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# Isolation and Characterization of Indole Acetic Acid Producing Root Endophytic Bacteria and Their Potential for Promoting Crop Growth

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## ABSTRACT

**Endophytic bacteria colonize in plant tissues and enhance plant growth by a wide variety of mechanisms. The objectives of this study were to examine the population of root endophytic bacteria in soybean and corn, and to identify IAA-producing endophytic bacterial strains and their growth promoting effect. The density of endophytic bacteria varied irrespective of crops, sampling times and soil amendments. A total of 119 and 277 bacterial isolates were isolated from soybean and corn roots, respectively. 39.6% of the total isolates showed IAA production in the range of 1~23 μg mL$^{-1}$ in culture medium supplemented with tryptophan. Fourteen isolates, designated as S1-S4 from soybean roots and C1-C10 from corn roots, had the capacity of producing IAA over 10 mg L$^{-1}$. Based on 16S rRNA gene sequence analysis, the fourteen isolates were closely related to *Psychrobacillus*, *Microbacterium, Lysinibacillus* and *Bacillus*. Pot experiment indicated that the growth-promoting effects varied among these 14 bacterial strains and not all of the strains were able to promote growth of the tested soybean and wheat plants. Strains *Microbacterium* sp. C4 and *Lysinibacillus* sp. C7 showed better performances in promoting soybean and wheat seedling growth.**

**Keywords:** Endophytic bacteria, Enhancement, IAA, Root, Plant growth.

## INTRODUCTION

Endophytic bacteria are bacteria that live inside plant tissues without gaining benefit or doing harm to the host (Hallmann *et al.,* 1997). They can be isolated from surface sterilized plant tissues or from internal plant tissues (Ferrando *et al.,* 2012, Mano *et al.,* 2006). Population densities of indigenous endophytic bacteria in roots are found to be about $10^5$ CFU g$^{-1}$ fresh weight which is higher than that of any other plant organs (Hallmann *et al.,* 1997). Many endophytic bacteria can affect plant growth through direct or indirect ways. Such as phytohormones production (Sheng *et al.,* 2008), nitrogen fixation (Kuklinsky-Sobral *et al.,* 2004), solubilization of soil phosphorus and iron (Kuklinsky-Sobral *et al.,* 2004, Sheng *et al.,* 2008), production of antibiotics or induction of systemic resistance in the plant host (Krechel *et al.,* 2002).

Previous studies have shown that endophytic bacteria in roots varied among plant species (Mcinroy *et al.,* 1995), environmental factors (Schulz *et al.,* 2006) and agricultural management (Seghers *et al.,* 2004). A large number of bacterial species were isolated from wheat, rice, potato and other crop roots, comprising 219 species representing 71 genera (Schulz *et al.,* 2006).

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Soybean (*Glycine max*) and corn (*Zea mays*) are two major crops in Northeast China. Citation proposed that bacteria producing Indole Acetic Acid (IAA) is a major way in affecting plant growth, which could increase the lateral root number and stimulated lateral root growth (Larcher *et al.,* 2003). The majority of endophytic bacteria, such as *Bacillus*, *Pseudomonas*, *Burkholderia* and *Enterobacteria* have been confirmed to synthesize IAA *in vitro* (Kuklinsky-Sobral, 2004). The objectives of this work were: (1) To examine the population of root endophytic bacteria in soybean and corn, (2) To identify IAA-producing endophytic bacterial strains, and their growth promoting effect.

## MATERIALS AND METHODS

### Collection of Root Samples

An experimental field was setup at Guangrong Village (47° 21' N,126° 49' E), Hailun City, Heilongjiang Province of Northeast China, which was established in the fall of 2004. The two simulated-erosion levels were established by removing topsoil with a bucket grader, operated at 0 and 30 cm depths. Two soil treatments, i.e., chemical fertilizer alone and chemical fertilizer plus manure were implemented. Further information about soil properties and site characteristics are described elsewhere (Sui *et al.,* 2013). Root samples of soybean (cv. Dongsheng-1) and corn (cv. Xinken-5) were collected on June 22$^{nd}$, July 10$^{th}$ and August 24$^{th}$ in 2012. Three plants of corn root samples and 9 plants of soybean root samples were collected and combined and put into a polyethylene bag at each sampling date, respectively, then the bags were transported to the laboratory and stored at –80ºC until use.

### Surface Sterilization of Root Samples

All root samples were washed several times using tap water to remove adhering soil particles and the majority of surface microbes. Then the roots were surface sterilized by sequent immersion in 70% (v/v) ethanol for 30 seconds, in sodium hypochlorite (0.1% (v/v) available chlorine) for 1 min and in 70% (v/v) ethanol for 30 seconds and then the roots were picked out and washed three times in sterilized distilled water for 1 min each time. 1 mL of the last rinsing liquid was plated on the 1/10 Nutrient Agar (NA) medium to confirm complete sterilization of the root surface (Hung *et al.,* 2007).

### Count and Isolation of Endophytic Bacteria from Crop Roots

A set of surface sterilized root samples were cut into 1-2 cm aseptically and then ground in a sterilized mortar thoroughly. The suspension was diluted tenfold series using sterile distilled water. One mL of each dilution was spread on 1/10 NA medium plates. The plates were incubated at 30°C for 7 days, and then the number of root endophytic bacteria was counted. After counting, based on the size, color and shape of bacterial colonies, the clear bacterial isolates were picked and purified by transferring 2-3 times on NA medium plates incubated at 30°C. All bacterial isolates were stored on NA slants at 4°C.

