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Isolation and characterization of phosphate solubilizing bacteria and their co-inoculation efficiency on tomato plant growth and phosphorous uptake

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Plant growth promoting traits which include indoleacetic acid (IAA), ammonia, siderophore and hydrogen cyanide (HCN) production were assessed in two phosphate solubilizing bacterial (PSB) isolates (*Pantoea agglomerans* and *Burkholderia anthina*) and their effect on growth and phosphorous uptake of tomato plants was investigated with a pot experiment conducted under green house conditions. The pots were arranged in a completely randomized block design with three replications per treatment. The experimental plan was based on eight treatments that is: (1) Soil without tri calcium phosphate (TCP) and bacteria inoculation (control), (2) soil + TCP, (3) Soil + *P. agglomerans*, (4) soil + *P. agglomerans* + TCP, (5) soil + *B. anthina*, (6) soil + *B. anthina* + TCP, (7) soil + *P. agglomerans* + *B. anthina*, and (8) soil + *P. agglomerans* + *B. anthina* +TCP. Both strains showed positive responses for all the tested plant growth promoting traits. IAA production was 10 and 7.5 µg/ml respectively for *P. agglomerans* and *B. anthina*. Both strains produced >80% siderophore and they were considered as efficient siderophore producers. Under green house conditions, both strains remarkably enhanced plant height, root length, shoot and root dry weight, phosphorous uptake and available phosphorous content of soil compared to the control. The increases were more pronounced in co-inoculation of PSB strains with TCP. Based on the results, it could be concluded that the strains possess great potential to be developed as biofertilizers to enhance soil fertility and plant growth. However, their performance under field conditions should be assessed before being recommended for commercial applications.

Key words: Phosphate solubilizing bacteria, phosphate solubilization, plant growth promoting traits.

INTRODUCTION

Phosphorus (P) is the second most important macro-nutrient required by plants, next to nitrogen. Compared to other essential macronutrients (with exception of nitrogen), P is one of the less-abundant (0.1% of total) elements in the lithosphere (Jones and Oburger, 2011), thus often regarded as a limiting nutrient in agricultural soils. Therefore, it becomes quite common to use chemical fertilizers in ensuring phosphorous requirement fertilizer to soil; a substantial amount of phosphorus is of

plants. Upon application as inorganic phosphorus rapidly transformed into less available forms by forming a complex with Al or Fe in acid soils or with Ca in calcareous soils (Toro, 2007) thus becomes unavailable to plants. Frequent application of chemical fertilizers, on the other hand, is recognized to be a costly affair and environmentally undesirable too.

Microorganisms which are capable of solubilizing insoluble phosphate, also called phosphate solubilizing microorganisms (PSMs) not only provide plants with phosphorus, but also facilitate the growth of plants through (a) fixing atmospheric nitrogen (Dobbelaere et al., 2002; Sahin et al., 2004); (b) accelerating the accessibility of other trace elements (Mittal et al., 2008);

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(c) producing plant hormones such as auxins (Jeon et al., 2003; Egamberdiyeva, 2005), cytokinins (Gracia de Salamone et al., 2001), and gibberellins (Gutierrez-Manero et al., 2001); (d) releasing siderophores (Wani et al., 2007), hydrogen cyanide (Kang et al., 2010), enzymes and/or fungicidal compounds such as chitinase, cellulose, protease (Dey et al., 2004; Lucy et al., 2004; Hamdali et al., 2008) which ensure antagonism against phytopathogenic microorganisms. Therefore, it is worth to believe that production of plant growth promoting substances by PSMs may effectively contribute to their effect on the enhancement of the plant performance (Hameeda et al., 2006a).

Due to phosphorous solubilizing ability from insoluble inorganic pools of total soil phosphorous, PSMs have been widely used as inoculants to increase phosphorous uptake and crop yield (Khalid et al., 2004; Hameeda et al., 2006b; Chen et al., 2008). Plant growth promotion and increased phosphorous availability due to inoculation of PSMs have been assessed in several studies under green house as well as field conditions (Reyes et al., 2002; Zaidi et al., 2003).

In the present study, thirty one phosphate solubilizing bacterial strains were isolated and out of them, two efficient PSB strains (*Pantoea agglomerans* and *Burkholderia anthina*) were employed in assessing plant growth promoting traits that is indoleacetic acid (IAA), ammonia, siderophore and hydrogen cyanide (HCN) production and their effect on growth and phosphorous uptake of tomato seedlings grown under green house conditions.

