微生物学通报
Microbiology
tongbao@im.ac.cn

OCT 20, 2008, 35(10): 1593~1599

研究报告

Isolation and Characterization of Antifungal Endophytic Bacteria from Soybean

ZHANG Shu-Mei* SHA Chang-Qing WANG Yu-Xia LI Jing
ZHAO Xiao-Yu ZHANG Xian-Cheng

(*Microbiological Institute of Heilongjiang Academy of Science*, *Harbin* 150010)

Abstract: Endophytic bacteria reside in most healthy plants; it can not be easily influenced by outer environment. Some endophytic bacteria are beneficial to host plants, such as growth promotion, disease prevention and nitrogen fixation *etc*. Therefore, endophytic bacteria are the potential microbial fungicides, it may be widely applied. In this study, endophytic bacteria were isolated from soybean cultivar Hefeng 25 that was a main soybean cultivar in Heilongjiang province, China. The results indicated that the density of endophytic bacteria varied in different tissues of the plant. It was 3.4×10^3 CFU/g in roots, 2.8×10^3 CFU/g in leaves, 2.9×10^2 CFU/g in stems and 1.4×10^2 CFU/g in seeds. The activity of 121 strains against *Fusarium oxysporum* f. sp. soybean, caused soybean root rot, were assayed. 25.6% of them showed antagonistic activity against *F. oxysporum* f. sp. soybean. One of them, strain TF28 isolated from soybean roots could inhibit the growth of many fungal pathogens. The inhibitory rates against *F. oxysporum* from different plant species were 80.2%-96.7%. Based on the morphological, physiological and biochemical characteristics as well as the sequence of 16S rRNA, strain TF28 was identified as *Bacillus amyloliquefaciens*.
Keywords: Antifungal endophytic bacteria, Screening, Characterization, Soybean

大豆内生细菌的分离及根腐病拮抗菌的筛选鉴定

张淑梅* 沙长青 王玉霞 李 晶 赵晓宇 张先成

(黑龙江省科学院微生物研究所 哈尔滨 150010)

摘 要: 内生细菌存在于健康植物体内, 一些内生细菌具有促生长、抗病和固氮等生物学功能。本项研究采用化学药剂表面灭菌方法从黑龙江省大豆品种合丰 25 的根、茎、叶和种子中分离到大量内生细菌, 其种群数量在根部最多, 为 3.4×10^3 CFU/g, 在叶部次之, 为 2.8×10^3 CFU/g, 在茎部和种子中最少, 为 2.9×10^2 CFU/g 和 1.4×10^2 CFU/g。从 121 株内生细菌中筛选到 31 株对大豆根腐病菌 *Fusarium oxysporum* f. sp. soybean 具有较强抑制作用的拮抗内生细菌, 其中菌株 TF28 抑菌谱广, 抑菌率高, 对不同植物的病原菌 *F. oxysporum* 的抑菌率为 80.2%~96.7%。经形态、生理生化和 16S rRNA 鉴定为解淀粉芽孢杆菌(*Bacillus amyloliquefaciens*)。

关键词: 拮抗内生细菌, 筛选, 鉴定, 大豆

Soybean is a valuable crop in China because it is widely used as the raw materials of making edible oil and food. However, some diseases limit the production of soybean. Soybean root rot disease is widespread in

Foundation item: International cooperation project of Heilongjiang (No. WC05B07)
***Corresponding author:** Tel: 86-451-84674876; ✉: zsmhlj@yahoo.com.cn
Received: March 31, 2008; **Accepted:** May 22, 2008

most soybean-producing countries, including America, Canada, Brazil, England, France, Germany, Australia, Japan, New Zealand, China, and so on [1]. The incidence of root rot disease in Heilongjiang province, a main agricultural area in China, is up to 75% annually. It can cause soybean yield to decrease 10%-20% and significant economy losses. *Fusarium* spp., *Pythium* spp. *and Rhizoctonia solani* are three major fungi which cause soybean root rot disease in Heilongjiang province. Among of them, *Fusarium oxysporum* is the major pathogen [2]. It is difficult to control *Fusarium oxysporum* root rot disease, because *F. oxysporum* is a kind of soil-borne pathogen that can infect many kinds of host plants throughout the growth period and cause the vascular wilt [3].

