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Full Length Research Paper

The growth promotion of mung bean (*Phaseolus radiatus*) by *Enterobacter asburiae* HPP16 in acidic soils

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A novel phosphate-solubilizing bacterium HPP16 from plant rhizosphere of Shandong University of Science and Technology districts of Qingdao (China) was isolated and the effects on promoting growth of mung bean (*Phaseolus radiatus*) seedlings in Campus and Jinshatan; two kinds of acidic soils were studied. HPP16 was identified as *Enterobacter asburiae* on the basis of 16S rDNA sequencing. It was Indole-3-acetic acid producer, synthesized siderophores and showed acid phosphatase activity. After mung bean was inoculated with HPP16, the germination rate and healthy stand in A-2 (inoculated with fermentation liquid) increased by 26 and 25% compared to A-4 (inoculated with the distilled water; negative control), and were 26 and 31.7% in B-2 (inoculated with fermentation liquid) compared to B-4 (inoculated with the distilled water, negative control). The individual plant height, fresh weight and dry weight in A-2 increased by 7, 10 and 6% compared to A-4, and increased by 8.5, 24 and 9% in B-2 compared to B-4. Mung bean could also increase to absorb K^+, Na^+ and Mg^{2+} and improve the production of endogenous indole acetic acid (IAA), and also it reduced the production of abscisic acid (ABA). Findings of this study suggest that HPP16 may be exploited for developing a potential source of biofertilizer.

Key words: Plant growth promoting rhizobacteria, indole-3-acetic acid, siderophore, *Enterobacter asburiae* HPP16, mung bean, abscisic acid, phosphate-solubilizing bacteria (PSB).

INTRODUCTION

Soil microorganisms that solubilize phosphate are important to provide P (phosphorus) nutrient for plants and play an important role in the development of sustainable agricultural practices (Gyaneshwar et al., 2002; Rodriguez et al., 2007). P is by far the least mobile and is the most limiting factor for plant growth after nitrogen (N_2) compared with the other major nutrients (Bieleski, 1973; Vance et al., 2000). Researchers have been increasingly aware of future depletion of economically recoverable phosphate reserves. Current global reserves may be depleted in future decades of years (Cordell et al., 2009). Increasing amounts of P derived from phosphate rock is used in modern agriculture, which is a non-renewable resource (Postma et al., 2010). There is an imperative need to look for alternative and renewable sources of P since P is an important element for plant growth.

It is well known that phosphate-solubilizing bacteria (PSB) play an important role in promoting plant growth because of their abilities of converting insoluble P to soluble form which can be readily absorbed by the plant roots (Mamta et al., 2010). PSB often produce phosphatases and organic acids to facilitate P dissolution from P compounds (Chen et al., 2006). Under normal circumstances, the soil is supplied with inorganic P in the

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form of chemical fertilizers. A vast proportion of the applied P has been bounded in phosphates in the soil with iron, aluminum and calcium, etc (Altomare et al., 1999). This fixed form of P is not efficiently taken up by the plants and thus causes many environmental problems such as soil salinity and eutrophication (Del Campillo et al., 1999). The environmental problems could be decreased by using PSB as biofertilizers associated with conventional chemical fertilizers. The use of biofertilizers may be one way to reduce production costs because fertilizer cost is a major component of crop production (Samina et al., 2010). In addition to P-solubilization, PSB may also produce other secondary metabolites to improve the plant productivity. Evidences related to plant growth was promoted by PSB through the production of IAA (Patten and Glick, 2002; Shahab et al., 2009) and siderophore (Koo and Cho, 2009) making the PSB more suitable for use as biofertilizers. PSB can be used as inoculants to increase crop yield by solubilizing insoluble P compounds in soils (Sundara et al., 2002; Dey et al., 2004)

Some strains of soil microorganisms such as *Azospirillum* sp., *Enterobacter* sp., *Pseudomonas* sp., *Klebsiella* sp., *Serratia* sp. and *Pantoea* sp. have been reported to be associated with a variety of grasses and other plants depicting growth promotion capabilities (Lemanceau, 1992; Okon and Labandera-Gonzalez, 1994; Koeppler et al., 1999; Kampfer et al., 2005; Mauricio et al., 2009). There are some reports related to the effects of PSB on the growth of soybean (Li et al., 2008), chickpea (Rojan et al., 2010; Munees and Mohd, 2010), maize (Oliveira et al., 2009) and wheat (Hamdali et al., 2008). There is however no major study on the effect of PSB *Enterobacter asburiae* on the growth of mung bean and no research has been done on the biomass enhancement of mung bean in acidic soil by PSB inoculation. This study was therefore carried out to isolate the PSB from the Campus soil and examine their effects on mung bean growth. The objectives of this study were: (1) to evaluate the chemical and physical properties of two kinds of soils; (2) to evaluate the morphological, physiological and biochemical characte-rization of HPP16 and 16S rDNA molecular identification; (3) to evaluate the effect of HPP16 on mineral phosphate solubilization and on indole acetic acid (IAA) and siderophore production; (4) to evaluate mung bean plant growth promoting potential of HPP16; (5) to evaluate the effect of HPP16 on mung bean seedlings uptaking ions.

MATERIALS AND METHODS

Bacterial strain and culture

The strain HPP16 used in this study was previously isolated from acidic soil in the campus of Shandong University of Science and Technology, and was grown on inorganic phosphorus medium [10 g glucose, 0.5 g $(NH_4)_2SO_4$, 0.3 g NaCl, 0.3 g KCl, 0.3 g $MgSO_4 \cdot 7H_2O$, 10 g $Ca_3(PO_4)_2$, 0.03 g $MnSO_4 \cdot 4H_2O$, pH 7.0 to 7.5]. HPP16 strain was cultured in inorganic phosphorus medium with shaking at 180 rpm and at 30°C for 48 h. Final cell concentration of HPP16 was maintained at 10^9 cfu ml^{-1} by constantly keeping it at 4°C.

Bacterial characterization

The morphological, physiological and biochemical properties of the isolated strain HPP16, that included Gram property, catalase test, V.P test, gelation hydrolysis test, methyl red test, nitrate reduction test, starch hydrolysis test, phenylalanine deaminase test, citrate test, cytochrome oxidase test, amino acid decarboxylase test, SIM agar test, glucose oxidation test, inositol fermentation test, dextrin crystallization test, cellulose hydrolysis test, cellulose production test, indole test, lactose test, urease test and TSI agar test, were determined as the standard methods according to Bergey's Manual of Determinative Bacteriology (Holt, 1994).

16S rDNA identification

Sequencing of 16S rDNA

Bacterial DNA was isolated by using the standard phenol-chloroform extraction method (Frederick et al., 1995). 16S rDNA PCR amplification were performed as stated by Ovreas et al. (1997) by using the universal primers. PCR product was purified with a PCR purification kit (from Bioer Technology Co., Ltd, China) and nucleotide sequence data under accession number GQ866854 and was deposited in Gen-Bank sequence database. The online program BLAST was used to find the related sequences with known taxonomic information in the databank at NCBI website (http://www.ncbi.nlm.nih.gov/BLAST) to accurately identify the strain HPP16.