MATERIALS AND METHODS

Isolation of bacterial strains

Soils used in isolating bacterial strains were collected from Chungchugnam-do province, Gongju-Gun area in South Korea. Field moist soil was mixed with sterile 0.85% NaCl solution and shaken for 30 min. Serial dilutions were inoculated using NBRIP (National Botanical Research Institute Phosphorus) agar plates containing 10 g glucose, 5 g Ca₃(PO₄)₂, 5 g MgCl₂·6H₂O, 0.25 g MgSO₄·7H₂O, 0.2 g KCl, 0.1 g (NH₄)₂SO₄ in 1 L distilled water (Nautiyal, 1999). The plates were incubated for 5 days at 30°C. The colonies with clear halos were considered to be phosphate solubilizing colonies. Predominant colonies were further purified by re-streaking on the fresh NBRIP agar plates at 30°C.

Strain identification

The partial sequencing of 16S rRNA for the bacterial strains was done with the help of DNA sequencing service, SOLGENT, Daejeon, South Korea using universal primers, 27F (5'-AGAGTTTGATCCTGGCTCAG -3') and 1492R (5'-GGTTACCTTGTTACGACTT -3') and PCR was performed with initial denaturation at 95°C for 2 min followed by 30 cycles with denaturation for 30 s at 94°C, annealing for 30 s at 58°C and extension for 45 s at 72°C. Final extension was held for 5 min at

72°C. The online program BLAST was used in identifying the related sequences with known taxonomic information available at the databank of NCBI (<http://www.ncbi.nlm.nih.gov/BLAST>). A phylogenetic tree was constructed using CLUSTAL X program (Thompson et al., 1997), which involved sequence alignment by neighbor joining method (Saitou and Nei, 1987) and maximum parsimony using the MEGA4 program (Kumar et al., 2001). Grouping of sequences was based on confidence values obtained by bootstrap analysis of 1,000 replicates. Gaps were edited in the BioEdit program and evolutionary distances were calculated using Kimura two parameter model. Reference sequences were retrieved from GenBank under the accession numbers indicated in the trees.

Assay of inorganic phosphate solubilizing ability

Bacterial strains were grown in sterilized liquid NBRIP medium (20 ml) at 30°C for two days with continuous shaking at 150 rpm. Bacterial suspension (1 × 10⁸ CFU ml⁻¹) was then transferred into a 500 ml flask (n=3 per strain) containing sterilized liquid NBRIP medium (200 ml) and incubated for 7 days with continuous shaking at 30°C. Sterilized uninoculated medium served as a control. Aliquot (10 ml) of each culture and control was taken two, five and seven days after inoculation and centrifuged at 8000 rpm for 15 min. The clear supernatant was used in determining the amount of phosphorous released into the medium. The pH of the culture medium was also recorded with a glass electrode equipped pH meter (CORNING 440). Phosphorus availability was determined using phospho-molybdate blue color method (Murphy and Riley, 1962).

Assay of other plant growth promoting traits

Production of indole acetic acid

IAA production was determined following the method described by Gutierrez et al. (2009). Bacterial strains grown in sterilized 100 ml liquid NBRIP medium containing 1 ml of 0.2% tryptopan were incubated for 72 h with continuous shaking at 30°C. A sterilized uninoculated medium was served as the control. Treated sample and control were taken into centrifugation tube for every 24 h and centrifuged 10 min at 12000 rpm. The clear supernatant of 1 ml was mixed with 4 ml of the Salkowski's reagent (50 ml of 35% perchloric acid and 1 ml of 0.05 M FeCl₃ solution). The mixture was incubated in the dark at 37°C for 30 min. Development of pink color indicates the IAA production and optical density was measured at 530 nm using UV spectrophotometer (Shimadzu UV-VIS).

Production of siderophore

Siderophore production was assayed qualitatively using chrome azurol S (CAS) blue agar as described by Schwyn and Neilands (1987). The bacterial strains were inoculated on the CAS agar plates and incubated at 3°C for 24 h. Orange halos around the colonies were recorded as the measurement of siderophore production. Quantitative estimation was done by CAS-shuttle assay (Payne, 1994). Culture supernatant (0.5 ml) was mixed with the same amount of CAS reagent (0.5 ml) and absorbance was measured at 630 nm against a reference consisting of equal volume of uninoculated broth and CAS reagent. Siderophore content in the aliquots were calculated using following formula.

$$\% \text{ Siderophore units} = \frac{\text{Ar-As}}{\text{As}} \times 100$$

Where, A_r is the absorbance of reference and A_s is the absorbance of the sample

ACC deaminase activity

Bacterial strains were assayed for 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity by testing their ability to grow on DF minimal medium (Dworkin and Foster, 1958) supplemented with 3 mmol ACC as the sole source of nitrogen (Penrose and Glick, 2003). Solid DF minimal medium containing ACC was inoculated with 10 μ l of starter culture (grown overnight at 30°C). Plates were then incubated at 30°C in dark and colony emergence was checked daily for consecutive 3 days.

