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ARTICLE

Isolation and characterization of rhizomicrobial isolates for phosphate solubilization and indole acetic acid production

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Abstract Recently, public concerns regarding the use of agrochemicals have increased due to the environmental impacts and potential risks to human health. The application of beneficial microorganisms is a novel technology to improve plant health and productivity and has therefore been extensively studied as an alternative strategy for biocontrol. In our study, 122 microbial isolates were obtained from the rhizosphere of *Panax ginseng* and subsequently tested in vitro for phosphate solubilization and indole acetic acid (IAA) production. Pikovskaya's medium was used to estimate rhizomicrobial isolates to solubilize tricalcium phosphate [Ca_3 $(PO_4)_2$]. Among all the investigated strains, 82 % of rhizospheric fungi showed phosphate solubilization activity; however, only 57.1 % of the rhizobacteria isolates showed phosphate solubilization ability. For IAA production, 64.7 % of the tested rhizofungi isolates were able to produce the phytohormone; however, only 47.62 % of the rhizobacteria isolates exhibited IAA production. Among all investigated species, *Pseudomonas fluorescence* and *Azotobacter chroococcum* showed the highest phosphate solubility demonstrating 885.4 and 863.4 μg mL^{-1}, respectively. *Mucor* sp. produced 42.3 μg mL^{-1} of IAA in Czapek's tryptophan medium, and the highest fungal species to solubilize the inorganic phosphate (237.5 μg mL^{-1}) was estimated by *Penicillium* sp. Rhizobacteria were more effective than rhizofungi in phosphate solubilization and IAA production. This study introduces some potent species in terms of phosphate solubilization and IAA production which could be likely to improve soils' quality and promote plant growth.

Keywords Indole acetic acid · Phosphate solubilization · Rhizobacteria · Rhizofungi

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Introduction

Root-associated bacteria and fungi have been known to benefit plants and are therefore referred to as plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi (PGPF), respectively (Kloepper et al. 1990; Reyes et al. 2002). The PGPR are organisms that possess some direct mechanisms to enhance plant growth (Kloepper et al. 1989). Biogeochemical cycles are important features of soil bacteria that provide plants with sources of nutrition and therefore improve crop production. Indigenous microflora is superior to improve crop performance due to traits pre-adapted to the local environment relative to introduced strains. A number of various bacteria enhance plant growth, including *Azotobacter* sp., *Pseudomonas* sp., *Bacillus* sp., and *Acetobacter* sp. (Turan et al. 2006). Microorganisms with phosphate-solubilizing capacity increase available phosphate and improve plant growth (Ponmurugan and Gopi 2006). Goldstein (1994) reported that soluble phosphate concentration in soil is often very limited approximately 1 μg mL^{-1} or less. The capacity of microbes to release metabolites, including organic acids, determines phosphorus-solubilizing activity, which converts phosphorus to soluble forms (Sagoe et al. 1998). Large numbers of saprophytic bacteria and fungi solubilize

