also inhibited another two model fungi.

The universality of soil fungistasis was significant. The mean of soil inhibition to *Pae. lilacinus*, *Po. chlamydospora*, and *C. rosea* were 92.2, 64.7, 79.9% in direct fungistasis experiment, and 75.5, 51.4, 58.1% in volatile experiment, respectively (Table 2). Though the mean of inhibition of volatile fungistasis was weaker than that of direct fungistasis, all of the means of inhibition to fungi were higher than 50%. And the Pearson correlation coefficients between direct fungistasis and volatile fungistasis to *Pae. lilacinus*, *Po. chlamydospora*, and *C. rosea*, were 0.76, 0.76, and 0.63, respectively, very significant at $P < 0.001$ (Fig. 1).

The degree of suppression of different soils to model fungi was various. The numbers of soils that strongly inhibited *Pae. lilacinus*, *Po. chlamydospora*, and *C. rosea*, were 141 (96.6%), 51 (34.9%), 102 (69.9%) in direct fungistasis assay, and 46 (31.5%), 10 (6.7%), 13 (8.9%) in volatile fungistasis assay, respectively (Table 2).

The sensitivities of the model fungi to soil fungistasis were significantly different ($P < 0.05$). From their spore germination data, we observed that conidia of *Pae. lilacinus* was most sensitive to soil fungistasis, *C. rosea* was intermediate, *Po. chlamydospora* was least.

To determine whether VOCs originated from soil microorganisms, all soils were autoclaved in volatile assay. Results showed fungistatic activity of the autoclaved treats were not significant. For model fungi, *Pae. lilacinus*, *Po. chlamydospora*, and *C. rosea*, the mean inhibition were 12.3, 7.6, 5.1%, respectively, and lower than 25% in all autoclaved soils. The difference between non- and autoclaved treatments was significant. For *Pae. lilacinus*,

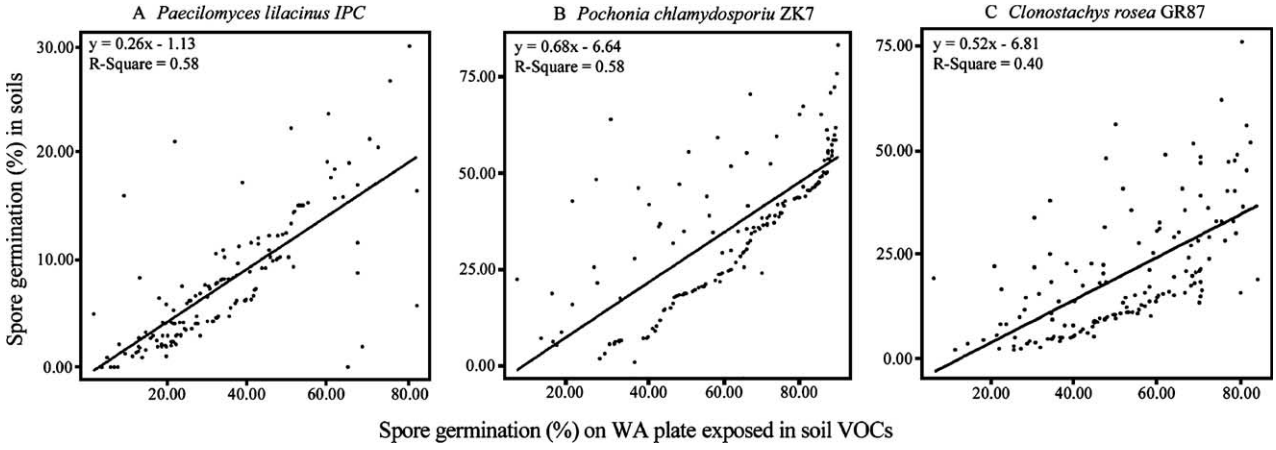

Fig. 1. The correlation between soil fungistasis and soil volatile fungistasis (A–C). Abscissa (x): germination rate of conidia of model fungi in soil direct fungistasis assay, Y-axis (y): germination rate of conidia of model fungi in soil volatile fungistasis assay. A total of 146 soils were assessed. The linear regression equation and correlation coefficient were calculated ($P < 0.001$).

Po. chlamydospora, *C. rosea*, the *t* values were 19.2, 7.8, and 12.2, respectively. $P<0.001$. This result suggested that the VOCs were produced mainly from microbial activity within these soils.

3.2. Soil VOCs detection, identification and commercial compounds fungistasis test

The VOCs from 30 strong, 30 moderate, 20 slight, and 12 no volatile fungistatic soils were detected. The SPME-GC/MS were used to detect volatile components. Peaks obtained from fungistatic and non-fungistatic soils were compared.