In order to prevent the infection of these pathogens, various chemical fungicides are widely used. However, these chemical agents are expensive, it can also cause serious environmental pollution and the resistance of pathogen to the agents so as to reduce fungicide efficacy [4]. In recent years, most studies focused on biological control of plant diseases, which is environment friendly and has been considered as an suitable alternative to chemical fungicides [5,6]. The microbal diversity seems provide an endless resource for biological control of plant disease. Many bacteria, including *Pseudomonas fluorescens, Pseudomonas putida* and *Bacillus* spp. *etc*, have been studied intensively as biological control agents of soil-borne plant pathogens[7-10]. However, because most of biological control strains were isolated from plant rhizospheric soil, so that they are difficulty to colonize in plant roots, their application as biological control agents are limited in practical production.

Endophytic bacteria live inside plant tissues without the damage to the host. Endophytic bacteria can be isolated from surface-sterilized plant tissues or from internal plant tissues. Some endophytes have been isolated from several different tissues of some plant species, such as rice, maize, wheat, sorghum, cotton and potato [11]. Endophytic bacteria can enter plant tissue through roots, flowers, stem and cotyledon[12]. Endophytic bacteria inside a plant may become localized at the point of entry or spread throughout the plant [13]. These bacteria can reside within cells, in the intercellular spaces or in the vascular system [14]. Endophytic bacteria have many biological functions due to their colonization and transformation characteristics in plant tissues. They can prevent pathogen infection or improve anti-disease capability of plant by competing with pathogens, producing antifungal substance and inducing host systemic resistance, *etc*. Consequently, endophytic bacteria are the potential biocontrol agent to control plant pathogens owing to their special characteristics [15]. Recently, it has been reported that endophytic bacteria are used to control plant disease of potato, rice, cotton, maize, cabbage, tomato, *etc* [16-17].

In this study, our objectives were to: 1) isolate endophytic bacteria strains from different tissues of local soybean cultivars; 2) assess the density of endophytic bacteria in different tissues; 3) screen antagonistic endophytic strains from endophytic bacteria and assess their antagonistic features against *F. oxysporum*; 4) obtain the antagonistic endophytic bacterium strain which showed strong inhibitory activity to *F. oxysporum* f. sp. soybean and identify it; 5) evaluate its antagonistic efficacy against different fungal pathogens *in vitro* and provide a potential antagonistic endophytic bacterium resource to control soybean root rot.

1 Materials and methods

1.1 Pathogen source

Twelve phytopathogenic fungi were used as indicators for screening antifungal activity of endophytic bacteria *in vitro* (Table 1). These phytopathogenic fungi are widely distributed worldwide and can cause serious plant diseases.

1.2 Plant source

Soybean cultivar Hefeng 25, which was widely grown in the Northeast of China, was used to isolate endophytic bacteria. The healthy soybean plant samples were collected from four different locations (Nehe, Tailai, Boli and Yanshou counties) in Heilongjiang province.

Table 1 Phytopathogen strains and their sources

Strains	Sources
F. oxysporum soybean	Isolated from soybean root by ourselves
Rhizoctonia solani soybean	Isolated from soybean root by ourselves
Phythium spp.	Isolated from soybean root by ourselves
F. oxysporum cucumber	Isolated from cucumber stem by ourselves
F. oxysporum watermelon	Isolated from watermelon stem by ourselves
Fusarium oxysporum niveum	Isolated from sweet melon stem by ourselves
Botrytis cinerea tomato	Provided by Russian experts
Alternaria solani pepper	Provided by Russian experts
Sclerotinia sclerotiorum soybean	Isolated from soybean stem by ourselves
Fusarium moniliforme sheld	Provided by Russian experts
Pyricularia oryzar Cav.	Purchased from Institute of Microbiology, Chinese Academy of Sciences
R. solani cucumber	Isolated from cucumber stem by ourselves

1.3 Sample collection

Four healthy soybean plants in the same field plot were collected from the soybean fields located in Nehe, Tailai, Boli and Yanshou counties in Heilongjiang province, respectively. Roots, stems, leaves and seeds were cut out respectively and placed in clean plastic bags, treated within 1 d after collection.

