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Fungistatic Intensity of Agricultural Soil Against Fungal Agents and Phylogenetic Analysis on the Actinobacteria Involved

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Abstract A total of 287 agricultural soil samples collected from 26 provinces or autonomous regions of China were tested on their ability to suppress the conidial germination of nine biocontrol fungal agents. These soil samples showed great differences in the degree to inhibit the germination of conidia ($22.8\% <$ mean inhibition rate $< 97.5\%$), but all exhibited fungistatic activities above the moderate levels (mean inhibition rate $> 50\%$) to most of tested fungi. Ten soil samples that have stronger fungistatic intensity (germination inhibition rate $> 68.3\%$) to the target fungi, *Trichoderma viride* and *Paecilomyces lilacinus*, were selected to evaluate their soil actinobacteria involved fungistasis in soil. Of the 1,000 isolates from those soil samples, 345 actinobacteria exhibited fungistatic activity to conidial germination of *T. viride* and *P. lilacinus* with germination inhibition rates higher than 10%. Sequences encoding 16S rRNA gene of the 345 actinobacteria were analyzed by ARDRA and resulted 44 different ARDRA types. Fifty-six isolates, at least one from each unique ARDRA type, were selected for 16S rDNA sequencing and phylogenetic analysis. Results indicated that the actinobacteria involved in the soil fungistasis had close phylogenetic relationship with the members of *Sterptomycetaceae*, *Microbacteriaceae*, *Micrococcaceae*, and *Nocardiacea*.

Introduction

Biological control of plant pathogens harnessing disease-suppressive microorganisms has been successfully applied to improve the plant health [12]. The application of biological control agents (BCAs) has played an important role in the agricultural practices. Although have not been used widely in the development of sustainable systems so far, the BCAs provide alternatives to chemical pesticides [2]. Soil is an extremely complex milieu, in which a number of factors, including soil texture, cation exchange capacity, organic matter content, pH, moisture, and the presence of a viable soil microflora, can influence the persistence and/or efficacy of BCAs. A prerequisite for using BCAs to control soilborne pathogens or pests is that fungal agents can germinate and grow normally and establish considerable population after being applied to soil. Unfortunately, most BCAs could not germinate and grow normally in soils due to the soil fungistasis [28] and thus reduced their efficacy.

Soil fungistasis represents the capability of most soils to suppress the germination and growth of fungi [5, 26]. The intensity of fungistasis has been found to vary depending on the physical and chemical properties of soils as well as

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on soil microbial activities [1, 17, 20]. Two popular hypotheses, the nutrient-deprivation [17] and antibiosis [22], have been proposed to explain the mechanism of soil fungistasis. The former states that exogenous energy sources may be unavailable for spores germinating due to strong competition from other soil microorganisms. The latter emphasizes that soil fungistasis can be caused by antifungal compounds produced by other soil microorganisms. Obviously, the soil microorganisms had been considered to play an important role in the formation of soil fungistasis in both the two different hypotheses. Previous studies had confirmed that fungistasis could be influenced by soil fungi [19] and soil bacteria [3, 9]. Actinobacteria is a group of gram positive bacteria with high DNA G + C content over 55%. More than 70% of bioactive compounds produced by these microorganisms have the potential to be applied in pharmacy, industry, agriculture, and environment. Members of this group are best known in biological control for their ability to produce various secondary metabolites, antibiotics and lytic enzymes. However, no study has been carried out to reveal the possible association between actinobacteria and fungistasis.

In addition, the severity and universality of soil fungistasis were characterized mainly using phytopathogens as targets [17], little attention has been paid to the inhibition of soil to a variety of BCAs. We previously characterized that the bacteria involved in the soil fungistasis by producing volatiles [29] and non-volatiles [15] to suppress the germination and growth of fungal agents. In this paper, we evaluated the fungistatic intensities of agricultural soils from 26 provinces and autonomous regions in China on the germination of 9 fungal agents, and presented a phylogenetic analysis on the actinobacteria involved in soil fungistasis.

