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Full Length Research Paper

Analysis of culturable fungal diversity in rhizosphere soil of healthy and diseased cotton in Southern Xinjiang

Luan Feng-gang[1,2], Wang Lei[1], Liu Ya-nan[1], Lou Yang-yang[1], Zhang Hai-yan[1]* and Zhang Li-li[1]

[1]Key Laboratory of Protection and Utilization of Biological Resources in Tarim Basin, Xinjiang Production and Construction Corps, Alar, Xinjiang 843300, China.
[2]College of Landscape and Art, Jiangxi Agricultrue University, Nanchang, Jiangxi 330045, China.

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The Southern Xinjiang is the main productive area of cotton, which is in long continuous cropping due to the limited land resources. The community structures of cultural fungi were analyzed in the rhizosphere soil from healthy and diseased cotton fields. A total of 236 soil fungal isolates were obtained by means of the soil plating and dilution plating method. Based on morphological characteristics and internal transcribed spacers (ITS) sequences, these fungal isolates were grouped into sixteen genera. Of these genera, *Aspergillus* was the dominant genus in these rhizosphere soils, followed by *Fusarium*. The isolates of *Trichoderma* species in the rhizosphere soils with healthy cotton significantly outnumbered those with diseased cotton, while the genera of *Fusarium* and *Verticillium* showed the opposite trend. The biocontrol potential of the soil fungi, especially the *Trichoderma* isolates, was evaluated *in vitro* against *Fusarium oxysporum* and *Verticillium dahliae* using the dual culture technique. The fungal isolates from different soils showed different antagonistic potential, which came mostly from *Aspergillus*. Moreover, 10 isolates of *Trichoderma* spp. were shown to have a marked inhibitory effect on the tested pathogens *in vitro*. All together, these data indicate that the biocontrol fungi on the plant pathogens can contribute significantly in sustaining and improving soil quality.

Key words: Continuous cropping, soil fungi, Fusarium wilt, Verticillium wilt, *Trichoderma*.

INTRODUCTION

Soil is the outer covering of the earth, which consists of loosely arranged layers of materials composed of inorganic and organic compounds in different stages of organization (Kapoor et al., 2002; Tate, 2000). Soil microorganisms are the most vigorous part of soil ecosystem and the most important biological group of soil organisms, which included five major taxonomic categories: algae, bacteria, fungi, protists, and viruses (Prescott et al., 1996; Giri et al., 2005). And the diversity of soil microbial communities can be key to the capacity of soils to suppress soil-borne plant diseases (van Elsas et al., 2002). Among them, soil fungi dominate all types of soils and represent the greatest diversity among soil microorganisms (Chen, 1981). Since the 19th century, many microbial processes including symbiotic and asymbiotic N_2 fixation, denitrification, and sulfate reduction and oxidation were studied, and more details have been achieved about soil fungi. Anderson and Domsch (1973) put forward the relative contributions of bacterial and fungal populations to soil respiration, which showed the bacterial and fungal contributions were circa 22 and 78% in the agricultural soil, respectively. Then, the interest in soil fungal diversity has grown rapidly in the

*Corresponding author. E-mail: zhanghaiyansw03@163.com (H-Y Z) or zhang63lyly@yahoo.com.cn.

scientific community.

Due to the influence of the environmental factors, the composition and distribution of soil fungal communities may vary significantly. For instance, the soil fungi showed good reproducibility and high sensitivity in two cultivated field, and the estimates of hyphal lengths on the different sampling time were greater in the organically cultivated soil than in the conventionally cultivated soil (Elmholt and Kjoller, 1987). The diversity and evenness of soil fungal allotment were inversely related to altitude. Within each site, differences among the soil horizons were primarily responsible for variation in the fungal community, and species groups were differentiated by their patterns of colonization of the soil profile (Bissett and Parkinson, 1979). The soil depths could influence soil fungi, because the micro-ecological environment of soil have difference on the availability of organic matter and the ratio between oxygen and carbon dioxide at various depth. Moreover, farm practices including crop rotation and fertilizer or pesticide applications also influence the nature and dominance of fungal species (Hawksworth, 1991a, b). 756 species of Phycomycetes, Ascomycetes, and Fungi Imperfecti were described in a book (A Manual of Soil Fungi, 2nd edition), and this book constituted a highly important contribution to the literature in the study of soil fungi (Gilman, 1957).

The class Fungi Imperfecti are commonly isolated from soils (Lynch, 1987), while the most familiar and economically important molds, including *Aspergillus* and *Penicillium*, are asexual forms of Ascomycota that are also abundant in soils: *Aspergillus* more commonly in tropical soils and *Penicillium* more commonly in cool temperate and boreal soils (Christensen, 1981). Among the filamentous Ascomycota are many of the most important soil-borne pathogens of crop plants, root and stem rots caused by *Cochliobolus*, *Gibberella*, *Gaeumannomyces*, *Phymatotrichopsis*, and *Sclerotinia (Agrios, 1997; Farr et al., 1989;* Holliday*, 2001*).