Production of hydrogen cyanide (HCN)

HCN production was assessed by growing the bacteria in 10% tryptic soy agar (TSA) supplemented with glycine (4.4 g/L). Filter paper soaked in picric acid and Na_2CO_3 (0.5 and 2%, respectively) solution was fixed to the underside of the lids of plates and incubated for five days at 30°C. A change in filter paper color from yellow to orange-brown was considered to be the indication of HCN production (Donate-Correa et al., 2005)

Production of ammonia

The bacterial isolates were tested for the production of ammonia in peptone water. Fresh cultures were inoculated into 10 ml peptone water and incubated for 48 h at 30°C. Nessler's reagent (0.5 ml) was added to each tube. Development of brown to yellow colour was considered to be a positive test for ammonia production (Cappucino and Sherman, 1992).

Single and mixed inoculation assay

Based on the performance of above, two efficient PSB strains identified as *Pantoea agglomerans* (PSB-1) and *Burkholderia anthina* (PSB-2) were selected for the pot trials. For this experiment, the bacterial strains were grown on nutrient agar initially. A single colony was transferred into 500 ml flasks containing nutrient broth and grown aerobically in flasks on a rotating shaker (150 rpm) for 48 h at 30°C. The bacterial suspension was then diluted in sterile distilled water to a final concentration of 10^8 CFU/ml, and resulting suspensions were used to treat four weeks old tomato seedlings (*Lycopersicon esculentum* Mill). For dual inoculation, equal volume (10^8 CFU/ml of each inoculant) of two cultures were mixed and used for treating tomato seedlings (same as for single inoculation).

The experiment was carried out in a green house located at the Chungnam National University, South Korea. The soil used as potting media was classified as sandy loam and had the following characteristics: pH 6.55, NH_4^+ -N 665 mg/kg, NO_3^- -N 660 mg/kg, P_2O_5 665 mg/kg, cation-exchange capacity (CEC) 10 Cmol^+ /L. One seedling was planted in each polyethylene pot (25 cm diameter, 35 cm height). Basal doses of nitrogen (320 mg/kg soil) and potassium (160 mg/kg soil) were applied in the form of urea and potassium chlorite, respectively. Tricalcium phosphate (TCP) was supplied as phosphate fertilizer at the dose of 350 mg/kg soil based on nutrient requirements of tomato plants.

The pots were arranged in a completely randomized block design with three replications (each having one seedling) per treatments. The experimental plan was based on eight treatments as follows; (1) Soil without TCP, PSB-1 and PSB-2; (2) soil + TCP; (3) soil + PSB-1; (4) soil + PSB-1 + TCP; (5) soil + PSB-2; (6) soil + PSB-2

+ TCP; (7) soil + PSB-1 + PSB-2; and (8) soil + PSB-1 + PSB-2 +TCP. Bacteria applications were performed following the syringing method (Aslantas et al., 2007). Accordingly, 50 ml of bacterial suspension was inoculated into the middle part of the seedling roots. Control plants received 50 ml of diluted LB broth with no bacteria. Tomato seedlings were watered daily to maintain the water holding capacity of the soil during the study period. Growth promoting effects of bacterial treatments were assessed by measuring plant height, shoot and root weight, and N and P nutrient uptake of tomato plants after eight weeks of planting.

Dry matter content of plants and phosphorous uptake

The root and shoot portions of tomato plants were separated and air dried for two days. They were then oven dried at 70°C to a constant weight. The shoot and root dry weights were recorded separately and the average dry weight of plants was expressed in g/plant. Plant samples were finely ground after drying and used to determine phosphorous content following Vanomolybdate phosphoric yellow color method as suggested by Jackson (1973).

Soil analysis

The samples of rhizosphere soil were aseptically separated from roots to measure soil pH, phosphorous content and population densities of PSB. Soil pH was measured in 1:2.5 soil : water suspension with a pH meter. Available phosphorous extracted by the bicarbonate method (Olsen et al., 1954) was determined following the molybdate blue color method. PSB population density was assessed using pour plate method. For that rhizosphere soil was collected by uprooting the plants. The soil adhering to the roots was serially diluted and aliquots of 0.1 ml of the sample from each of these dilutions were spread on to a Petri dish containing NBRIP medium. The plates were incubated for three days in an incubator at 30°C. The colonies with clear halos were counted at the end of the incubation.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using SAS package (SAS, 1999). The Duncan's Multiple Range Test (DMRT) was applied to test the significance of treatment means at $P \leq 0.05$.

