African Journal of Microbiology Research Vol. 7(10), pp. 875-884, 5 March, 2013
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.1500
ISSN 1996-0808 ©2013 Academic Journals

Full Length Research Paper

Screening and diversity of plant growth promoting endophytic bacteria from peanut

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Accepted 21 February, 2013

In order to study potential abilities to promote the growth of the plants and population diversity of endophytic bacteria in peanut plant at four different growth stages, 94 endophytic bacteria strains were isolated. Result indicate that numbers of endophytic bacteria of different growth stages ranged from 2.6 to 7.1×10^4 cfu/g FW with the maximum presented at the full pod stage. Based on 16S- restriction fragment length polymorphism (RFLP) and 16S rDNA sequences, these 94 strains were identified as 14 genera among which *Bacillus* and *Pantoea* were the dominant genera. The genus of the endophytic bacteria varied tremendously during annual growth period with the highest species richness at the full pod stage. Of the 15 antibacterial strains for the five phytopathogenic fungi, 12 strains were known to express antimicrobial activity against *Fusarium solani, Colletotrichum gloeosporioides, Botrytis cinerea Pers* and *Pseudoperonospora cubensis,* but only three against *Sclerotium rolfsii. Bacillus* was dominant of these antibacterial strains. Among the 48 indole acetic acid (IAA) producing strains, *Pantoea agglomerans, Bacillus megaterium* and *Enterobacter asburiae* were remarkable for their high levels of IAA production and only three siderophore-producing bacteria were isolated which belong to *Pseudomonas* spp. At the same time, growth-promoting effects of three strains (Y21, F10 and H2) were proved by treatment of peanut seedling with bacterial supernatants. This study revealed the diversity of endophytic bacteria in peanuts at different growth stages. The obtained isolates have potential applications as inoculants that can adapt to poor soils and peanut. Basis is also provided for the manufacture of a multifunction bacterium agent.

Key words: Peanut, endophytic bacteria, growth-promoting, antifungal activity.

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is one of the most important crops in China because it is widely used in edible oils and food. The sown area of peanut in China is the second largest globally, next only to India (Xu et al., 2010). However, peanut production is limited by several diseases. About 24 infectious peanut diseases have been reported in China, including 17 fungal diseases. The use of fertilizers and pesticides is the main method of controlling these diseases and improving plant productivity. Recently, increasing attention has been paid to the use of biocontrol agents as an environmentally friendly approach. Plant growth-promoting rhizobacteria (PGPR) are the main types of such agents. PGPR can directly affect plant growth, such as via the synthesis of different phytohormones and nitrogen fixation, by providing the plant with a compound produced by the bacterium or promoting the uptake of certain nutrients from the environment. PGPR can also indirectly affect plant growth by decreesing or preventing the deleterious effects of one or more phytopathogenic organisms. However, bacteria that colonize the internal tissues of plants seem to have an ecological advantage over bacteria that can only colonize plants

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Abbreviations: IAA, indole acetic acid; **PGPR,** plant growth-promoting rhizobacteria; **RFLP,** restriction fragment length polymorphism; **CAS,** chrome Azural S.

epiphytically. The internal tissues of plants possibly provide a more uniform and protective environment for microorganisms than plant surfaces. For example, many studies have reported that endophytic diazotrophs are more advantageous than root-associated diazotrophs (Boddey et al., 1995; McInroy and Kloepper, 1995; Triplett, 1996). Therefore, endophytic bacteria have gradually become some of the most important resources of biological control strains.

Bacteria that can be isolated from surface-disinfected tissues or screened from internal plant tissues without causing harm to the plant are called endophytic bacteria (Kobayashi and Palumbo, 2000; Sheng et al., 2011; Sun et al., 2008). Many studies have demonstrated that endophytic bacteria widely exist in plant tissues, such as stems, roots, flowers, leaves, and seeds (Kobayashi and Palumbo, 2000). Based on their impact on host plants, endophytic bacteria can be divided into three groups, namely, plant-growth promoting, plant-growth inhibiting, and plant-growth neutral (Bai et al., 2002). As plant-growing promoting bacteria, endophytic bacteria can be used to control plant pathogens, insects, and nematodes (Ryan et al., 2008). They can also enhance plant growth by activating a number of similar mechanisms, including indole acetic acid (IAA) production (Palaniappan et al., 2010), phosphate solubilisation activity (Wakelin et al. 2004), and siderophore (Kloepper et al., 1980). There are also many mechanisms by which endophytic bacteria can promote plant growth and health. The most important one is the antagonistic effect of endophytic bacteria against fungal pathogens, which is considered to promote plant growth indirectly. They are able to interfere with pathogen growth, survival, or infection by the following mechanisms: inhibition of growth by biosurfactants, such as antibiotics, toxins, and surface-active compounds; competetion for colonisation sites, nutrients, and minerals; and competition for the synthesis of chitinase and β-1,3-glucanase that is involved in cell wall degradation (Chernin and Chet, 2002; Souza et al., 2003; Whipps, 2001). Endophytic bacteria have been isolated from a variety of plants because they ubiquitously inhabit most plant species (from crop plants to woody trees), such as cotton, wheat, rice, sugarcane, and poplar tree (Chen et al., 1995; Jha and Khumar, 2009; Naik et al., 2009; Magnani et al., 2010; Taghavi et al., 2009). The diversity of endophytes found in many plant tissues has been described in numerous studies. For example, several genera have been isolated from legume tissues, including *Aerobacter*, *Aeromonas*, *Agrobacterium*, *Bacillus*, *Chryseomonas*, *Curtobacterium*, *Enterobacter*, *Erwinia*, *Flavimonas*, *Pseudomonas* and *Sphingomonas* (Elvira-Recuenco and Vuurde, 2000; Gagné et al., 1987; Oehrle et al., 2000; Sturz et al., 1997).