40–70 peaks were observed in total ion current chromatograms of soil VOCs detection from strong antifungal soils, 30–60 peaks from moderate, 20–40 peaks from slight, and 20–40 peaks from non-antifungal soils. The stronger the soils inhibited fungi, the more kinds of VOCs the soils produced.

The VOCs, trimethylamine, 3-methyl-2-pentanone, dimethyl disulfide, methyl pyrazine, 2,5-dimethyl-pyrazine, *N*,*N*-dimethyloctylamine and nonadecane, were found in fungistatic soils, but were not found in soils which showed no fungistasis. Benzaldehyde was observed in almost all samples, but its relative concentration in fungistatic soils was higher than that in non-fungistatic soil samples. Fig. 2 gave a representative example of this result. Compared with standard compounds of GC/MS, we also found that none of VOCs' concentrations in natural soil samples could exceed 200 mg l^{-1}, calculated as VOC mass with reference to the volume of soil sample.

These additional VOCs produced by strong inhibition soils very likely play essential role in fungistasis. To test their function in fungistasis, the commercial compounds of some VOCs, i.e. trimethylamine, 3-methyl-2-pentanone, dimethyl disulfide, methyl pyrazine, 2,5-dimethyl-pyrazine, *N*,*N*-dimethyloctylamine and nonadecane were tested for their antifungal activity to model fungi. The results were shown in Table 3 and Fig. 3.

Filter paper treatments and autoclaved soil treatments gave similar results of antifungal tests. Trimethylamine, benzaldehyde, *N*,*N*-dimethyloctylamine suppressed the test fungi intensively at low concentration. Increased concentration of these compounds (from 1 to 15 mg l^{-1} as shown in Fig. 3) increased inhibition to conidia germination of test fungi. When the concentration of trimethylamine, benzaldehyde, *N*,*N*-dimethyloctylamine in sealed plates reached approximate 6–9, 4–12, 6–9 mg l^{-1}, respectively, in filter paper treatments, germination of spores of model fungi on WA plates were completely inhibited. However, their suppression was not significantly different for model fungi ($P>0.05$). This indicated that trimethylamine, benzaldehyde, *N*,*N*-dimethyloctylamine were important compounds for fungistasis.

The sensitivity of different model fungi to trimethylamine, *N*,*N*-dimethyloctylamine was not significantly

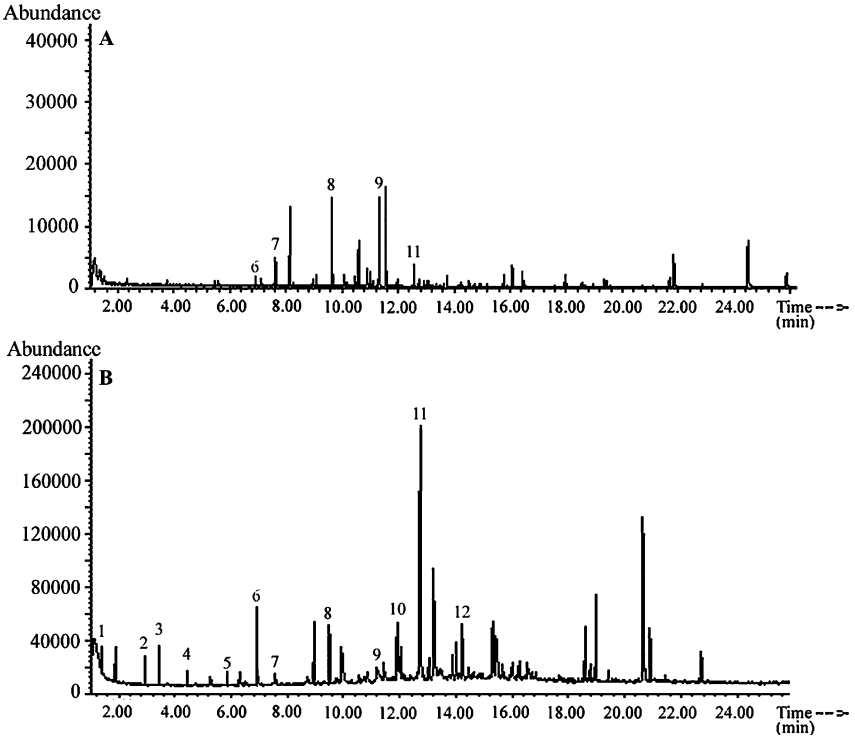

Fig. 2. A representative example of SPME total ion current chromatograms showing VOCs identified from soils with EI modes: (A) soil sample showing no fungistatic activity to *Pae. lilacinus* IPC, *Po. chlamydospora* ZK7, and *C. rosea* GR87; (B) soil sample with strong fungistatic activity. Key: (1) trimethylamine, (2) 3-methyl-2-pentanone, (3) dimethyl disulfide, (4) methyl pyrazine, (5) 2,5-dimethyl-pyrazine, (6) benzaldehyde, (7) octanal, (8) nonanal, (9) decanal, (10) *N*,*N*-dimethyloctylamine, (11) (*E*)-2-decenal, (12) nonadecane.