### Screening the Ability of IAA Production

IAA production was measured using a modified quantification method developed by Bric *et al.* (1991). Briefly, the isolated endophytic bacterial strains were cultured for 24 hours in 1 mL of Nutrient Broth (NB) medium. Then 10 μL of bacterial inoculums were transferred into the same medium supplemented with 100 μg mL$^{-1}$ of *L*-tryptophan (Sigma-Aldrich). Cultivation was performed in the dark at 30°C on a shaker (180 rpm) for 7 days. The bacterial cells were removed from the culture medium by centrifugation (8,000 rpm, 10 minutes). 200 μL of the supernatant was added to an ELISA plate, then 400 μL of the Salkowski reagent



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(49 mL of 35% $HClO_4$+1 mL of 0.5 M $FeCl_3$) was added (Tsavkelova *et al.,* 2007). The mixture was kept at room temperature for 35 minutes and then the absorbance at 490 nm was measured. The uninoculated tryptophan-containing medium mixed with the Salkowski reagent was used in parallel as a blank. A standard curve was prepared from serial dilutions of IAA stock solution. The presented results are means of three independent cultivations.

### Phylogenetic Analysis of IAA Producing Endophytic Bacteria

The endophytic bacteria producing the quantity of IAA≥ 10 μg $mL^{-1}$ were identified by 16S rRNA gene sequencing. The 16S rRNA gene sequence was amplified by PCR with the primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGCTACCTTGTTACGACTT-3') (Muyzer *et al.,* 1993). The PCR products were clone sequenced at the BGI Genome Center (Beijing, China). Phylogenetic data were obtained by alignment of the different 16S rRNA gene sequences retrieved from the BLAST algorithm (National Center for Biotechnology Information [http://www.ncbi.nlm/nih.gov]), using the software ClustalW 1.8 (Thompson *et al.,* 1994) and EzbioCloud (Kim *et al.,* 2011) with the default parameters. A neighbor-joining phylogenetic tree was constructed using MOLECULAR EVOLUTIONARY GENETIC ANALYSIS software (MEGA 4.0) (Tamura *et al.,* 2007) with 1,000 bootstrap replicates.

### Crop Growth Promoting Experiment

The identified endophytic bacteria producing IAA≥ 10 μg $mL^{-1}$ were used for growth promoting experiment. Pure cultured bacterial strains were cultured for 2 days at 30°C in NB medium. At the late exponential growth phase bacterial cells were harvested by centrifugation (8,000×g, 10 minutes), and washed twice with 0.85% sterile NaCl solution and resuspended to the concentration of $10^8$ CFU $mL^{-1}$. Seeds of soybean (*Glycine max* cv. Hefeng 55) and wheat (*Triticum aestivum* cv. Longmai 26) were surface-sterilized by submerging in 70% ethanol for 3 minutes, then rinsing 3 times with sterile distilled water, and subsequently treating the seeds with a solution of 20% (v/v) commercial bleach and 0.1% SDS for 30 minutes. The seeds were then washed with sterile distilled water five times in order to remove the bleach (Dobbelaere *et al.,* 1999). After germination in sterilized vermiculite, the radicles of soybean and wheat were carefully removed and a tiny wound was made with a sterilized scalpel blade. The wounded radicles were subsequently dipped in prepared bacterial inoculum for 3 hours. Uninoculated control was treated the same way but the seedlings were dipped in bacteria-free 0.85% NaCl solution. Four soybean seedlings and six wheat seedlings per treatment were transplanted in 10 cm diameter plastic pots filled with a 2:1 (v/v) mixture of sterile soil and sand, respectively. Each treatment had four replicates and was cultivated at 26°C. Seedlings were irrigated with 1 mL of prepared bacterial inoculum near the root in the first three days, and then watered daily. The control seedlings were without bacterial inoculum and watered daily from the very beginning. After 30 days growth, shoot height, shoot fresh and dry weight and root fresh and dry weight were measured. Also root length, root area and root volume were measured with Win-RHIZO system (Regent Inc., Quebec, Canada). The differences of these parameters were compared according to Tukey's multiple range tests at 5% significant level using the SPSS 16.0 (SPSS, Inc., Chicago, IL).

## RESULTS AND DISCUSSION

### Populations of Culturable Endophytic Bacteria

Populations of endophytic bacteria are dynamic, varied, and diverse (Sturz *et al.,*



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1998). Although we acknowledged that the plate counting method had inherent limitations in investigating the root endophytic bacteria density, this method was being widely used in endophytic microbial studies (Mcinroy *et al.,* 1995, Surette *et al.,* 2003). We observed that the population of root endophytic bacteria ranged from $0.79\times10^4$ to $19.95\times10^4$ CFU $g^{-1}$ fresh weight irrespective of crops, sampling dates and soil amendments (Figure 1). This is in agreement with the range reported by Schulz *et al.* (2006). We also found that the population of endophytic bacteria on July 10$^{th}$ was greater than samples on June 22$^{nd}$ and August 24$^{th}$ in soybean and corn roots, respectively. Earlier studies clearly showed that the densities in root endophytic bacteria were strongly dependent on numerous biotic and abiotic factors that often interact (Hallmann *et al.,* 1997), such as plant species, plant growth phase, certain nutrients and other factors (Kuklinsky-Sobral *et al.,* 2004, Mcinroy *et al.,* 1995). The present study revealed that severity of soil erosion could influence endophytic bacterial populations (Figure 1). Greater population of root endophytic bacteria was found more in non-eroded soil than in the eroded soil, the difference was made up by manure application for soybean roots. The reduction in soil nitrogen, phosphorus reserve and availability (Bakker *et al.,* 2004), as well as soil key micro-nutrients (Izaurralde *et al.,* 2006) by soil erosion might be part of the reasons affecting the endophytic bacteria colonization and growth.