Materials and Methods

Soil Sampling

In total, 287 soil samples used in this study were collected from agricultural fields with various plants in 26 provinces or autonomous regions of China. Approximately 1 kg of soil was sampled randomly from the top layer (2–15 cm) over an area of more than 10 m^2 per field. The soil was sieved through a 2-mm sieve, and stored in glass bottles at 4°C until used.

Fungal Isolates and Preparation of Conidial Suspension

Three nematophagous fungi (*Paecilomyces lilacinus*, *Pochonia chlamydosporia*, *Lecanicillium psalliotae*), five mycoparasites (*Clonostachys rosea*, *Trichoderma viride*, *T. harzianum*, *T. koningii* and *T. hamatum*), and one entomopathogenic fungus (*Beauveria bassiana*) were used in this study. The nine biological control fungi were provided by MSCYNU (Microorganism Store Center of Yunnan University) and cultured on potato dextrose agar (PDA) plates for 7 days at 25°C. Conidia were scraped off from fresh colony and suspended in sterile distilled water. To remove mycelial fragments, the suspension was filtered through six layers of lens cleaning paper towels and rinsed twice by centrifugation at 8,000 rpm at room temperature for 10 min. The conidial concentration of suspension was adjusted to approximately 10^6 spores per milliliter using a hemocytometer.

Isolation and Determination of Fungistatic Actinobacteria from Soil

The susceptibility of conidia to soil fungistasis was determined following the procedures described by Li et al. [15]. Ten of the 287 samples from different locations and originally with strong fungistatic capacity (GIR $\geq$ 75%) to *T. viride* and *P. lilacinus* were selected as the substances for actinobacteria isolation and their fungistatic intensities were measured again after being stored at 4°C for 2 months (Table 2). The actinobacteria were isolated using ISP5 medium (ISP5: L-asparagine 1 g, glycerol 10 g, K_2HPO_4 1 g, trace salts 1 ml, agar 15 g, pH 7.2, added to 1000 ml with double-distilled water, where the trace salts included $FeSO_4{\cdot}7H_2O$ 0.2 g, $MnCl_2{\cdot}2H_2O$ 0.1 g, $ZnSO_4{\cdot}7H_2O$ 0.1 g, added to 100 ml with double-distilled water). Fungistatic intensity of candidate actinobacteria was determined following the procedures described by Li et al. [15] using *T. viride* and *P. lilacinus* as fungal targets.

The fungistatic intensity of soil or actinobacteria to conidial germination was expressed using the germination inhibition rate (GIR) and calculated according to the following formula: GIR = (germination in the negative control − germination in the treatment)/germination in the negative control × 100%. The GIR of more than 75% was considered as strong inhibition, 50–75% as moderate, 25–50% as low, and 10–25% as slight.

Phylogenetic Analysis of the Actinobacteriaa Involved in Soil Fungistasis

Genomic DNA of actinobacteria was extracted using a bacterial genomic DNA extraction kit (BioTeke Corporation, China, Cat#:DP2001) and their 16S rRNA genes were amplified by PCR using the combination of primer FA (5′ CAG AGT TTG ATC CTG GCT 3′) and FB(5′ AGG AGG TGA TCC AGC CGC 3′). The amplified products were purified with agarose gel DNA purification kit (TakaRa, Cat#: DV805A) and used for amplified rDNA restriction

analysis (ARDRA) using endonucleases *Rsa*I, *Alu*I, and *Hae*III [15]. One to six representative isolates from each unique ARDRA type were submitted to Beijing Genomics Institute for sequencing. The resulting sequences of 16S rRNA gene were compared with those available in GenBank by using BLAST to determine their phylogenetic affiliation. Phylogenetic analysis was performed using the PAUP (Phylogenetic Analysis Using Parsimony) software (version 4.0 b8) [24] after multiple alignment of data by CLUSTAL_X [25], with gaps treated as missing data. Clustering was done by using the neighbor-joining method [23]. Bootstrap analysis was used to evaluate the tree topology of the neighbor-joining data by performing 1,000 replicates [11]. The 16S rRNA gene sequences of isolates had been deposited in the NCBI nucleotide sequence database.