phosphorus in scarcely soluble soil phosphates, mainly by chelation-mediated mechanisms (Whitelaw 2000). Phosphate-solubilizing bacteria enhanced *Cicer arietinum* seedling growth (Sharma et al. 2007). Moreover, co-inoculation of phosphate-solubilizing microorganisms and plant growth-promoting rhizobacteria reduced the application of phosphate by 50 % without affecting corn yield (Yazdani et al. 2009). Phosphate solubilization takes place via different microbial actions, including organic acid production and proton extrusion (Surange et al. 1995; Dutton and Evans 1996). Microorganisms increase the phosphate available to plants by mineralizing organic phosphates in soil and by solubilizing precipitated phosphates (Pradhan and Sukla 2005; Chen et al. 2006). Various bacteria and fungi are known to solubilize phosphates and increase plant yield. Phosphate-solubilizing microorganisms in soil solubilize insoluble phosphates, mainly by secreting organic acids, which chelate with calcium ions lowering the pH (Arti and Patel 2003). Strains from the bacterial genera *Pseudomonas* and *Bacillus*, as well as the fungal genera *Penicillium* and *Aspergillus*, are the most effective phosphate solubilizers (Whitelaw 2000). Chen et al. (2006) reported that *Arthrobacter ureafaciens*, *Phyllobacterium myrsinacearum*, *Rhodococcus erythropolis*, and *Delftia* sp. have the capacity to solubilize considerable amounts of tricalcium phosphate in the medium by organic acid secretion. Rhizomicroorganisms solubilize phosphorus and produce phytohormones, which are essential for enhancing crop productivity. Biosynthesis and phytohormone release of indole acetic acid (IAA) is an additional important PGPR mechanism, which enhances plant growth (Seshadri et al. 2002). It was hypothesized that plant growth was regulated by 'a substance, which transmits its effects from one part of the plant to another' (Kevin 2003). Later, this 'substance' was termed auxin ('auxein' in Greek means 'to grow'), and auxins were identified as IAA (Kevin 2003; Spaepen et al. 2007). One of the most important traits identified in plant growth-promoting microorganisms (PGPM) was the capacity to produce phytohormones (Husen 2003). Therefore, many studies examined the production of the phytohormone IAA in phosphate-solubilizing fungi. Either symbiotic or non-symbiotic PGPR, which act on the production of plant hormones, including auxins and gibberellins, promote plant growth directly. Hayat et al. (2010) reported that several bacterial genera produced indole-3-ethanol or indole-3-acetic acid (IAA), compounds belonging to auxins, and the results indicated that not only plants but also microorganisms, including bacteria and fungi, synthesize IAA (Kevin 2003). Plant growth can be directly enhanced via phytohormone synthesis (Xie et al. 1996), as well as the solubilization of inorganic phosphate and mineralization of organic phosphate, which makes phosphorous available to plants (Khan et al. 2014). Previous studies (e.g., Hontzeas et al. 2004) demonstrated that PGPF performed one or several mechanisms to promote plant growth, including phytohormone production, mineral solubilization, and antagonism, toward phytopathogens. The aim of the present work was to evaluate phytohormone biosynthesis and phosphate solubilization in indigenous bacteria and fungi isolated from the rhizosphere of *Panax ginseng* (Korean ginseng) and to select promising strains for future experiments to determine strain efficacy as plant growth-promoting rhizomicrobes.

Materials and methods

Bacterial and fungal isolates

A total of 105 bacterial isolates and 17 fungal isolates were taken from the rhizospheric soil supporting *P. ginseng*. All strains were purified and screened to examine plant growth promotion PGPR traits of inorganic phosphate solubilization and IAA productivity.

Isolation of rhizobacteria

Rhizosphere soils were collected from *P. ginseng* (Korean ginseng) fields. Plants were uprooted, placed in zipper bags, and transported to the laboratory. Non-rhizosphere soil from ginseng plants was removed by gentle root shaking, and the soil tightly adhering to the roots (rhizosphere soil) was separated. Dilution plate method was applied for isolation under aseptic conditions, and trypticase soy agar was used to culture bacteria (Atlas 2004). Additional streaking on fresh plates was performed to selected colonies. Fungi were isolated on Czapek's solution agar containing 30 g saccharose, 2 g sodium nitrate, 1 g dipotassium phosphate, 0.5 g magnesium sulfate, 0.5 g potassium chloride, 0.01 g ferrous sulfate, and 15 g agar. Additional isolate purification and multiplication was conducted by streaking on 2 % malt extract agar plates grown at 27 °C and stored at 5 °C prior to further screening.

Rhizosphere isolates identification

Fungal strains were identified by direct microscopic examination, and culture features were identified according to Domsch et al. (1980) and Moubasher (1993). Bacterial colonies with different characters were tested biochemically and identified using the Bergey's manual of systematic Bacteriology (Holt 1986). Moreover, in order to confirm the identification, the 16S rDNA of some strains was amplified with the 16F27 (forward) (5′-AGAGTTTGATCCTGGCTCAG-3′) and 16R1542 (reverse) (5′-AAGGAGGTGATCCAGCCGCA-3′) universal primers according to Gürtler and

Stanisich (1996). The partial 16S rRNA gene sequence was compared with the full sequence available in the GenBank database using a BLAST search to identify the isolated bacteria.