## Screening of Endophytic Bacteria for Producing IAA

IAA is the main auxin in controlling many important physiological processes such as cell enlargement, division and tissue differentiation (Tsavkelova *et al.,* 2007, Samavat *et al.,* 2011). Sarwar *et al.* (1995) stated that some bacteria with the ability to produce IAA may be useful in increasing the growth of crops. Previous studies had clearly shown that the bacterial IAA yield varied with bacterial strains, culture conditions, amount of tryptophan supplemented and method of analysis (Tambalo *et al.,* 2006). In this study, 119 bacterial strains from soybean roots and 277 strains from corn roots were randomly selected for screening their abilities of producing IAA. Among the 396 isolates selected, we found 157 isolates (54 from soybean and 103 from corn), i.e.

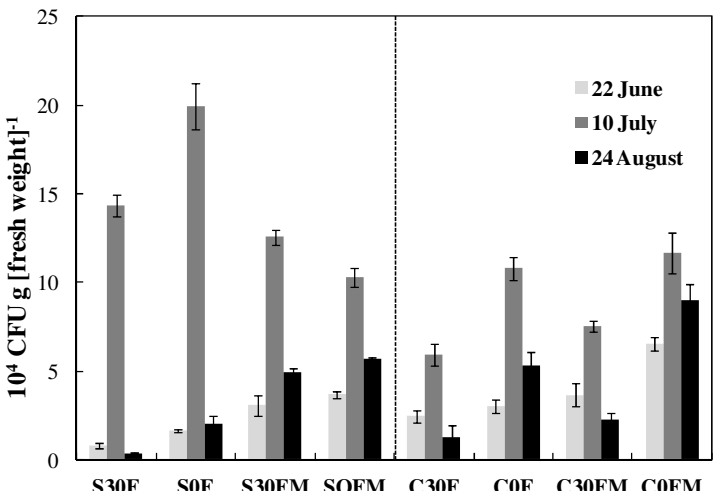


**Figure 1.** The number of culturable root endophytic bacteria in soybean and corn roots. S and C indicate Soybean and Corn root samples, respectively; 0 and 30 indicate non-eroded and 30 cm topsoil removal conditions, respectively. F and FM indicate chemical Fertilizer alone and chemical Fertilizer plus Manure, respectively.



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39.6% of the isolates selected showed IAA-producing ability in liquid culture supplemented with tryptophan in the range of 1 to 23 μg $mL^{-1}$. One isolate (C1) produced IAA as high as 20 μg $mL^{-1}$, and 13 isolates (S1-S4 isolated from soybean; C2-C10 isolated from corn) produced IAA ranging from 10 to 20 μg $mL^{-1}$ (Table 1). Sixty seven isolates, i.e. 16.9% of the total isolates showed IAA-producing ability less than 1 μg $mL^{-1}$. A similar amount of IAA production was reported by Zhang *et al.* (2011). Given IAA could stimulate plant growth in the range of 10-100 μg $mL^{-1}$ (Tsavkelova *et al.,* 2007), the 14 isolates with the capacity of IAA production greater than 10 μg·$mL^{-1}$ were shortlisted for further studies.

## Phylogenetic Identification of IAA-producing Endophytic Bacteria

The 16S rRNA gene sequences of 14 isolates have been registered in GenBank under accession numbers KP334978-KP334991. Based on the sequencing information (Table 1) and the phylogenetic tree of Figure 2, the putative Plant Growth Promoting Bacterial (PGPB) isolates were identified into four genera: *Microbacterium*, *Psychrobacillus*, *Lysinibacillus* and *Bacillus*. Most bacteria belonging to these genera have been reported as PGPB in several studies (Li *et al.,* 2008, Sgroy *et al.,* 2009, Registeri *et al.,* 2012). Nine of the 14 isolates were identified as *Microbacterium*, suggesting that this bacterial genus is a significant constituent for IAA-producing endophytic bacteria (Table 1). Many *Microbacterium* isolates from plant samples, soils and rhizospheres were known to be IAA producers (Tsavkelova *et al.,* 2007). For example, three species of *Microbacterium* isolated from Korean rice cultivars produced IAA in a range of 4 to 19 μg $mL^{-1}$, which had been tested with abilities of increasing rice height, dry weight and antagonistic effects against fungal pathogens (Ji *et al.,* 2014). Genus of *Bacillus* and *Lysinibacillus* were also often observed in plant roots (Li *et al.,* 2008, Schulz *et al.,* 2006, Vendan *et al.,* 2010). Idris *et al.* (2007) demonstrated the relationship of plant growth efficiency with IAA synthesizing ability of *Bacillus* sp. strain. Notably, we observed that the most active IAA producers in this work belonged to the genus *Psychrobacillus* (Table 1).

## Effects of IAA-producing Endophytic Bacteria on Crop Growth

Endophytic bacteria can have remarkable effects on plant growth in a variety of plants (Krechel *et al.,* 2002, Kuklinsky-Sobral *et al.,* 2004, Sheng *et al.,* 2008). In this study, the effect of 14 IAA-producing endophytic bacteria on the early growth of soybean and wheat was investigated by pot experiment, and parts of promoting growth effects were illustrated in Figure 3. In general, all the 14 isolates had positive effects on growth parameters of crops. For soybean, the average plant height was 9.6% higher than that of the non-inoculated. The greatest in shoot height and dry weight was obtained in *Lysinibacillus* sp. C7. In terms of root dry weight and length, the highest was determined in *Bacillus* sp. C6 and *Microbacterium* sp. C2, respectively, while the lowest was observed in *Lysinibacillus* sp. C9 and *Psychrobacillus* sp. S1 respectively. For wheat, a similar trend was found in the 14 isolates. The increase in shoot height by tested strains ranged from 3.5 to 18.5%. Bacterial inoculations also significantly affected wheat root growth, especially with *Microbacterium* sp. C2, *Microbacterium* sp. C4 and *Lysinibacillus* sp. C7 (Table 2). In general, *Microbacterium* (C4) and *Lysinibacillus* (C7) strains isolated from the corn roots had potential function on both soybean and wheat seedling growth.