Mineral phosphate solubilization

Mineral phosphate solubilization was assayed on agar plates containing insoluble tricalcium phosphate (Goldstein and Liu, 1987). The plates were incubated at 30°C. Development of a clear zone around the colony was evaluated at 48 h.

Phosphatase activity

The acid phosphatase activity in the culture medium used for P solubilization experiment was measured by the method based on the hydrolysis of p-nitrophenyl phosphate as described by Tabatabai and Bremner (1971). Culture filtrate was incubated with p-nitrophenyl phosphate and modified universal buffer. After 1 h, the hydrolysis reaction of p-nitrophenyl phosphate by phosphatase was terminated by adding 0.5 M $CaCl_2$ and 0.5 M NaOH solution. The mixture was centrifuged and the yellow of supernatant was measured at 410 nm.

Quantitative analysis of the ability of phosphate-solubilizing bacteria

The concentration of phosphate (P) was determined with the molybdenum blue colorimetric method. The organophosphorus of nucleic acids will be transformed into inorganic phosphorus under strong acid digestion. A yellow complex was formed between inorganic phosphorus and ammonium molybdate. The concen-tration of the afterwards formed blue complex by reduction of the yellow complex was measured at 660 nm using a spectrophotometer.

Table **1.** Chemical and physical properties of soil layer (20 cm under the soil surface).

Soil sample	Ct	Nt	P	K	Mg	Na	Ca	Total salt	pH
Campus Soil (g/kg)	4.6	0.6	0.2232	0.1304	0.7330	0.1615	0.3606	1.7960	6.17
Jinshatan Soil (g/kg)	18.8	1.9	0.8370	0.1706	0.6318	0.2053	0.2594	3.3394	5.76

A standard curve was established with a solution of potassium phosphate (1~5 $mg \cdot L^{-1}$ PO_4^{3-}). Supernatant samples of 1.5 ml of each culture were collected everyday during seven days, and then centrifuged through a centrifuge at 10,000 rpm for 5 min to remove cell debris. Then, the available phosphorus content was calculated according to the standard curve derived.

IAA and siderophore production

To test the productivity of IAA by the isolates, each isolate was inoculated in 5 ml of inorganic phosphorus medium, supplemented with 0.5 mg/ml of L -tryptophan, and incubated on a rotary shaker (180 rpm) at 30°C. The composition of the inorganic phosphorus medium was as mentioned earlier. The resulting culture suspension by centrifugation was mixed with Salkowski's reagent (150 ml of concentrated H_2SO_4, 250 ml of distilled water, 7.5 ml of 0.5 M $FeCl_3 \cdot 6H_2O$) with a 1:2 (v/v) ratio, and was allowed to stand at room temperature for 20 min. The pink color developed, indicating IAA production, was measured at 530 nm with a spectrophotometer (WFJ 7200-Visible Spectrophotometer, UNICO Instrument Co., China). The absorbance was converted into the concentration of IAA using a standard curve prepared with IAA (C_8H_6N-CH_2COOH; Sigma, www.solarbio.cn). Each test was carried out in triplicate.

The activity of the siderophore produced by the isolates was determined by using blue agar plates which contained chrome azurol S (CAS) (Schwyn et al., 1987). An orange halo around the colony on the CAS agar was indicative of siderophore excretion. Each colony was inoculated on the center of a CAS agar plate, with the increasing surface area of the orange halo around the colony periodically measured during incubation at 30°C.

Mung bean variety, bacterial strain and soil type

Campus and Jinshatan soil samples for the experiment were collected from the campus of the Shandong University of Science and Technology and Jinshatan beach, the eastern part of Shandong province, Qingdao, China. 50 kg was collected from the roots of the tree at a distance of 20 cm under soil surface. The chemical properties of the two different kinds of soils are presented in Table 1. The total organic content (Ct) and the total nitrogen content (Nt) were determined by elemental analysis. Molybdenum blue colorimetry method was used to determine the total phosphorous (P) content of the soil. An atomic absorption spectrophotometer was employed to measure the content of calcium (Ca), extractable magnesium (Mg), potassium (K) and sodium (Na). The pH of soil was measured by an Electrometer. The total salt in the soil was determined by the conductance method. The electrical conductivity of the campus and Jinshatan soils were 0.1426 and 0.3248 ms cm^{-1}, respectively. Mung bean variety Weilv 4 and bacterial strain HPP16 were used for the inoculation experiment. Mung bean seeds were obtained from Weifang Seed Company in Shandong province of China.

Plant growth and inoculation in illumination incubator

The strain HPP16 was cultured in inorganic phosphorus liquid medium and shaken at 180 rpm at 37°C for 48 h and then the bacterial cells were pelleted by centrifugation at 5000 × *g* for 5 min. Bacterial suspension was prepared by washing bacterial cells twice with saline solution (0.16 M NaCl) and twice with sterile Milli-Q water, re-suspending and adjusting to 10^9 cfu ml^{-1} with a spectrophotometer (Mayak et al., 2004).

Mung bean seeds were sterilized with 70% alcohol for 30 s and 5% hydrogen peroxide solution for 2 h, and then washed with sterile distilled water (Caitriona et al., 2004). The disinfected seeds were immersed in bacterial suspension for 6 h and were then sown in Campus and Jinshatan soil in pots. 15 mung bean seeds were sown per pot. There were four treatments of seedlings growth in Campus soil, and these were A-1 (inoculated with bacterial suspension), A-2 (inoculated with fermentation liquid), A-3 (the culture medium; positive control) and A-4 (the distilled water; negative control). The other four treatments of seedlings growth in Jinshatan soil were B-1 (inoculated with bacterial suspension), B-2 (inoculated with fermentation liquid), B-3 (the culture medium; positive control) and B-4 (the distilled water; negative control). Each treatment was replicated ten times.

Plants were placed in illumination incubator (Jiangsu Jintan Shuangjie Instrument Company, China, model GPX-250B) with a day/night temperature of 25/18°C and with 100 umol photons/m^2 s^{-1} of light supplied for 12 h during the daytime. The seed germination rate was measured after two days. Individual plant height, fresh weight (FW), dry weight (DW), the K^+, Na^+, Ca^{2+}, Mg^{2+} and Fe^{3+} contents and the endogenous IAA and ABA contents of mung bean plants were examined after two weeks of germination. The biomass of the samples were dried at 60°C for 10 h to get a constant weight and then an atomic absorption spectrophotometer was employed to calculate the K^+, Na^+, Ca^{2+}, Mg^{2+} and Fe^{3+} extractable contents of dry mung bean plants.