Phosphate-solubilizing activity

Tricalcium phosphate [$Ca_3(PO_4)_2$] was considered a model compound for measuring the potential or relative rates of microbial solubilization. Precipitated [$Ca_3(PO_4)_2$] solubilization on Pikovskaya's agar medium (g L^{-1}; yeast extract 0.5, dextrose 10, calcium phosphate 5, ammonium sulfate 0.5, potassium chloride 0.2, magnesium sulfate 0.1, manganese sulfate 0.0001, ferrous sulfate 0.0001, agar 15) was used for isolation of phosphate-solubilizing microorganisms (Pikovskaya 1948). Rhizosphere microorganisms on precipitated calcium phosphate agar produced clear zones around colonies if colonies were capable of solubilizing calcium phosphate. Phosphate solubilization was estimated quantitatively by an inoculating loop full of spore suspension isolates in 10 mL of Pikovskaya's broth at an initial pH adjusted to 7.0; bacteria were incubated at 30 °C for 3 days and fungi at 27 °C for 7 days. Ion Chromatography was used to analyze the soluble inorganic phosphorus concentration.

IAA production by rhizobacteria

Luria–Bertani (LB) agar medium supplemented with 0.5 mM tryptophan, sodium dodecyl sulfate, and 1 % glycerol was prepared and plated. The bottom of each plate was divided into 16 squares with a marker. Rhizosphere isolates were grown in LB broth overnight. Using sterile tooth picks, isolates were spotted separately in the divided squares. The spotted plates were overlaid immediately with sterile Whatman No. 1 filter paper disks of the inner diameter size. The cultures covered with filter paper were incubated at 30 °C for 2 days. Following appropriate growth of 2 mm of colony diameter, the filter papers were removed from the plates and treated with Salkowski's reagent (2 % 0.5 M $FeCl_3$ solution in 35 % perchloric acid) in a Petri dish. The filter paper disks were examined for the appearance pink color, which indicated IAA-producing isolates. The colorimetric method was conducted for quantitative analyses of IAA production by rhizosphere microbes. Minimal salt (MS) medium was used with L-tryptophan (Frankenberger and Poth 1988) for IAA-producing microbes. The ability of inoculant to synthesize IAA in broth of MS medium–tryptophan was measured colorimetrically according to Gordon and Weber (1951). Three-day-old bacterial LB broth (100 µL) was transferred to 10 mL MS broth containing 2 mg mL^{-1} of L-tryptophan. The cultures were incubated at 30 °C with shaking at 150 rpm. After 2 days, the cultures were centrifuged for 10 min at 13,000×*g*. One milliliter of the supernatant was mixed with 2 mL of Salkowski's reagent (0.01 M $FeCl_3$ in 35 % $HClO_4$). The test tube contents were left undisturbed for 30 min for color development. Salkowski's reagent shows variable color responses based on the IAA amount biosynthesized by rhizosphere microbes. Color also was developed in standard IAA solutions; therefore, a standard curve was applied to calculate IAA production in rhizosphere microbes. Pink color appearance indicated IAA production and absorbance was measured at 530 nm (Fig. 1).

IAA production by rhizofungi

Seventeen different fungal isolates were quantitatively tested to produce indole-3-acetic acid IAA following Brick et al. (1991). Fungi were grown in synthetic Czapek-Dox broth amended with tryptophan (1000 µg mL^{-1}) instead of $NaNO_3$. Fungal isolates were inoculated with a loop full of actively growing mycelium spore suspension in Sabouraud media into 10 mL of Czapek-Dox broth media, incubated at 30 °C, and agitated at 150 rpm. Following 7 days, each culture was centrifuged at 13,000×*g* for 10 min. Salkowski's reagent (0.01 M $FeCl_3$ in 35 % $HClO_4$) was used to identify indole derivatives. One milliliter of each fungal culture (10 mL) was mixed with an equal volume of Salkowski's reagent and incubated in the dark for 30 min. The optical density was measured using a spectrophotometer at 530 nm. A standard curve of prepared IAA concentration was designed to calculate the equivalent IAA concentration produced by each fungal strain in the bioassay media.