Biosynthesis of IAA is considered very crucial in plant growth and development (Ali *et al.,* 2009). Therefore, the bacteria with the ability of IAA production can be used as a basic criterion for screening



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**Table 1.** Indole Acetic Acid (IAA) producing root endophytic bacteria and their closest relatives determined with 16S rRNA gene sequences.

| Strains | Source of isolates | | | | IAA production (mg $L^{-1}$) | Seq (bp) | Closest relatives | | Similarity (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Plant species | Depth of topsoil removal (cm) | Soil amendment[a] | Sampling time | | | Microorganisms | Accession number | BLAST | ClustalW | EzBioCloud |
| S1 | Soybean | 0 | Fertilizer | June 22th | 13.4 | 1516 | *Psychrobacillus psychrodurans* ATCC BAA-796$^T$ | AJ277984 | 99 | 98 | 98.79 |
| S2 | Soybean | 30 | Fertilizer | July 10th | 12.6 | 1486 | *Microbacterium foliorum* CIP 107137$^T$ | AJ249780 | 99 | 98 | 99.11 |
| S3 | Soybean | 30 | Fertilizer+Manure | July 10th | 12.5 | 1485 | *Microbacterium oxydans* DSM 20578$^T$ | Y17227 | 99 | 98 | 99.59 |
| S4 | Soybean | 30 | Fertilizer+Manure | June 22th | 12.0 | 1484 | *Microbacterium oxydans* DSM 20578$^T$ | Y17227 | 99 | 98 | 99.52 |
| C1 | Corn | 30 | Fertilizer | June 22th | 23.0 | 1515 | *Psychrobacillus psychrodurans* DSM 11713$^T$ | AJ277984 | 99 | 98 | 98.72 |
| C2 | Corn | 30 | Fertilizer | June 22th | 14.6 | 1531 | *Microbacterium oxydans* DSM 20578$^T$ | Y17227 | 99 | 99 | 99.66 |
| C3 | Corn | 0 | Fertilizer+Manure | June 22th | 14.3 | 1485 | *Microbacterium hydrocarbonoxydans* BNP48$^T$ | AJ698726 | 99 | 98 | 99.59 |
| C4 | Corn | 0 | Fertilizer+Manure | June 22th | 10.9 | 1486 | *Microbacterium foliorum* DSM 12966$^T$ | AJ249780 | 99 | 98 | 99.45 |
| C5 | Corn | 0 | Fertilizer+Manure | June 22th | 10.9 | 1485 | *Microbacterium oxydans* DSM 20578$^T$ | Y17227 | 99 | 98 | 99.66 |
| C6 | Corn | 0 | Fertilizer | July 10th | 10.8 | 1513 | *Bacillus aryabhattai* B8W22$^T$ | EF114313 | 99 | 99 | 99.93 |
| C7 | Corn | 30 | Fertilizer | July 10th | 10.3 | 1514 | *Lysinibacillus fusiformis* ATCC 7055$^T$ | AF169537 | 99 | 99 | 99.32 |
| C8 | Corn | 30 | Fertilizer+Manure | July 10th | 10.3 | 1485 | *Microbacterium oxydans* DSM 20578$^T$ | Y17227 | 99 | 98 | 99.72 |
| C9 | Corn | 30 | Fertilizer+Manure | July 10th | 10.2 | 1510 | *Lysinibacillus chungkukjangi* KACC 16626$^T$ | JX217747 | 98 | 98 | 98.12 |
| C10 | Corn | 0 | Fertilizer+Manure | June 22th | 10.1 | 1488 | *Microbacterium suwonense* M1T8B9$^T$ | GQ246683 | 99 | 99 | 99.16 |

[a] Fertilizer: 57 kg N $ha^{-1}$; 101 kg $P_2O_5$ $ha^{-1}$; 24.4 kg $K_2O$ $ha^{-1}$ applied before seeding, and 69 kg N $ha^{-1}$ top dressed at tasseling stage of corn; Fertilizer+Manure: Fertilizer as above proportion plus 15,000 kg $ha^{-1}$ (dry weight basis) of manure containing 730 g $kg^{-1}$ organic material; 7.4 g $kg^{-1}$ total N; 12.2 g $kg^{-T}$ total P, and 10.2 g $kg^{-1}$ total K.

**Table 2.** Effects of tested IAA producing root endophytic bacterial strains on seedling growth of soybean and wheat in pot experiment.

