Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 20(2008) 871–877
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Soil fungistasis and its relations to soil microbial composition and diversity: A case study of a series of soils with different fungistasis

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Received 6 September 2007; revised 9 October 2007; accepted 12 November 2007

Abstract

Fungistasis is one of the important approaches to control soil-borne plant pathogens. Some hypotheses about the mechanisms for soil fungistasis had been established, which mainly focused on the soil bacterial community composition, structure, diversity as well as function. In this study, the bacterial community composition and diversity of a series of soils treated by autoclaving, which coming from the same original soil sample and showing gradient fungistasis to the target soil-borne pathogen fungi *Fusarium graminearum*, was investigated by soil bacterial 16S rDNA-PCR (polymerase chain reaction) cloning, restriction fragment length polymorphism (RFLP), and sequencing. The results showed that the soil fungistasis capacity was closely correlated with soil bacterial community composition and diversity, such as soil fungistasis declined with the decrease of soil bacterial diversity. Meanwhile, the bacterial community composition and structure were significantly different along the gradient of soil fungistasis tested. α-*Proteobacteria*, β-*Proteobacteria*, *Flexibacter*, and some uncultured soil bacteria were contributed to soil fungistasis in combination with some other special bacteria (*Pseudomonas* and *Acidobacteria*) which were know to be key species in suppression of fungal growth.

Key words: soil fungistasis; 16S rDNA clone library; microbial diversity; bacterial community composition

Introduction

Fungistasis was first described by Dobbs and Hinson in 1953, which represents the capability of most soils to control the germination and growth of soil-borne fungi. Since then, many studies have been conducted on the physical and chemical traits of soil, fungal characteristics, community composition, and metabolic activities of other soil microbes, and environmental changes, to understand the mechanisms of soil fungistasis (Lockwood, 1964, 1977; Dobbs and Gash, 1965; Romine and Baker, 1973; Mondal and Hyakumachi, 1998; Alabouvette, 1999). Recently, the roles of soil microbes in soil fungistasis were mentioned by several studies (Yang *et al*., 2001; 2001; Benizri *et al*., 2005; Garbeva *et al*., 2006; Prez-Piqueres *et al*., 2006). De Boer *et al*. (2003) demonstrated that disease suppressive element could be eliminated through soil pasteurization, and could be transferred to a conducive soil through the introduction of very small amounts of the suppressive soil. suppressive soil. Vermicompost added to various container media significantly inhibited the infection of tomato plants by *Fusarium oxysporum* f. sp. *Lycopersici*, and microbe is suggested for the suppressiveness of it (Szczech, 1999).

To date, the studies on disease suppressive element mainly focused on some special bacteria, especially *pseudomonas* and their metabolites with antibiosis (Raaijmakers *et al*., 1997; Raaijmakers and Weller, 1998; Sharifi-Tehrani *et al*., 1998; Siddiqui and Shaukat, 2002; Liu *et al*., 2006). De Boer *et al*. (2007) discovered that the mixtures of apparently non-antagonistic soil bacteria could exhibit a strong negative effect on the growth of fungi *in vitro*. In addition, other microbes could enhance the could enhance the antibiotic production of antagonistic soil bacteria (Becker *et al*., 1997; Pierson *et al*., 1998; Lutz *et al*., 2004; Maurhofer *et al*., 2004), which meant that soil microbial community composition is as important as antagonistic microbes in soil fungistasis. However, besides the relatively clear fungistasis of some special bacteria, such as pseudomonads, the influences or functions of bacterial community composition on fungistasis are

Soil microbial community is influenced by many factors. When Gelsomino *et al*. (1999) and Da Silva *et al*. (2003) suggested that suggested that soil type largely determined the structure of bacterial communities, and that similar soil types tended to select similar communities. Steenwerth *et al*. (2003) and Clegg *et al*. (2003) indicated that management practice has an impact on the community structure of specific bacterial groups. At the same time, many studies found that plant type was a major determinant of the communities in soil, because plants are the main providers of specific carbon and energy sources (Germida