RESULTS

Identification of phosphate solubilizing bacterial strains

Selected two bacterial strains had a marked solubilizing ability of insoluble phosphate as visualized by the clear zone developed around the colonies after three days of incubation. According to 16S rRNA sequence analysis, the strains, which showed close proximity with *Pantoea agglomerans* DSM3493 (99.33%) and *Burkholderia anthina* R4183 (99.56%) were identified as *Pantoea agglomerans* (PSB-1) and *Burkholderia anthina* (PSB-2). A phylogenetic tree was constructed with 16S rRNA sequence of strain with other *Pantoea* and *Burkholderia*

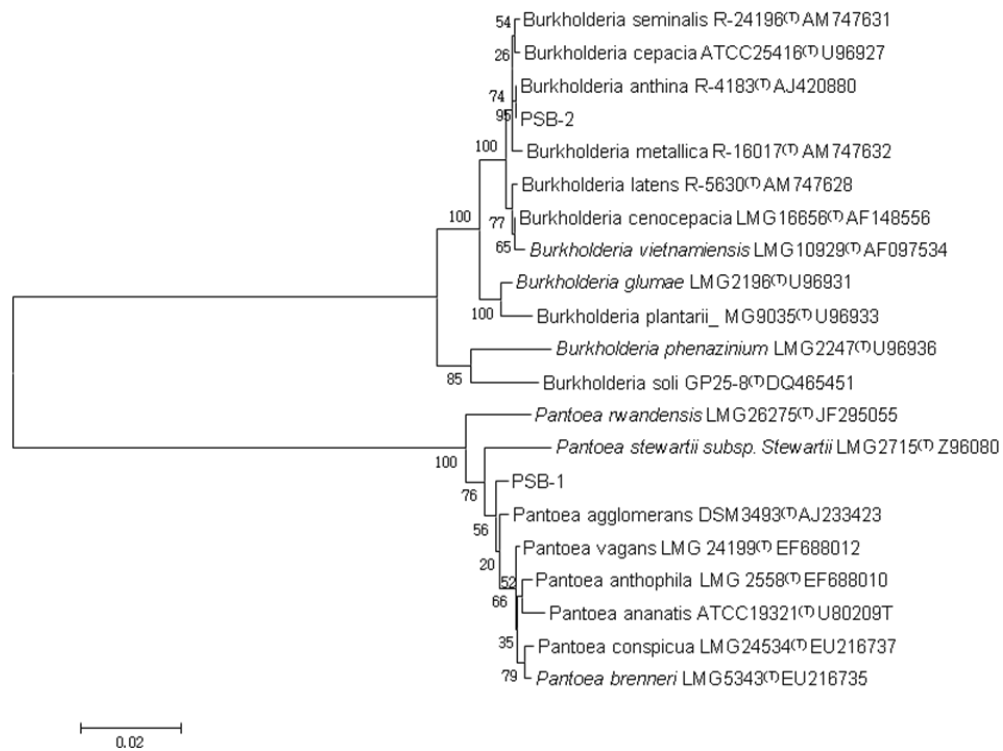


Figure 1. Phylogenetic tree based on 16S rDNA gene sequences, showing the position of *P. agglomerans* or *B. anthina* strains with respect to related species. The scale bar indicates 0.02 substitutions per nucleotide position and accession numbers are given in parenthesis.

species using neighbor-joining method (Figure 1).

Assay of inorganic phosphate solubilizing ability

As depicted in Figure 2, phosphate solubilizing ability of both strains was shown to be more or less similar. A rapid increase of available phosphorus contents (> 600 µg/ml of culture filtrate) in the medium was observed during the first 2 days of the incubation, then remained high for several days and decreased towards the end of the incubation. There was no significant change in the content of soluble phosphorus under the control, which only resulted in a negligible slight increment throughout the incubation period. Both strains lowered the pH (from initial pH 7 to 3.83 and 3.82 in medium containing *P. agglomerans* and *B. anthina* respectively) of the NBRIP medium as compared with the control, where it remained constant.

Assay of other plant growth promoting traits

Both strains showed positive responses for all the tested plant growth promotion traits that is IAA production,

siderophore production, ACC deaminase activity, ammonia production and HCN production. However, as shown in Figure 3, IAA production was comparatively lower (10 and 7.5 µg/ml respectively for *P. agglomerans* and *B. anthina*) than the other traits. *P. agglomerans* exhibited the highest IAA production within the first 24 h; whereas *B. anthina* exhibited the highest IAA production within the first 48 h followed by reduction as incubation progressed. Both strains formed orange halos around the colonies on CAS agar thus were considered to be good siderophore producers. As depicted in Figure 4, high proportion (> 80%) of total siderophore produced by *B. anthina* was reported within the first 24 h; whereas *P. agglomerans* showed a slow response thus took 72 h to exhibit the highest siderophore production.