Results

To determine efficient PGPR, strains with multiple plant growth promotion activities were determined by isolating a total of 105 bacterial strains and 17 fungal strains from the rhizospheric soil supporting *P. ginseng* in the vicinity of Kangwon National University, Chuncheon City, South Korea (37°87472N, 127°73417E). These isolates were biochemically characterized and screened in vitro for plant growth-promoting features to elicit IAA and calcium phosphate solubilization.

Phosphate-solubilizing activity

Azotobacter chroococcum, *P. putida*, and *E. coli* isolates collected from *P. ginseng* rhizosphere showed 100 % capability to solubilize calcium phosphate (Table 1). This result was followed by *P. fluorescens* which revealed 77 % capability for inorganic phosphate solubilization.

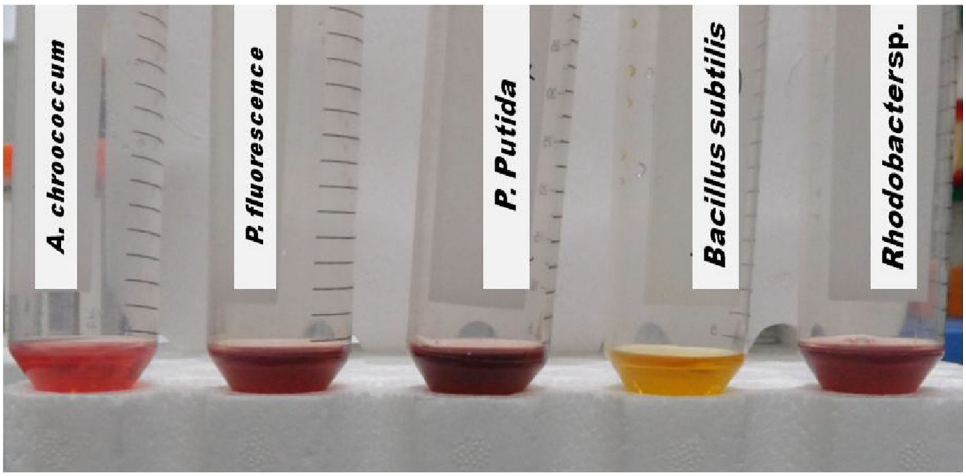

Fig. 1 Variable color responses depending on the IAA amount using Salkowski's reagent biosynthesized by rhizosphere microbes prior to the colorimetric quantification according to Gordon and Weber (1951)

Table 1 Screening of phosphate-solubilizing and IAA-producing bacteria isolated from rhizosphere of *P. ginseng*

Species	Phosphate solubilization		IAA production	
	No. of isolates (frequency)	No. of phosphate-solubilizing strains (percentage)	No. of isolates (frequency)	No. of IAA-producing strains (percentage)
Arthrobacter sp.	1	0 (0)	1	0 (0)
Azotobacter chroococcum	2	2 (100)	2	2 (100)
Bacillus sp.	6	3 (50)	6	1 (16)
Brevibacterium sp.	1	0 (0)	1	0 (0)
Burkholderia sp.	5	0 (0)	5	2 (40)
E. coli	4	4 (100)	4	1 (25)
Lactobacillus sp.	10	7 (70)	10	5 (50)
Listeria sp.	1	0 (0)	1	0 (0)
Pseudomonas aeruginosa	19	8 (42)	19	13 (68)
P. fluorescens	18	14 (77)	18	12 (66)
P. plecoglossicida	2	1 (50)	2	0 (0)
P. putida	6	6 (100)	6	4 (66)
Rhodobacter sp.	9	6 (66)	9	4 (44)
Rhodopseudomonas sp.	2	0 (0)	2	1 (50)
Staphylococcus sp.	10	6 (60)	10	2 (20)
Streptomyces sp.	9	3 (33)	9	3 (33)
Total	105 (100 %)	60 (57.1)	105 (100 %)	50 (47.62)