Extraction and estimation of the IAA and ABA content

The IAA and ABA content of mung bean seedlings were measured by the method of high performance liquid chromatogram (HPLC) (Li et al., 2002; Javier et al., 2004). 5.0 g mung bean fresh leaves were calculated and sheared to pieces, and then soaked overnight by 67% acetone. The soaking liquid remove plant pigments by diatomite adsorption. The supernatant after centrifugation was adjusted to pH 2.8 and extracted three times by ethyl acetate. The water phase again was adjusted to pH 8.0, and again extracted three times by ethyl acetate. Ester phase was merged and evaporated, washed with methanol after full drying, and dissolved in methanol. The dissolving liquid was used to analyze the concentration of ABA and IAA. Each value was the mean of three replicates.

Mung bean plant growth promotion by HPP16

To evaluate the effect of strain HPP16 on the mung bean seeds under flowerpot conditions, we carried out eight types of treatments. The first four types of treatments were that mung bean seeds were: cultivated in Campus soil, inoculated with bacterial suspension, fermentation liquid, the culture (positive control) and distilled water (negative control), named A-1, A-2, A-3 and A-4, respectively. The

Table **2.** Physiochemical characteristics of HPP16.

Characteristic	HPP16	Characteristic	HPP16
Gelatin (GEL)	+	SIM agar	-
Glucose oxidation	+	Methyl red	-
Inositol fermentation	+	Gram staining	-
Catalase	+	Dextrin crystallization	-
Nitrate reduction	+	Hydrolysis of cellulose	-
Starch hydrolysis	+	Amino acid decarboxylase (Gly)	-
V.P	+	Indole	-
Production of cellulose	+	Amino acid decarboxylase (Leu)	-
Citrate	+	Urease test	-
Lactose test	+	Phenylalanine deaminase	-
TSI agar	+	Cytochrome oxidase test	-
Growth at 28℃	+	Amino acid decarboxylase	-
Growth at 29℃	+	Colony morphology	Dome
Growth at 30℃	+	Size of colony(d)	> 2 mm
Salt tolerance (0.2%)	+		

bacterial suspension was prepared by the fermentation liquid pelleted by centrifugation for 5 min at 4,000 rpm and the harvested cells were washed twice by distilled water and resuspended (OD_{600} = 0.8 - 0.9). The other four groups were that mung beans were cultivated in Jinshatan soil, were also inoculated with bacterial suspension, fermentation liquid, the culture (positive control) and distilled water (negative control), named B-1, B-2, B-3 and B-4, respectively. There were ten flowerpots in each group, and 15 mung bean seeds in each flowerpot. The whole experiments were repeated three times.

Data analysis

All data were expressed as an average of three replicates. The data collected were analyzed statistically using SPSS 17 software. The effects of the strain HPP16 on treatment plants were considered significant according to the magnitude of the F value ($P < 0.05$). F test was applied to determine the level of significance for treatments, and mean separation was determined according to Fisher protected least significant difference (LSD) test.

RESULTS

Chemical and physical properties of soil layer

We analyzed two kinds of soils, one named Campus soil, and the other named Jinshatan soil (Table 1). These two kinds of soils have a common characteristic (they are acidic*)*. The pH values of Campus soil and Jinshatan soil were 6.17 and 5.76. The potassium and sodium contents of the Jinshatan soil were just above those of the Campus soil; increased by 0.0402 and 0.0438 g/kg, respectively. The magnesium and calcium contents of the Campus soil were 0.1012 and 0.1012 g/kg higher than those of the Jinshatan soil. The Ct, Nt, P and total salt contents of the Jinshatan soil were obviously higher than those of the campus soil. The Ct and P contents of the Jinshatan soil were four times the contents of the Campus soil. The soils total phosphorus analyzed was available. The Nt content of the Jinshatan soil was more than three times the contents of the Campus soil. Total salt content was about twice of the content of the Campus soil.

Characterization and molecular identification of HPP16

The strain HPP16 was characterized and identified by using standard morphological, physiological and bio-chemical tests. The characteristics of the colonies of HPP16 were described in Table 2. On the basis of these features, HPP16 was precisely identified as *Enterobacter* sp. To further characterize the strain HPP16, 16S rDNA sequence analysis was used. 16S rDNA of HPP16 was sequenced and found approximately 1140 bp in size. PCR direct sequencing was done and sequences of 16S

rDNA of the strain HPP16 were submitted to Gen-Bank (Gen-Bank accession number GQ866854). A similar

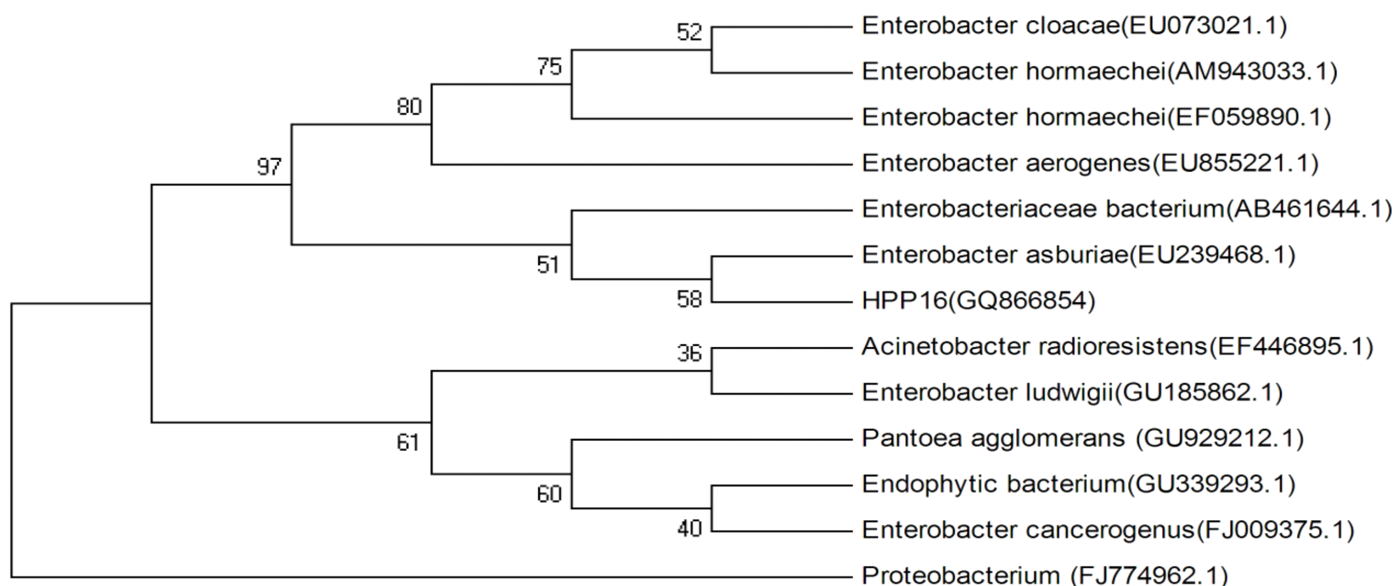

Figure 1. Phylogenetic tree drawn from neighbor-joining analysis based on the 16SrDNA sequence alignment.

search was performed by using the BLASTn program that indicated that the strain HPP16 shared a close relationship with the DNA sequence of *E. asburiae* (16S: 99% similarity with the reference strain EU 239468.1). Such high similar values confirmed the strain HPP16 as *E. asburiae*; as 16S rDNA sequence similarity was more than 98%, it can be considered as the same species (Figure 1).