Studies on the diversity, phylogeny, and ability to invade peanut nodules of endophytic bacteria screened from the root nodules have been reported (Ibáñez et al., 2009; Yang and Zhou, 2008). We carried out a study on the diversity and phylogeny changes of peanut endophytic bacteria isolated at different growth stages by the restriction fragment length polymorphism (RFLP) analysis of 16S ribosomal RNA (rRNA) and 16S rRNA sequencing. The antimicrobial activities, IAA production, and siderophore production of the collected endophytic bacteria were assessed by the plate confrontation method, Salkowski colorimetric technique, and CAS (Chrome Azural S) agar plates, respectively. This study aimed to provide basic information on the dynamic changes in peanut endophytes. Information on using endophytic bacteria for enhancing crop development is provided.

MATERIALS AND METHODS

Sampling

Healthy peanut plants were sampled at four growth stages, namely, young seedling (Y), beginning peg (B), full pod (F), and harvest maturity (H) stages, in the same field plot in Da Guan village, Tai'an, Shandong, China.

Surface sterilisation of plants and isolation of endophytic bacteria

Whole plants were washed with tap water to remove attached clay, and the roots, stems, leaves, and seeds were cut out and mixed. About 10 g of the mixed sample was weighed, immersed in 75% ethanol for 3 min, rinsed in 3% sodium hypochlorite (NaClO) for 2 min, dipped in 75% ethanol for 30 s, and finally washed five times with sterile distilled water. To verify the success of the sterilisation process, 100 µl of the final rinsing liquid was set on R2A (Kawai et al., 2002) medium plates (0.05% proteose peptone, 0.05% starch, 0.05% glucose, 0.05% yeast extract, 0.05% casein hydrolysate, 0.03% dipotassium phosphate, 0.03% sodium pyruvate, 0.0024% magnesium sulphate anhydrous, 2% w/v agar, pH 7.2 ± 0.2). The plates were incubated at 28°C for 1 day to 3 days to check the surface sterilisation efficacy. All surface-sterilised samples were placed in a sterilised mortar and thoroughly ground after adding 10 ml of sterile distilled water. After diluting the suspension 10-fold with sterile distilled water, about 100 µl of each dilution was spread onto R2A agar medium. Based on the morphology of bacterial colonies, the isolates were obtained after incubation at 28°C for 2 days to 3 days. Glycerol was then added and the purified isolates were kept frozen at -80 °C.

Total DNA extraction and 16S rRNA gene PCR amplification

The total DNA of the bacterial isolates was prepared according to the procedures of Murray and Thompson (1980). 16S rRNA gene PCR amplification was carried out with a set of universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGCTACCTTGTTA CGACTTCACCCC-3'), using a Biometra TGRADIENT thermocycler. The PCR conditions were as follows: initial denaturation at 95°C for 4 min followed by 32 cycles of denaturation at 94 C for 45 s, annealing at 56°C for 1 min and extension at 72°C for 1.5 min followed by a final extension at 72°C for 10 min. A 1500 bp product was obtained after monitoring by 1% agarose (w/v) gel electrophoresis at 110 V for 30 min in 1×Tris-boric acid-EDTA (TBE) buffer, ethidium bromide staining, and UV transillumination.

Table 1. Numbers of endophytic bacteria screened out at different growth stages.