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et al., 1998; Grayston *et al*., 1998; Grayston *et al*., 1998; Kaiser *et al*., 2001; Marschner *et al*., 2001; Kowalchuk *et al*., 2002; Garbeav *et al*., 2006). Marschner *et al*. (2001) found that plant species, soil types, and root zone location affected bacterial community structures in the rhizosphere. Owing to those complicated factors, it is difficult for us to sample comparable soils with different fungistasis from natural environment to study the relationship between soil fungistasis and bacterial community composition. As a result, it is necessary to construct a model soil, which will avoid these complex

In this study, a series of soils with the gradient of fungistasis ranging from high to low were established by autoclaving at different temperatures. Bacterial 16S rDNA-PCR (polymerase chain reaction) cloning, Restriction fragment length polymorphism (RFLP), and sequencing of soil genomic DNA were employed to analyze the populations of the series of soils. The soils were tested to characterize the difference of bacterial community composition and diversity of the gradient fungistasis soils samples between bacterial community composition and level of soil fungistasis.

1 Materials and methods

1.1 Soil sampling

The soil sample (0–15 cm) was collected from a fallow land at the Shenyang Experimental Station of Ecology, Chinese Academy of Sciences. The sample was immediately brought to lab, sieved through a 2-mm mesh, and treated to establish a series of soil samples with gradient fungistasis to target fungi. The physical and chemical properties of soil sample are presented in Table 1.

1.2 Soil treatment

After mixing, the soil sample was divided into 4 groups and three replications were obtained in each group. One group did not receive pasteurization treatments and the others received 4-min pasteurization treatments at 100, 110, and 121°C, respectively (Fig.1). Then, an agar disk (potato dextrose agar, 1-cm diameter) from the growing margin of *Fusarium graminearum* (a primary soil-borne plant pathogen in Northeast China) colony was inverted and placed centrally on top of soil in a petri dish. After 3 d of incubation at 25°C, the extension of the mycelium was determined and soil samples were collected. The series of soils with gradient fungistasis from high, medium, low, and bally low were assessed by determining the diameter of hyphal extension. Statistical analyses to evaluate the distinction were performed with the software package SPSS 13.0 for Windows (SPSS Inc., USA, 2004) by comparison in ANOVA using the Fisher's LSD test at P level of 1%.

In this article, the relative fungal control rate (RFCR) of high fungistasis soil was set at 1, and the bally low was 0. The RFCR was calculated as:

$$\mathrm{RFCR} = 1 - (D_n - D_0)/(D_1 - D_0) \times 100\%$$

where, D_n is the diameter of hyphal extension in different soils, D_1 and D_0 are the hyphal diameter of high and bally low fungistasis soils, respectively.

1.3 Total community DNA extraction

DNA was extracted from soil samples after 3 d of incubation following pasteurization. DNA, from triplicate 0.5 g soil subsamples, was extracted using the Fast DNA SPIN kit for soil (Bio101, Inc., USA) according to the manufacturer's protocol.