Mineral phosphate-solubilizing ability of the bacterium

Strain HPP16 was tested for its phosphate-solubilizing ability. Upon incubation of the microorganism on to the solid plates containing insoluble phosphate, HPP16 strains were detected by the formation of clear halos around their colonies (Figure 2). To that end, the effect of solubilization was evaluated on a medium of tricalcium phosphate with concentration of 10 mg ml^{-1} which contained glucose as a sole carbon source. HPP16 showed a good phosphate-solubilizing ability as the formation of clear halos around their colonies. In the study on the culture in flask scale of PSB, stain HPP16 recorded high solubilization efficiency (Figure 3). The maximum solubilization efficiency was observed at 96 h. Acid phosphatase activity produced by the isolated bacteria was about 0.1130 mM as measured by p-nitrophenol production.

IAA and siderophore production

Strain HPP16 possessed the ability of IAA production and siderophores synthesis (Figure 4). The IAA concentration in the culture suspension increased with the growth of HPP16 and was 170 mg l^{-1} after 35 h (Figure 4A). The capability of synthesizing IAA is an important feature of a strain to be considered as a PGPR; it is well known that the hormone participates in plant growth promotion by increasing the radical surface of the inoculated plants. Figure 4A shows that the strain HPP16 synthesizes IAA at a high level. The results of the IAA biosynthesis obtained from strain HPP16 supported the notion that we were dealing with a PGPR. Therefore, strain HPP16 was further characterized and it was observed that the area of orange halo around HPP16 colony on to the CAS-agar plate increased linearly during the incubation, thus indicating that HPP16 could synthesize siderophores (Figure 4B).

Effect of HPP16 on germination rate of mung bean

Mung bean seeds soaked in HPP16 bacteria fermentation liquid were planted in these two kinds of soils to test the effects of HPP16 strain on mung bean plant promoting growth in different composition of soils. We could get two facts in common: one fact was that mung bean germination rates in A-1 and A-2 were obviously higher than A-3 and A-4 and mung bean germination rates in B-1 and B-2 were higher than B-3 and B-4; the other common fact was that mung bean germination rate in A-4 was obviously higher than A-3 germination rate and B-4 rate was obviously higher than that of B-3 (Figure 5). The growth trends of mung bean in these two

kinds of different soils were almost identical but the germination rate in the Jinshatan soil (B group) was higher than that in Campus soil (A group).

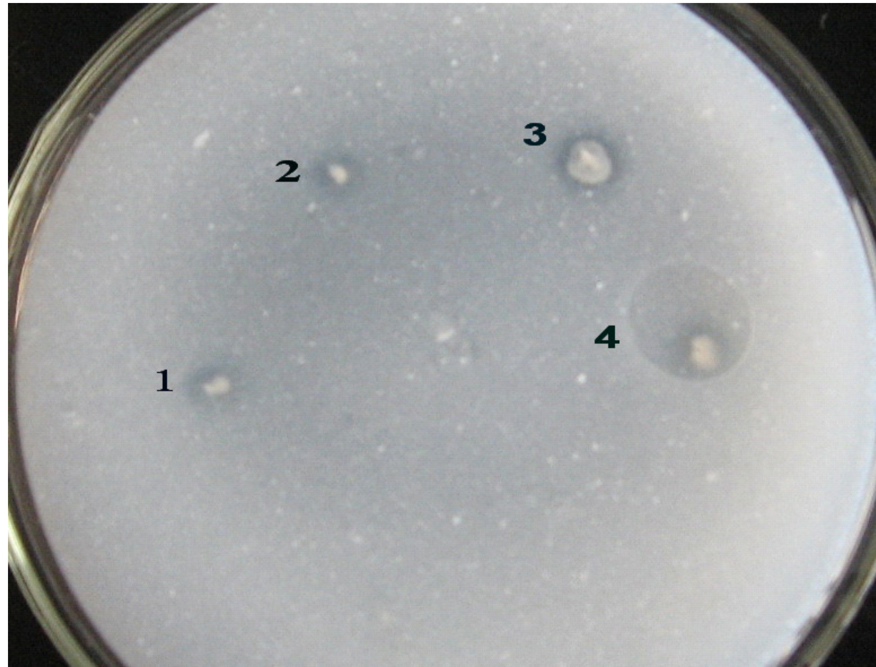

Figure 2. Extra cellular solubilization of tricalcium phosphate present in the medium at concentrations 10 mg ml^{-1}. All the spots (1, 2, 3 and 4) are of same bacteria.