Statistical Analysis

The data of soil fungistasis were analyzed using the software SPSS 11.0 (Inc., Chicago, IL, USA). Means were compared by the test of least significant difference (LSD). Unless otherwise indicated, rejection level was set when $P < 0.05$.

Results

Fungistatic Intensity of Agricultural Soils to the Conidial Germination of Biocontrol Fungi

The fungistatic intensities of the 287 soil samples collected from 26 provinces or autonomous regions of China were determined by using nine fungal agents as targets. It was shown that soil samples varied greatly in their fungistatic activities to the fungi, and all exhibited their fungistatic activities above the moderate level (GIR > 50%) to conidial germination of all tested fungi except *L. psalliotae*, *T. koningii*, and *B. bassiana*, on which their GIRs were all less than 50% (Table 1). The most sensitive groups included *P. lilacinus*, *T. harzianum*, *T. viride*, and *T. hamatum*, of which all tested soil samples showed strong fungistatic activities to all the four fungi with the mean inhibition rates more than 95%. Then followed *C. rosea* and *P. chamydosporia*, on which the mean inhibition rates were 70.8 and 72.7%, respectively. The least sensitive group included *L. psalliotae*, *T. koningii* and *B. bassiana* where soils only exhibited moderate or slight fungistatic activities. The mean inhibition rates to the three fungi were 22.8, 28.4, and 24.6%, respectively (Table 1). Among all the four tested species of the genus *Trichoderma*, unlike the other three which were significantly sensitive to soil fungistasis, *T. koningii* expressed a less sensitive level. This suggested that there were obvious differences among the species of the same genus responding soil fungistasis.

Isolation and Determination of the Soil Actinobacteria Involved in Soil Fungistasis

Though the fungistatic intensities decreased slightly after 2 mouths of storage period, the 10 soil samples selected still showed high fungistatic activity (Table 2). Sample A, B, C, E, G, H, and J strongly inhibited the conidial germination of *T. viride* and *P. lilacinus*, with GIRs of 83.4–92.7% and 84.3–93.5%, respectively. The other three samples (D, F, and I), respectively from the provinces of Tibet, Hubei and Qinghai, showed moderate inhibition to the two fungal species (68.3% < GI < 73.2%).

In total, 1,000 strains of actinobacteria were isolated randomly from the 10 soil samples mentioned above (100 from each sample). Fungistatic intensities test using *T. viride* and *P. lilacinus* as targets showed 345 isolates (34.5% of total) exhibited fungistatic activity to conidial germination of the both tested fungi. Of these, the number of isolates with the strong (GIR > 75%), the moderate (75% > GIR > 50%), the low (50% > GIR > 25%) and the slight (25% > GIR > 10%) inhibitory activity were 45, 221, 62, and 17, respectively.

Phylogenetic Analysis on the Soil Actinobacteria Involved in Soil Fungistasis

The 16S rRNA gene of the 345 fungistatic actinobacteria were amplified using a pair of primers FA and FB, giving rise to a single band of about 1,500 bp in length. All PCR products were analyzed by ARDRA using separate enzymatic digestions with endonucleases *Rsa*I, *Alu*I, and *Hae*III. In total, 44 different ARDRA types were generated (Table 3). The five largest groups, type R10, R13, R24, R25, and R26, together accounted for 27.6% (46, 29, 26, 22, and 20 of isolates from each, respectively) of the isolates analyzed. The groups of R01, R18, R20, R27, R31, R32, R33, R34, R36, and R43, which included 14–17 isolates, were also relatively large. Of the remaining 34 ARDRA patterns were represented by 1–13 isolates.

Based on the ARDRA patterns, 56 isolates were selected for 16S rDNA sequencing. The resulting sequences were used to determine their approximate phylogenetic affiliations. Phylogenetic analyses placed the 345 fungistatic isolates into the following four groups of the class *Actinobacteria*: *Sterptomycetaceae* (47.8% of the 345 isolates), the largest group, followed by *Microbacteriaceae* (20.0%), *Micrococcaceae* (18.9%) and *Nocardiaceae* (13.3%) (Fig. 1).