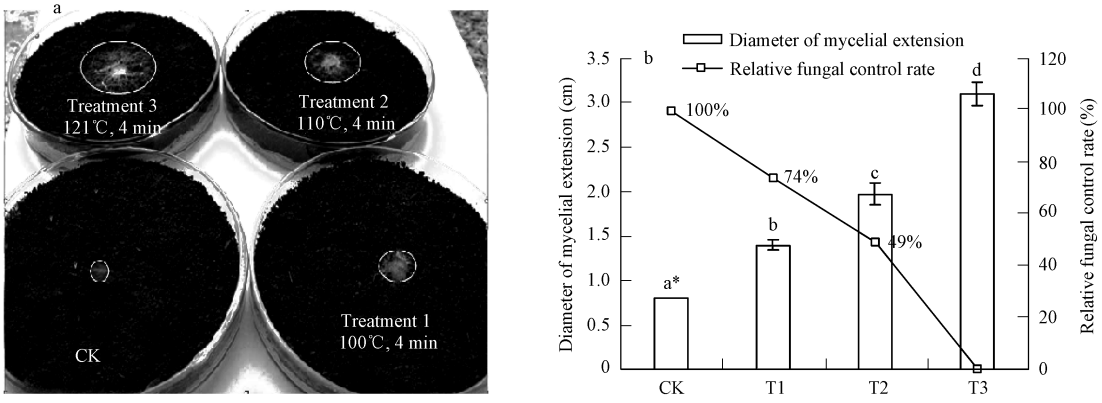

Fig. 1 Abilities of different treated soils to control target fungal (*Fusarium graminearum*) growth (a) and the effect of pasteurized treatments on hyphal extension ability (b). CK: natural soil; T1: Treatment 1; T2: Treatment 2; T3: Treatment 3. Treatments 1, 2, and 3 were the soil samples autoclaved at 100, 110, and 121°C respectively for 4 min. *Different letters following means ± standard deviation in the column indicate significant differences by Fisher's LSD ($P < 0.01$).

Table 1 Main physico-chemical properties of soil sample

Soil type	pH	Moisture content (%)	Organic matter (g/kg)	Total nitrogen (g/kg)	Total phosphorus (g/kg)	Total potassium (g/kg)	Physical composition (%)		
							Sand	Slit	Clay
Aquic brown soil	6.21	11.2	15.9	0.83	0.41	0.93	14.1	62.8	23.1

1.4 PCR amplification

Genomic DNA coding for the partial 16S rDNA gene was amplified with the primer pair 27F and 1492R (Martin-Laurent *et al*., 2001). PCR reaction was performed in a total volume of 50 μl reaction mixture containing 1 × reaction buffer, 200 mmol/L dNTP, 1.0 mmol/L of each primer, 2 units Ex *Taq* DNA polymerase (TaKaRa Bio Inc., Japan) in thermocycler (PTC 200 gradient cycler, MJ Research, USA) at 94°C for 5 min, followed by 30 cycles at 94°C for 1 min, 52°C for 1 min, and 72°C for 2 min and followed a final extension period of 10 min at 72°C. The PCR products were then separated by electrophoresis on a 1% agarose gel, excised, purified with Agarose Gel DNA Purification Kit Ver.2.0 (TaKaRa Bio Inc., Japan) as recommended by the manufacturer.

1.5 Cloning and RFLP analyzing

DNA fragments were individually ligated with vector pMD19-T DNA (TaKaRa Bio Inc., Japan) for 1 h at 16°C. The ligated DNA (10 μl) were mixed with 100 μl competent cells of *E. coli* JM109 (TaKaRa Bio Inc., Japan) in ice-cold tubes for 30 min, at 42°C for 45 s, and in ice again for 2–3 min, followed by addition of 890 μl SOC medium (2% Bacto Tryptone, 0.5% yeast extract, 0.05% NaCl, 2.5 mmol/L KCl, 10 mmol/L $MgCl_2$, 20 mmol/L glucose). DNA was then transferred to cells and transformed cells were allowed to recover for 1 h at 37°C with shaking at 200 r/min before plating on selective LB media containing Amp (100 μg /ml), X-Gal (40 μg/ml), and IPTG (24 μg/ml).

Clone inserts were grouped into operational taxonomic units (OTUs) by restriction RFLP banding profiles. White colonies were randomly picked and screened directly for inserts by performing colony PCR with primers RV-M (TaKaRa Bio Inc., Japan) and M13-47 (TaKaRa Bio Inc., Japan) for the vector. The PCR products were run on 1% agarose gel along with DL2000 (TaKaRa Bio Inc., Japan) marker and stained with SYBR Green I (TaKaRa Bio Inc., Japan) to estimate the size of the bands. Without further purification, the PCR products were digested with the restriction endonucleases, *Taq* I and *Hinf*, for 12 h under the standard conditions suggested by the manufacturer (Takara Bio Inc., Japan). Restriction bands were separated in 10% (*W/V*) polyacrylamide gel.

The presence/absence of each restriction fragment was recorded in a binary data matrix for further statistical analysis (Yeh *et al*., 1997). Software POPGENE (version 1.31), the UPGMA (unweighted pair group method arithmetic average) cluster analysis was used to reveal the composition similarity among the populations.

1.6 Rarefaction analysis and diversity indices

Rarefaction analysis and calculation of diversity indices were applied to 16S rDNA sequences being defined as operational taxonomic units (OTUs). Rarefaction curve were produced by using the freeware software Analytic Rarefaction (Holland, 2003). Coverage index (*C*), Shannon-Weaver diversity index (*H′*), and Evenness index (*E*) values were calculated as previously described (Hughes and Bohannan, 2004).