Up till now, morphological characters provide the basis of current fungal systematics. In some cases, morphological criteria present some problems and fail to resolve taxonomic relationships. For several decades, various molecular techniques, especially the polymerase chain reaction (PCR)-based molecular techniques, have been applied successfully in fungal systematics, and the molecular methods combining morphological characters have resolved many taxonomic questions at different taxonomic levels (Gouy and Li, 1989; Bruns et al., 1991; Spatafora, 1995; Liew et al., 2000; Jeewon et al., 2002, 2003a, b, 2004; Duong et al., 2004; Cai et al., 2005). Altogether, molecular techniques can provide substantial support for fungal systematics research and offer insight into this far inaccessible systems, for instance in analyses of population and community structures, for elucidation of phylogenetic relationships, or for the characterization and monitoring of specific strains in the environment. Comparing with 18S rDNA (small subunit; SSU) and 28S rDNA (large subunit; LSU), the internal transcribed spacers (ITS) and intergenic spacer (IGS) regions evolve faster and are highly variable and therefore valuable for comparing fungal species at the intraspecific level. Sequence comparisons of selected regions within the rDNA have been useful for inferring phylogenetic relationships among fungi for several reasons. Universal single primers that are complementary to several regions within this gene are ready available (White et al., 1990, Vilgalys and Hester, 1990).

The diversity of soil microbial communities can be key to the capacity of soils to suppress soil-borne plant diseases (van Elsas et al., 2002). And the diversity of fungal communities in agricultural soils were rich, which have unique features and composition. Viaud et al. (2000) assessed ITS- restriction fragment length polymorphism (RFLP) both with cultural soil fungi and with total environmental DNA extracted from the same soil sample, and the results showed that the culture-independent molecular tool provides an efficient approach to assess fungal diversity. The fungi in the soil sample were identified to Ascomycetes, Basidiomycete, Zygomycete, Chromista and Protozoa (Viaud et al., 2000). The ITS-RFLP was tested as an easy method to identify isolates of filamentous fungi on grapes by Diguta et al. (2011), and the results showed that the technique was a rapid and reliable method. van Elsas et al. (2002) assessed the fungal diversity by the method of oligonucleotide fingerprinting of rRNA genes (OFRG). The results showed that 88% of clones in the two main clusters were affiliated with Ascomycota, while 12% belonged to Basidiomycota. Smaller assemblages of clones had high sequence identities of the Alternaria, Ascobolus, Chaetomium, Cryptococcus, and Rhizoctonia clades.

Xinjiang Autonomous Region is the largest cotton growing regions, which accounts for 25% of the whole areas in China with a total yield of 40%. The suitable planting area of cotton in Xinjiang are about 16 000 km^2 and the area of cotton planting accounts for 60%, thus the continuous cropping cotton planting area in Xinjiang has been a striking problem. In the course of long term co-evolution, the soil microbials form the co-existence and mutual restriction relationship with each other, and all the microbials and plants composed of the balance of dynamic ecosystem. However, the soil-borne diseases would occured when the balance is broken. Most investigation results for the continuous cropping showed that about 70% fields with continuous cropping obstacle were due to the soil infectious diseases (Liang et al., 2007). The reason for the barrier of monoculture of different crops maybe different, and all the reasons were summarized as following: (1) soil nutrient deficiencies; (2) soil abnormal reaction; (3) the deterioration of soil physical characteristics; (4) harmful substance from plants; (5) the soil microbial change, and emphasized the soil microbial change was the major factor of the continuous cropping obstacle (Hoestra, 1987).

Among different plant biotic stresses, diseases caused by soil-borne fungal pathogens are important (Sudini et al., 2011). The relative abundance of soil-borne fungi in a given soil can play a defining role in the manifestation of soil-borne diseases or may influence other organisms to induce plant diseases and, therefore, losses to crop production. Describing the soil microbial community in agricultural crops is an important step in understanding plant disease complexes and, ultimately, their management. And many soil fungi can be used to control plant diseases and pests, which exhibited much more efficient than the chemicals (Wei et al., 2005) Manka and Fruzynska Jozwiak (1996) isolated some fungi from forestry soil, such as *Trichodema viride*, *Trichodema harzianum*, *Penicillium adametzii*, *Penicillium funiculosum*, and all above fungi exhibited high inhibitory effect on *Fusarium oxysporum* f. sp. *Dianthi*.

However, the continuous cropping obstacle does not appear in some cotton plots of Southern Xinjiang, even the continuous cropping years up to 30 years. Moreover, the main cotton pathogens of *F. oxysporum* and *Verticillium dahliae* were soil residents and were difficult to eradicate. Application of biological control may possibly be used in the eradication of these pathogenic reservoirs. For this purpose, samples were collected from soils of the healthy and diseased cotton rhizosphere to investigate the community composition of cultivatable fungi. The cultivable soil fungi were compared with the help of molecular tools, and the beneficial fungi against the pathogens of Verticillium wilt and Fusarium wilt of cotton were also selected and analysed.

MATERIALS AND METHODS

Study area and sampling procedure

This study was conducted within Regimental Farm No.3, Agricultural Division No.1, the Xinjiang Production and Construction Corps, China. The study area is the main cotton-product region in the neighborhood of 40.375° N and 80.136° S latitude with continuous cropping for 15 years, which belongs to typical desert oasis with typical continental and arid climate (Zheng and Yu, 1996). According to symptoms of Verticillium wilt and Fusarium wilt of cotton, diseased and healthy cotton plants were selected. The soil samples were collected from rhizosphere soils of cotton at the boll-opening period. After the surface soil (approximately 1 to 2 cm) was removed, the soil attached to the roots was used as rhizosphere soil samples. All the samples were preserved at 4°C until they were used for isolation of soil fungi.