Growth stage	Numbers of isolate	Total genera	Population density (cfu/g FW)
Young seedling	22	6	3.8×10^4
Beginning peg	28	5	6.8×10^4
Full pod	30	8	7.1×10^4
Harvest maturity	14	6	2.6×10^4

RFLP analysis of 16S rRNA gene sequencing and evolutionary analysis

Two enzymes, *Msp*I and *Hae*III (TakaRa Biotechnology, China), were used in the enzymatic reactions. The digestions were performed at 37°C for 4 h after PCR product purification using a 3S Spin PCR Product Purification Kit (Shenergy Biocolor Bioscience and Technology Company, China). Subsequently, reaction mixture (20 µl) was run on 3% (w/v) agarose gel in 1×TBE buffer for 4 h at 100 V. The agarose gels were stained, visualised, and imaged as previously described. The NTSYS 2.1 programme was used in the computer evaluation of the RFLP analysis-generated fingerprints. Based on the results of 16S-RFLP analysis cluster, the 16S rRNA gene PCR products of the representative strains were sequenced at Sangon Biotechnology (China), and were deduced by a BLAST search to determine the closest sequence. A phylogenetic tree was constructed by the method of Jukes and Cantor (1969) to calculate the evolutionary distances. The bootstrap analysis of 1000 resamplings was performed by the software MEGA 4 (Tamura et al., 2007).

Screening of IAA-producing bacterial isolates and quantitive analysis

Modified R2A medium with L-tryptophan (200 mg/L) was used to screen IAA-producing bacteria. The bacterial isolates were cultured at 28 °C and centrifuged at 180 rpm for 3 days. About 50 µl of the bacterial liquid cultures were mixed with the same volume of Salkowski reagent on a white board. The IAA-producing bacteria were then separated based on their characteristic colour (pink to red) after being kept in the dark for 0.5 h. The concentrations of the IAA-positive strain cultures were measured at 600 nm before centrifugation at 10 000 rpm for 10 min. The amount of IAA produced by the bacteria was determined using the procedures of Glickmann and Dessaux (1995). A standard curve was obtained using pure IAA.

Siderophore production

Chrome azurol S agar plates were used to screen siderophore-producing bacteria per the method described by Schwyn and Neilands (1987). The bacteria were spot-inoculated on the plates and incubated at 28°C for 2 to 7 days. The development of a yellow-orange halo around the colonies indicated siderophore production. The plates were soaked for 3 days in 6 M HCl to remove traces of iron. Double distilled-deionised water was used to prepare all media and clean the soaked plates.

***In vitro* antagonistic spectrum bioassay**

Five phytopathogenic fungi (*Sclerotium rolfsii*, *Fusarium solani*, *Colletotrichum gloeosporioides*, *Botrytis cinerea,* and *Pseudoperonospora cubensis*) were used to assay the antagonistic spectrum of the endophytic bacteria. The bacterial isolates were pointed onto the margin of the fungal colony using sterile toothpicks and incubated at 28°C for 2 days to 4 days. Growth inhibition was calcu-lated using the formula R_1/R_2, where R_1 is the maximum radius of the fungal colony away from the bacterial colony and R_2 is the radius of the bacterial colony.

Evaluation of the growth-promoting activity of the endophytic strains

The peanut seeds were soaked in water at 20°C for 12 to 20 h in Petri dish. Garden soils mixed vermiculite (2:1) were sterilized at 121°C for 2 h and transferred in a pot. There is one seed in every pot. The pots were covered with plastic wrap at 25 to 28°C. Then sterilized distilled water was used to moisten the mixture soil.

We choose high level IAA strain F10, H2 and low level strain Y21 randomly for the pot experiment. Strains were cultured in the R2A medium at 28°C, 180 rpm for 3 days. Sterilized distilled water was used to dilute the suspensions to a final concentration of approximately 10^8 CFU/mL.

Four experimental treatments were set up with five replicates. The control treatment was dealt with sterilized R2A medium. 20 mL suspensions or sterilized R2A medium was used for every treatment. 15 days after cultivation, 3 to 5 leaves emerged. The root length (RL), shoot length (SL) and leaf number (LN) were measured and used as the criterion for growth promoting effect of the strains.

RESULTS

Endophytic isolates from peanut plant

From peanut plants at four growth stages, 94 isolates were obtained based on phenotypic characterisation (Table 1). The number of endophytic bacteria was coun-ted and the densities were found to range from 2.6×10^4 to 7.1×10^4 cfu/g FW, showing significant differences. The largest density was observed in the full pod stage, and the smallest density number was in harvest maturity stage.

16S-RFLP analysis, sequencing and phylogeny analysis

By 16S-RFLP fingerprints (Figure 1) of the restriction enzymes, a dendrogram was constructed based on the similar banding patterns we obtained (Figure 2). The 94 isolates were divided into 29 clusters or genotypic groups at 100% similarity, and clustered together at 83% similarity. There were three large groups, namely, group 1 with nine isolates, group 5 with 15 isolates, and group 18 with 18 isolates. There were 7 small groups that consisted only of a single isolate, and the other 47 isolates corresponded to the other 19 clusters.