1.7 Sequencing of 16S rDNA clones

The RFLP patterns of each library were grouped visually and some representative clones were selected for sequencing: Group 1, the unique clones of CK (natural soil); Group 2, the clones only existed in CK and Treatment 1 but not in Treatment 2 and Treatment 3; Group 3, the clones existed in CK and Treatment 2 but not in Treatment 3; Group 4, the unique clones of Treatment 3; and Group 5, the common clones of all treatments. Group 1, 2, and 3 would be the most probable parts which contributed to fungistasis, whereas Groups 4 and 5 had less contribution to soil fungistasis, compared with the others.

OTU representatives in every group were fully sequenced in Shanghai Sangon Biological Engineering Technology and Services Co., Ltd., China. Vector primers M13-47 was used for sequencing. All sequences were checked for possible chimeric artifacts by the Check-Chimera program (Maidak *et al*., 1999). Sequences were aligned by DNAMAN (Lynnon Biosoft, version 4.0), and compared to available databases using the BLAST (Basic Local Alignment Search Tool) network service to determine their approximate phylogenetic affiliations.

1.8 Nucleotide sequence accession numbers

The sequences of the 16S rDNA clones have been submitted to GeneBank under the following accession numbers: EF526220-EF526294.

2 Results

2.1 Soil samples with gradient fungistasis

As shown in Fig.1, the suppression of hyphal growth of target fungi *F. graminearum* was significantly relieved with the increase of treating temperature from CK to 121°C ($P < 0.01$). Treatment 3 was bally low fungistasis, and its hyphal diameter reached to 3.11 cm, the RFCR value was 0 as we defined. And CK was high fungistasis, its RFCR value was 100%, Treatment 1 and Treatment 2 was low and medium fungistasis, respectively.

2.2 RFLP analysis of 16S rRNA clone library

Rarefaction analysis was applied to evaluate whether screening clones were sufficient to estimate diversity within the clone libraries. The expected number of OTUs was plotted versus the number of 16S rDNA clones in the clone libraries. The calculated rarefaction curves were close to saturation, indicating that the clone libraries were large enough to reflect the bacterial diversity of samples (Fig.2). In agreement of this observation, the *C* (Coverage index) values were 75.0%, 89.9%, 91.6%, and 90.3% (Table 2).

The Shannon index represents bacterial diversity when Evenness index denotes distribution of all bacteria in samples. Along decreasing of Shannon index and Evenness index, the fungistasis was reduced. But the index of Treatment 3 was obviously higher than that of Treatment

Table 2 Comparison of 16S rDNA clone libraries of different fungistasis soils

Treatment	Fungistasis	Number of clones	Number of OTUs	Number of unique OTUs	Coverge index (*C*) (%)	Shannon index (*H′*)	Evenness index (*E*)
CK	High	164	76	39	75.0	5.837	0.844
T1	Medium	198	46	23	89.9	4.322	0.772
T2	Low	190	34	7	91.6	3.286	0.646
T3	Bally low	195	41	16	90.3	3.882	0.725

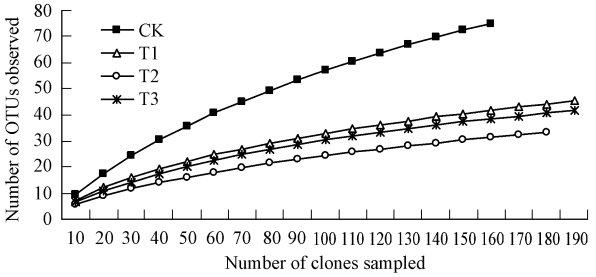

Fig. 2 Rarefaction curves for OTUs of 16S rDNA clones. The expected number of OTUs is plotted versus the number of clones.

2, and the unique OTU number of Treatment 3 was also greater than that of Treatment 2 (Table 2).