Isolation and morphological identification

Fungal isolates of soil samples were isolated by using the soil plate and dilution plates methods. For soil samples collected from rhizosphere of healthy and diseased cotton were isolated on selective medium of Rose Bengal Agar described by Fang (1998), which consisted of peptone (5 g), glucose (10 g), KH_2PO_4 (1 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), Rose Bengal (0.033 g), agar (20 g) and chloromycetin (0.1 g) in 1 L of deionized water. Sub-sample of soil (10 g) was placed into 90 ml of sterile water, and the sample was homogenized using a surface-sanitized warning blender. The homogenate was diluted three times in a 10-fold dilution series in sterile water, 100 µl from each dilution was spread onto the semi-selective medium, and the cultures were maintained at 25°C in dark. Cultures were examined after 3 days and daily thereafter for 4 additional days. Conidia characteristics of different fungi were transferred onto potato dextrose agar (PDA), the cultures were purified. Then, the main macro-factors included colony color, colony morphology, colony growth rate in PDA, and Leica inverted biomicroscopy was adopted for observation and photo taking. The fungi were identified with the typical characteristics according to related references (Ellis, 1971, 1976; Nagamani et al., 2006; Gilman and Joseph, 1998).

Genomic DNA extraction

The genomic DNA was extracted by the method of benzyl chloride described by Zhu et al. (1994) with some modifications. Briefly, the method included the following steps. (1) to a 1.5 ml Eppendorf tube containing 500 µl of lysis buffer (100 mM Tris-HCl [pH 8.0], 40 mM EDTA [pH 8.0]), 100 µl of 10% (w/v) sodium dodecyl sulfate and 300 µl of benzyl chloride, a small lump of freeze-drying mycelia was add by using a sterile toothpick, with which the lump of mycelia was disrupted. The tube was then left to water bath heating for 1 h in constant temperature (65°C). (2) The tube was spun at 10,000 ×g for 15 min, and the supernatant was transferred to another 1.5 ml Eppendorf tube, and 600 µl of phenol/chloroform/isoamyl alcohol was added (25:24:1). After mixing up the sample by upside down, the tube was spun once again. (3) After transferring the supernatant to a new 1.5 ml Eppendorf tube, 550 µl of isoamyl alcohol and 50 µl of 3 M NaAc were added. The tube was mixed by inversion briefly and left for -20°C over night. (4) The tube was spun at 10,000 ×g for 8 min, while the supernatant was discarded. The resultant DNA pellet was washed twice with 800 µl of 75% ethanol. After the pellet was spun at > 10,000 rpm for 2 min, the supernatant was discarded. The DNA pellet was air dried and dissolved in 50 µl of sterile diethylprocarbonate (DEPC) water. The concentration and quality of the purified DNA was evaluated both by 0.8% agarose gel electrophoresis and by spectrophotometry.

DNA amplification and sequencing

Using aliquots of extracted DNA, the region of the ribosomal repeat from 3′ end of 18S rDNA to 5′ end of the 28S rDNA with partial sequence, spanning ITS1, 5.8S rDNA and ITS2. Primer sequence used were 5-TCCTCCGCTTATTGATATGC-3□ (ITS4) and 5□-GGAAGTAAAAGTCGTAACAAGG-3□ (ITS5) (White et al., 1990). All primers were custom synthesized by Shanghai Sangon Biologic Engineering & Technology and Service Co. Ltd, China. PCR reaction were performed in 25-µl reaction volumes containing 20 ng genomic DNA, 1X reaction buffer, 0.5 mM dNTPs, 2 mM $MgCl_2$, 0.4 µM of each primer and 0.625U Taq DNA polymerase (Dream Taq™, Fermentas Life Science). The conditions of amplification were 2 min at 94°C, followed by 35 cycles for 30 s at 94°C, 30 s at 52°C, 1 min at 72°C and a final step for 10 min at 72°C. PCR products were detected by electrophoresis in 0.8% Tris-Acetate electrophoresis (TAE) agarose gels containing 10 µg ml^{-1} ethidium bromide. Nucleotide sequences of PCR products were performed by Shanghai Invitrogen Biotechnology Co., Ltd.

Dual culture

The inhibitory effects by soil fungi, especially to *Trichoderma* species on the pathogens of *F. oxysporum* and *V. dahliae* were evaluated with the following techniques as described by Fang

Table 1. Phylogenetic relation to nearest neighbors of strains isolated from rhizosphere soil of cotton.