Lactobacillus sp. and *Rhodobacter* sp. rhizosphere isolates showed 70 and 66 % capacity to solubilize phosphate on Pikovskaya's agar, respectively. *Staphylococcus* sp., *Bacillus* sp., and *P. aeruginosa* isolates solubilized inorganic calcium phosphate at 60, 50, and 42 %, respectively. Rhizobacteria action on precipitated calcium phosphate agar determined positive phosphate solubilization strains (Fig. 2). Generally, the percentage of rhizobacteria isolates exhibiting phosphate solubilization activity was 57.1 % (Table 1). However, results indicated an overall 82 % phosphate solubilization activity in rhizofungi isolates (Table 2). *Aspergillus* sp., *Penicillium* sp., and *Trichoderma* sp. were common species isolated from *P. ginseng* rhizosphere plants. *Mucor* sp., *Saccharomyces* sp., and *Trichoderma* sp. isolates all showed 100 % inorganic phosphate solubilization capacity. *Aspergillus niger* and *Penicillium* sp. isolates exhibited 75 and 60 % calcium phosphate solubilization capacity, respectively (Table 2).

Fig. 2 Formation of clear zones by rhizobacteria on precipitated calcium phosphate agar

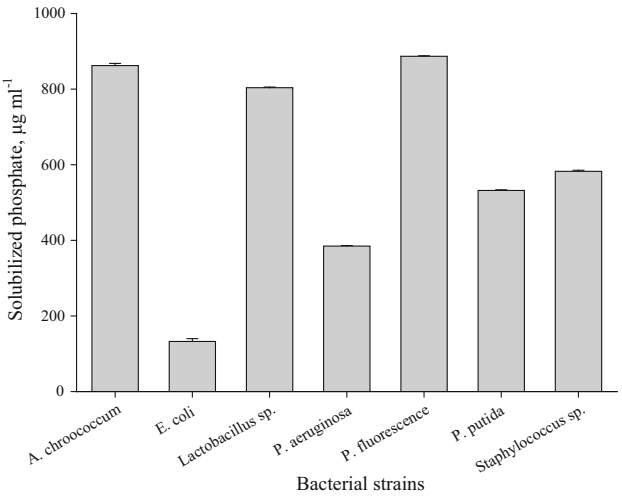

Fig. 3 Phosphate solubilization efficacy by different rhizobacterial isolates

Azotobacter chroococcum and *Lactobacillus* sp. showed high qualitative and quantitative capacity to solubilize inorganic calcium phosphate. The highest phosphate solubilization efficacy was demonstrated in *P. fluorescence* and *A. chroococcum* (Fig. 3). *Penicillium* sp. showed the maximum phosphate solubilization levels of 316.1 µg mL^{-1} followed by *Mucor* sp. (237.3 µg mL^{-1}) on Pikovskaya's agar (Fig. 4).

IAA production activity

All isolates of *A. chroococcum* produced the phytohormone IAA (100 %), followed by *P. aeruginosa* in which 68 % of its isolates were able to produce IAA. *P. fluorescens* and *P. putida* isolates exhibited 66 % IAA production. However, both *Lactobacillus* sp. and *Rhodopseudomonas* sp. isolates produced only 50 % IAA on LB agar tryptophan medium. Results indicated that the isolates *Arthrobacter* sp., *Listeria* sp., and *P. plecoglossicida* were not able to synthesize the