Growth of tomato plants and phosphorous uptake

As shown in Table 1, plant height, root length, and dry weight of shoot and root was higher in tomato plants inoculated with *P. agglomerans* or *B. anthina* or co-inoculated with both strains compared to non-inoculated tomato plants. Growth was found to be further enhanced when soils inoculated with *P. agglomerans* or *B. anthina*

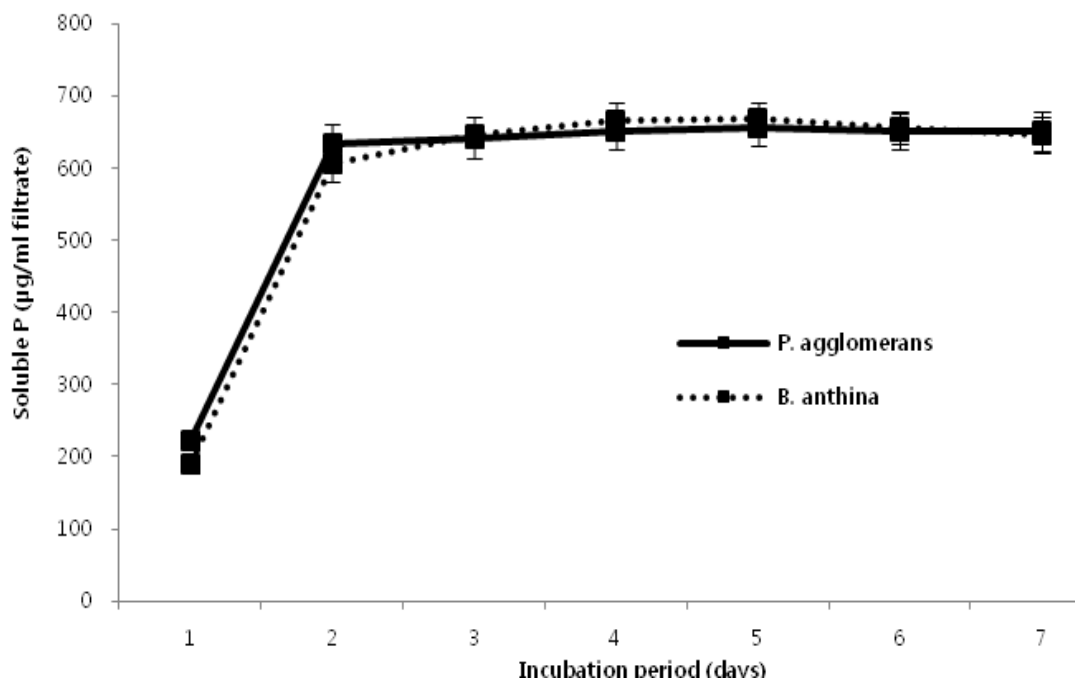


Figure 2. Phosphate solubilization ($\mu\text{g/ml}$ of filtrate) by *Pantoea agglomerans* and *Burkholderia anthina* strains. Values are the means ($n = 3$) \pm standard deviation.

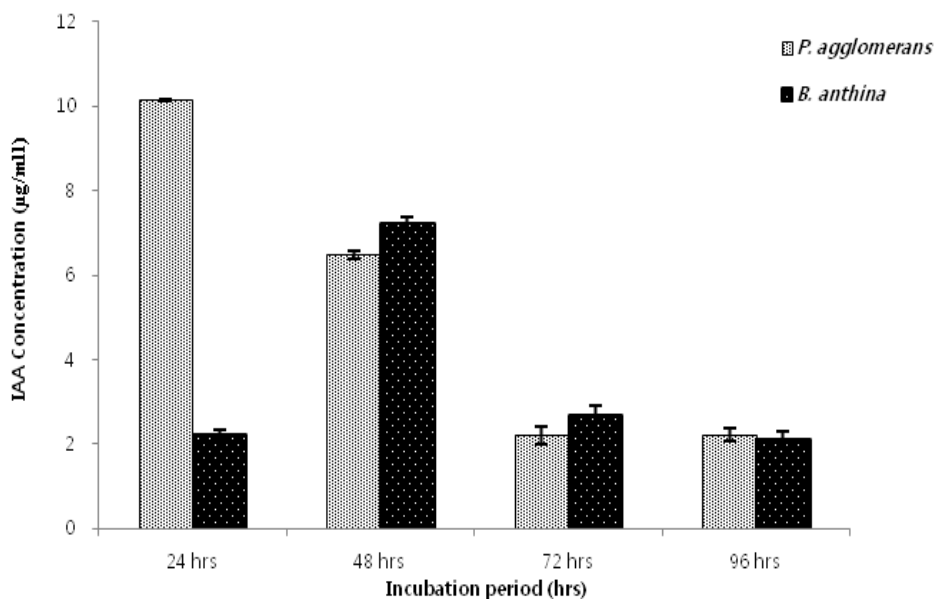


Figure 3. IAA production ($\mu\text{g/ml}$) by *Pantoea agglomerans* and *Burkholderia anthina* strains. Values are the means ($n = 3$) \pm standard deviation.

or both strains with TCP. However, no significant differences were found between TCP treated co-inoculated plants and TCP treated single inoculated plants, except shoot biomass, which is significantly ($P \leq$

0.05) higher in co-inoculated plants with TCP than any other treatment.