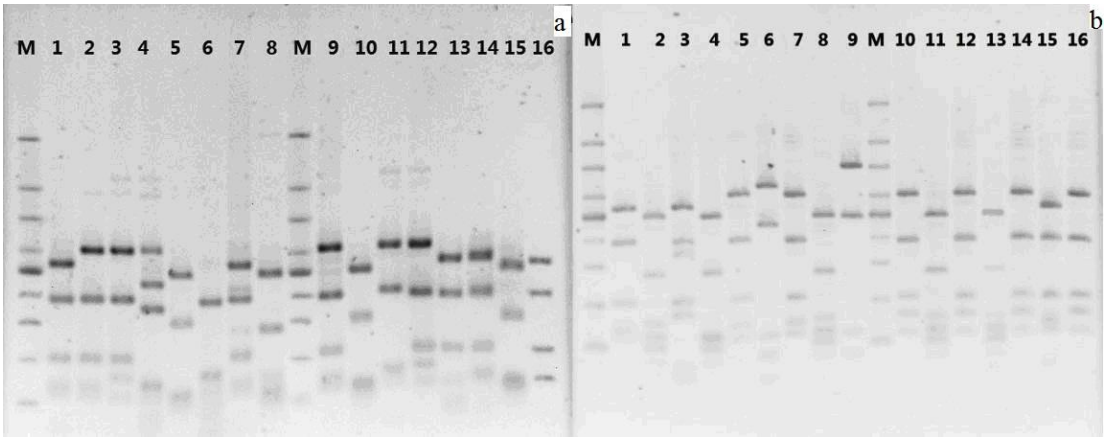

Figure 1. 16S-RFLP patterns of some endophytic bacteria. M, 100 bp DNA marker, size in order as 1500, 1000, 800, 600, 500, 400, 300, 200 and 100 bp; (a) 16S-RFLP patterns of endophytic bacteria from young seedling stage digested with *Msp*I; (b) 16S-RFLP patterns of endophytic bacteria from beginning of peg stage digested with *Msp*I. Lane 1-16 represent different isolates.

A total of 31 representative isolates from each cluster were sequenced and analysed using the NCBI BLASTn programme to retrieve the annotated sequence (Table 2). There were 31 isolates found to be clustered into 14 different genera, which were further divided into at least 25 different species. There were 6, 5, 8, and 6 different genera obtained from the young seedling, beginning peg, full pod, and harvest maturity stages, respectively. *Bacillus* and *Pantoea* were the dominant genera in all growth stages, and the numbers of isolates from these two genera were 44 and 18, respectively. Many distinctive genera were observed in some growth stages, such as *Enterobacter*, *Exiguobacterium*, *Acinetobacter*, *Flavobacterium*, *Rhizobium*, *Microbacterium*, and *Burkholderia*.

IAA production, siderophore production, and antimicrobial activities

A total of 48 endophytic bacteria were able to produce IAA. There were 14, 13, 12, and 9 isolates from the young seedling, beginning peg, full pod, and harvest maturity stages, respectively. IAA production ranged from 3.8 to 169.48 $mg \cdot L^{-1} \cdot OD600^{-1}$ (Table 3).

Quantitative analysis revealed that the high-level (>50 $mg \cdot L^{-1} \cdot OD600^{-1}$) IAA-producing strains were *Bacillus megaterium*, *Pantoea agglomerans*, *Enterobacter asburiae*, *Rhizobium* sp., *B. megaterium*, and *Rhizobium* sp. from the harvest maturity stage, *E. asburiae* from the young seedling stage, and *P. agglomerans* from all four stages. The low-level IAA-producing strains isolated from the Young seedling stage accounted for a greater proportion than those from the other three stages. Only three siderophore-producing endophytic bacteria (Y17, Y21, and F23) formed faint orange halos on the CAS agar plates.

All the above mentioned isolates were subjected to antagonism experiments to screen the antagonistic bacteria (Figure 3).

There were 14 endophytic isolates active against at least one of the five pathogenic fungi (Table 4). The antagonistic spectrum and effect of the endophytic bacteria from different stages significantly varied. There were eight, two, and four antagonistic bacteria screened from the young seedling, beginning peg, and full pod stages, respectively. Most antagonistic bacteria exerted antagonist effects on four pathogenic fungi, namely, *F. solani*, *C. gloeosporioides*, *B. cinerea Pers*, and *P. cubensis*. Some isolates such as Y21 and F23 exhibited weak antifungal activity against *S. rolfsii*, but had no antifungal activity against the other four pathogenic fungi. Surprisingly, no antagonist species was found from the samples at the harvest maturity stage.

Effect of the endophytic strains on growth of peanut seedlings

The tested endophytic bacteria improved the growth of peanut plants and showed different growth-promoting effects (Figure 4). Maximum SL, LN and RL were observed following treatment with H2, which were 46.3, 40.7 and 85.2% higher than the control. Strain H2 was identified as *B. megaterium*. Treatment with strain F10 and Y21 also increased RL (53.8; 3.8%), SL (34.3;11.6%) and LN (23.7; 0.8%) of peanut seedling respectively.