1.4 Surface sterilization of sample

Quantitative soybean roots, stems, leaves and seeds (1.0 cm in diameter) were cut out and washed thoroughly with tap water to remove the attached soil, then sterilized by dipping in 70% of ethanol for 5 min following 2% of sodium hypochlorite for 10 min. Each sample was rinsed 4 times with sterile distilled water in a sterile petri dish to remove surface sterilization agents. 100 μL of the last rinsing liquid were spreaded on the LB medium plate. The plate was incubated at 30°C for 2 d to 3 d to check the surface sterilization efficacy.

1.5 Isolation of endophytic bacteria

All surface sterilized samples were cut into a sterilized mortar with a sterile blade, and ground thoroughly after adding 10 mL sterile distilled water. The suspension was diluted tenfold series using sterile distilled water. 100 μL of each dilution was spreaded on the LB medium plate. The plate was incubated at 30°C for 1 d to 3 d. According to the morphology of bacterial colonies, including size, color, shape *etc*, the clear bacterial isolates were picked and purified by transferring for 2 to 3 times. The amount of endophytic bacteria from different tissues was counted. All isolates were incubated in the LB broth at 30°C for 18 h. The bacterial liquid cultures were kept at −70°C after adding glycerol.

1.6 Screening of antagonistic endophytic bacteria

F. oxysporum soybean was used for screening antifungal endophytic bacteria. The fungus was identified to cause soybean root rot.

To assay the antifungal activity of endophytic bacterial isolates, the confrontation culture method was used. Four different endophytic bacterium strains were streaked on four sites of the PDA medium. After incubation at 28°C for 2 d, *F. oxysporum* cake (5 mm in diameter) was inoculated between bacteria. The distance of inoculation site between endophytic bacterium and fungus was 30 mm, The plates containing endophytic bacteria and fungia were cultured at the same culture conditions for 7 d. Then, the distance between the edge of endophytic bacteria colony and the edge of fungal colony was measured. The antifungal activity was calculated according to the distance between bacterium and fungus. Each treatment was repeated 4 times.

1.7 *In vitro* antagonistic spectrum assay

Twelve phytopathogenic fungi were used for assaying antagonistic spectrum of antifungal endophytic bacteria.

The mycelial growth inhibition method was used to test the antifungal endophytic bacteria effects on different pathogens. 200 μL of bacterial liquid cultures,

whose concentration is 3.0×10^7 CFU/mL, were mixed with PDA medium and poured into petri dishes. Then, 5 mm diameters pathogen cakes were inoculated on its centre, cultured at 28°C for 7 d. The diameters of pathogen colonies were measured after 7 d. The inhibitory rate against pathogens was calculated. Each treatment was repeated 4 times. Sterile distilled water was used as control.

1.8 Identification of endophytic bacterial strain TF28

Strain TF28 was identified by combining the method of morphological, physiological and biochemical characteristics with the sequence analysis of 16S rRNA.

According to Bergey`s Manual of Determinative Bacteriology (edition Ⅷ), the physiological and biochemical characterristics of strain TF28 was determined.

16S rRNA sequence analysis method was as following: total genomic DNA was extracted from strain TF28 liquid cultures in LB broth using boiled method. The following universal bacteria primers were used for PCR amplification of 16S rDNA (Forward Primer 5′-AGAGTTTGATCCTGGCTCAG-3′ and reverse primer 5′-GGTTACCTTGTTACGACTT-3′). PCR was conducted in a Gene Amp^{R}PCR System 9700 using the following protocol: initial denaturation at 94°C for 5min, followed by 30 cycles of denaturating at 94°C for 1min, annealing at 55°C for 1 min, and an extension at 72°C for 1min, eventually by an additional extension at 72°C for 10 min. The PCR products were sequenced by Shanghai GeneCore BioTechnologies Co., Ltd. The 16S rDNA sequences were compared at Blast program of NCBI website. Phylogenetic tree was reconstructed by neighbor-joining method.

1.9 Statistical analysis

All data were analyzed using statistical analysis software SPSS13.0.

2 Results

2.1 Effectiveness of surface sterilization

After surface sterilization, all samples were immersed in 5 mL sterile distilled water, respectively. 0.1 mL of suspension was spreaded on LB medium plate. No microbes appear on LB medium after incubation at 30°C for 2 d to 3 d. The results indicated that epiphytic bacteria of all samples could not grow after surface sterilization.