Group of Sterptomycetaceae contained 165 isolates represented by 15 ARDRA types (R4, R05, R07, R10, R12,

Table 1 Fungistatic intensity of 287 agricultural soil samples from China using nine different biological control fungi as targets

Fungi	Proportion of soil samples with fungistatic activities (%)				Mean GIR ± SD (%)
	Strong[a]	Moderate	Low	Slight	
P. lilacinus	100.0	0.0	0.0	0.0	96.8 ± 1.6
T. harzianum	100.0	0.0	0.0	0.0	96.5 ± 1.7
C. rosea	27.9	72.1	0.0	0.0	70.8 ± 6.9
L. psalliotae	0.0	0.0	40.1	59.9	22.8 ± 7.1
T. koningii	0.0	2.8	64.5	32.8	28.4 ± 9.9
T. viride	100.0	0.0	0.0	0.0	97.1 ± 1.7
T. hamatum	100.0	0.0	0.0	0.0	97.5 ± 1.0
P. chamydosporaa	41.1	58.9	0.0	0.0	72.7 ± 7.3
B. bassiana	0.0	0.0	51.2	48.8	24.6 ± 7.1

[a] Degree of fungistasis: Strong, 100–75% inhibition; moderate, 75–50% inhibition; low, 50–25% inhibition; slight, 25–10% inhibition

Table 2 Soil sampling locations and their fungistatic intensities

Soil sample	Sampling location	GIR (Mean ± SD %)	
		T. viride	*P. lilacinus*
A	Hainan Province (109.34E, 19.31N)	92.7 ± 1.8	93.5 ± 0.7
B	Guangxi Autonomous Region (108.18E, 22.39N)	86.6 ± 1.2	85.4 ± 0.9
C	Guangdong Province (113.2E, 23.07N)	89.4 ± 0.6	91.0 ± 1.1
D	Tibet Autonomous Region (94.21E, 29.4N)	68.3 ± 1.1	73.2 ± 0.8
E	Zhejiang Province (120.1E, 30.16N)	87.9 ± 0.7	84.3 ± 1.7
F	Hubei Province (114.22E, 30.33N)	72.3 ± 0.5	69.9 ± 2.6
G	Sichuan Province (104.05E, 30.42N)	83.4 ± 0.9	87.0 ± 1.4
H	Shandong Province (117.05E, 36.12N)	87.1 ± 0.4	88.8 ± 0.6
I	Qinghai Autonomous Region (101.41E, 36.37N)	70.0 ± 2.4	70.9 ± 1.3
J	Liaoning Province (121.36E, 38.54N)	90.6 ± 1.7	91.2 ± 0.9

R13, R17, R20, R23, R25, R37, R40, R42, R43, and R44) and were phylogenetically associated with two genera of *Sterptomycetaceae* (97–100% similarities): *Streptomyces* (164 isolates, 14 types) and *Kitasatospora* (1, 1). Among them, the members of types R10 (31 isolates), R25 (22), R40 (19), R13 (18), and R23 (16) were the most predominant and phylogenetically associated with the species *Streptomyces flavotricini*, *Streptomyces flavoviridis*, *Streptomyces variabilis*, *Streptomyces exfoliates*, and *Streptomyces cirratus*, respectively.

The *Microbacteriaceae* group included 69 fungistatic isolates represented by nine ARDRA types (R16, R18, R21, R26, R31, R34, R35, R36, and R38) and was phylogenetically associated with two genera of *Microbacteriaceae*. The genus *Microbacterium* contained 57 isolates and was represented by seven ARDRA types. The other genus *Curtobacterium* included 12 isolates and exhibited two ARDRA types. Among them, the members of types R36 (13 isolates), R34 (10), and R31 (9) were the most predominant and phylogenetically associated with the species *Microbacterium kitamiense, Microbacterium aurum*, and *Microbacterium esteraromaticum*, respectively.