DISCUSSION

Endophytic bacteria associated with peanut have been frequently isolated and identified. But this paper provides the change in endophytic bacteria diversity of peanut from different growth phases. We screened plant growth

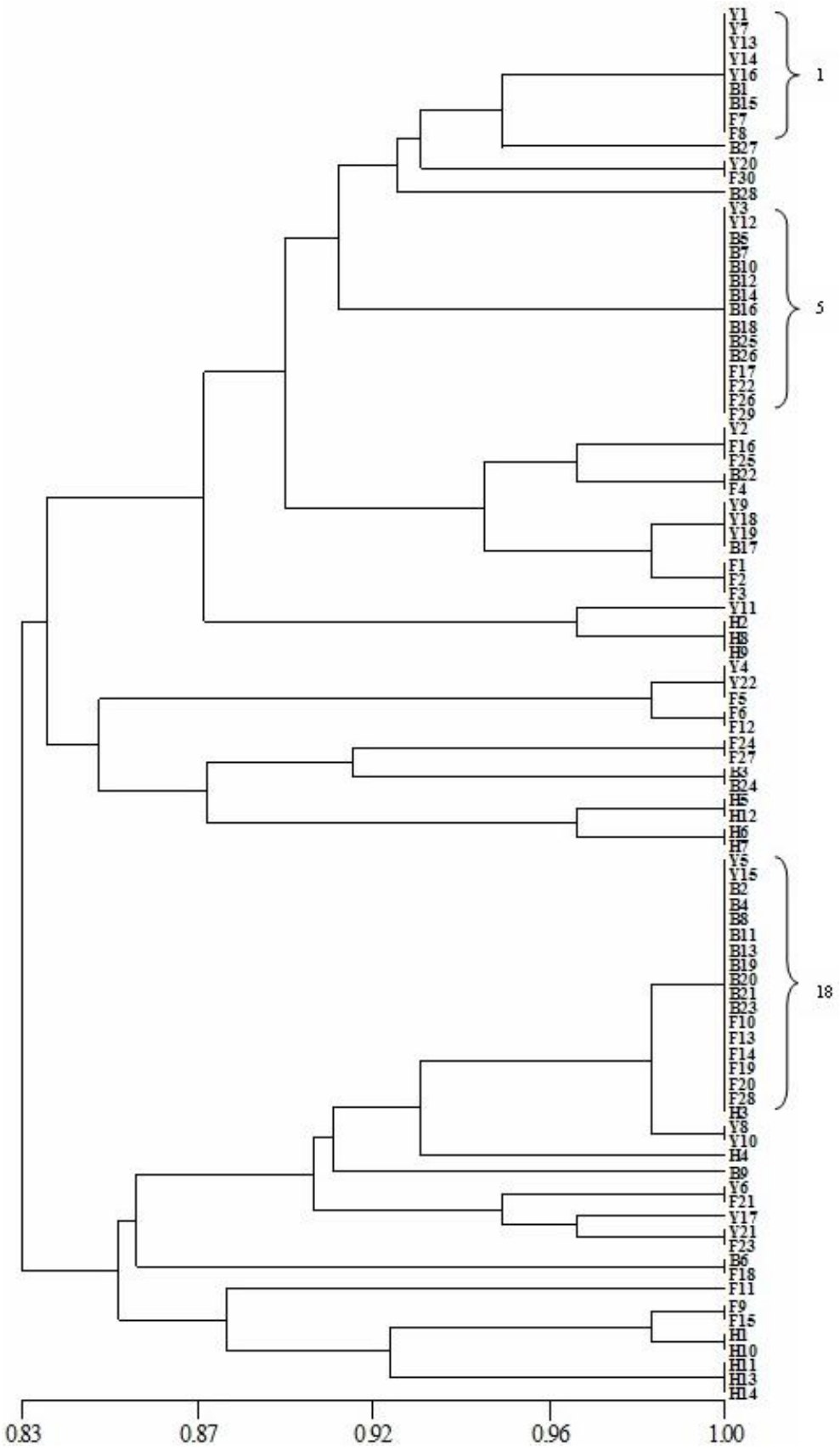

Figure 2. Dendrogram showing the relationship of 94 endophytic bacteria based on 16S-RFLP fingerprints using cluster analysis.

Table 2. Endophytic bacteria obtained in this study representing each 16S-RFLP group and their closest affiliation based on 16S rRNA gene sequencing.