2.2 Isolation of endophytic bacteria

Endophytic bacteria were isolated from the roots, the leaves, the stems and the seeds of soybean cultivar Hefeng 25. Based on the morphology of colony, including shape, size and color, 121 endophytic bacteria were isolated from the different tissues of soybean collected from four different locations of Heilongjiang province. The density of endophytic bacteria varied in different tissues while it kept stable in same tissues from four different plantation places. It was 3.4×10^3 CFU/g in roots, 2.8×10^3 CFU/g in leaves, 2.9×10^2 CFU/g in stems and 1.4×10^2 CFU/g in seeds, respectively (Table2).

2.3 Screening of antagonistic endophytic bacteria

The inhibitory activity of 121 endophytic bacteria strains against *F. oxysporum* soybean were measured by confrontation culture method. The results indicated that 31 strains showed the antagonism against *F. oxysporum* soybean. The isolation frequency of antagonistic endophytic bacterium was 25.6%. Among 31 antagonistic endophytic bacterium strains, 5 strains exhibited strongest inhibitory activity to *F. oxysporum* soybean, which were significantly different at $P_{0.01}$ level. 12 strains exhibited stronger inhibitory activity to *F. oxysporum* soybean, which were significantly different at $P_{0.05}$ level. 14 strains exhibited weak inhibitory activity to *F. oxysporum* soybean, which were not significantly different at $P_{0.05}$ level. In 31 antagonistic endophytic bacterium strains, 19 strains isolated from soybean roots, 9 strains isolated from soybean leaves, and 3 strains isolated from soybean stems. The results suggested most of antifungal endophytic bacteria colonized in soybean roots and could protect their host plants from invasion of *F. oxysporum* soybean. It was considered that antifungal endophytic bacteria

Table 2 Density of endophytic bacteria in different tissues from four locations (10^3 CFU/g)

Tissue \ Site	Yanshou	Tailai	Nehe	Boli	Mean
Roots	3.21±0.0151	3.70±0.1183	3.32±0.0119	3.54±0.0238	3.44±0.0422
Leaves	2.52±0.0294	3.20±0.0201	3.04±0.0170	2.63±0.0238	2.84±0.0225
Stems	0.30±0.0158	0.32±0.0875	0.27±0.0170	0.28±0.0165	2.92±0.0342
Seeds	0.14±0.0085	0.16±0.0132	0.12±0.0193	0.13±0.0165	0.14±0.0143

Notes: Data are the mean of four samples. ± Notation represents the standard errors of the mean

play an important role on protecting their host plant from invasion of *F. oxysporum* soybean because of high isolation frequency of antagonistic endophytic bacterium from roots. Strain TF28, one of all antagonistic endophytic bacteria strains, exhibited the strongest activity to suppress the growth of *F. oxysporum* soybean. Its inhibition zone was 1.5 cm width (Table 3, 4). Buren reported 32% of strains in 192 endophytes had antagonistic activity against potato disease [19]. In this study, we isolated 31 antagonistic endophtes from 121 endophtes. The isolation percentage of antagonistic endophtes was 25.6%.

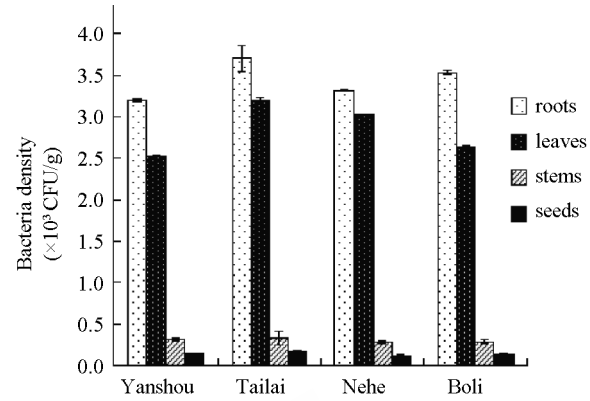

Fig. 1 Density of endophytic bacteria in different tissues from four locations

Notes: Bar represents the standard errors of the means

Table 3 The effect of antagonistic endophytic bacteria on *F. oxysporum* soybean on PDA plates