Group *Micrococcaceae* contained 65 fungistatic isolates represented by 11 ARDRA types (R29, R19, R32, R31, R09, R34, R25, R06, R35, R14, and R11). These isolates were phylogenetically related to four genera of *Micrococcaceae* (97–100% similarities): *Arthrobacter* (43 isolates, 6 types), *Micrococcus* (2, 1), *Kocuria* (11, 2), and *Curtobacterium* (9, 2). In this group, the types R32 (11 isolates), R33 (9), R11 (7), and R19 (7) were the most predominant and phylogenetically related with the species *Arthrobacter arilaitensis*, *Arthrobacter sulfonivorans*, *Arthrobacter polychromogenes*, *Kocuria rhizophila*, respectively.

The last group, *Nocardiaceae*, included 46 isolates represented by nine ARDRA types (R01, R02, R03, R08, R15, R22, R27, R39, and R41) and phylogenetically associated with two genera of *Nocardiaceae* (97–100% similarities): *Nocardia* (42 isolates, 7 types) and *Rhodococcus* (4, 2 types). In this group, types R1 (11 isolates), R27 (10), and R41 (9) were the most predominant and

Table 3 List of actinobacteria isolates obtained in this study representing each ARDRA group and their closest affiliations according to their partial 16S rRNA gene

Isolate [accession no.]	GIR (%)*	ARDRA type	The closest relative in the database [accession no.]
MH167 [GQ495659]	56.4	R01	*Nocardiaceae bacterium* Ben-13 [EF028121]
MH124 [FJ626642]	33.9	R02	*Nocardia cummidelens* 173896 [EU570344]
MH165 [GQ495657]	46.8	R02	*Nocardia cummidelens* 173707 [EU593592]
MH166 [GQ495658]	65.1	R03	*Nocardia seriolae* KN0205 [AB255699]
MH160 [GQ495652]	82.3	R04	*Kitasatospora phosalacinea* NBRC 14372 [AB184596]
MH118 [FJ626640]	60.2	R05	*Streptomyces purpureus* NBRC 13927 [AB184547]
MH137 [FJ626620]	23.1	R06	*Arthrobacter dextranolyticus* T6 [AB117515]
MH120 [FJ626633]	58.8	R07	*Streptomyces anulatus* HBUM82831 [FJ486289]
MH163 [GQ495655]	81.6	R08	*Nocardia cyriacigeorgica* ATCC 14759 [EF127493]
MH159 [GQ495651]	78.9	R09	*Citricoccus alkalitolerans* YIM 70010 [AY376164]
MH117 [FJ626631]	32.2	R10	*Streptomyces flavotricini* NBRC 12770 [AB184132]
MH101 [FJ626649]	44.4	R10	*Streptomyces flavotricini* NBRC 12770 [AB184132]
MH140 [FJ626624]	35.9	R11	*Arthrobacter polychromogenes* 4 [EF154244]
MH115 [FJ626646]	85.8	R12	*Streptomyces gardneri* NRRL B-5615T [DQ442500]
MH130 [FJ626658]	87.2	R13	*Streptomyces exfoliatus* NRRL B-2494 [DQ026647]
MH121 [FJ626648]	78.8	R13	*Streptomyces exfoliatus* NBRC 13191 [AB184324]
MH139 [FJ626623]	43.6	R14	*Arthrobacter oxydans* RC28 [FJ263367]
MH168 [GQ495660]	44.2	R15	*Rhodococcus equi* CUB 1261 [DQ157912]
MH149 [GQ495641]	44.6	R16	*Microbacterium lacticum* 3388 [EU714364]
MH102 [FJ626635]	23.8	R17	*Streptomyces albireticuli* CSSP396 [AY999748]
MH156 [GQ495648]	59.1	R18	*Curtobacterium luteum* DSM 20542 [X77437]
MH134 [FJ626626]	35.9	R19	*Kocuria rhizophila* KL-057 [AY030341]
MH143 [FJ626653]	55.1	R20	*Streptomyces erythrochromogenes* NBRC 3304 [AB184746]
MH151 [GQ495643]	75.8	R21	*Microbacterium paraoxydans* 3131 [EU714354]
MH169 [GQ495661]	77.9	R22	*Rhodococcus ruber* R6 [EF581125]
MH145 [FJ626661]	32.1	R23	*Streptomyces cirratus* CSSP547 [AY999794]
MH135 [FJ626618]	25.6	R24	*Arthrobacter nitroguajacolicus* MN13 [FM213384]
MH136 [FJ626622]	46.2	R24	*Arthrobacter nitroguajacolicus* MNPB4 [FM213395]
MH144 [FJ626656]	38.2	R24	*Arthrobacter nitroguajacolicus* CCM 4924T [AJ512504]
MH131 [FJ626659]	17.6	R25	*Streptomyces flavoviridis* NBRC 12772 [AB184842]
MH104 [FJ626650]	61.9	R25	*Streptomyces flavoviridis* HBUM82119 [EU841558]
MH106 [FJ626652]	33.3	R25	*Streptomyces flavoviridis* HBUM82119 [EU841558]
MH109 [FJ626630]	45.8	R26	*Curtobacterium flaccumfaciens* pv. *flaccumfaciens* [FJ418770]
MH157 [GQ495649]	33.3	R26	*Curtobacterium flaccumfaciens* SAFR-008 [AY167854]
MH155 [GQ495647]	58.9	R26	*Curtobacterium flaccumfaciens* pv. *basellae* BR-10 [AY273210]
MH162 [GQ495654]	69.8	R27	*Nocardia farcinica* ATCC 3318 [DQ659906]
MH110 [FJ626625]	43.8	R28	*Kocuria himachalensis* K07-05 [AY987383]
MH158 [GQ495650]	66.6	R29	*Citricoccus muralis* 4-0 [AJ344143]
MH132 [FJ626629]	28.2	R30	*Micrococcus luteus* CV39 [AJ717368]
MH126 [FJ626651]	46.4	R30	*Micrococcus luteus* [AB167385]
MH154 [GQ495646]	51.3	R31	*Microbacterium esteraromaticum* XJ-7 [FJ527721]
MH127 [FJ626619]	77.1	R32	*Arthrobacter arilaitensis* DS37 [EU834260]
MH138 [FJ626621]	34.6	R33	*Arthrobacter sulfonivorans* DSM 14002 [AF235091]
MH148 [GQ495640]	70.1	R34	*Microbacterium aurum* 2588 [EU714343]
MH152 [GQ495644]	39.5	R35	*Microbacterium trichothecenolyticum* 154 [EU730939]
MH153 [GQ495645]	48.9	R36	*Microbacterium kitamiense* DS69 [EU834283]
MH116 [FJ626639]	73.7	R37	*Streptomyces spororaveus* HBUM174519 [EU877063]