Table 3
The antifungal activity of commercial products of VOCs to model fungi

Compounds	The lowest dosage of complete inhibition[a] ($mg\,l^{-1}$)					
	Pae. lilacinus		*Po. chlamydospora*		*C. rosea*	
	A[b]	B	A	B	A	B
Trimethylamine	6±0.2	15±0.2	9±0.4	31±0.5	9±0.4	28±0.6
3-Methyl-2-pentanone	420±4.0	NI	NI	NI	790±6.2	NI
Dimethyl disulfide	1200±9.5	NI	NI	NI	NI	NI
Benzaldehyde	4±0.3	12±0.4	6±0.2	24±0.8	12±0.1	37±0.9
Methyl pyrazine	NI	NI	NI	NI	NI	NI
2,5-Dimethyl-pyrazine	NI	NI	NI	NI	NI	NI
N,N-Dimethyloctylamine	9±0.2	23±0.7	6±0.2	27±0.7	9±0.2	32±0.4
Nonadecane	NI	NI	400±5.5	NI	NI	NI

[a] Inhibition % ≥99%.
[b] Treatment: A, test compounds were added on the filter paper; B, compounds were mixed into soils. NI, Not inhibition.

different ($P>0.05$). To benzaldehyde significant difference only lies between *Pae. lilacinus* and *C. rosea* ($0.01<P<0.05$), no significant difference between *Pae. lilacinus* and *Po. chlamydospora*, *Po. chlamydospora* and *C. rosea* ($P>0.05$).

However, methyl pyrazine, 2,5-dimethyl-pyrazine, and nonadecane did not show positive antifungal activity. 3-Methyl-2-pentanone, dimethyl disulfide showed antifungal activity at very high concentration. 3-Methyl-2-pentanone inhibited *Pae. lilacinus* and *C. rosea* at concentration of 420, 790 $mg\,l^{-1}$, respectively; and no inhibition to *Po. chlamydospora*. Dimethyl disulfide inhibited *Pae. lilacinus* at concentration of 1200 $mg\,l^{-1}$, no inhibition to *Po. chlamydospora* and *C. rosea*. Considering soil VOC cannot reach a very high concentration (more than 200 $mg\,l^{-1}$) in natural condition, 3-methyl-2-pentanone, dimethyl disulfide, methyl pyrazine, 2,5-dimethyl-pyrazine, and nonadecane could be excluded as causes of fungistasis in natural soil.

4. Discussion

Soil fungistasis occurs widely in soils (Dobbs and Hinson, 1953; Lockwood, 1964, 1977; Sharapov and Kalvish, 1984; Ann, 1994). In our study, not only the soil direct fungistasis phenomena, but also the soil volatile fungistasis phenomena were observed in widespread soils, though the sensitivities of three model fungi, *Pae. lilacinus* IPC, *Po. chlamydospora* ZK7 and *C. rosea* GR87 to soil fungistasis are different.

Steiner and Lockwood (1969) considered that the sensitivity correlates the spore size. The more sensitive the fungus, the smaller was its spore size. Among model fungi, spore size of *Pae. lilacinus* is the smallest; *Po. chlamydospora* intermediate; *C. rosea*, biggest. However, the data on mean inhibition of spore germination in soil direct and indirect fungistasis showed the order of the sensitivity is *Pae. lilacinus*>*C. rosea*>*Po. Chlamydospora*. In the test of commercial volatile