Dendrogram of bacterial composition of soils (Fig.3) analyzed by popgene (Yeh *et al*. 1997) based on RFLP patterns. It shows that CK, Treatment 1, and Treatment 2 had greater composition similarity and clustered together, and Treatment 3 which had the lowest fungistasis belongs to another group. Treatments 1 and 2 clustered much closer than the natural soil in the first cluster. This indicates that the disturbed soils at 100 and 110°C had higher similarity of bacterial community composition, and they were also mainly similar to the natural soil though some soil bacterial populations had changed. Treatment 3, the most interfered soil, had far genetic distance with the other soils. High temperature treatment changed soil bacterial community composition and relieved suppression of hyphal growth of *F. graminearum* (Fig.1a). The natural soil had the strongest effect on indicative fungal growth. Treatments 1 and 2 can control fungal growth to a certain extent, and the RFCR was 74% and 49%, respectively, the bacterial community composition were similar with that of the natural soil (RFCR was 100%). Treatment 3 represented the lowest fungistasis (RFCR was 0), and the bacterial community composition was far from that of the natural soil (Fig.1b).

2.3 Bacterial community composition by sequencing

After excision of 3 chimeric sequences which all belonged to Group 4, a total number of 75 16S rDNA clones were sequenced and their phylogenetic affiliations were estimated by BLAST search. The bacterial community developments of 4 samples were significantly different (Table 3). The 16S rDNA clones in Group 1 and Group 2 were dominated by uncultured species, and *Bacillus* in the Group 3, 4, 5. Clones belonged to *Pseudomonas*, *Myxococcales*, *Acidobacteria*, *Bacteroidetes*, *Rubrobacter*, *Pseudonocardia*, *Nitrospira*, *Mesorhizobium*, and *Verrucomicrobia* were recovered only from the Group 1 (CK), while clones belonging to *Flexibacter* were only detected in Group 2.

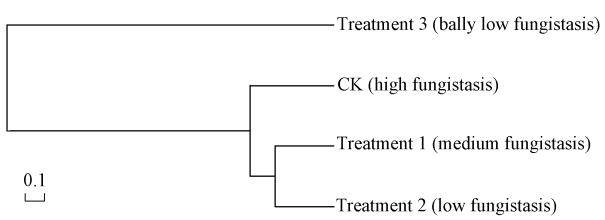

Fig. 3 Dendrogram of bacterial composition for gradient fungistasis soils.

3 Discussions

It was reported that soil type, cultural practice, and plant type were able to affect soil microbial diversity and community composition, and thus the level of suppressiveness of plant diseases. All evidence suggest that suppression of soil-born pathogenic fungi is a complicated system, but the soil bacterial community composition was the

Table 3 Summary of bacterial community composition

Genus (closest match)	Clones belonging to group respectively samples (%)			
	CK	T1	T2	T3
Group 1				
Pseudomonas (10)	16.13	–	–	–
α-*Proteobacteria* (6)	9.68	–	–	–
β-*Proteobacteria* (1)	1.61	–	–	–
Myxococcales (6)	9.68	–	–	–
Acidobacteria (4)	6.45	–	–	–
Bacteroidetes (2)	3.23	–	–	–
Rubrobacter (2)	3.23	–	–	–
Pseudonocardia (1)	1.61	–	–	–
Nitrospira (1)	1.61	–	–	–
Mesorhizobium (1)	1.61	–	–	–
Verrucomicrobia (1)	1.61	–	–	–
Bacteroidetes (1)	1.61	–	–	–
Uncultured (26)	**41.94**	–	–	–
Group 2				
β-*Proteobacteria* (10/11)	40	33.33	–	–
Flexibacter (3/1)	12	3.03	–	–
Uncultured (12/21)	**48**	**63.64**	–	–
Group 3				
Bacillus/Paenibacillus (11/14)	57.90	–	43.75	–
α-*Proteobacterium* (2/1)	10.53	–	3.13	–
Uncultured (6/17)	**31.57**	–	**53.12**	–
Group 4				
Bacillus/Paenibacillus (21)	–	–	–	91.30
Uncultured (2)	–	–	–	**8.70**
Group 5				
Bacillus (9/12/8/9)	64.29	92.31	88.89	90
Bosea (5/1/1/1)	35.71	7.69	11.11	10