Table 3 continued

Isolate [accession no.]	GIR (%)*	ARDRA type	The closest relative in the database [accession no.]
MH150 [GQ495642]	60.1	R38	*Microbacterium oxydans* 3227 [EU714357]
MH164 [GQ495656]	53.3	R39	*Nocardia nova* SEI2b [DQ840029]
MH103 [FJ626636]	42.9	R40	*Streptomyces variabilis* HBUM174059 [EU841659]
MH142 [FJ626637]	43.6	R40	*Streptomyces variabilis* HBUM174059 [EU841659]
MH105 [FJ626660]	35.4	R40	*Streptomyces variabilis* HBUM174059 [EU841659]
MH161 [GQ495653]	78.5	R41	*Nocardia beijingensis* IFM 0908 [AB094653]
MH123 [FJ626641]	33.9	R42	*Streptomyces viridochromogenes* HBUM174912 [EU841620]
MH114 [FJ626645]	50.1	R43	*Streptomyces sporovirgulis* L0801 [EU781491]
MH129 [FJ626654]	53.4	R44	*Streptomyces diastatochromogenes* ATCC 12309 [AB026218]

* The values of GIR were measured by using the fungus *T. viride* as target

phylogenetically associated with the species of *Nocardiaceae bacterium*, *Nocardia farcinica*, and *Nocardia beijingensis*, respectively.