Group	Number	Sequenced isolate	Accession number	Closest NCBI strain and accession number	Similarity (%)
1	9	Y1	JQ579620	*Bacillus subtillis* AJ276351.1	99
		Y14	JQ579621		
2	1	B27	JQ579622	*Bacillus licheniformis* CP000002.3	99
3	2	F30	JQ579634	*Bacillus luciferensis* AJ419629.1	99
4	1	B28	JQ579624	*Bacillus arbutinivorans* AF519469.1	99
5	15	F22	JQ579625	*Bacillus nealsonii* EU656111.1	99
6	3	F16	JQ579626	*Bacillus anthracis* AB190217.1	99
7	2	F4	JQ579627	*Bacillus mycoides* AB021192.1	99
8	4	B17	JQ579628	*Bacillus thuringiensis* D16281.1	99
9	3	F2	JQ579629	*Bacillus cereus* AE016877.1	99
10	1	Y11	JQ579630	*Bacillus fusiformis* AF169537.1	99
11	3	H2	JQ579631	*Bacillus megaterium* D16273.1	99
12	3	Y22	JQ579632	*Paenibacillus hunanensis* EU741036.2	99
13	2	F12	JQ579633	*Paenibacillus borealis* AJ011322.1	99
14	2	F24	JQ579623	*Paenibacillus illinoisensis* FN422001.1	99
15	2	B3	JQ579635	*Exiguobacterium indicum* AJ846291.1	99
16	2	H5	JQ579636	*Rhizobium* sp.DQ419569.1	99
17	2	H6	JQ579637	*Microbacterium* sp.EU741023.1	99
18	18	Y5	JQ613283	*Pantoea agglomerans* AJ233423.1	99
		B20	JQ613284	*Pantoea agglomerans* AJ233423.1	99
19	2	Y8	JQ579638	*Enterobacter asburiae* AB004744.1	99
20	1	H4	JQ579639	*Burkholderia* sp. DQ835011.1	99
21	1	B9	JQ579640	*Acinetobacter calcoaceticus* AJ888983.1	99
22	2	Y6	JQ579641	*Arthrobacter nicotinovorans* X80743.1	99
23	1	Y17	JQ579642	*Pseudomonas koreensis* AF468452.1	99
24	2	F23	JQ579643	*Pseudomonas aeruginosa* X06684.1	99
25	2	B6	JQ579644	*Stenotrophomonas maltophilia* AB294553.1	99
26	1	F11	JQ579648	*Flavobacterium anhuiense* EU046269.1	99
27	2	F9	JQ579645	*Sphingomonas trueperi* X97776.1	99
28	2	H1	JQ579646	*Sphingomonas azotifigens* AB033947.1	99
29	3	H11	JQ579647	*Sphingomonas kaistensis* AY769083.1	99
Total	94				

Groups, 16S-RFLP groups; NO, the number of endophytic bacteria; accession no, GenBank accession number.

promoting bacteria, including IAA producing bacteria, siderophore producing bacteria and antagonistic bacteria. Then, we evaluated the effect of the Y21, F10 and H2 on growth of peamut seedlings under pot experimental condition.

A large population of endophytic bacteria of diverse genera and species were found in the peanut plants by bacterial density detection and molecular analysis. Distinctive changes in the quantity of endophytic occupation were observed with a peak at the full pod stage and a

Table 3. IAA production of endophytic isolates.

Endophytic bacteria	Isolate	IAA production* ($mg \cdot L^{-1} \cdot OD_{600}^{-1}$)	Endophytic bacteria	Isolate	IAA production* ($mg \cdot L^{-1} \cdot OD_{600}^{-1}$)
Pantoea agglomerans	Y5	94.56 ± 0.2	*Bacillus subtillis*	Y1	5.39 ± 0.1
	Y15	91.93 ± 0.7		Y7	5.16 ± 0.2
	B2	88.85 ± 0.4		Y13	5.54 ± 0.4
	B4	135.95 ± 0.5		Y14	5.57 ± 0.1
	B8	121.79 ± 0.6		Y16	5.47 ± 0.3
	B11	133.79 ± 0.3		B1	5.38 ± 0.6
	B13	73.17 ± 0.9		B15	4.94 ± 1
	B19	145.68 ± 0.2		F7	5.55 ± 0.3
	B20	128.03 ± 1		F8	5.6 ± 0.6
	B21	63.85 ± 0.3	*Rhizobium* sp.	H5	57.72 ± 0.2
	B23	128.09 ± 0.4		H12	62.15 ± 0.2
	F10	133.53 ± 0.6	*Bacillus fusiformis*	Y11	8.33 ± 0.5
	F13	126.76 ± 0.7	*Sphingomonas azotifigens*	H11	17.75 ± 0.4
	F14	138.36 ± 0.2		H13	17.35 ± 0.2
	F19	131.27 ± 0.7		H14	18.82 ± 0.1
	F20	129.58 ± 0.7	*Sphingomonas trueperi*	F9	13.76 ± 0.5
	F28	127.04 ± 0.3		F15	14.02 ± 0.8
	H3	140.27 ± 0.3	*Enterobacter asburiae*	Y8	125.36 ± 0.3
	Y9	4.04 ± 0.6		Y10	125.69 ± 0.4
Bacillus thuringiensis	Y18	3.91 ± 0.6	*Bacillus megaterium*	H2	166.98 ± 0.4
	Y19	3.8 ± 0.2		H8	169.48 ± 0.1
	B17	3.89 ± 0.7		H9	161.73 ± 0.4
Flavobacterium anhuiense	F11	16.14 ± 0.3	*Pseudomonas aeruginosa*	Y21	9.83 ± 0.2
Bacillus arbutinivorans	B28	14.46 ± 0.5		F23	9.83 ± 0.5

*Average ± standard error from triplicate samples. The high-level (>50 $mg \cdot L^{-1} \cdot OD600^{-1}$) IAA-producing strains are shown in boldface.

