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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 22nd, July 10th and August 24th 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 0.79×10^4 to 19.95×10^4 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 10th was greater than samples on June 22nd and August 24th 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.

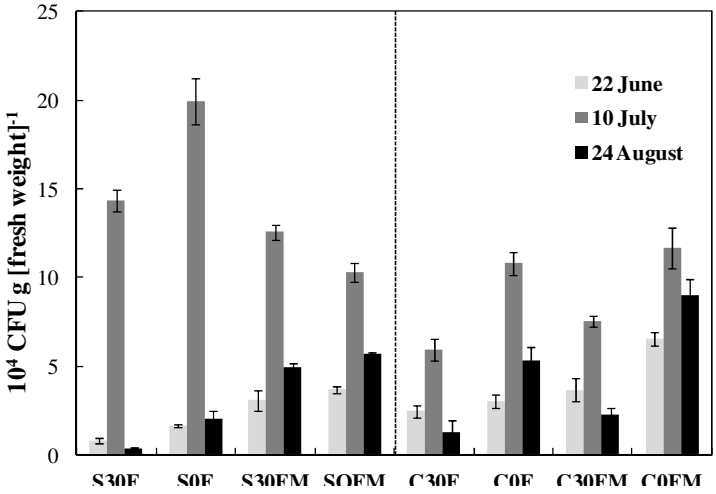


Figure 1. The number of culturable root endophytic bacteria in soybean and corn roots. S and C indicate Soybean and Corn root samples, respectively; 0 and 30 indicate non-eroded and 30 cm topsoil removal conditions, respectively. F and FM indicate chemical Fertilizer alone and chemical Fertilizer plus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

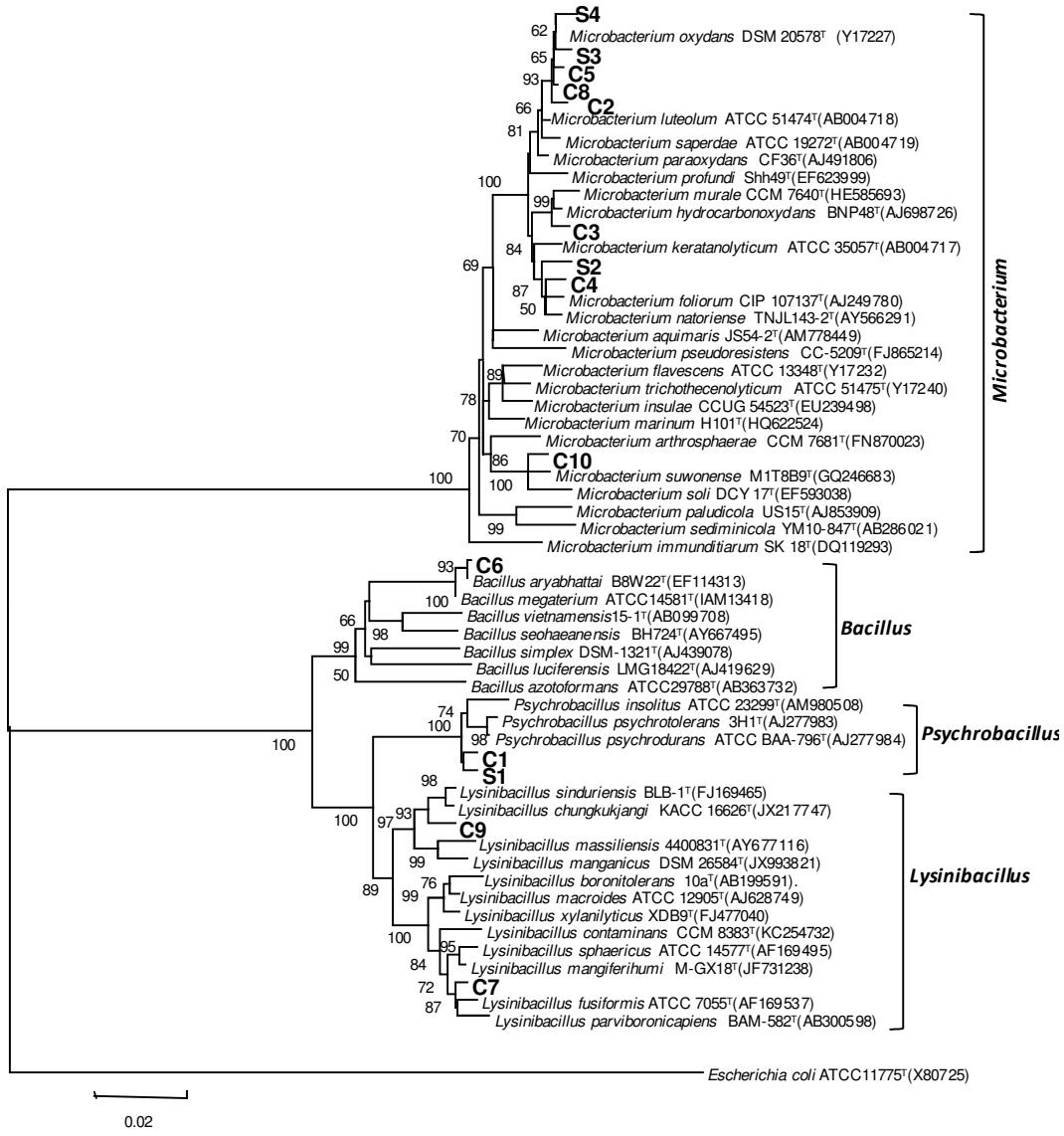


Figure 2

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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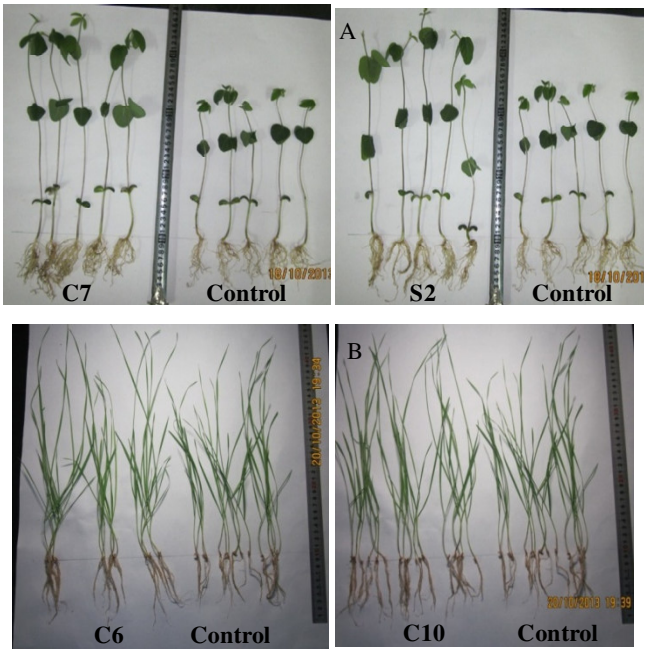


Figure 3. Effect of tested root endophytic bacteria on seedling growth of soybean (A) and wheat (B). C6, C7, C10, and S2 are the name of the tested bacterial strains.

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

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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 1-1~23 µg mL 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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