GIRs of the 56 sequenced isolates ranged from 17.6% of *Streptomyces flavoviridis* MH131 to 87.2% of *Streptomyces exfoliatus* MH130 (Table 3). Among the above four groups, *Nocardiaceae* showed the highest fungistatic ability to fungus *T. viride*, which had an average GIR of 60.7%. The fungistatic intensity of *Microbacteriaceae* (average GIR = 53.4%) and *Sterptomycetaceae* (51.7%) were similar and higher than that of *Micrococcaceae* (44.6%).

Discussion

Soil fungistasis is a widespread phenomenon occurring in biological control [4, 17]. Most of the fungal agents could not germinate and grow normally in soil due to this effect. Fungistatic intensity varies with the changing of physical, chemical properties of soil [12, 20, 21] and microbial community composition in soil [3, 15, 17, 27, 28]. However, no such report has being presented on the intensity and universality of soil fungistasis against biocontrol fungi by using a variety of soil samples. In this study, it was shown that almost all soil samples from across China could inhibit the nine tested biological control fungi more or less (Table 1), suggesting that soil fungistasis should be taken into account when the biocontrol fungi are developed and applied in the agricultural soils. Among the tested fungi used in this study, two nematophagous fungi, *P. chlamydosporia* and *P. lilacinus*, have been developed into commercial products and used to control parasitic nematodes in the field for years [2, 16]. However, these fungal agents usually displayed unstable control effects [28]. Our study had indicated that the germination of the two fungi was severely inhibited in all of the soil samples. These repressed conidia could keep dormant for 3 to 6 months and then lose their vigor gradually (Data not shown).

Mycoparasites of the genus *Trichoderma* had the potential to control the soil borne pathogens [6, 7, 13, 14] and were one of the most exploited fungal agents used for the management of plant diseases [8, 10, 18]. Previous studies had indicated that different species within a genus or different isolates of a species would vary their sensitivities to soil fungistasis [17]. In this study, *Trichoderma*, *T. hamatum*, *T. viride*, and *T. harzianum* were more sensitive to soil fungistasis than *T. koningii*. It would be important to take the resistance capability of different species as a criterion in the selection of fungal control agents.

Concerning the formation mechanism of soil fungistasis, two popular hypotheses, nutrient-deprivation and antibiosis, had been proposed [17]. Enough evidences from previous studies supported that soil microorganisms were one of the vital factors in the development of soil fungistasis by excreting antifungal compounds or competing for nutrition. De Boer et al. [3] suggested that microbial community composition was an important factor determining soil fungistasis and that the presence of antifungal activity of Pseudomonads could be important in this aspect. Zou et al. [29] characterized that the community composition of bacteria involved in the soil fungistasis by producing the organic volatiles such as acetamide, benzaldehyde, benzothiazole, L-butanamine, methanamine, phenylacetaldehyde, and L-decene. Li et al. [15] reported the composition of soil bacteria producing non-volatile fungistatic substances, including the members from groups of *Actinobacterales*, *Bacillales*, and *Gammaproteobacteria*. In this study, we firstly characterized the soil actinobacteria as fungictatic antagonists against fungal agents. These fungistatic actinobacteria primarily came from *Sterptomycetaceae* (47.8%), followed by *Microbacteriaceae* (20.0%), *Micrococcaceae* (18.9%), and *Nocardiaceae* (13.3%), and varied largely in their abilities to inhibit the canidial