As shown in Table 2, phosphorous uptake by tomato plants also showed a similar trend as plant height, root

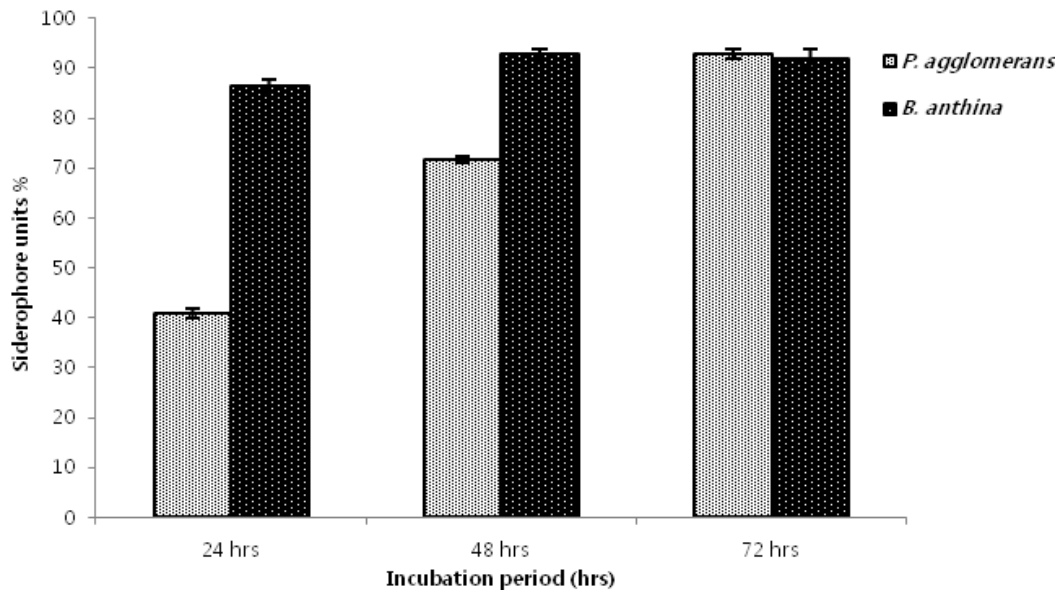


Figure 4. Siderophore production (%) by *Pantoea agglomerans* and *Burkholderia anthina* strains. Values are the means ($n = 3$) \pm standard deviation.

Table 1. Effect of *Pantoea agglomerans* and *Burkholderia anthina* on growth of tomato plants.

Treatment	Plant height (cm)	Root length (cm)	Shoot dry matter (g/plant)	Root dry matter (g/plant)
Soil without TCP, PSB-1 and PSB-2	124.33 ^c \pm 1.75	30.33 ^c \pm 0.98	42.84 ^c \pm 1.34	33.72 ^c \pm 2.54
Soil + TCP	122.67 ^c \pm 2.31	31.67 ^c \pm 1.35	42.38 ^c \pm 1.89	35.15 ^c \pm 1.76
Soil + PSB-1	134.01 ^{cb} \pm 1.65	34.67 ^{cb} \pm 0.93	42.26 ^{bc} \pm 0.62	40.84 ^{cb} \pm 2.65
Soil + PSB-1 + TCP	148.33 ^a \pm 1.58	38.01 ^{ab} \pm 0.84	48.32 ^b \pm 2.15	53.24 ^{ab} \pm 1.68
Soil + PSB-2	139.67 ^{ab} \pm 2.64	34.33 ^{cb} \pm 0.84	45.91 ^{bc} \pm 0.91	37.47 ^c \pm 3.01
Soil + PSB-2 + TCP	142.67 ^{ab} \pm 1.34	37.33 ^{ab} \pm 1.27	48.52 ^b \pm 0.75	53.62 ^{ab} \pm 1.94
Soil + PSB-1 + PSB-2	134.33 ^{cb} \pm 1.32	36.67 ^{ab} \pm 1.18	44.18 ^c \pm 1.11	44.36 ^{cb} \pm 1.35
Soil + PSB-1 + PSB-2+TCP	146.01 ^{ab} \pm 0.95	40.33 ^a \pm 1.28	54.91 ^a \pm 1.49	66.92 ^a \pm 2.84

Values are given as means \pm SD for triplicate samples. Within each column, means followed by same letter(s) are not significantly different at $P \leq 0.05$.

length and shoot-root dry weight. The phosphorous contents in shoots as well as in roots were higher in PSB inoculated plants than non-inoculated tomato plants regardless the single or co-inoculation. Maximum phosphorous uptake was recorded when co-inoculation of two PSB strains combine with TCP.