Strains	Isolated tissues	Inhibition zone (cm)	Inhibition activity	Strains	Isolated tissues	Inhibition zone (cm)	Inhibition activity
TF21	roots	0.8±0.03	++	TF98	roots	1.0±0.07	+++
TF32	leaves	0.7±0.06	++	TF91	stems	0.4±0.13	+
TF28	roots	1.5±0.03	+++	TF89	leaves	0.3±0.03	+
TF42	leaves	0.2±0.07	+	TF102	roots	0.9±0.06	++
TF46	roots	0.3±0.04	+	TF121	roots	0.5±0.09	++
TF51	leaves	0.1±0.12	+	TF114	stems	0.4±0.14	+
TF38	roots	1.2±0.09	+++	TF71	leaves	0.6±0.09	++
TF66	roots	0.1±0.13	+	TF63	roots	1.0±0.03	+++
TF75	roots	0.4±0.04	+	TF112	roots	0.3±0.05	+
TF71	leaves	0.5±0.05	++	TF108	roots	0.8±0.10	++
TF94	roots	0.6±0.16	++	TF119	roots	1.1±0.08	+++
TF2	leaves	0.6±0.15	++	TF29	leaves	0.3±0.06	+
TF14	roots	0.2±0.08	+	TF18	stems	0.2±0.13	+
TF12	roots	0.7±0.10	++	TF10	roots	0.9±0.05	++
TF47	leaves	0.2±0.19	+	TF58	roots	0.6±0.04	++
TF36	roots	0.3±0.13	+				

Notes: Data are the mean of four replicates; +++ notation indicates inhibitory activity to pathogen of bacterial strains are significantly different at $P_{0.01}$ level; ++ notation indicates inhibitory activity to pathogen of bacterial strains are significantly different at $P_{0.05}$ level; + notation indicates inhibitory activity to pathogen of bacterial strains are not significantly different at $P_{0.05}$ level; ± notation represents the standard errors of the mean

Table 4 Classification of inhibitory activity of endophytic antagonistic bacteria to *F. oxysporum* soybean

Suppression action	Inhibition zone (cm)	No.	Percentage (%)
Strong (+++)	≥1.0	5	4.1
Middle (++)	≥0.5; <1.0	12	9.9
Weak (+)	≥0.1; <0.5	14	11.5
–	0	90	74.4

Notes: No. indicates the number of strains. Percentage indicates the number of endophytic antagonistic bacteria account for the percentage of all strains

2.4 *In vitro* antagonistic spectrum assay

The antagonistic effects of strain TF28 on twelve kinds of pathogens were studied by the mycelial growth inhibition method (Table 5). The results showed strain TF28 had the broad-spectrum activity. It had the strongest antagonistic activity to soybean root rot pathogens, including *F. oxysporum* soybean, *Rhizoctonia solani* soybean *and Phythium* spp. The inhibitory rates to three pathogens were 93.8%, 95.6%, 96.7%, respectively. Moreover, strain TF28 also had strong inhibitory activity against *F. oxysporum* from

Table 5 Antagonistic activity of endophytic bacteria strain TF28 to different pathogens on PDA plates

Strains	Control (mm)	Treatment (mm)	Inhibitory rates (%)
F. oxysporum soybean	84.1±0.09	7.6±0.04	96.7a
Rhizoctonia solani soybean	83.6±0.12	9.8±0.08	93.8ab
Phythium spp.	85.4±0.04	8.5±0.09	95.6a
F. oxysporum cucumber	85.6±0.10	20.9±0.12	80.2c
F. oxysporum watermelon	82.4±0.07	16.8±0.06	84.7bc
F. oxysporum niveum	81.6±0.08	10.3±0.06	93.1ab
Botrytis cinerea tomato	79.3±0.07	19.2±0.09	80.9c
Alternaria solani pepper	80.2±0.09	18.9±0.13	81.5c
Sclerotinia sclerotiorum soybean	82.4±0.11	20.1±0.07	80.5c
Fusarium moniliforme sheld	83.2±0.09	17.6±0.06	83.8bc
Pyricularia oryzar Cav.	83.1±0.03	22.1±0.03	78.1c
R. solani cucumber	83.5±0.04	16.2±0.02	85.7b

Notes: Data are the mean of four replicates; ± notations represent the standard errors of the mean; column with the same letters are not significantly different ($P_{0.05}$) based on SSR analysis

different plants. The inhibitory rates to different *F. oxysporum* were 80.2%-96.7%. Strain TF28 might be a potential biocontrol bacterium against soilborne pathogens, because it can colonize in soybean roots and had broad-spectrum activity.