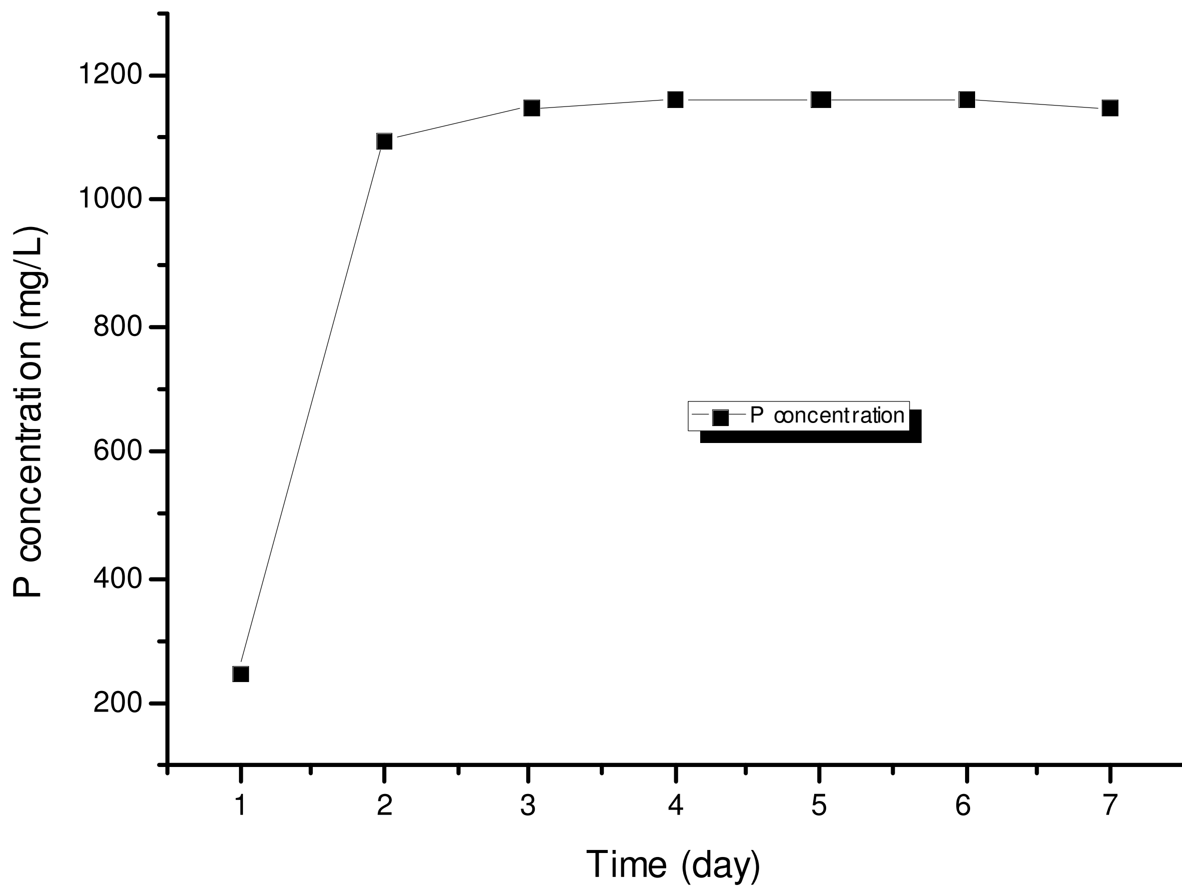

Figure 3. Solubilization efficiency of HPP16 in the inorganic phosphorus medium.

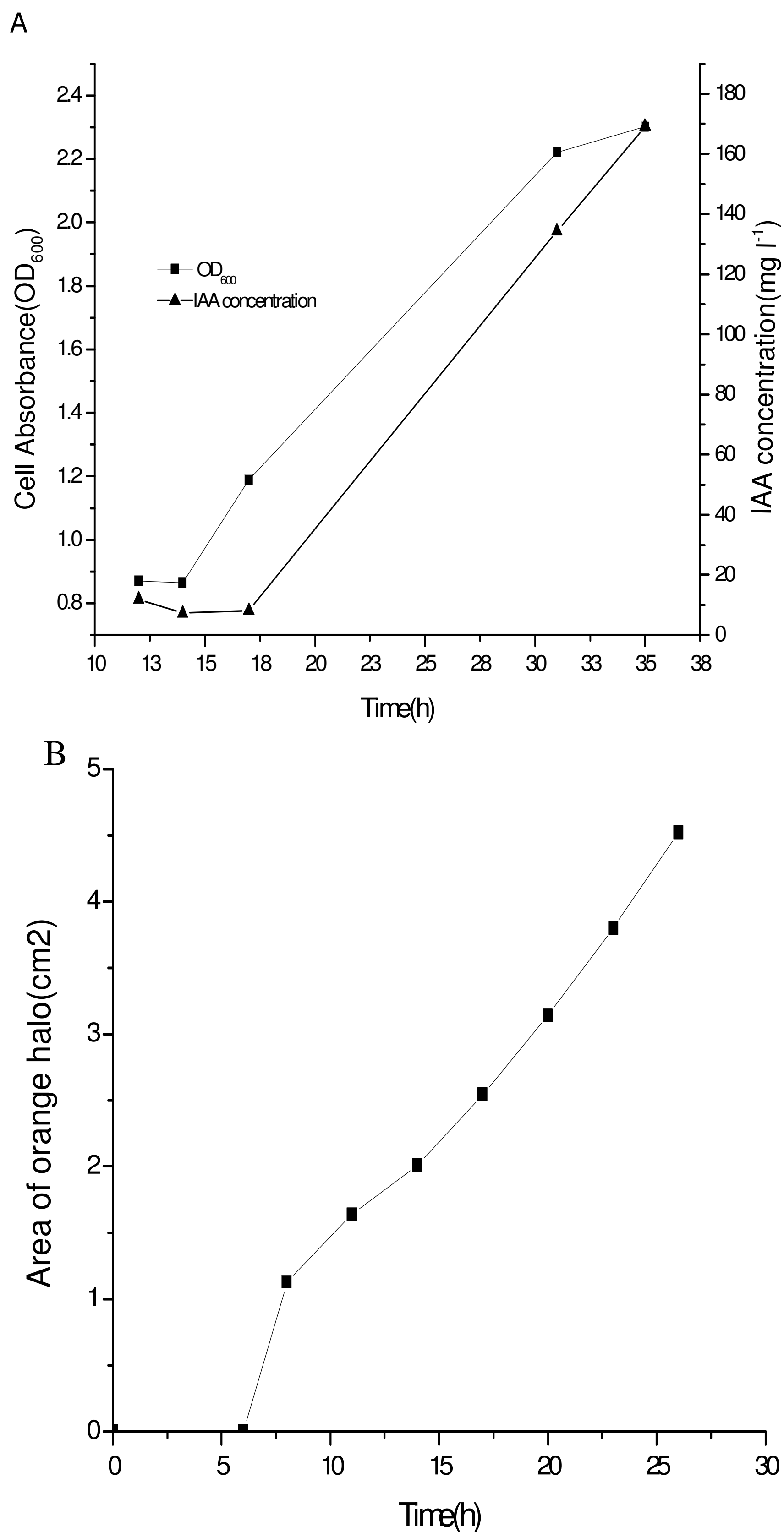

Figure 4. Time course of the IAA concentration in the inorganic phosphorus

medium containing L -tryptophan (A) and orange halo area in the CAS-agar plate (B).