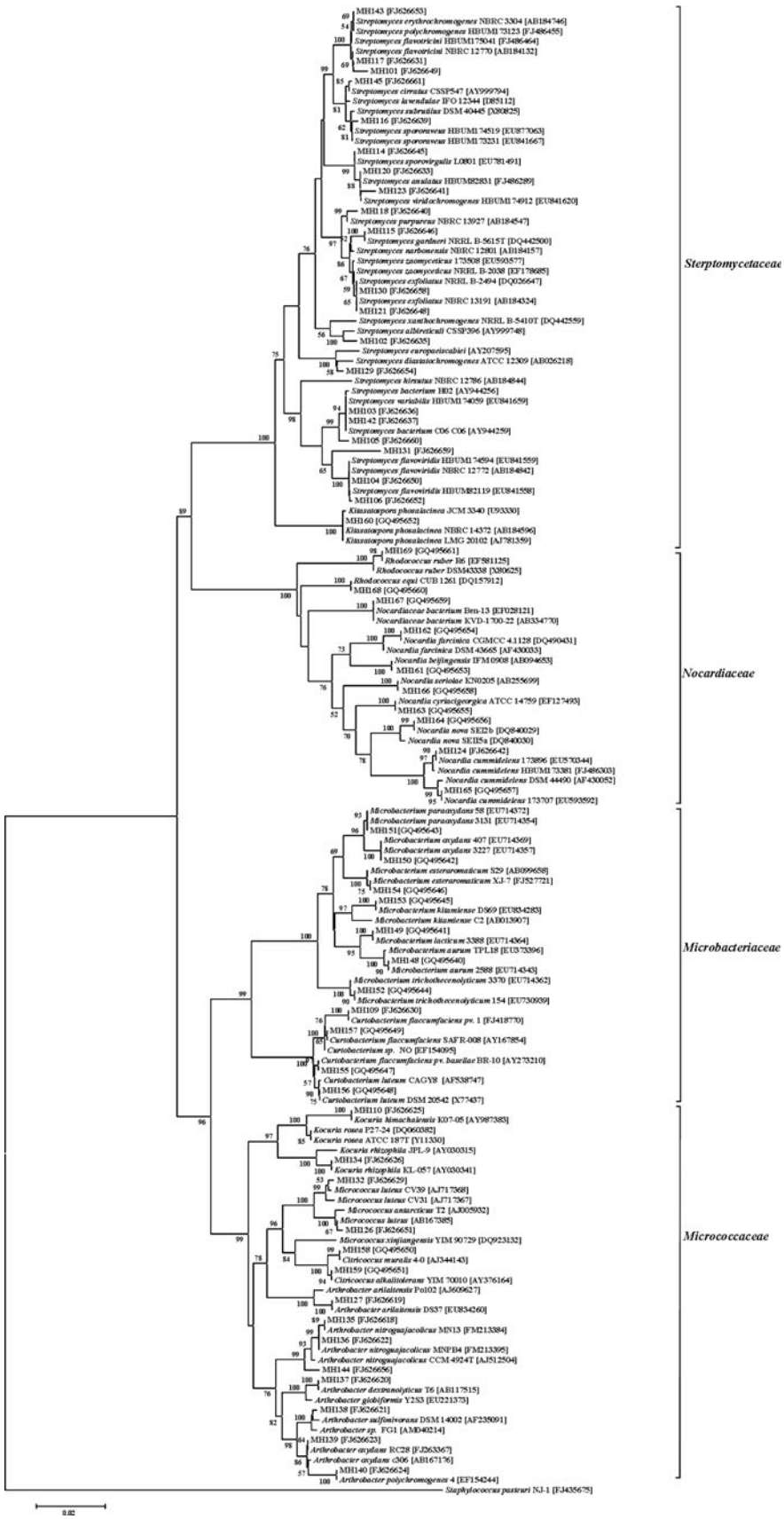

Fig. 1 16S rRNA-based dendrograms showing phylogenetic relationships of fungistatic actinobacteria (shown in *bold*) to closely related sequences from GenBank. Bootstrap values ($n = 1000$ replicates) higher than 50% are reported as percentages. The *scale bar* represents the number of changes per nucleotide position. *Staphylococcus pasteuri* NJ-1 (FJ435675) is used as the outgroup. Accession numbers are given at the end of each sequence

germination with GIRs from 17.6 to 87.2% (Table 3). This suggested that the multifarious soil microorganisms contributed to the formation of soil fungistasis, and the contribution of these involved indigenes was unequal. In addition, the fungistatic compounds from soil actinobacteria are still unknown and need to be characterized in the future.

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