| Strains | Shoots | | | | | | Roots | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Soybean | | | Wheat | | | Soybean | | | | | Wheat | | | | |
| | Height (cm) | Fresh weight (g $plant^{-1}$) | Dry weight (mg $plant^{-1}$) | Height (cm) | Fresh weight (mg $plant^{-1}$) | Dry weight (mg $plant^{-1}$) | Fresh weight (g $plant^{-1}$) | Dry weight (mg $plant^{-1}$) | Length (cm) | Area ($cm^2$) | Volume ($mm^3$) | Fresh weight (mg $plant^{-1}$) | Dry weight (mg $plant^{-1}$) | Length (cm) | Area ($cm^2$) | Volume ($mm^3$) |
| S1 | 30.3* | 1.49±0.37 | 211±53* | 31.0 | 136±16 | 32±2 | 0.61±0.55* | 40±7 | 167.9 | 27.6±3.5 | 362.7±55.8 | 50±5* | 14±1* | 135.7 | 11.9±2.1* | 84±5* |
| S2 | 29.1* | 1.76±0.34* | 226±37* | 33.7* | 136±14 | 31±2 | 0.48±0.17 | 44±7 | 212.3 | 31.3±5.2 | 367.7±49.1 | 50±6* | 11±1 | 158.8* | 12.4±1.2* | 77±8* |
| S3 | 25.4 | 1.24±0.22 | 183±27 | 30.2 | 131±9 | 29±3 | 0.48±0.09 | 50±7* | 224.6 | 44.2±6.9* | 521.0±90.1* | 40±3 | 14±0* | 123.8 | 10.5±0.1 | 72±3* |
| S4 | 25.8 | 1.43±0.27 | 190±22 | 26.2 | 98±10 | 24±3 | 0.61±0.19* | 50±6* | 233.5 | 39.1±6.7 | 520.1±86.0* | 36±5 | 11±2 | 71.7 | 7.2±0.7 | 60±10 |
| C1 | 24.9 | 1.29±0.22 | 170±16 | 29.8 | 128±39 | 27±6 | 0.64±0.17* | 46±4* | 188.9 | 36.0±5.8 | 546.1±83.0* | 75±13* | 13±1 | 110.2 | 9.7±4.0 | 71±12* |
| C2 | 27.5 | 1.58±0.23 | 203±22 | 30.7 | 123±10 | 29±3 | 0.65±0.14* | 53±7* | 266.3 | 43.3±3.4* | 561.2±27.6* | 44±6 | 13±1 | 132.2 | 10.9±0.6 | 77±4* |
| C3 | 28.3* | 1.49±0.29 | 206±27 | 28.4 | 111±14 | 26±3 | 0.45±0.07 | 43±3 | 195.8 | 31.8±5.2 | 410.5±84.1 | 42±13 | 13±0 | 108.6 | 9.4±1.9 | 68±11 |
| C4 | 29.5* | 1.72±0.25* | 228±23* | 33.0* | 142±21* | 32±5 | 0.63±0.16* | 52±8* | 237.1 | 38.0±7.7 | 487.8±100.3* | 69±9* | 13±1* | 148.4* | 12.5±1.2* | 84±13* |
| C5 | 28.5* | 1.74±0.14* | 216±17 | 29.6 | 130±17 | 29±5 | 0.51±0.10 | 45±6 | 176.6 | 32.8±2.4 | 489.8±95.9* | 44±4 | 11±3 | 144 | 11.2±1.5* | 69±11 |
| C6 | 23.0 | 1.62±0.18 | 199±22 | 33.0* | 147±28* | 33±3* | 0.72±0.30* | 54±7* | 205.5 | 37.8±7.4 | 552.2±109.3* | 47±11* | 12±3 | 151.2* | 12.2±2.6* | 79±9* |
| C7 | 31.5* | 1.83±0.16* | 229±27* | 31.3 | 136±3 | 31±1 | 0.68±0.14* | 48±4* | 264.0 | 43.1±5.6* | 560.1±72.3* | 54±2* | 12±1 | 140.1 | 12.0±1.3* | 82±4* |
| C8 | 26.4 | 1.44±0.33 | 185±26 | 31.4 | 143±17* | 31±7 | 0.59±0.23* | 47±3* | 185.6 | 35.2±2.5 | 530.5±57.2* | 44±9 | 14±1* | 145.5 | 11.5±1.5* | 72±14* |
| C9 | 26.0 | 1.51±0.29 | 185±42 | 30.7 | 134±32 | 31±6 | 0.39±0.11 | 40±5 | 212.6 | 37.7±5.6 | 400.6±50.2 | 55±14* | 12±1 | 151.0* | 13.6±5.6* | 97±15* |
| C10 | 28.9* | 1.51±0.19 | 187±25 | 33.9* | 143±8* | 34±5* | 0.57±0.08 | 49±4* | 242.1 | 36.4±4.9 | 437.2±52.8 | 46±3* | 13±1* | 160.9* | 12.5±0.6* | 77±2* |
| Uninoculated | 25.1 | 1.48±0.30 | 184±34 | 28.6 | 109±9 | 26±2 | 0.40±0.13 | 36±2 | 212.2 | 30.5±2.4 | 348.1±24.1 | 31±7 | 11±1 | 94.7 | 7.2±1.4 | 44±8 |

* Indicates a mean value significantly different between tested endophytic bacteria and uninoculated control at $P < 0.05$ (Tukey's test) level.