Changes in pH, available soil phosphorous and PSB population in tomato rhizosphere

The effect of PSB inoculation on soil pH, available phosphorous content and total PSB population in the tomato rhizosphere is presented in Table 3. Inoculation of

P. agglomerans or *B. anthina* or co-inoculation of isolates significantly ($P \leq 0.05$) decreased soil pH and increased soil available phosphorous content compared to uninoculated soil. The population of PSB in tomato rhizosphere (as measured by CFU/g soil) increased significantly ($P \leq 0.05$) with the inoculation of single or combination of two bacterial strains. PSB population further increased with the addition of TCP.

DISCUSSION

It is of urgent need to establish sustainable agricultural practices which could maintain long-term ecological balance

Table 2. Effect of *Pantoea agglomerans* and *Burkholderia anthina* on phosphorous uptake by tomato plants.

Treatment	P content in shoot (mg/plant)	P content in root (mg/plant)	Total P uptake (mg/plant)
Soil without TCP, PSB-1 and PSB-2	138.73 ^c ±1.58	10.04 ^f ±1.04	148.76 ^e ±3.11
Soil + TCP	140.22 ^c ±2.13	10.19 ^f ±0.34	150.41 ^{ed} ±3.21
Soil + PSB-1	142.17 ^c ±1.35	18.95 ^d ±0.13	161.12 ^c ±4.52
Soil + PSB-1 + TCP	176.71 ^d ±1.58	25.99 ^b ±1.06	202.71 ^b ±3.57
Soil + PSB-2	143.22 ^c ±2.17	12.36 ^e ±0.67	155.58 ^{ecb} ±1.56
Soil + PSB-2 + TCP	182.32 ^{ab} ±1.84	20.82 ^c ±1.52	203.15 ^b ±3.51
Soil + PSB-1 + PSB-2	145.02 ^c ±2.84	12.51 ^e ±1.37	157.33 ^{cd} ±1.24
Soil + PSB-1 + PSB-2+TCP	187.48 ^a ±1.57	28.91 ^a ±1.01	216.41 ^a ±4.84

Values are given as means ± SD for triplicate samples. Within each column, means followed by same letter(s) are not significantly different at $P \leq 0.05$.

Table 3. Effect of *Pantoea agglomerans* and *Burkholderia anthina* on soil pH, available phosphorous content and population of phosphate solubilizing bacteria in rhizosphere soil of tomato plants.

Treatment	Soil pH	Soil available P (mg/kg)	No of PSB (CFU/g soil)
Soil without TCP, PSB-1 and PSB-2	6.52 ^a ±0.48	108.18 ^d ±2.13	1.13×10 ^{2(a)}
Soil + TCP	6.57 ^a ±0.22	110.69 ^d ±1.87	1.17×10 ^{2(a)}
Soil + PSB-1	6.11 ^b ±0.34	139.62 ^{cd} ±2.11	4.55×10 ^{4(b)}
Soil + PSB-1 + TCP	6.08 ^b ±0.54	183.65 ^{ab} ±1.57	5.77×10 ^{5(b)}
Soil + PSB-2	5.98 ^b ±0.27	138.36 ^{cd} ±2.54	5.64×10 ^{4(b)}
Soil + PSB-2 + TCP	6.10 ^b ±0.34	168.55 ^{cb} ±3.24	5.15×10 ^{5(b)}
Soil + PSB-1 + PSB-2	6.09 ^b ±0.44	153.46 ^{cb} ±1.28	6.58×10 ^{6(b)}
Soil + PSB-1 + PSB-2+TCP	5.99 ^b ±0.37	210.06 ^a ±2.67	8.71×10 ^{6(b)}

Values are given as means ± SD for triplicate samples. Within each column, means followed by same letter(s) are not significantly different at $P \leq 0.05$.

of the soil system. In this context, PSB are reckoned to be one of the possible alternatives for chemical fertilizers. In the present study, rhizosphere soil samples from tomato plants were screened for the isolation of PSB. Among the 31 phosphate solubilizing isolates, two efficient PSB were selected for further studies. According to 16S rRNA sequence analysis, the strains were identified as *Pantoea agglomerans* (PSB-1) and *Burkholderia anthina* (PSB-2). Previous reports also described some *Burkholderia* and *Pantoea* strains as efficient phosphate solubilizers (Peix et al., 2001; Caballero-Mellado et al., 2007; Viruel et al., 2011; Khalimi et al., 2012; Silini-Cherif, 2012).