Accession of strain	Fragment length (bp)	Reference taxa (accession number)	Query coverage	E value	Max ident (%)
11103	583	*Doratomyces microsporus* (GU566278)	98	0.0	99
8	516	*Fusarium oxysporum* (JN222394)	100	0.0	100
22110	535	*Metarhizium anisopliae* (FJ177505)	98	0.0	100
22210	546	*Alternaria tenuissima* (FJ827038)	97	0.0	100
12211	565	*Aspergillus ochraceus* (GU358697)	99	0.0	99
12012	598	*Trichoderma* spp. (JQ863230)	99	0.0	99
22213	767	*Ochroconis tshawytschae* (AB161066)	98	0.0	99
22212	580	*Myrothecium verrucaria* (FJ235085)	95	0.0	99
22010	564	*Bionectria ochroleuca* (HQ115729)	98	0.0	99
21206	544	*Chaetomium* spp. (HQ649928)	99	0.0	99
11302	550	*Cladosporium cladosporioides* (EU622927)	97	0.0	99
12303	530	*Beauveria bassiana* (AJ560682)	98	0.0	100
21010	500	*Verticillium dahliae* (JF810409)	100	0.0	100
12117	521	*Penicillium oxalicum* (JQ946374)	99	0.0	100
21117	583	*Stachybotrys chartarum* (HQ286790)	96	0.0	99
22201	543	*Sclerotinia nivalis* (JN415131)	99	0.0	99

(1998). The pathogens of *F. oxysporum* and *V. dahliae* were originally isolated from the diseased cotton showing the typical symptom and their hyphal growth and morphology were examined with a Leica inverted biomicroscopy (Leica®, Leica Microsystems CMS GmbH, Germany). Before carrying out the inhibitory experiments, the discs (5 mm) of fungal pathogens were cultured on PDA media at 25°C for 3 days in the dark. In order to check whether the antagonist was able to overgrow the pathogens of *F. oxysporum* and *V. dahliae*, four agar discs (5 mm) of soil fungi were removed from non-sporulating regions of the mycelium, and were placed equidistantly at the margin of Petri dishes (90 mm) around the pathogens. Petri dishes only with pathogenic fungi were used as the control. Four replicates were used for each experiment. The inhibition of mycelial growth was measured after an incubation period of 3 days.

Data analysis

Growth data were analyzed using Statistical Package for Social Sciences (SPSS) Version 15.0 (SPSS Inc., Chicago, IL, USA) with guidance (Pallant, 2007). Nucleotide sequences for the isolates were searched for their similarity index by using BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

RESULTS

Fungal species and community composition

Based on the statistical results, a total of 236 fungal isolates were separated from rhizosphere soil samples from healthy and diseased cotton; in each sample the number of soil fungal isolates was 103 (healthy) and 133 (diseased), respectively. The identification of soil fungi were determined with the help of morphological characters and 5.8S-ITS rDNA sequences analysis. The results showed that all the isolates belonged to 16 genera, 11 families, and 7 orders (Table 1). Of the 236 fungal isolates, 31.4% (74 isolates) belonged to *Aspergillus*, which was the dominant genus in the rhizosphere soils of healthy and diseased cotton (Table 1). Four soil fungal genera, including *Chaetomium*, *Myrothecium*, *Stachybotrys* and *Ochroconis*, were only detected in the diseased soil. The quantity of genera and isolates detected from the diseased soil were higher than the healthy, which suggested there were more diversity in the diseased soil. Moreover, the isolates percentage of *Fusarium* and *Verticillium* which had pathogenicity in the diseased soil were higher than in the healthy soil, this showed that *Fusarium* and *Verticillium* fungus maybe the major pathogens which caused cotton diseases in Southern Xinjiang.

Evaluation of antagonistic activity in dual cultures

The quantity of antagonistic fungi on *F. oxysporum* and *V. dahliae* is as shown in Figure 3, and the number of antagonistic fungi of different genus were distinctly different. The results showed that the number of *Aspergillus* isolates was dominant genus with antagonistic effects on either *F. oxysporum* or *V. dahliae* (Figure 2). Moreover, four genera, including *Cladosporium*, *Verticillium*, *Ochroconis* and *Sclerotinia*, showed no antagonistic effects on *F. oxysporum* or *V. dahliae*.

Ten *Trichoderma* isolates were selected through indoor plate confrontation test, and the results showed that there were no significant differences of antagonistic activities