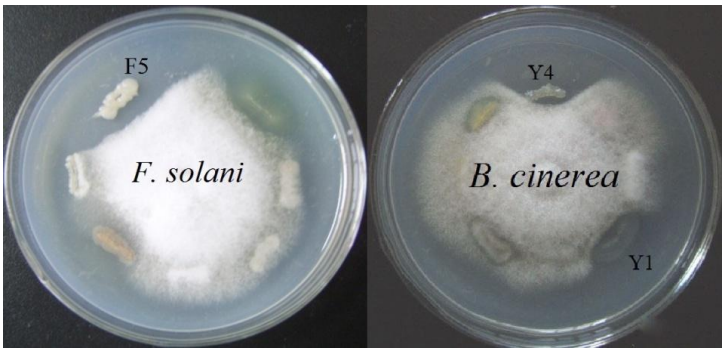

Figure 3. Screening of antagonistic bacteria used plate confrontation method in PDA (Potato Dextrose Agar) plates. In the middle of the agar plates are the pathogenic fungi around the pathogenic fungi are parts of the endophytic bacteria.

nadir at the harvest maturity stage. The 94 isolates were separated into 14 different genera based on the results of 16S rRNA sequencing analysis. The genera that presented at the young seedling stage were *Bacillus*, *Enterobacter*, *Pantoea*, *Paenibacillus*, *Pseudomonas*, and *Arthrobacter*; those at the beginning peg stage were

Table 4. Antifungal activities of the endophytic isolates.

Isolate	*S. rolfsii*	*F. solani*	*C. gloeosporioides*	*B. cinerea*	*P. cubensis*
Y1	-	++	+	+	+
Y4	-	+++	+	+++	++
Y7	-	+	+	+	+
Y13	-	+	+	+	+
Y14	-	++	++	++	++
Y16	-	++	++	++	++
Y21	+	-	-	-	-
Y22	-	+++	++	+	++
B1	-	++	+	+	+
B15	-	++	+	+	+
F5	-	+++	+	+++	+++
F7	-	++	+	+	+
F8	-	++	+	+	+
F23	+	-	-	-	-

Inhibition: **+**, $0 < R_1/R_2 < 1$; ++, $1 \leq R_1/R_2 < 2$; +++, $2 \leq R_1/R_2 < 2.5$; -, $R_1/R_2 < 0$.

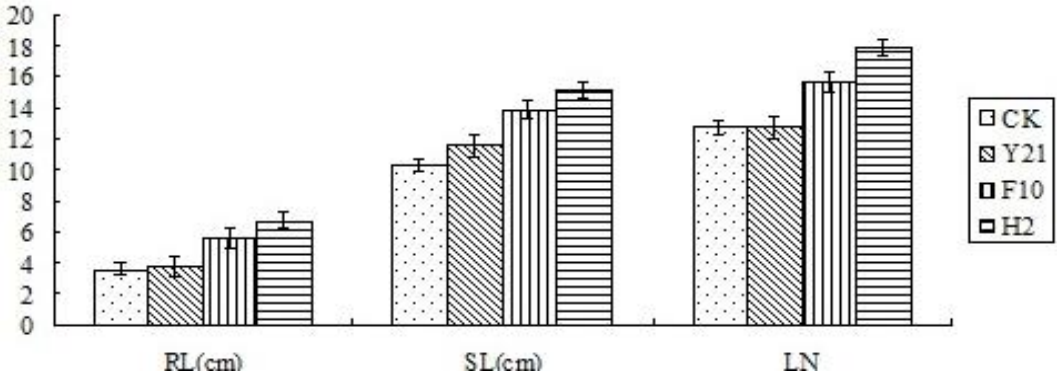

Figure 4. Growth-promoting activities of strains Y21, F10 and H2. RL, root length; SL, stem length; LN, leaf number.