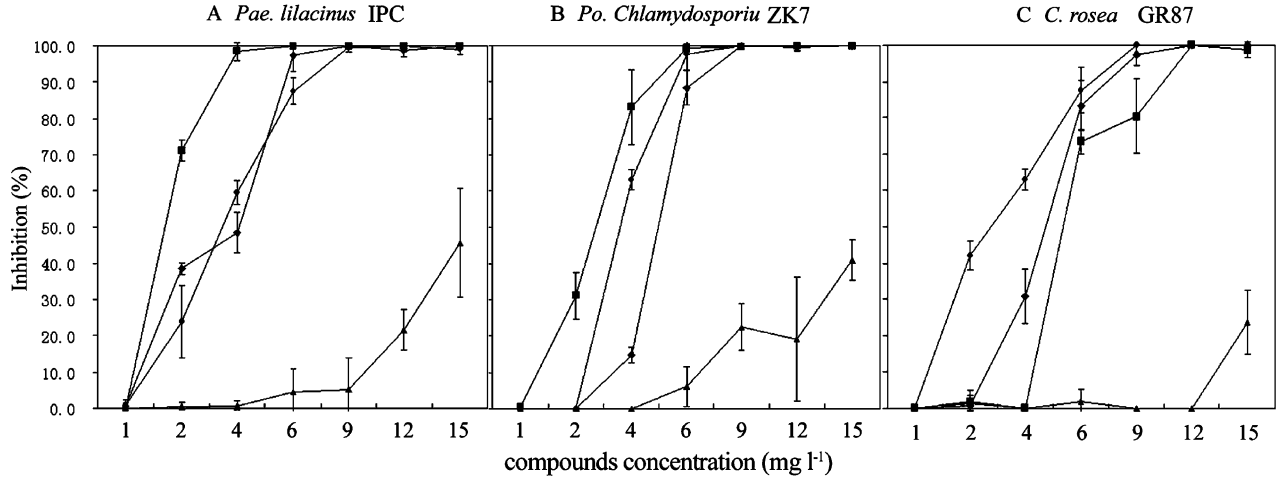

Fig. 3. Effects of several commercial volatile compounds on spore germination of three model fungi (A–C). Spores of three test fungi on WA plate were exposed to the volatile compounds at 25 °C for 24 h, and the inhibition was calculated (inhibition %=(germination of control−that of treatment)/control×100). Symbols: ♦, trimethylamine; ■, benzaldehyde; ●, *N,N*-dimethyloctylamine; ▲, nonadecane. Bar is SD.

compounds, the sensitivity of model fungi to trimethylamine, benzaldehyde, *N*,*N*-dimethyloctylamine was not significantly different ($P>0.05$) except to benzaldehyde between *Pae. lilacinus* and *C. rosea* ($0.01<P<0.05$). So our results did not vary according to their view.

In many fungistasis cases, soil VOCs served an important role (Balis, 1976; Herrington et al., 1987; Liebman and Epstein, 1992; Mackie and Wheatley, 1999). These were coherent with our results. Firstly, VOCs of many soils inhibited fungi strongly as evaluated by the low germination rate of model fungi in soil VOCs fungistasis tests. Secondly, the direct soil fungistasis and soil VOCs fungistasis had a clearly positive correlation. Soils, which showed strong direct antifungal activity, also generated many volatile fungistatic organic compounds. The most popular explanation for soil fungistasis thought that strong competition by soil microorganisms limited carbon availability to the germination spores of fungi (Arora et al., 1983; Ho and Ko, 1986). But in our soil volatile fungistasis test, fungi did not contact with soil, but fungistasis still occurred. It suggested that the occurrence of volatile fungistasis may not need the direct competition between soil microorganisms and adscititious fungi, and may have different mechanism with direct fungistasis.

Hora and Baker (1974) suggested that volatile fungistatic compounds were produced primarily by abiotic factors. But our results that autoclaved treatments, which killed the microorganisms but did not damage the structure and property of VOCs, could annul the suppression of soil VOCs to fungi, not only suggests the importance of microbial activities in fungistatic process, but also indicates that soil fungistatic VOCs were produced mainly by microorganisms in most soils.

Through identification and comparison of VOCs produced by volatile fungistatic soils and non-fungistatic soils, the following VOCs were specifically found in fungistatic soils: trimethylamine, 3-methyl-2-pentanone, dimethyl disulfide, methyl pyrazine, 2,5-dimethyl-pyrazine, benzaldehyde, *N*,*N*-dimethyloctylamine and nonadecane. Further, antifungal test of above compounds showed that trimethylamine, benzaldehyde, *N*,*N*-dimethyloctylamine suppressed the spore germination of model fungi strongly and their suppression was not significantly different for model fungi ($P>0.05$), indicating these three compounds made important contributions to soil volatile fungistasis. Trimethylamine was ever reported to inhibit arthrospore germination in *Geotrichum candidum* (Robinson et al., 1989). The other two compounds were first reported in this paper. Trimethylamine, *N*,*N*-dimethyloctylamine and another reported fungistatic compound ammonia (Ko et al., 1974), all belong to the amine group. They may change soil pH to become more alkaline. It is considered that high pH values can lead to fungistasis and ammonia production (Lockwood, 1977; Kao and Ko, 1983; Ann, 1994; Liebman and Epstein, 1992), so the generation of alkaline volatile compounds may be a reasonable explanation for VOCs fungistasis. The reason why benzaldehyde had strong antifungal activity was not clearly known. Of course, many VOCs, which have not been identified, may participate in soil volatile fungistatic process by synergistic effects.

In addition, the detection method for VOCs used in this study may be useful in further study on identification and characterization of the volatile fungistatic compounds.

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