The major mechanism associated with the solubilization of insoluble phosphate is the production of organic acids, accompanied by acidification of the medium (Puente et al., 2004). Reductions in releasing rate of soluble phosphorous during the later stages of the incubation might be due to the depletion of nutrients in the culture medium, in particular, carbon source needed

for the production of organic acids (Kang et al., 2002; Kim et al., 2005; Chaihar and Lumyong, 2009). However, as reported by Varsha-Narsian et al. (1994) availability of soluble phosphorus in the culture medium might also have an inhibitory effect on further phosphate solubilization. Excretory toxic products may also be responsible for such decline in P-solubilization. The inverse relationship between pH and soluble phosphorus concentration observed in the present study suggested that acidification of the medium could facilitate the inorganic phosphate solubilization. The present results are also in agreement with other researchers (Yasmin and Bano, 2011; Yu et al., 2011), who reported similar negative relationships.

IAA stimulates a rapid response (example increased cell elongation) as well as a long-term response (example cell division and differentiation) in plants (Ahmad et al., 2008). Furthermore, IAA stimulates lateral root formation which in turn could facilitate high root surface area for nutrient absorption from soil (Compant et al., 2010).

Therefore, IAA production by microbes could have definite effect on growth of the host plant. Similar to these findings, IAA production by PSB strains such as *Achromobacter xylosoxidans* and *Klebsiella* SN 1.1 have also been reported (Jha and Kumar, 2009; Chaiharu and Lumyong, 2011). ACC deaminase (1-amino cyclopropane-1-carboxylic acid deaminase), an enzyme produced by many growth promoting microorganisms is involved in the stimulation of root elongation in seedlings (Lie et al., 2000). Both strains, through the production of ACC deaminase, displayed their capability to grow in N-free basal medium. It has been reported that microbial IAA promotes root growth either directly by stimulating plant cell elongation or cell division or indirectly by its influence on the ACC deaminase activity (Patten and Glick, 2002).

The increased plant height and root length could be associated with cell elongation and multiplication induced by greater absorption of nutrients, particularly phosphorous. It can also be attributed to strains' ability to produce phytohormones such as IAA. The present results are in line with the study of Rudresh et al. (2005) and Gull et al. (2004) who investigated phosphorous uptake and growth promotion of chickpea plants (*Cicer aritenium* L.) in growth chamber and green house experiments. Yu et al. (2011) reported that *Pseudomonas chlororaphis* and *Pseudomonas fluorescens* remarkably increased plant height, shoot and root dry weight, and phosphorous and nitrogen uptake of walnut seedlings. Furthermore those increments were higher when combined inoculation of PSB strains with TCP than without TCP. Selvaraj et al. (2008) also observed increased root elongation and biomass production of Chinese cabbage after seed bacterization with PSB strains, although they had no effect on the phosphorous uptake of plants. Similarly, De Freitas et al. (1997) also observed that PSB strains of *Bacillus* and *Xanthomonas* significantly increased the height and biomass of canola plants though they were unable to increase phosphorous content in canola plants compared to un-inoculated plants. This suggests that PSB can enhance plant growth without substantial contribution from increased phosphorous uptake. In addition, there are some similar reports on enhanced dry matter content of maize and groundnut due to inoculation of PSB (Hameeda et al., 2006a; Pandey et al., 2006).

Significant ($P \leq 0.05$) decreased in soil pH and increased available phosphorous content of soil after inoculation of PSB strains *P. agglomerans* or *B. anthina* or co-inoculation of isolates is in agreement with the study of Yu et al. (2011) who observed similar results after soil inoculation with *Pseudomonas chlororaphis* and *Pseudomonas fluorescens*. In accordance with our results, they also observed lowest pH and highest phosphorous concentration in soil when PSB co-inoculation than single inoculation. Contrary to these findings, Hariprasad and Niranjana (2009) noticed

stabilized pH in the rhizosphere soil samples when inoculated with PSB. This could be due to the buffering capacity of soil coupled with the inability of bacteria to secrete high concentrations of organic acids (Gyaneshwar et al., 2002).

Conclusion

Both tested strains tend to enhance the growth of tomato (as measured by plant height, root length, shoot and root dry weight). The strains also improved the uptake of phosphorous by tomato plants and the available phosphorous content in the soil compared to the control. Combined inoculation of two PSB strains with TCP further enhanced the growth of tomato plants implying that both strains could act synergistically with each other in promoting the growth. Further studies under field conditions would be ideal in confirming the present findings and also in recommending the strains for commercial applications.

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