2.5 Identification of endophytic bacterial isolate TF28

The morphology of strain TF28 was single cell, rod–shaped, endospore-forming and Gram-positive. The colony was milk-white color, coarse surface and untidy margin. According to the characteristics of *Bacillus* in Bergey's Mannual of Determinitive Bacteriolgy[20], the physiological and biochemical characteristics of strain TF28 were determined (Table 6) .

Table 6 Physiological and biochemical characters of strain TF28

Character	Strain TF28
Gelatin hydrolysis	+
Starch hydrolysis	+
V.P reaction	+
Catalase reaction	+
Methyl red reaction	+
Nitrate reduction	+
Citrate utilization	+
Glucose utilization:acid produced	+
Aerogenesis	−
Litmus milk peptonization	+
Aerobic growth	−

Notes: +: Notation represents positive; −: Notation represents negative

767 bp 16S rDNA fragment was sequenced. Compared with the bacterial rDNA sequence in Genbank (Fig. 2). The homology of strain TF28 with some bacteria, such as *Bacillus amyloliquefaciens* GH40, *Bacillus amyloliquefaciens* GH53, was 98%. The result showed that evolutionary distance of strain TF28 was closest to *Bacillus*. Strain TF28 was identified as *Bacillus amyloliquefaciens* with the morphological, physiological and biochemical characteristics and the 16S rRNA sequence analysis.

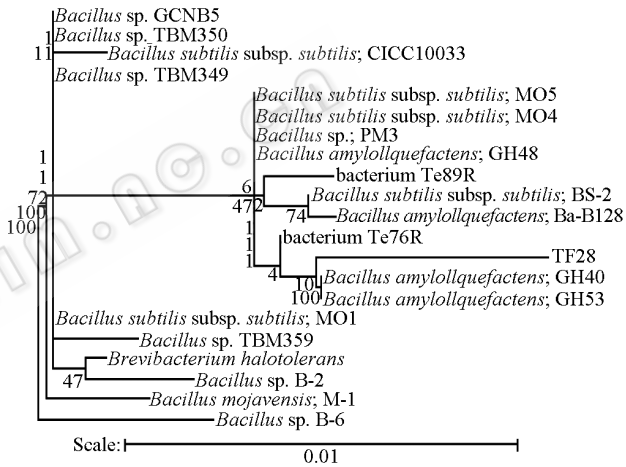

Fig. 2 The Phylogenetic tree of strain TF28

3 Discussion

(1) At present, most researchers use surface sterilization to isolate endophytic bacteria. In the process of endophytic bacterium isolation, surface-sterilized time is the key step. If plant sample is sterilized for too short or too long time, it will influence the precise for measuring the density of endophytic bacteria. In this study, using 70% ethanol and 2% sodium hypochlorite to sterilize experimental materials for 15 min can ensure that isolated bacteria were not epiphytes but endophytic bacteria.

(2) It has been reported that a number of facultative endophytes have been isolated from some plant species, such as rice, maize, wheat, sorghum, cotton and potato *etc*. However, few were reported on the

study of soybean endophytic bacteria. The study revealed that a large number of indigenous endophytic bacteria resided within soybean plants. The density of endophytic bacteria varied in different tissues while it kept stable in same tissues from different plantation places. It was the highest in roots, low in leaves than in roots, and the lowest in stems and seeds. The study accords with previous research about other plant species. Lamb T.G reported bacteria density was the highest in roots, low in the stems and leaves [18]. It was the first report on the isolation and description of endophytic bacterium density from soybean cultivar in Heilongjiang province.

(3) The study on antifungal activity of endophytic bacteria against pathogens showed that 25.6% of endophytic bacteria can inhibit the growth of *F. oxysporum* soybean that caused soybean root rot. One of them, strain TF28 isolated from soybean roots had broad spectrum and the strongest antifungal activity. It was identified as *B. amyloliquefaciens* according to the morphological, physiological and biochemical characteristics and the 16S rRNA sequence analysis. It was reported that most of antifungal endophytic bacterium species was *Bacillus subtilis*. It was the first report that endophytic *B. Amyloliquefaciens* which can inhibit muitiple pathogens isolated from soybean cultivar. Therefore, we concluded that isolate TF28 might be the potential biocontrol bacterium. However, the antagonistic activity of isolate TF28 against mutiple pathogens was only measured *in vitro*, the further detailed studies on it as biocontrol agents in greenhouse are needed. Furthermore, its antagonism mechanism against pathogens need be elucidated.