Bacillus, *Pantoea*, *Exiguobacterium*, *Acinetobacter*, and *Stenotrophomonas*; those at the full pod stage were *Bacillus*, *Paenibacillus*, *Pantoea*, *Arthrobacter*, *Pseudomonas*, *Stenotrophomonas*, *Sphingomonas*, and *Flavobacterium*; and those at the harvest maturity stage were *Bacillus*, *Rhizobium*, *Microbacterium*, *Pantoea*, and *Burkholderia*. The most dominant genera were *Bacillus* and *Pantoea*, which were present at all four growth stages. Many isolates were also found in only one of the four growth stages. For example, *Rhizobium*, *Microbacterium*, and *Burkholderia* were found only in the harvest maturity stage, *Exiguobacterium* and *Acinetobacter* only in the beginning peg stage; and *Enterobacter* and *Flavobacterium* only in the young seedling and full pod stages, respectively. Kuklinsky-Sobral et al. (2004) have attributed these changes to the influences of seasons, host growth phase, or host genotype on the population density and taxonomic diversity of endophytic bacteria. Thus, one sensible explanation for this result may be that the abundant nutrients at the full pod stage provide the best condition for the growth of endophytic bacteria. Given its physiological characteristics, the *Bacillus* has become the most suitable flora to the host plant, as verified by a study on bacterial communities present in 14 maize Chinese cultivars and tropical maize (Figueiredo et al., 2009; Gao et al., 2004). The presence of these growth-stage specific bacteria can be explained by the fact that interactions with other bacteria or the different requirements of each microorganism were the main reasons for this phenomenon.

There were 14, 12, 12, and nine IAA-producing bacteria isolated from the young seedling, beginning peg, full pod, and harvest maturity stages, respectively. These IAA-producing bacteria belonged to 4, 2, 5, and 4 genera respectively. For all growth stages, *Pantoea* and *Bacillus* were the dominant genera. *Enterobacter*, *Flavobacterium*,

and Rhizobium were found only in the full pod and harvest maturity stages. The dominant, although low-level, IAA-producing bacterium at the young seedling stage was *Bacillus*. There were also four strains classified as *P. agglomerans* and *E. asburiae* that can produce high levels of IAA. For the beginning peg and full pod stages, the major IAA-producing bacterium was *P. agglomerans*, which was determined as the most abundant species. *P. agglomerans* was also found to be the second strongest ability to produce IAA after *B. megaterium*, which was only found in the harvest maturity stage. As the most active IAA producer, *B. megaterium* has been reported by Sturz et al. (1997) and confirmed to promote the growth of red clover plants either individually or in combination with *Rhizobium leguminosarum*. Considering that high and low IAA concentrations suppress and promote plant growth, respectively, *Bacillus* sp. is better for the growth of seedlings than *P. agglomerans*, which is suited for luxuriant growth. However, at different growth phases, the same genus showed different IAA production abilities. This finding can be explained on one hand by many of the isolated bacteria promoting the growth and fitness of their host by modulating IAA homeostasis (Long et al., 2008). On the other hand, the IAA production of strains is susceptible to the composition of the exogenous nutrient content.

Among the 94 endophytic bacteria, only isolates Y17, Y21, and F23, which belonged to *Pseudomonas*, were able to produce siderophores. These three isolates were also IAA-producing bacteria with antagonistic activities, except Y17. Bacteria belonging to this genus have been previously described by several authors as siderophore-producing bacteria (Alexander and Zuberer, 1991; Tian et al., 2009). Under experimental pot conditions, the strain H2 inhibited the growth of the peanut seedlings firstly, and then it promoted peanut growth obviously. Maybe high concentrations of IAA in fermentation broth could inhibit plant growth.

The antagonistic bacteria were from three genera, namely, *Bacillus*, *Paenibacillus*, and *Pseudomonas*. Table 2 shows that the highest diversity of antagonistic bacteria was at the young seedling stage, and *B. subtillis* was the most dominant species at all growth stages except the harvest maturity stage. *B. subtillis* demonstrated anti-microbial activity against the other four fungal pathogens except *S. rolfsii*. But Thasanaa et al. (2010) reported that the subtilis SSE4 culture filtrate exhibited antifungal activities to *C. gloeosporioides* and *S. rolfsii*. As a plant pathogen basidiomycete, the formation of sclerotia possibly renders *S. rolfsii* more difficult to control than other fungal pathogens. *P. aeruginosa* (Y21 and F23) appeared to exert effective antagonistic effects only against *S. rolfsii*.

The significant diversity change at the four growth processes was also investigated. Except for *Bacillus* and *Pantoea,* many genera were present in one or more particular growth stages. However, these generea were absent in others. One reasonable explanation for these dynamic changes may be the interactions of endophytic bacteria or different nutritional requirements of each microorganism that allow them to inhabit the plants. Climate conditions and human activities can also be responsible for such changes. Many strains were believed to be valuable to the development and application of bacterium agents, such as *B. megaterium* strains that presented the highest level of IAA production, as well as the siderophore-producing strain F23 that also had a weak ability to produce IAA and inhibit the growth of *S. rolfsii*. Further investigations should be performed to elucidate the endophytic colonisation of functional bacteria as well as their interactions with host plants.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (31100005), China.

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