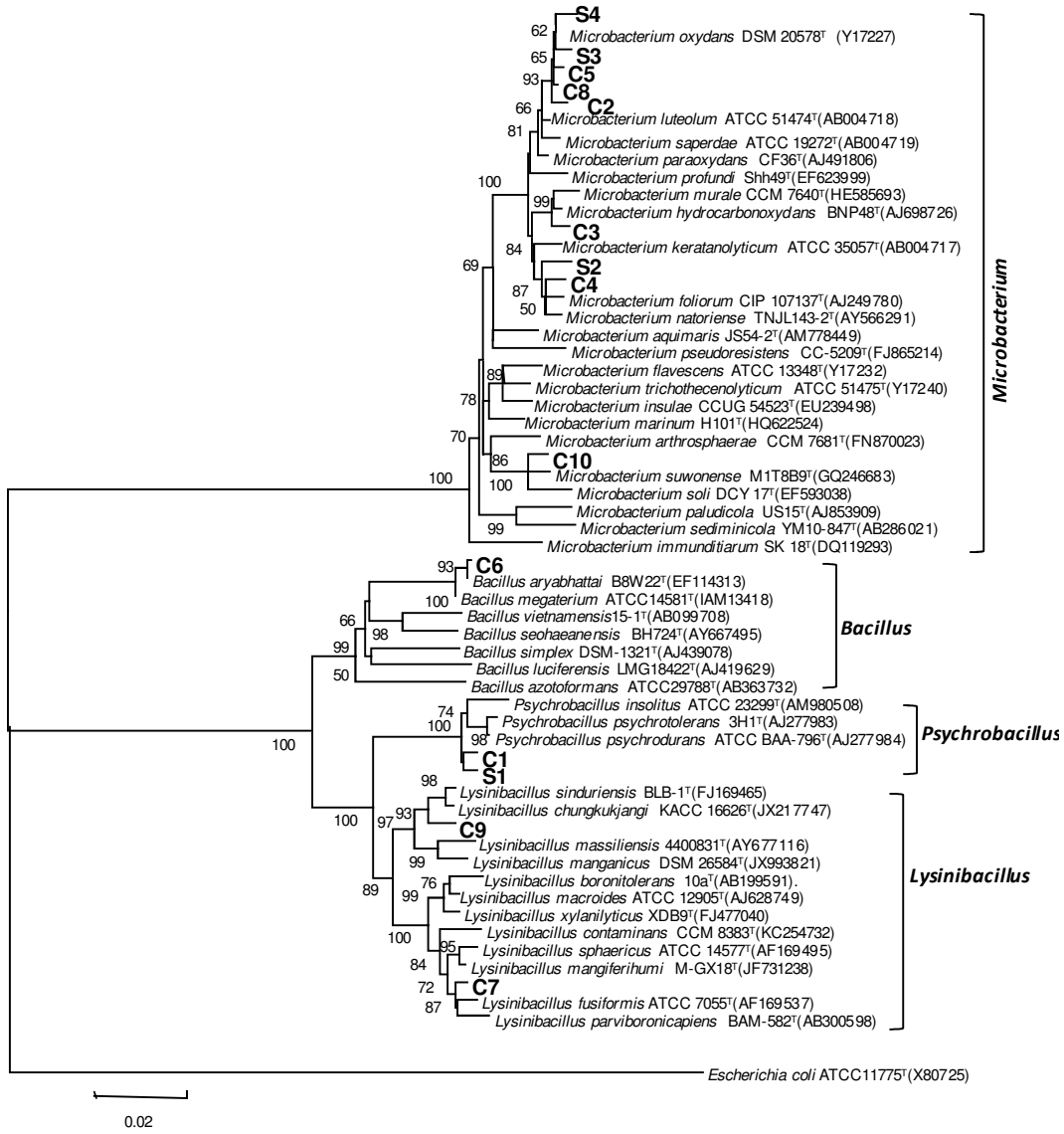


Figure 2

**Figure 2.** Neighbour-joining phylogenetic tree for the sequences of the 16S rRNA gene showing the relationships among root endophytic bacteria produced high level of IAA and related type strains. Bootstrap values (%) are based on 1,000 replicates.

effective PGPB in the commercial production of a bio-fertilizer for crops. IAA production bacteria have been extensively studied as bio-fertilizers (Ali *et al.,* 2009, Naveed *et al.,* 2014). However, to be an effective PGPB, bacteria must be able to colonize roots at a population sufficient to produce the beneficial effects. The colonization abilities varied in different plants and cultivation conditions. Future study should focus on establishing the colonizing ability of *Microbacterium* (C4) and *Lysinibacillus* (C7) in different conditions and thus investigating their role in promoting crop growth in the field condition.



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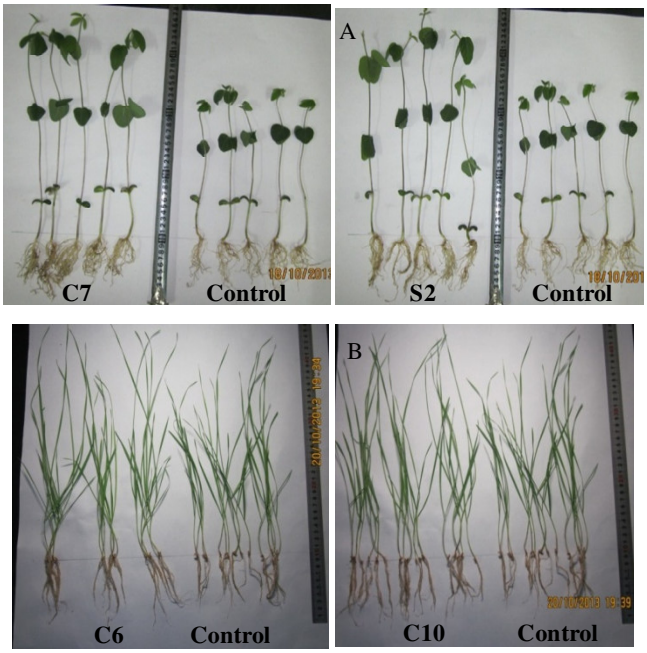


**Figure 3.** Effect of tested root endophytic bacteria on seedling growth of soybean (A) and wheat (B). C6, C7, C10, and S2 are the name of the tested bacterial strains.