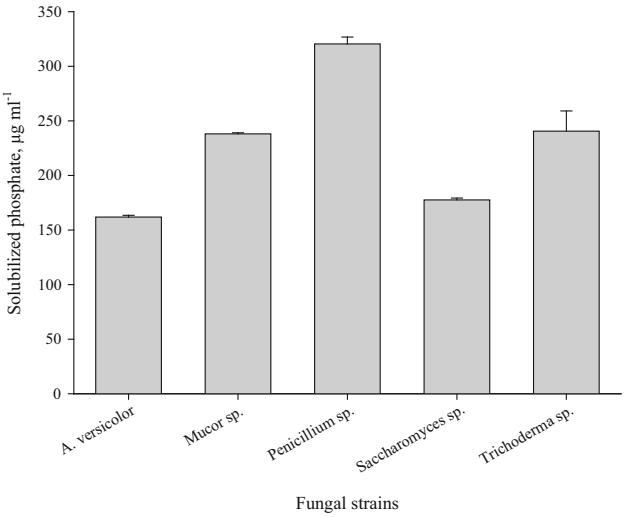

Fig. 4 Different capacities of tricalcium phosphate solubilization by several rhizofungal isolates

Table 2 Screening of phosphate-solubilizing and IAA-producing fungi isolated from rhizosphere of *P. ginseng*

Species	Phosphate solubilization		IAA production	
	No. of isolates (frequency)	No. of phosphate-solubilizing strains (percentage)	No. of isolates (frequency)	No. of IAA-producing strains (percentage)
Aspergillus sp.	4	3 (75)	4	4 (100 %)
Mucor sp.	1	1 (100)	1	1 (100 %)
Penicillium sp.	5	3 (60)	5	2 (40)
Saccharomyces sp.	5	5 (100)	5	3 (60)
Trichoderma sp.	2	2 (100)	2	1 (50)
Total	17 (100 %)	14 (82)	17 (100 %)	11 (64.7)

phytohormone IAA. Overall, 50 out of 105 rhizobacterial strains showed IAA productivity in vitro with a percentage value of 47.62 (Table 1). *Aspergillus* sp. and *Mucor* sp. rhizosphere exhibited 100 % IAA production in Czapek-Dox broth fortified by tryptophan (1000 μg mL^{-1}). *Saccharomyces* sp. and *Trichoderma* sp. strains isolated from *P. ginseng* rhizosphere showed the capacity to produce IAA of 60 and 50 %, respectively. However, *Penicillium* sp. isolates showed only a 40 % capacity to produce IAA under the same conditions. Overall, 11 of 17 rhizosphere fungal isolates (64.7 %) produced IAA in Czapek-Dox tryptophan (Table 2). The highest IAA productivity was detected in *A. chroococcum*, *Burkholderia* sp., *P. putida*, *Rhodobacter* sp., and *Streptomyces* sp. at a level of 47 μg mL^{-1}. These results were followed by *E. coli*, *Lactobacillus* sp., *P. aeruginosa*, *P. fluorescens*, and *Staphylococcus* sp., which produced IAA of 29.7, 23.5, 31.0, 37.2, and 34.1 μg mL^{-1} in LB tryptophan medium broth, respectively (Table 3). The lowest IAA production value was found in *B. subtilis*, which produced only 0.2 μg mL^{-1}. *A. niger* and *Saccharomyces* sp. showed the highest IAA productivity at the levels of 47 μg mL^{-1} in Czapek-Dox medium fortified with tryptophan, followed by *Mucor* sp., which produced 42.3 μg mL^{-1} of IAA in the same medium. Low IAA productivity of 0.2 and 1.9 μg mL^{-1} was detected in *A. terreus* and *Trichoderma* sp., respectively. IAA production in *Penicillium* sp. ranged from 2 to 4.5 μg mL^{-1} (Table 4). Three *P. fluorescens*, two *Rhodobacter* sp., two *Burkholderia* sp., two *P. putida*, and one *A. chroococcum* isolates showed high levels of IAA (47 μg mL^{-1}) production in 2 mg mL^{-1} of tryptophan. *P. fluorescens* showed strong phosphate solubilization and IAA production of 885.4 and 47.02 μg mL^{-1}, respectively, followed by *A. chroococcum* with phosphate solubilization and IAA production values of 863.42 and 46.68 μg mL^{-1}, respectively.