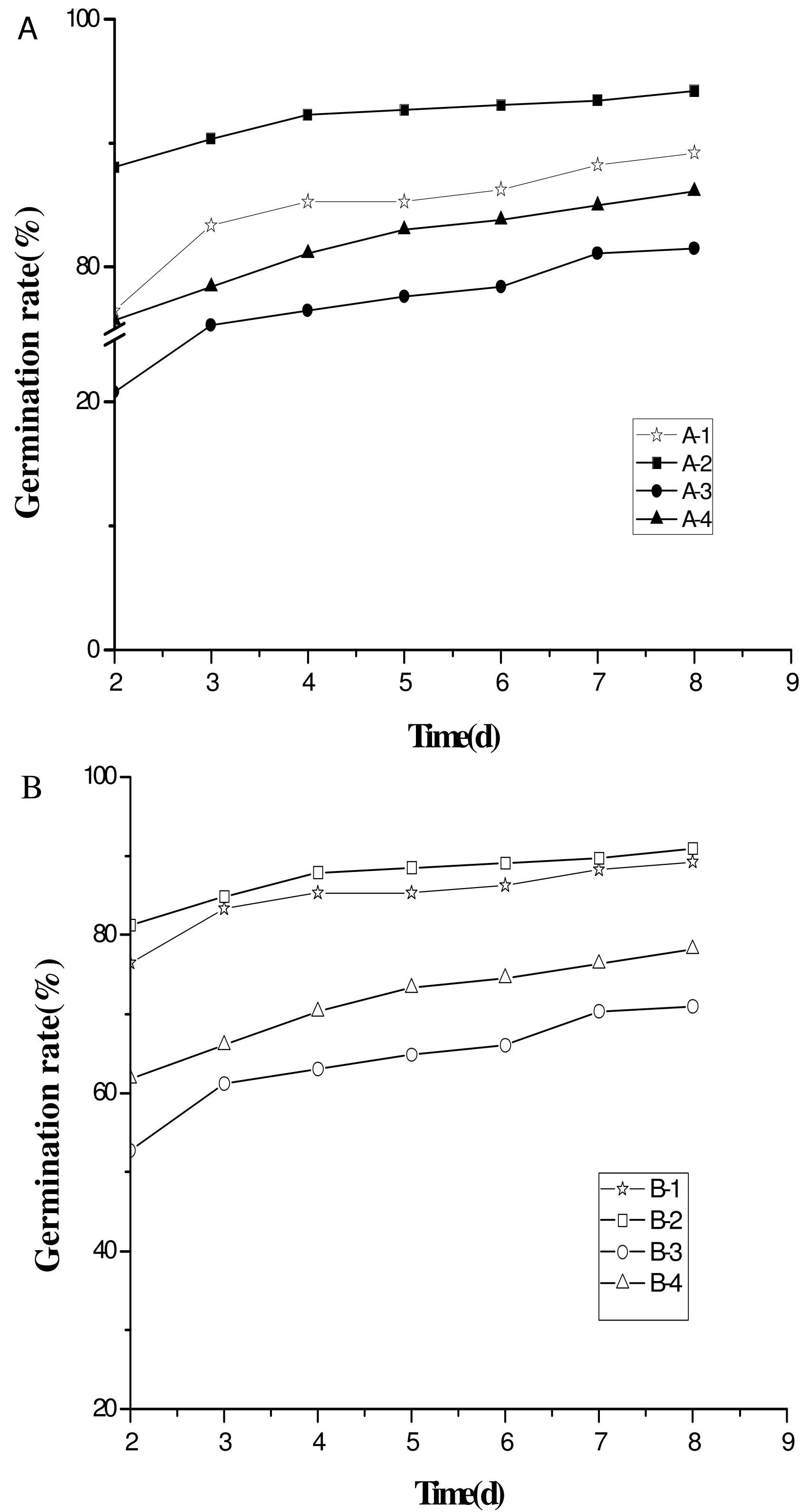

Figure 5. Effect of HPP16 on germination rate of mung bean after planted two days in two kinds of soils. Treatments: A. Campus soil; B. Jinshatan soil.(1) A-1, B-1 inoculated with bacterial suspension, (2) A-2, B-2 inoculated with fermentation

liquid, (3) A-3, B-3 inoculated with the culture, (4) A-4, B-4 inoculated with distilled water (control).

Table 3. Effect of HPP16 on mung bean growth.

Treatment	Average dry weight (g/plant)	Average fresh weight (g/plant)	Individual plant height (cm/plant)
A-1	0.0340 ± 0.0002	0.5350 ± 0.0002	19.86 ± 0.4546
A-2	0.0350 ± 0.0001*	0.5501 ± 0.0002*	19.98 ± 2.4367*
A-3	0.0318 ± 0.0001	0.4401 ± 0.0025	18.19 ± 1.6480
A-4	0.0330 ± 0.0002	0.5001 ± 0.0002	19.64 ± 0.8861
B-1	0.0336 ± 0.0014	0.5058 ± 0.0002	19.67 ± 3.9623
B-2	0.0357 ± 0.0002*	0.5900 ± 0.0002*	21.01 ± 1.1843*
B-3	0.0322 ± 0.0001	0.4481 ± 0.0002	19.06 ± 0.5535
B-4	0.0329 ± 0.0001	0.4750 ± 0.0005	19.36 ± 0.9603

Mung bean seeds were cultivated in Campus soil, inoculated with bacterial suspension, fermentation liquid, the culture and distilled water, named A-1, A-2, A-3 and A-4, respectively; mung bean seeds were cultivated in Jinshatan soil, also inoculated with bacterial suspension, fermentation liquid, the culture and distilled water, named B-1, B-2, B-3 and B-4, respectively. Average standard error of the mean was from three separate treatments. Values with * indicate significant difference at $P < 0.05$.

Table 4. Effect of HPP16 on Ca, K, Fe, Mg and Na contents accumulation of mung bean seedlings.

Treatment	K (mg/g)	Na (mg/g)	Mg (mg/g)	Ca (mg/g)	Fe (mg/g)
A-1	8.4210 ± 0.0219	0.3634 ± 0.0019	0.3203 ± 0.0078	0.0505 ± 0.0478	0.4053 ± 0.0449
A-2	11.1520 ± 0.0218*	0.6367 ± 0.0045*	0.9962 ± 0.0053*	0.3789 ± 0.0740	0.8281 ± 0.2133
A-3	8.4650 ± 0.0168	0.5458 ± 0.0023	0.6172 ± 0.0247	0.4673 ± 0.0505	1.1335 ± 0.0738
A-4(CK)	8.3010 ± 0.0273	0.1462 ± 0.0016	0.1172 ± 0.0040	1.6040 ± 0.0695*	1.1805 ± 0.0738*
B-1	11.6670 ± 0.0220	0.1802 ± 0.0034	0.0756 ± 0.0039	0.4800 ± 0.0318	1.3215 ± 0.1325
B-2	15.4500 ± 0.0277*	0.5239 ± 0.0008*	0.5261 ± 0.0040*	1.2630 ± 0.0623	1.4624 ± 0.0448
B-3	12.9610 ± 0.0336	0.2120 ± 0.0035	0.3073 ± 0.0040	1.4900 ± 0.0578	1.5681 ± 0.0294
B-4(CK)	9.6050 ± 0.0319	0.1189 ± 0.0016	0.0209 ± 0.0026	1.6670 ± 0.0624*	1.6504 ± 0.0778*

Mung bean seeds were cultivated in Campus soil, inoculated with bacterial suspension, fermentation liquid, the culture and distilled water, named A-1, A-2, A-3 and A-4, respectively; mung bean seeds were cultivated in Jinshatan soil, also inoculated with bacterial suspension, fermentation liquid, the culture and distilled water, named B-1, B-2, B-3 and B-4, respectively. Average standard error of the mean was from three separate treatments. Values with * indicate significant difference at $P < 0.05$.