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## REFERENCES

1. Ali, B., Sabri, A. N., Ljung, K. and Hasnain, S. 2009. Auxin Production by Plant Associated Bacteria: Impact on Endogenous IAA Content and Growth of *Triticum aestivum* L. *Lett. Appl. Microbiol.*, **48(5):** 542-547.
2. Bakker, M. M., Govers, G. and Rounsevell, M. D. A. 2004. The Crop Productivity–erosion Relationship: an Analysis Based on Experimental Work. *Catena*, **57(1):** 55-76.
3. Bric, J. M., Bostock, R. M. and Silverstone, S. E. 1991. Rapid *In situ* Assay for Indoleacetic Acid Production by Bacteria Immobilized on a Nitrocellulose Membrane. *Appl. Environ. Microbiol.,* **57(2):** 535-538.
4. Dobbelaere, S., Croonenborghs, A., Thys, A., Broek, A. V. and Vanderleyden, J. 1999. Phytostimulatory Effect of *Azospirillum brasilense* Wild Type and Mutant Strains Altered in IAA Production on Wheat. *Plant Soil,* **212(2):** 153-162.
5. Ferrando, L., Mañay, J. F. and Scavino, A. F. 2012. Molecular and Culture-dependent Analyses Revealed Similarities in the Endophytic Bacterial Community Composition of Leaves from Three Rice (*Oryza sativa*) Varieties. *FEMS Microbiol. Ecol.,* **80(3):** 696-708.
6. Hallmann, J., Quadt-Hallmann, A., Mahaffee, W. and Kloepper, J. 1997. Bacterial Endophytes in Agricultural Crops. *Can. J. Microbiol.,* **43(10):** 895-914.
7. Hung, P. Q., Kumar, S. M., Govindsamy, V. and Annapurna, K. 2007. Isolation and Characterization of Endophytic Bacteria from Wild and Cultivated Soybean Varieties. *Biol. Fertil. Soil.,* **44(1):** 155-162.



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[ DOR: 20.1001.1.16807073.2016.18.5.21.7 ]

8. Idris, E. E. S., Iglesias, D. J., Talon, M. and Borriss, R. 2007. Tryptophan-dependent Production of Indole-3-Acetic Acid (IAA) Affects Level of Plant Growth Promotion by *Bacillus amyloliquefaciens* FZB42. *Mol. Plant Microbe. Interact.,* **20(6):** 619-626.
9. Izaurralde, R. C., Malhi, S. S., Nyborg, M., Solberg, E. D. and Jakas, Q. 2006. Crop Performance and Soil Properties in Two Artificially Eroded Soils in North-central Alberta. *Agron. J.*, **98:** 1298-1311.
10. Ji, S. H., Gururani, M. A. and Chun, S. -C. 2014. Isolation and Characterization of Plant Growth Promoting Endophytic Diazotrophic Bacteria from Korean Rice Cultivars. *Microbiol. Res.,* **169(1):** 83-98.
11. Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H., Yi, H., Won, S. and Chun, J. 2011. Introducing EzTaxon-e: A Prokaryotic 16S rRNA Gene Sequence Database with Phylotypes that Represent Uncultured Species. *Int. J. Syst. Evol. Microbiol.,* **62:** 716-721.
12. Krechel, A., Faupel, A., Hallmann, J., Ulrich, A. and Berg, G. 2002. Potato-associated Bacteria and Their Antagonistic Potential towards Plant-pathogenic Fungi and the Plant-parasitic Nematode *Meloidogyne incognita* (Kofoid and White) Chitwood. *Can. J. Microbiol.,* **48(9):** 772-786.
13. Kuklinsky-Sobral, J., Araújo, W. L., Mendes, R., Geraldi, I. O., Pizzirani-Kleiner, A. A. and Azevedo, J. L. 2004. Isolation and Characterization of Soybean-associated Bacteria and Their Potential for Plant Growth Promotion. *Environ. Microbiol.,* **6(12):** 1244-1251.
14. Larcher, M., Muller, B., Mantelin, S., Rapior, S. and Cleyet-Marel, J. C. 2003. Early Modifications of *Brassica napus* Root System Architecture Induced by a Plant Growth-promoting *Phyllobacterium* Strain. *New Phytol.,* **160(1):** 119-125.
15. Li, J. H., Wang, E. T., Chen, W. F. and Chen, W. X. 2008. Genetic Diversity and Potential for Promotion of Plant Growth Detected in Nodule Endophytic Bacteria of Soybean Grown in Heilongjiang Province of China. *Soil Biol. Biochem.,* **40(1):** 238-246.
16. Mano, H., Tanaka, F., Watanabe, A., Kaga, H., Okunishi, S. and Morisaki, H. 2006. Culturable Surface and Endophytic Bacterial Flora of the Maturing Seeds of Rice Plants (*Oryza sativa*) Cultivated in a Paddy Field. *Microbes. Environ.,* **21(2):** 86-100.
17. Mcinroy, J. A. and Kloepper, J. W. 1995. Survey of Indigenous Bacterial Endophytes from Cotton and Sweet Corn. *Plant Soil,* **173(2):** 337-342.
18. Muyzer, G., De Waal, E. C. and Uitterlinden, A. G. 1993. Profiling of Complex Microbial Populations by Denaturing Gradient Gel Electrophoresis Analysis of Polymerase Chain Reaction-amplified Genes Coding for 16S rRNA. *Appl. Environ. Microbiol.*, **59(3):** 695-700.
19. Naveed, M., Qureshi, M. A., Zahir, Z. A., Hussain, M. B., Sessitsch, A. and Mitter, B. 2014. *L*-Tryptophan-dependent Biosynthesis of Indole-3-Acetic Acid (IAA) Improves Plant Growth and Colonization of Maize by *Burkholderia phytofirmans* PsJN. *Ann. Microbiol.*, **65(3):** 1381-1389.
20. Registeri, R., Taghavi, S. M. and Banihashemi, Z. 2012. Effect of Root Colonizing Bacteria on Plant Growth and Fusarium Wilt in *Cucumis melo*. *J. Agr. Sci. Tech. (Iran),* **14(5):** 1121-1131.
21. Samavat, S., Besharati, H. and Behboudi, K. 2011. Interactions of *Rhizobia* Cultural Filtrates with *Pseudomonas fluorescens* on Bean Damping-off Control. *J. Agr. Sci. Tech. (Iran)*, **13:** 965-976.
22. Sarwar, M. and Kremer, R. 1995. Determination of Bacterially Derived Auxins Using a Microplate Method. *Lett. Appl. Microbiol.,* **20(5):** 282-285.
23. Schulz, B. J., Boyle, C. J. and Sieber, T. N. 2006. *Microbial Root Endophytes*. Springer, New York, PP.17-22.
24. Seghers, D., Wittebolle, L., Top, E. M., Verstraete, W. and Siciliano, S. D. 2004. Impact of Agricultural Practices on the *Zea mays* L. Endophytic Community. *Appl. Environ. Microbiol.,* **70(3):** 1475-1482.
25. Sgroy, V., Cassán, F., Masciarelli, O., Del Papa, M. F., Lagares, A. and Luna, V. 2009. Isolation and Characterization of Endophytic Plant Growth-Promoting (PGPB) or Stress Homeostasis-regulating (PSHB) Bacteria Associated to the Halophyte *Prosopis strombulifera*. *Appl. Microbiol. Biotechnol.,* **85(2):** 371-381.
26. Sheng, X. F., Xia, J. J., Jiang, C. Y., He, L. Y. and Qian, M. 2008. Characterization of Heavy Metal-resistant Endophytic Bacteria from Rape (*Brassica napus*) Roots and Their Potential in Promoting the Growth and Lead