Acknowledgements

We thank Prof. Wang Ao-Xue, Prof. YANG Zhi-Xing and ZHANG Duo-ying for reading and revising the manuscripts and thank my colleague ZHANG Yun-Yu.

REFERENCES

[1] Fu CX. Occurrence of soybean root rot disease and its comprehensive control strategy. *Soybean Bulletin*, 2006, **5**: 13–15.

[2] Tong ZH, Wang XS. Screened for antagonistic isolates against soybean root rot disease in the laboratory and greenhouse. *Chinese Journal of Biological Control*, 1997, **14**(1): 25–27.

[3] Wang ZY, Li DB. Genetic diversity of *Fusarium oxysporum*. *Chinese Journal of Phytopathology*, 2000, **30**(3): 193–199.

[4] Cook RJ, Bruckart WL, Coulson JR, *et al*. Safety of microorganisms intended for pest and plant disease control: a framework for scientific evaluation. *Biological Control*, 1996, **7**: 333–351.

[5] Hadar Y, Harman GE, Taylor GE. Evaluation of *Trichoderma koningii* and *T. harzianum* from New York for biological control of seed rot caused by *Pythium* spp. *Phytopathology*, 1984, **74**: 106–110.

[6] Chet I, Inbar J. Biological control of fungal pathogens. *Applied Biochemistry and Biotechnology*, 1994, **48**(1): 431–437.

[7] Emmert ABE, Handelsman J. Biocontrol of plant disease: a(Gram-)positive perspective. *FEMS Microbiology Letters*, 1999, **171**(1): 1–9.

[8] Mari M, Guizzardi M, Pratella GC. Biological control of gray mold in pears by antagonistic bacteria. *Biological Control*, 1996, **7**(1): 30–37.

[9] Khan MR, Fischer S, Egan D, *et al*. Biological control of *Fusarium* seeding blight disease of wheat and barley. *Phytopathology*, 2006, **96**(4): 386–394.

[10] Brian B, Gardener M. Biological control of plant pathogens: Research, commercialization, and application in the USA. *APSnet feature: Biological Control of Plant Pathogens*, 2002.

[11] Sharma PK, Sarita S, Prell J. Isolation and characterization of an endophytic bacterium related to *Rhizobium agrobacterium* from wheat (*Triticum aestivum* L.) roots. *Current Science*, 2005, **89**(4): 607–610.

[12] Bacon CW, White JF. Microbial endophytes. New York: Marcel Dekker.Inc., 2000, pp.199–233.

[13] Hallmann J, Quadt HA, Mahafee WF. Bacterial endophytes in agricultural crops. *Can J Microbiol*, 1997, **43**: 895–914.

[14] Zinniel DK, Lambrecht P, Harris NB, *et al*. Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. *Applied and Environmental Microbiology*, 2002, **68**(5): 2198–2208.

[15] Kleopper JW, Schipper B, Bakker PM. Proposed elimination of the term endorhizosphere. *Phytopathology*, 1992, **82**: 26–727.

[16] Chris P, Chanway DC. Bacterial endophytes. New York: Marcel Dekker Inc., 2002, pp.43–46.

[17] Sturz AV, Christie BR, Nowak J. Bacterial endophytes potential role in developing sustainable systems of crop production. *Critical Review in Plant Science*, 2000, **19**(1): 1–30.

[18] Lamb TG, Tonkyn DW, Kluepfel DA. Movement of *Psedomonas aureofaciens* from the rhizosphere to aerial plant tissue. *Can J Microbiol*, 1996, **42**: 1112–1120.

[19] Buren AM, Andre C, Jshimaru CA. Biological control of bacterial ring rot pathogen by endophytic bacterial isolated from potato. *Phytopathology*, 1993, **83**: 1406.

[20] Buchanan RE, Gibbons NE. Bergey's manual of determinative bacteriology. Eighth edition. Baltimore: Willians & Wilkins Company, 1974, p.751.