Effect of HPP16 on biomass of mung bean seedlings

In Campus soil and Jinshatan soil, the fresh weight, dry weight and plant height of the whole mung bean plants treated with HPP16 increased. The promoting effects of the strain HPP16 on mung bean seedlings emergence in these two kinds of acidic soils were evaluated (Table 3). The results indicate that mung bean plants inoculated with bacterial suspension (A-1, B-1), and fermentation liquid (A-2, B-2) increased the biomass (dry weight, fresh weight and the individual plant height of mung bean seedlings) as compared with those inoculated with the culture and distilled water (A-3 , B-3 and A-4 , B-4).

In Campus soil, the average plant height of A-1, and A-2 treatments were 19.86 and 19.98 cm, respectively, while the untreated plants (inoculated with the culture and distilled water, A-3 and A-4) only had an average length of 18.19 and 19.64 cm. Individual plant height in A-1 and A-2 increased by 6.5 and 7%, respectively compared with A-4, and increased by 9.2 and 9.8%, respectively as compared with A-3. Dry weight increased by 3 and 6.1%, respectively compared with A-4, and increased by 6.9 and 10.1% compared with A-3. Fresh weight increased by 7 and 10%, respectively compared with A-4, and increased by 21.6 and 25% compared with A-3.

In Jinshatan soil, the individual plant height in B-1 and B-2 increased by 1.6 and 8.5% respectively as compared with B-4, and increased by 3.2 and 10.2% compared with A-3. Dry weight in B-1 and B-2 increased by 2 and 8.5%, respectively compared with B-4, and increased by 4.3 and 10.9% compared with A-3. Fresh weight in B-1 and B-2 increased by 7 and 24%, respectively, compared with B-4, and increased by 12.9 and 31.7% compared with A-3. The strain HPP16 showed significant improvement in the growth of mung bean seedlings (Table 4) but individual plant height, dry weight and fresh weight in A-3 decreased by 7.4, 3.6 and 12%, respectively compared with B-4. The individual plant height, dry weight and fresh

weight in B-3 decreased by 1.5, 2 and 5.7%, respectively, compared with B-4.

Table 5. The contents of the endogenous IAA and ABA (µg /g FW).

Plant hormone	A-1	A-2	A-3	A-4(CK)	B-1	B-2	B-3	B-4(CK)
IAA	229.16 ± 0.47	30.63 ± 0.52*	23.21 ± 0.32	26.79 ± 0.11	77.97 ± 2.81	83.27 ± 0.89*	49.40 ± 1.64	73.42 ± 2.47
ABA	0.48 ± 0.01	0.45 ± 0.01	0.55 ± 0.01*	0.495 ± 0.01	0.91 ± 0.02	0.77 ± 0.01	1.17 ± 0.04*	1.01 ± 0.02

Mung bean seeds were cultivated with Campus soil, inoculated with bacterial suspension, fermentation liquid, the culture and distilled water (control), named A-1, A-2, A-3 and A-4, respectively; mung bean seeds were cultivated with Jinshatan soil, also inoculated with bacterial suspension, fermentation liquid, the culture and distilled water (control), named B-1, B-2, B-3 and B-4, respectively. Average standard error of the mean was from three separate treatments. Values with the * indicate significant difference at $P < 0.05$.

Effect of HPP16 on mung bean seedlings to uptake ions

The contents of K^+, Na^+, Mg^{2+}, Ca^{2+} and Fe^{3+} elements of mung bean seedlings in different treatments were recorded four weeks after germination. The results show that HPP16 could affect ions accumulation in mung bean seedlings (Table 4). The order of K^+ concentration of mung bean plants cultivated in Campus soil was A-2> A-3 > A-1> A-4 (Table 5), which was consistent with the orders of Na^+ and Mg^{2+} concentration. This result indicates that HPP16 helped mung bean seedlings to uptake some kinds of ions. K^+ concentration of mung bean plants in A-1, A-2 and A-3 increased by 1.4, 34.3 and 16.4%, respectively as compared to the control [A-4(CK)]. Na^+ concentration increased by 149, 335 and 273%, respectively as compared to the control (A-4(CK)). Mg^{2+} concentration increased by 173, 750 and 427%, respectively. The order of Ca^{2+} concentration of mung bean plants was A-4> A-3 > A-2> A-1 (Table 5), which was consistent with the order of Fe^{3+} concentration. Ca^{2+} concentration of mung bean plants in A-1, A-2 and A-3 decreased by 97, 76 and 71%, respectively as compared to the control (A-4(CK)). Fe^{3+} concentration decreased by 66, 30 and 4%, respectively.

Ions accumulation of mung bean plants in the Jinshatan soil was similar with that in the Campus soil. The order of K^+ concentration of mung bean plants was B-2> B-3 > B-1> B-4 (Table 5), which was consistent with the orders of Na^+ and Mg^{2+} concentration. K^+ concentration of mung bean plants in B-1, B-2 and B-3 were increased by 22, 61 and 35%, respectively as compared to the control (B-4(CK)). Na^+ concentration were increased by 53, 340 and 78%, respectively. Mg^{2+} concentration increased by 262, 2417 and 1370%, respectively. The order of Ca^{2+} concentration the same as Fe^{3+} concentration was B-4> B-3 > B-2> B-1 (Table 5). Ca^{2+} concentration in B-1, B-2 and B-3 were decreased by 71%, 24% and 11% respectively. Fe^{3+} concentration were decreased by 20%, 11% and 5% respectively. From these results we could infer that there was less Ca^{2+} content with more Mg^{2+} content in mung bean plants, which indicated the calcium ions played hostile role in magnesium ions accumulation. The order of Fe^{3+} content of mung bean plants was opposite to the order of phosphorus content of inoculated liquid. These facts proved that phosphorus could interfere with the iron absorption.

Effect of HPP16 on the endogenous IAA and ABA contents of mung bean seedlings

The endogenous IAA and ABA contents of mung bean seedlings in different treatments were recorded after four weeks of germination. The results show that different concentrations of phosphorus could affect endogenous IAA and ABA contents of mung bean seedlings. In Campus soil, the endogenous IAA of the whole mung bean plants treated with HPP16 increased. The promoting effect of the strain to mung bean seedlings emergence in the Campus soil was evaluated (Table 5). The endogenous IAA contents of mung bean seedlings in A-1 and A-2 were increased by 9 and 14%, respectively as compared to the plants inoculated with distilled water [A-4(CK)]. IAA contents A-3 decreased by 13%. The endogenous ABA contents of mung bean seedlings in A-1 and A-2 decreased by 3 and 9%, respectively. ABA content in A-3 was increased by 11% (Table 5). In Jinshatan soil, the endogenous IAA of the whole mung bean plants treated with HPP16 also increased. The endogenous IAA contents of mung bean seedlings in B-1 and B-2 were increased by 6 and 13%, respectively as compared to the plants inoculated with distilled water [B-4(CK). IAA content in B-3 decreased by 33%. More also, the endogenous ABA contents of mung bean seedlings in B-1 and B-2 decreased by 10 and 24%, respectively. ABA content in B-3 increased by 16%.