Group 1: the unique clones of CK; Group 2: the clones only existed in CK and T1; Group 3: the clones existed in CK and T2; Group 4: the unique clones of T3; Group 5: the common clones of all treatments. Bold numbers are the percentage of Uncultured clones in each group.

most important of all factors (Kowalchuk *et al*., 2003; Schönfeld *et al*., 2003; Gorissen *et al*., 2004; Benizri *et al*., 2005; Garbeav *et al*., 2006). To elucidate the relationship between soil suppression and soil bacteria, as well as the mechanism of soil fungistasis, a series of series of soils with the bacterial community composition as the only variable were tested. Here, the series of soils with a gradient of soils with a gradient of fungistasisi was established in laboratory. *F. graminearum* (sexual stage: *Gibberella zeae*) is the dominant pathogen responsible for wheat scab and maize stalk rotten epidemics in Northeast China. Therefore, it was chosen as the target fungi to evaluate soil fungistasis.

Originally, partial sterilization by microwave, autoclaving by a gradient temperature from 40 to 90°C ascending 10°C per treatment, and autoclaving (30 min at 121°C) followed by 0%, 25%, 50%, and 75% inoculation with nonsterile soil were applied on soil sample in this study. However, the treatments mentioned above couldn't make significantly different fungistasis by determination of *F. graminearum* extension in the Petri dish (data not shown).

Finally, a series of soils were obtained by partial sterilization at 100, 110, 121°C for 4 min respectively and the CK by measuring the relief of suppression of hyphal growth of *F. graminearum*. The bacterial community composition and diversity were investigated by soil metagenomic 16S rDNA clone library, RFLP, and sequencing for all soil samples with different fungistasis. Along with the RFCR decrease, the Shannon-Weaver index and Evenness index were reduced except Treatment 3, which had the lowest fungistasis (Table 2) and the bacterial community composition was changed significantly (Fig.3).

Soil fungistasis was significantly correlated with Shannon-Weaver index and Evenness index. With the index decreasing, the soil fungistasis was weakened. But slightly higher Shannon index and a few more unique OTUs were found in Treatment 3 than Treatment 2 (Table 2). The possible reason is that high temperature (121°C, 4 min) killed predominant soil bacteria. It resulted in vigorous proliferating of infrequent species which can not be detected by molecular biological method in natural soil because of the disappearing of competitive stress. However, those species were not the primary populations to control fungal growth, so the diversity of Treatment 3 was higher whereas fungistasis was declined.

The natural soil was healthy and strongly suppressive to plant pathogens. In Fig.3, all fungistasis declined treatments resulted in a large shift in the bacterial community composition, and Treatment 3 was much more different from the CK. The most likely explanation is that the balance of the initial soil bacteria community composition was destroyed, and new predominant species and balance of bacterial species were presented. Therefore, soil microbial diversity and community composition can be one of the major factors to determine soil fungistasis.

In this study, the clones affiliated with the genera *Bacillus* dominated in the Groups 3, 4, and 5. *Bacillus* is an important species in bio-control by producing large quatities of anti-microbial metabolites, most notably the non-ribsomally synthesized cyclic lipopeptides surfactin, iturin, and fengcin, as well as several modified small peptides, proteins, and volatiles (Emmert and Handelsman, 1999). They have been used successfully to control a diverse selection of plant pathogenic fungi and bacteria (Sharga and Lyon, 1998). Due to the heat resistance of *Bacillus*, some of them would be enriched with rising temperature even under 121°C autoclaving. It is still uncertain whether *Bacillus* plays a key role in soil fungistasis in this study. However, the clones belonged to Groups 4 and 5, affiliated with the genera *Bacillus/Paenibacillus*, *Bosea*, and uncultured bacteria, would have no effect on soil fungal suppressiveness. Because of their polulations were presented only in Treatment 3 (Group 4), and common clones of all treatments (Group 5). The results indicated that not all *Bacillus* species played an important role in soil fungistasis.