Table 3 UV spectrometry detection of IAA production by potent bacterial strains using Salkowski's reagent

Bacterial species	IAA production (μg mL^{-1})
Azotobacter chroococcum	46.68a
Azotobacter chroococcum	32.29b
Bacillus subtilis	0.175f
Bacillus sp.	0.33ef
Burkholderia sp.	47.3a
Burkholderia sp.	47.67a
E. coli	29.76bc
Lactobacillus sp.	16.12de
Lactobacillus sp.	17.59d
Lactobacillus sp.	11.42e
Lactobacillus sp.	23.48c
P. aeruginosa	31.03b
P. aeruginosa	47.14a
P. fluorescens	46.96a
P. fluorescens	37.2ab
P. fluorescens	35.91b
P. fluorescens	47.02a
P. fluorescens	46.57a
P. putida	47.54a
P. putida	47.65a
Rhodobacter sp.	47.63a
Rhodobacter sp.	47.14a
Staphylococcus sp.	34.14ab
Streptomyces sp.	47.65a

The mean difference is significant at the $P < 0.5$ level

Discussion

Plant health and soil fertility are highly dependent on plant–bacterial interactions. Rhizobacteria are capable of increasing plant growth by colonizing plant roots. PGPR are also termed plant health-promoting rhizobacteria and also interact with plants as nodule-promoting rhizobacteria. Plant growth stimulation by rhizobacteria has been demonstrated in both laboratory and field trials. Strains of *P. putida* and *P. fluorescens* have enhanced root and shoot length in canola, lettuce, and tomato (Hall et al. 1996; Glick et al. 1997). Phosphorus in insoluble compounds is unavailable to plants, and many rhizobacteria and rhizofungi are capable of solubilizing insoluble phosphates, mostly by secreting chelating organic acids (Richardson 2001; Vessey et al. 2004). It was found that not only plants but also microorganisms including bacteria and fungi are able to synthesize IAA (Kevin 2003; Hontzeas et al. 2004). Actually, IAA introduced by rhizobacteria affects plant metabolic processes because the endogenous pool of plant

Table 4 UV spectrometry detection of IAA production by potent fungal strains using Salkowski's reagent

Fungal species	IAA production (μg mL^{-1})
Aspergillus niger	47.28a
A. terreus	0.19d
A. versicolor	2.49cd
Mucor sp.	42.31a
Penicillium sp.	4.56bc
Penicillium sp.	2.54b
Penicillium sp.	3.69bcd
Saccharomyces sp.	47.63a
Trichoderma sp.	1.87cd

The mean difference is significant at the $P < 0.5$ level

IAA may be modulated by the interference of IAA that has been secreted by soil bacteria (Glick 2012). Consequently, IAA acts as a mutual signaling molecule interfering gene expression in many rhizobacteria. Therefore, IAA plays a very important role in microbe–plant interactions (Spaepen et al. 2007).