The results therefore show that the orders of IAA and ABA concentration in Campus soil were the same as the orders in Jinshatan soil, and that IAA concentration in A-1, and B-1 were higher than that of the control. This indicate that HPP16 showed significant promoting effect on the growth of mung bean seedlings in the two kinds of soils (Table 5). The facts that ABA concentration in A-3 and B-3 were the highest indicate that excessive amounts of phosphorus could produce adverse effect on the growth of mung bean plant.

DISCUSSION

The efficiency of PGPR in growth promotion is well documented in different kinds of crops (Ramamoorthy et al., 2001). Our experimental results confirmed earlier studies and indicate a future possibility of use of the strain HPP16 in promoting growth and yield of commercial crops. Our results evaluated that the strain HPP16 isolated from acidic soil in Qingdao show its capacity of promoting the growth of mung bean seedlings in the acidic soil short of soluble phosphate. After treatment with the strain HPP16 in the two kinds of acidic soils, germination rate, and growth parameters (plant height, fresh weight and dry weight) of the mung bean seedlings were promoted more rapidly than the untreated plants (Tables 3 and 4). The two groups (A and B) experimental results were exactly the same with each other but the germination rate, and growth parameters (plant height, fresh weight and dry weight) of the mung bean seedlings in the Jinshatan soil (B group) were higher than those in the Campus soil (A group). The reason may be that the Ct, Nt, P and total salt contents of the Jinshatan soil were obviously far higher than those of the campus soil (Table 1).

The germination rate, and growth parameters (plant height, fresh weight and dry weight) in A-3 and B-3 were obviously lower than those in A-4 and B-4. The reason may be that the culture contained a large number of phosphorus elements. So the growth of mung bean seedlings in A-3 and B-3 containing excess of phosphorus was impeded. The inoculated liquids in A-1, and B-1 contained only HPP16 strains and water; HPP16 strains could change insoluble phosphorus into soluble phosphorus to be absorbed by plants. While the inoculated liquids in A-2, and B-2 contained fermentation liquid with HPP16 strains, there were a large number of phosphorus elements and may be a quantity of plants hormone IAA. So, the growth of mung bean seedlings was greatly promoted in A-2 and B-2. Earlier studies on PGPR reported that rhizobacteria are potential growth enhancers in different kinds of crops such as tomato, sorghum (Baldani et al., 1986; Bashan et al., 1989) and others. For example, *P. putida* has a stimulating effect on canola seedlings growth (Xie et al., 1996). In this study, mung bean seedlings treated with HPP16 showed great growth promotion in Campus soil and Jinshatan soil in illumination incubator.

Further analysis revealed that K^+, Na^+ and Mg^{2+} contents of mung bean plants in A-1(B-1), A-2(B-2) and A-3(B-3) were higher than those of the control. The reason was that there were a large quantity of K^+, Na^+ and Mg^{2+} in A-2(B-2) and A-3(B-3), which came from the culture medium of HPP16. There were 0.3 g NaCl, 0.3 g KCl, and 0.3 g $MgSO_4 \cdot 7H_2O$ in the culture medium. So K^+, Na^+ and Mg^{2+} content of mung bean plants in A-2(B-2) and A-3(B-3) were higher than those of the control. Yang et al. (2009) reported that efficiency of high-affinity K^+ transporter 1 (HKT1) gene isolated by transcriptome analysis in Arabidopsis under salt stress was inhibited which controls Na^+ import. HKT1 has an effect to adjust Na^+ and K^+ levels but K^+, Na^+ and Mg^{2+} contents of mung bean plants in A-1 (B-1) were also higher than those of the control. There was only HPP16 strain in the inoculated liquid of A-1 (B-1) treatment. The result indicates that strain HPP16 supports mung bean seedlings to absorb K^+, Na^+ and Mg^{2+}. On the contrary, Ca^{2+} and Fe^{3+} contents of mung bean plants in A-1 (B-1), A-2(B-2) and A-3(B-3) were lower than those of the control. The result was opposite to K^+, Na^+ and Mg^{2+} contents of mung bean plants in A-1(B-1), A-2(B-2) and A-3(B-3). One reason was that there were a mass of phosphate groups which could react with Ca^{2+} and Fe^{3+} to generate precipitation. Meanwhile, the strain HPP16 proved to be siderophore producer could compete with mung bean seedlings to absorb Fe^{3+}. Another reason may be that Mg^{2+} ions accumulation interfered with Ca^{2+}, that is to say Mg^{2+} ions played antagonism role in Ca^{2+} ions accumulating. Fe^{3+} content of mung bean plants in A-1 (B-1), A-2(B-2) and A-3(B-3) lower than the control suggested that phosphorus could interfere with the Fe^{3+} absorption. In general, HPP16 caused plant to uptake some kinds of mineral ions cultivated in soils not rich in soluble P elements, thus seeming to be very effective in promoting plants growth.

Our results also reveal that HPP16 affected mung bean plants hormone balance by increasing IAA and decreasing abscisic acid ABA contents of mung bean seedlings (Table 5). Generally, PGPR have been reported to influence plant growth by changing the host plant endogenous pool of phytohormones (Baldani et al., 1986; Cowan et al., 1999). IAA is a plant growth hormone, while ABA is a well-known stress-inducible plant hormone and growth inhibitor, thus it is considered as a physiological stress symbol in numerous higher plants (Yao et al., 2010). In this study, the significant increase in IAA concentration of the mung bean plants suggested strain HPP16 seemed to be very effective in protecting mung bean plants from growth inhibition caused by phosphorus deficiency. Those unexpected low level of IAA and high level of ABA found in A-3 and B-3 could be attributed to excess amounts of phosphorus. Mung bean plants produced some ABA hormone under excess phosphorus stress. The fact that IAA concentration in A-1, and B-1 were higher than that of the control indicated that HPP16 strain significantly influenced IAA and ABA biosynthesis in mung bean plants and consequently promoted mung bean growth.

In summary, no previous studies about HPP16 promoting mung bean growth were found. Mung bean seedlings inoculated by HPP16 can increase germination rate, biomass, nutrient uptake and IAA concentration. One possible reason could be the expanded and elongated root system leading to improved uptake of water and nutrients. The enhanced root growth along with its increased branching system and a higher number of

root tips and production of phytohormones may be regarded as the reasons of PGPR promoting growth

(Lixia et al., 2010;) all of which need further analysis. The results show that inoculation of HPP16 also led to significant increase of the mung bean's absorbability of K^+, Na^+, Mg^{2+} and decreased the absorption of Ca^{2+} and Fe^{3+}. Increased production of IAA and inhibited production of ABA may be a good means of improving mung bean growth in the acidic soil. Leguminous plants are highly sensitive to phosphorus deficiency and HPP16 as one kind of phosphorus-solubilizing bacteria will certainly play positive role in the growth of leguminous plants. Our results are therefore of great importance to promote mung bean growth by the strain HPP16, although the mechanisms through which the bacterium improve plant growth requires further study.

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