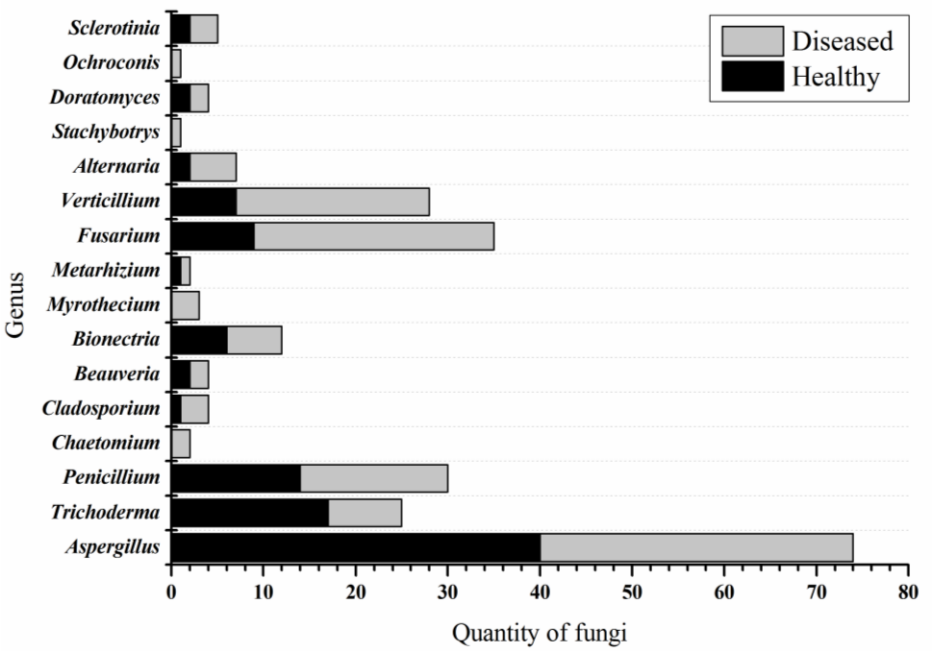

Figure 1. Fungal community composition in rhizosphere soil of healthy and diseased cotton.

against *F. oxysporum* (Figure 3) and *V. dahlia* (Figure 4) *in vitro* tests after 7 days. Among them, the isolate 11005 and 11008 exhibited the stronger antibiosis capacity to *F. oxysporum,* which growing covered the petri dishes after 6 days.

DISCUSSION

In subsistence agricultural systems, crop yields are directly dependent on the inherent soil fertility and on microbial processes that govern the mineralization and mobilization of nutrients required for plant growth (Choudhary et al., 2011). The use of rhizosphere fungi, specifically rhizofungi for biocontrol of soil-borne pathogens has been well documented (Lorito 2004). Due to their ability to control plant diseases and to increase crop production in an environmentally friendly manner, the mycofungicides based on the rhizosphere fungi have been promoted for agricultural use (Yang et al. 1999). In recent years, several mycofungicides have been patented and registered for plant diseases control. Formulation of mycofungicides includes wettable powers and granules based on *Chaetomium globosum*, *Ch. cupreum*, *Trichodema harzianum*, etc (Kaewchai et al., 2009). Moreover, the genus *Fusarium* includes both plant pathogenic and non-pathogenic races (Larkin and Fravel, 1999), and the non-pathogenic species are known to have effective biocontrol activity (Whipps, 2001; Harman et al., 2004; Kvas et al., 2009).

The fungal composition between the rhizosphere soil of healthy and diseased cotton have been compared in this study, and the antagonistic effect on the pathogens of Fusarium wilt and Verticillium wilt were also be discussed. Most fungal species isolated in this study were common soil fungi that are widely distributed in temperate soil. A total of 236 isolates of soil fungi, belonging to 16 genera, were isolated from the healthy and diseased cotton rhizosphere. The results shown in Figure 1 suggested that the abundance in the latter was higher than the former. Not only the total number, but also the antagonistic amount of *Aspergillus* was the highest, which suggested that the isolates of this genus played an important role for the balance of soil microecological system. Moreover, the amount of *Fusarium* and *Verticillium* isolates in the diseased cotton rhizosphere were obviously higher than the healthy cotton, which suggested that *F. oxysporum* and *V. dahliae* were the main pathogens of the cotton in the study areas.

Among the rhizosphere fungi, species of the genus *Trichoderma* are ubiquitous soil-borne fungi that exhibit antagonism towards a number of economically important plant-pathogenic fungi and oomycetes (Thornton, 2008). As early as 1930s, species of genus *Trichodema* showed antagonistic activity on the plant pathogens (Weindling, 1932). Since then, more and more species of *Trichodema* were isolated and identified. To date, there are 33 species recoded and described (Samuels et al., 2011). The main species includes *Trichodema hamatum*, *T. harzianum*, *Trichodema koningii*, *T. viride*, etc.

In this study, most isolates of *Trichodema* exhibited high antagonistic activity against *F. oxysporum* and *V.*