Pseudomonas was presented only in the natural soil which exhibited the highest fungistasis. Some studies have suggested that *Pseudomonas* was correlated with soil fungistasis (Mazzola, 1999, 2002; De Boer *et al*., 2003; Weller *et al*., 2002; Bergsma-Vlami, 2005; Duffy and Défago, 1999; Girlanda *et al*., 2001). A series of antifungal compounds were synthesized and secreted by *Pseudomonas*, including phenazine-1-carboxylic acid (PCA), 2,4-diacetylphloroglucinol (2,4-DAPG), pyoluteorin (PLT), pyrrolnitrin (PRN), which contributed to soil suppressiveness (Raaijmakers and Weller, 1998; Sharifi-Tehrani *et al*., 1998; Siddiqui and Shaukat, 2002; Liu *et al*., 2006). Therefore, the presence of *Pseudomonas* could be essential for the development of fungistasis in nature soil.

Hunter *et al*. (2006) studied the differences in microbial activity and microbial population of peat associated with suppression of damping-off disease caused by *P. sylvaticum*. They found that *Acidobacteria* was potentially associated with suppression for its increasing specifically in the suppressive peat. In this study, Group 1 had close affinity with the genera of *Acidobacteria* and the natural soil represents the highest fungistasis, which was in good accordance with the conclusion from Hunter's study that *Acidobacteria* likely plays an important role in soil fungistasis.

As shown in Fig.1, the difference of fungal control ability between CK and Treatment 1 was just 26%, suggesting that all of the clones belonged to the Group 1 contributed to only 26% of the whole fungistasis, and the remaining fungistasis between Treatment 1 and Treatment 3 was 74%. Correspondingly, Groups 1, 2, and 3 contributed to the whole fungistasis, so the sequences of Groups 2 and 3 accounted for the rest of 74% fungistasis. The clones, which were affiliated with the genera β-*Proteobacteria* and uncultured bacteria, dominated in the Group 2. However, the clones dominated in the Group 3 were associated with *Bacillus/Paenibacillus* and uncultured bacteria. The current study has not showed whether α-*Proteobacteria*, β-*Proteobacteria*, and *Flexibacter* existed in the Groups 2 and 3 was antagonistic bacteria. Their real functions on fungistasis are unclear. However, De Boer *et al*.

(2007) found that apparently non-antagonistic soil bacteria may be important contributors to soil suppressiveness and fungistasis in a community context. They mixed strains of four soil bacteria that exhibited little or invisible antifungal activity on different agar media. Also tested for antagonism on a nutrient-poor agar medium against the plant pathogenic fungi *Fusarium culmorum* and *Rhizoctonia solani* and the saprotrophic fungus *Trichoderma harzianum*, bacterial mixture strongly reduced the fungal growth. This means that α-*Proteobacteria*, β-*Proteobacteria*, and *Flexibacter* in this study contributed to soil fungistasis indirectly or directly despite of that they were antagonistic bacteria.

The dominance of uncultured clones in Group 2 (48%, 63.64% in CK and Treatment 1, respectively) and Group 3 (31.57%, 53.12% in CK and Treatment 2, respectively) means that uncultured bacteria play an important role in soil fungistasis. It is known that uncultured microorganisms represent the majority of the entire soil microbial community (Dauga *et al*., 2005). They could be accounted for soil suppression by influencing soil microbial diversity and community structure. In addition, some uncultured bacteria could produce and secrete antifungal compounds but we have not separated them still. Further work on fungistasis should pay more attention to uncultured microorganisms.

4 Conclusions

In this study, a series of soils samples with a gradient fungistasis were obtained by autoclaving. After DNA extraction, 16S rDNA clone library construction, RFLP, UPGMA analysis and sequencing of the soils, we can draw the conclusion that soil fungistasis is closely related to bacterial community composition. The higher the bacterial population diversity is and the more similar the bacterial community composition is to natural soil, the stonger fungistasis is resulted. Our study further confirmed that *Pseudomonas* and *Acidobacteria* are necessary for the fungistasis, α-*Proteobacteria*, β-*Proteobacteria*, *Flexibacter* and uncultured soil bacteria may contribute to soil fungistasis directly or indirectly. In short, the results in this article suggested that the bacterial community composition is important to fungistasis, and provides further insights into the soil fungistasis and better understanding of the relationship between the bacterial community composition and fungistasis. This will be meaningful for future controlling of soil borne plant pathogens.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 30770405).

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