In the present study, more than one hundred rhizobacteria isolates and 17 fungal rhizosphere strains were isolated from *P. ginseng* plants, a wildly cultivated and economically important species in South Korea, where the climate and soil produce the world's finest perennial herb in the *Araliaceae*. The isolated microbial strains showed high variability to biosolubilize the phytohormone IAA and inorganic calcium phosphate, among either fungal or bacterial isolates in defined culture media. The primary objective of this study was to evaluate phytohormone biosynthesis and phosphate solubilization by rhizospherical bacterial and fungal species isolated from the Korean economic crop *P. ginseng*. Therefore, the strains/species that exhibited the highest activities were selected for quantitative evaluation. In the present investigation, rhizobacterial isolates demonstrated higher IAA productivity and inorganic calcium phosphate solubilization relative to rhizofungal isolates. Alam et al. (2002) demonstrated that bacteria were more efficient in phosphorus solubilization than fungi, consistent with our results. According to our results, the *A. chroococcum* strain we examined showed distinctive activity in terms of phosphate solubilization and IAA production. *A. chroococcum* isolates also showed 100 % IAA production capacity, followed by *P. aeruginosa*, where results showed that 68 % of the isolates produced IAA. *P. fluorescens* and *P. putida* isolates showed 66 % IAA production. IAA is one of the most common and well-characterized phytohormones. Patten and Glick (1996) estimated that 80 % of all rhizosphere bacteria produce the phytohormone. However, our results showed that only 50 out of 105 rhizosphere bacterial isolates exhibited IAA production with an overall 47.62 % (Table 3). PGPR mechanisms that promote plant growth are not yet fully understood; however, many different traits of these bacteria are well known for growth promotion activities (Cattelan et al. 1999). Among these mechanisms are plant hormones, including IAA, gibberellic acid, production of siderophores, dissolved phosphates, and other nutrients. Azotobacter fixes dinitrogen and therefore promotes plant growth. The use of phosphate-solubilizing bacteria was reported to increase plant growth, but only in some cases. Therefore, other mechanisms might be involved in growth response (De Freitas et al. 1997). The rhizosphere population and phosphate-solubilizing bacteria depend on the soil physical–chemical properties, (e.g., organic matter and phosphate content) as well as agricultural activities which occur on a farm (Kim et al. 1998). Our results indicated that *Arthrobacter* sp., *Listeria* sp., and *P. plecoglossicida* isolates did not show IAA production. The overall rhizobacteria isolates showed IAA productivity in 50 out of 105 rhizosphere bacterial isolates in 47.62 % (Table 3). In addition, Rodríguez et al. (1996) reported that a strain of *B. cepacia* did not exhibit IAA production, but displayed a significant mineral phosphate solubilization, and moderate phosphatase activity improved tomato growth. Our results showed phosphate solubilization activity in *Brevibacterium* sp. and *Burkholderia* sp. *Bacillus* sp., *Staphylococcus* sp., and *Streptomyces* sp. strains exhibited moderate activity in phosphate solubilization and IAA productivity. Phosphate-solubilizing bacteria have the potential to increase available plant phosphate levels, especially in soils with large amounts of precipitated phosphate (Goldstein 1986). These bacteria solubilize phosphate by secreting organic acids; however, it is not the only way in which phosphate is solubilized (De Freitas et al. 1997; Kim et al. 1998). Phosphate is an important plant nutrient, which is involved in cell division, photosynthesis, energy generation, and nutrient uptake. Phosphate-solubilizing microbes enable phosphate to be available for plant absorption after solubilization. Several soil bacteria, particularly those belonging to the genera Pseudomonas and Bacillus, and fungi belonging to the genera Penicillium and Aspergillus possess the capacity to convert insoluble soil phosphates into available forms by secreting acids, including formic, acetic, propionic, lactic, and glycolic. These acids lower the pH and elicit the dissolution of bound forms of phosphate (Tomar et al. 1998). According to our data, *Penicillium* sp. showed the highest phosphate solubilization level followed by *Mucor* sp. on Pikovskaya's agar (Fig. 4). Among soil bacterial communities, ectorhizospheric strains from *Pseudomonads* and *Bacilli* have been described as effective phosphate solubilizers (Igual et al. 2001). The present work demonstrated that 77 % of *P. fluorescens* were able to solubilize inorganic phosphate. *Lactobacillus* sp. and *Rhodobacter* sp. rhizosphere isolates showed 70 and 66 % capacity, respectively, to solubilize phosphate on Pikovskaya's agar. *Staphylococcus* sp., *Bacillus* sp., and *P. aeruginosa* isolates solubilized inorganic calcium phosphate at 60, 50, and 42 %, respectively. Strains from the genera *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Agrobacterium*, *Micrococcus*, and *Erwinia* had the capacity to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate and rock phosphate (Rodríguez et al. 1996; Rodriguez and Fraga 1999).

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