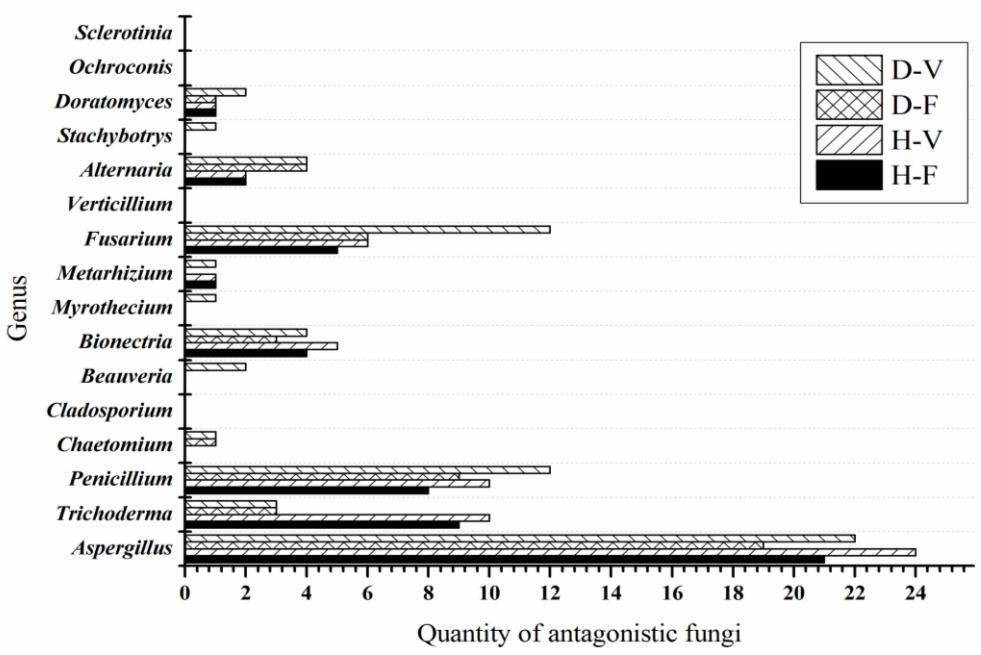

Figure 2. Comparison of dual cultures of different fungal genera against the pathogens of *F. oxysporum* and *V. dahliae*. (D) antagonistic fungi in the diseased cotton rhizosphere; (H) antagonistic fungi in the healthy cotton rhizosphere; (V) *V. dahliae*; (F) *F. oxysporum*.

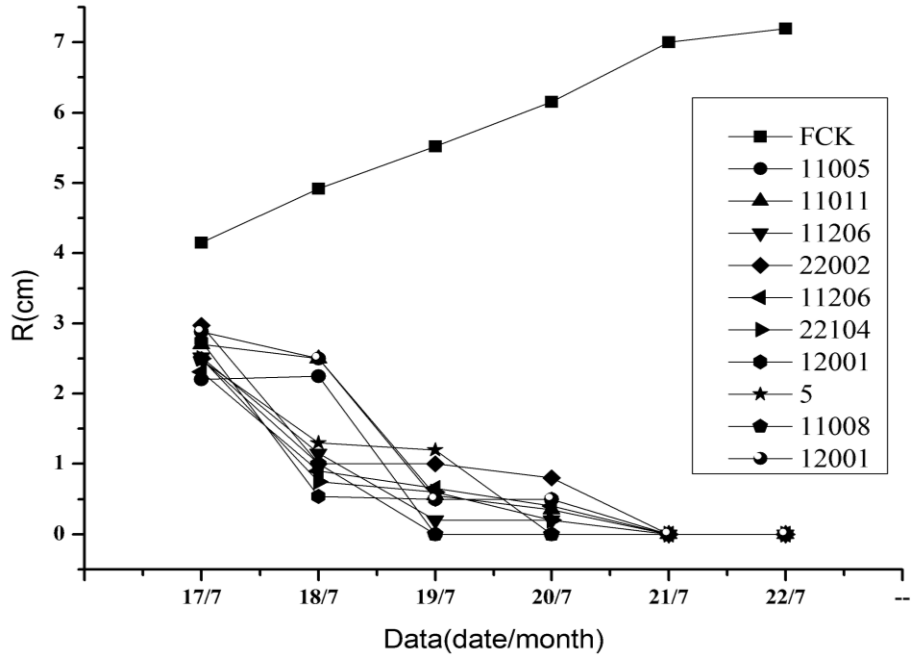

Figure 3. Antagonist effects of 10 *Trichoderma* isolates on *F. oxysporum*. FCK: the control isolate *F. oxysporum*.

dahliae, and the amount of antagonistic fungi in the diseased cotton rhizosphere were also higher than the healthy cotton. The results of indoor plate confrontation test suggested that the beneficial fungi of *Trichodema*

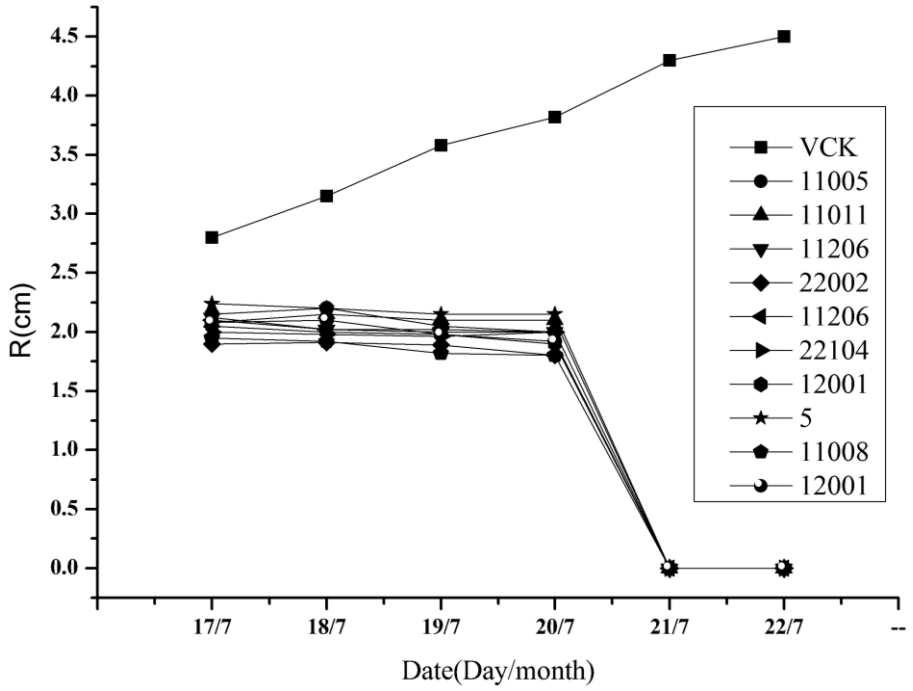

Figure 4. Antagonist effects of 10 *Trichoderma* isolates on *V. dahliae*. VCK: the control isolate *V. dahliae*.