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[ DOR: 20.1001.1.16807073.2016.18.5.21.7 ]

Accumulation of Rape. *Environ. Pollut.,* **156(3):** 1164-1170.
27. Sturz, A., Christie, B. and Matheson, B. 1998. Associations of Bacterial Endophyte Populations from Red Clover and Potato Crops with Potential for Beneficial Allelopathy. *Can. J. Microbiol.,* **44(2):** 162-167.
28. Sui, Y., Jiao, X., Chen, W., Liu, X., Zhang, X. and Ding, G. 2013. Labile Organic Matter Content and Distribution as Affected by Six-year Soil Amendments to Eroded Chinese Mollisols. *Chin.Geogr. Sci.,* **23(2):** 692-699.
29. Surette, M. A., Sturz, A. V., Lada, R. R. and Nowak, J. 2003. Bacterial Endophytes in Processing Carrots (*Daucus carota* L. var. Sativus): Their Localization, Population Density, Biodiversity and Their Effects on Plant Growth. *Plant Soil,* **253(2):** 381-390.
30. Difuntorum-Tambalo, D., Paterno, E., Barraquio, W. and Duka, I. 2006. Identification of an Indole-3-acetic Acid-producing Plant Growth-Promoting Bacterium (PGPB) Isolated from the Roots of *Centrosema pubescens Benth*. *Philipp. Agric. Scientist,* **89:** 149-156.
31. Tamura, K., Dudley, J., Nei, M. and Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. *Mol. Biol. Evol*., **24(8):** 1596–1599.
32. Thompson, J. D., Higgins, D. G. and Gibson, T. J. 1994. CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment through Sequence Weighting, Position-specific Gap Penalties and Weight Matrix Choice. *Nucleic Acid. Res.,* **22(22):** 4673-4680.
33. Tsavkelova, E. A., Cherdyntseva, T. A., Botina, S. G. and Netrusov, A. I. 2007. Bacteria Associated with Orchid Roots and Microbial Production of Auxin. *Microbiol. Res.,* **162(1):** 69-76.
34. Vendan, R. T., Yu, Y. J., Lee, S. H. and Rhee, Y. H. 2010. Diversity of Endophytic Bacteria in Ginseng and Their Potential for Plant Growth Promotion. *J. Microbiol.,* **48(5):** 559-565.
35. Zhang, Y. F, He, L. Y., Chen, Z. J., Zhang, W. H., Wang, Q. Y., Qian, M. and Sheng, X. F. 2011. Characterization of Lead-resistant and ACC Deaminase-producing Endophytic Bacteria and Their Potential in Promoting Lead Accumulation of Rape. *J. Hazard. Mater.,* **186(2):** 1720-1725.

# جداسازی و شناسایی باکتری های اندوفیت ریشه تولید کننده ایندول استیک اسید و پتانسیل آن ها برای بهبود رشد گیاه زراعی

ج. یو، ژ. ه. یو گ. ه. وانگ، و ز. ب. لیو

## چکیده

باکتری های اندوفیت در بافتهای گیاهی استقرار می یابند و توسط طیف گسترده ای از مکانیسم ها باعث رشد گیاه می شوند. هدف این مطالعه بررسی جمعیت این باکتری ها در سویا و ذرت ، شناسایی سویه های باکتری های تولید کننده IAA و اثر آن ها بر روی بهبود رشد می باشد. تراکم باکتری های اندوفیت بدون در نظر گرفتن محصولات زراعی، زمان نمونه برداری و اصلاحات خاک متفاوت است. در کل به ترتیب ۱۱۹ و ۲۷۷ ایزوله باکتری از ریشه های ذرت و سویا جدا شده است .در ۳۹.۶٪ از کل این ایزوله ها، تولید IAA در محیط کشت غنی شده با تریپتوفان در مقدار 1-1 µg mL~23 نشان



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داده شد. ۱۴ ایزوله، به نام های S1-S4 از ریشه های سویا، C1-C10 از ریشه های ذرت، دارای پتانسیل تولید IAAدر مقدار بیش از ۱۰ mg L-1 هستند. براساس تجزیه تحلیل توالی ژن 16S rRNA ، این چهارده ایزوله خیلی به *Psychrobacillus*, *Microbacterium*, *Lysinibacillus* و *Bacillus* مرتبط هستند. آزمایشات گلدانی نشان داد که اثرات بهبود کننده رشد در میان این ۱۴ استرین باکتری متفاوت است و همه استرین ها قادر به بهبود رشد این گیاهان گندم و سویای تست شده نبودند. استرین های*Microbacterium sp. C4* و *Lysinibacillus sp. C7* عملکرد بهتری در بهبود رشد نهال های سویا و گندم نشان دادند.



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