could suppress the growing development of plant pathogens, and have some effect on the healthy soil, which can partially explain that there were no continuous cropping obstacles of cotton in Southern Xinjiang. Moreover, there were some strains of *Fusarium* that exhibit antagonistic activity against the pathogens of *F. oxysporum* and *V. dahliae*. The results of this study provide some insight into the cropping obstacles of cotton. However, some questions remain to be answered in future, in particular, how the beneficial fungi interact with pathogens during the process of cotton.

It is well known that majority of microorganisms do not form visible colonies through laboratory cultivation in most cases. Thus, the methods based on the culturable microorganisms are inadequate to quantify the active cells in environmental samples (Amann et al., 1995). Molecular ecological approaches applying tools, such as PhyloChip, 454-pyrosequencing, allow for capture the broad range of microbial diversity without the need for microbial cultivation. Thus, further studies based on cultivation-independent analyses of community structures using profiling and/or large scale sequencing approaches could offer ways to investigate and understand the genetics, biology and ecology of the antagonistic fungi.

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REFERENCES

Agrios GN (1997). Plant Pathology, Academic Press. San Diego, USA.

Amann RI, Ludwig W, Schlerfer KH (1995). Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. Microbiol. Rev. 59:143-169.

Anderson J, Domsch K (1973). Quantification Of Bacterial And Fungal Contributions To Soil Respiration. Arch. Microbiol. 93:113-127.

Bissett J, Parkinson D (1979). Fungal community structure in some alpine soils. Can. J. Bot. 57:1630-1641.

Bruns TD, White TJ, Taylor JW (1991). Fungal molecular systematics. Ann. Rev. Ecol. Syst. 22:525-564.

Cai L, Jeewon R, Hyde KD (2005). Phylogenetic evaluation and taxonomic revision of schizothecium based on ribosomal DNA and protein coding genes. Fungal Divers. 19:1-21.

Chen HK (1981). Soil Microbiology. Shanghai Scientific & Technical Publishers.

Choudhary DK, Sharma KP, Gaur RK (2011). Biotechnological perspectives of microbes in agro-ecosystems. Biotechnol Lett. 33:1905-1910.

Christensen M (1981). Species diversity and dominance in fungal communities. In: Wicklow Er Al. (Eds). The Fungal Community. Marcel Dekker, New York pp. 201-232.

Diguta CF, Vincent B, Guilloux-Benatier M, Alexandre H, Rousseaux S (2011). PCR ITS-RFLP: A Useful Method For Identifying Filamentous Fungi Isolates On Grapes. Food Microbiol. 28:1145-1154.

Duong LM, Lumyong S, Hyde KD, Jeewon R (2004). Emarcea castanopsidicola gen. et sp. nov. from thailand, a new xylariaceous taxon based on morphology and DNA sequences. Stud. Mycol. 50:253-260.

Ellis M (1971). Dematiaceous Hyphomycetes, Commonwealth Mycological Institute. Kew, Surrey, England, pp 1-106.

Ellis MB (1976). More Dematiaceous Hyphomycetes, Commonwealth Mycological Institute, pp. 1-507.
Elmholt S, Kjoller A (1987). Measurement of the length of fungal hyphae by the membrane filter technique as a method for comparing fungal occurrence in cultivated field soils. Soil Biol. Biochem. 19:679-682.
Fang ZD (1998). Research method of plant pathology (3rd), China Agriculture Press, Beijing pp. 122-155.
Farr DF, Bills GF, Chamuris GP, Rossman AY (1989). Fungi on plants and plant products in the United States, APS Press.
Gilman J (1957). A Manual Of Soil Fungi (2nd Edition). Iowa State College Press, Ames pp. 1-450.
Gilman JC, Joseph C (1998). A manual of soil fungi. Daya Publishing House pp. 1-392.
Giri B, Giang PH, Kumari R, Prasad R, Varma A (2005). Microbial diversity in soils. In: Buscot F (Ed) Microorganisms in soils: Roles in genesis and functions, Springer Verlag, pp. 19-55.
Gouy M, Li W (1989). Molecular phylogeny of the kingdoms Animalia, Plantae, and Fungi. Mol. Biol. Evol. 6:109-122.
Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004). *Trichoderma* Species - opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol. 2:43-56.
Hawksworth DL (1991a). The biodiversity of microorganisms and invertebrates: Its role in sustainable agriculture. CAB Int. pp. 1-300.
Hawksworth DL (1991b). The fungal dimension of biodiversity: magnitude, significance, and conservation. Mycol. Res. 95:641-655.
Hoestra H (1987). General remarks on replant disease. In: Trees Worpwf (Ed) ISHS. Acta Hortic. pp. 11-16.
Holliday P (2001). A dictionary of plant pathology, Cambridge University Press pp 1-562.
Jeewon R, Liew E, Hyde K (2004). Phylogenetic evaluation of species nomenclature of pestalotiopsis in relation to host association. Fungal Divers. 17:39-55.
Jeewon R, Liew ECY, Hyde KD (2002). Phylogenetic relationships of pestalotiopsis and allied genera inferred from ribosomal DNA sequences and morphological characters. Mol. Phylogenet. Evol. 25:378-392.
Jeewon R, Liew ECY, Hyde KD (2003a). Molecular systematics of the Amphisphaeriaceae based on cladistic analyses of partial LSU rDNA gene sequences. Mycol. Res. 107:1392-1402.
Jeewon R, Liew ECY, Simpson JA, John Hodgkiss I, Hyde KD (2003b). Phylogenetic significance of morphological characters in the taxonomy of *Pestalotiopsis* species. Mol. Phylogenet. Evol. 27:372-383.
Kaewchai S, Soytong K, Hyde K (2009). Mycofungicides and fungal biofertilizers. Fungal Divers. 38:25-50.
Kapoor R, Giri B, Mukerji KG (2002). Soil factors in relation to distribution and occurrence of vesicular Arbuscular Mycorrhiza. In: Mukerji KG Et Al. (Eds) Techniques In Mycorrhizal studies. Kluwer Academic Publishers pp. 51-85.
Kvas M, Marasas WFO, Wingfield BD, Wingfield MJ, Steenkamp ET (2009). Diversity and evolution of *Fusarium* species in the Gibberella Fujikuroi Complex. Fungal Divers. 34:1-21.
Larkin RP, Fravel DR (1999). Mechanisms of action and dose-response relationships governing biological control of Fusarium Wilt of tomato by nonpathogenic *Fusarium* spp. Phytopathology 89:1152-1161.
Liang Z, Zhou B, Zhong X, Yang T, Ma X (2007). Yield reduction and technical measures on the long fibre cotton area in Xinjiang. China Cotton pp. 36-37.
Liew ECY, Aptroot A, Hyde KD (2000). Phylogenetic significance of the Pseudoparaphyses In Loculoascomycete Taxonomy. Mol. Phylogenet. Evol. 16:392-402.
Lorito M (2004). Novel understanding of Trichoderma interaction mechanisms. J Zhejiang University Agric. Life Sci. 30:387-387.
Lynch J (1987). Soil Biology: Accomplishments and Potential. Soil Sci. Soc. Am. J. 51:1409.
Manka M, Fruzynska JD (1996). Biocontrol of greenhouse carnation Fusarium Wilt with saprophytic forest soil fungi. Folia Hortic. 8:93-105.
Nagamani A, Kunwar IK, Manoharachary C (2006). Handbook of soil fungi. IK International pp. 1-500.
Pallant J (2007). SPSS survival manual: A step by step guide to data analysis using SPSS for Windows Version 15, Open University Press, pp. 1-352.
Prescott L, Harley J, Klein D (1996). The diversity of the microbial world. Microbiology. WCB Publishers, Dubuque, Iowa.
Samuels G, Chaverri P, Farr D, Mccray E (2011). Trichoderma online. systematic Botany and mycology laboratory, ARS, USDA. Retrieved September 17, From /Taxadescriptions/Keys/Trichodermaindex.Cfm.
Spatafora JW (1995) Ascomal evolution of filamentous Ascomycetes: Evidence from molecular data. Can. J. Bot. 73:811-815.
Sudini H, Arias CR, Liles MR, Bowen KL, Huettel RN (2011). Comparison of soil fungal community structure in different peanut rotation sequences using ribosomal intergenic spacer analysis in relation to aflatoxin-producing fungi. Phytopathology 101:52-57.
Tate RL (2000). Soil Microbiology, John Wiley pp. 1-508.
Thornton CR (2008). Tracking fungi in soil with monoclonal antibodies. Eur. J. Plant Pathol. 121:347-353.
Van Elsas JD, Garbeva P, Salles J (2002). Effects of agronomical measures on the microbial diversity of soils as related to the suppression of soil-borne plant pathogens. Biodegradation 13:29-40.
Viaud M, Pasquier A, Brygoo Y (2000). Diversity of soil fungi studied by PCR-RFLP of ITS. Mycol. Res. 104:1027-1032.
Vilgalys R, Hester M (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal dna from several *Cryptococcus* Species. J. Bacteriol. 172:4238.
Weindling R (1932). *Trichoderma lignorum* as a parasite of other soil fungi. Phytopathology. 22:837-845.
Whipps JM (2001). Microbial interactions and biocontrol in the rhizosphere. J. Exp. Bot. 52(Suppl 1):487-511.
White T, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA (Eds) PCR Protocols: A guide to methods and applications. Academic Press, pp. 315-322.
Yang HT, Tang WH, Ryder M (1999). Trichoderma and biological control of plant diseases. Shandong Sci. 12:7-15.
Zheng LZ, Yu PP (1996). Annals of regimental farm No.3, Agricultural Division No.1, The Xinjiang Production and Construction Corps. Xinjiang Peoples Publishing House, Urumqi, China.
Zhu H, Qu F, Zhu LH (1994). Isolation Of Genomic DNAs from fungi using Benzyl chloride. Acta Mycol. Sinica 13:34-40.