Rhizosphere mediated nutrient management in *Allium hookeri* Thwaites by using phosphate solubilizing rhizobacteria and tricalcium phosphate amended soil

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**ABSTRACT**

This work describes integrated nutrient management for cultivation of *Allium hookeri* by using phosphate solubilizing bacteria (PSB) applied in rhizosphere, along with tricalcium phosphate (TCP). *Arthrobacter luteolus* S4C7, *Enterobacter asburiae* S5C7, *Klebsiella pneumoniae* S4C9, S4C10 and S6C7, and *K. quasipneumoniae* S6C2, were isolated from rhizosphere of *Allium hookeri* Thwaites, and were found to release substantial amount of soluble phosphate (124.8–266.4 μg/mL) from TCP in vitro conditions. These isolates were experimented for plant growth promoting attributes, including IAA, siderophore, and nitrogen-fixation. Treatment with PSB resulted in enhanced growth of *A. hookeri* Th., which was even better with TCP amendment with PSB. *K. quasipneumoniae* S6C2 resulted in 39.1% and 533.3% increase (*p* ≤ 0.05) of root length and weight respectively. The treatment with these isolates, in TCP amended soil also resulted in 200–250% increase in available P in soil, which was maximum for *K. quasipneumoniae* (1.866 mg/g).

**Introduction**

Phosphorus (P) is the second most important macro-nutrient and an essential growth factor limiting nutrient for plant growth, as it is required in important metabolic pathways like photosynthesis, biological oxidation, nutrient uptake and cell division (Epstein 1972; Illmer and Schinner 1992; Holford 1997). P is present in soil in abundance, in both organic and inorganic forms, but the majority of P is immobilized and rendered unavailable for plant uptake as it complexes with cations of Ca, Fe, and Al, depending on the type of soils. Thus, only the phosphate in a soluble ionic form (Pi) is effective as a mineral nutrient (Kucey et al. 1989; Ae et al. 1991). Plants are unable to utilize precipitated form of phosphorous. However, organic matter, on the other hand, is an important reservoir of immobilized phosphate that accounts for 20–80% of soil phosphorous (Richardson 2001) and only a small portion (~0.1%) is available to plants. Conversion of the insoluble forms of phosphorous to a form accessible by plants, like orthophosphate, is an important trait of phosphate solubilizing rhizobacteria (PSRB) in increasing growth and yield of crop plant. Application of phosphate solubilizing bacteria increases soil fertility due to their ability to convert insoluble P to soluble P by releasing organic acids, chelation and ion exchange (Omar 1998; Narula et al. 2000; Whitelaw 2000). The main active strains in this conversion belong to a range of genera, including *Pseudomonas, Mycobacterium, Arthrobacter, Serratia, Chryseobacterium, Gordonia, Phyllobacterium, Delftia, Enterobacter, Pantoea, Klebsiella, Micrococcus, Bacillus, Flavobacterium, Rhizobium, Mesorhizobium* and *Sinorhizobium* (Asea et al. 1988; Salih et al. 1989; Rodriguez and Fraga 1999; Chen et al. 2006; Chung et al. 2005).

Rhizospheric bacteria are known to play a very significant role in plant growth promotion by different mechanisms, one of which is the ability to dissolve poorly soluble fixed P, and such bacteria are known as phosphate-solubilizing bacteria (PSB). Their role is to convert these insoluble phosphates into soluble forms through the process of acidification, chelation, and production of low molecular weight organic acids such as acetic, gluconic, 2-ketogluconic, glycolic, isobutyric, isovaleric, lactic, malonic, oxalic, fumaric and succinic by lowering the pH of the surrounding (Bolan et al. 1994; Goldstein 1995; de Freitas et al. 1997; Sharma et al. 2005). These acids are produced in the periplasm of many gram-negative bacteria through a non-phosphorylated direct oxidation pathway of glucose (Matsusshita et al. 2002). Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with the roots that benefit the plant growth with direct and/or indirect mechanisms (Glick 1995; Ahmad et al. 2008). The mechanisms of PGPR includes fixation of atmospheric nitrogen to ammonia, production of IAA, solubilization of phosphorus, siderophore production, antibiotics, lytic enzymes etc. (Compant et al. 2005).

*Allium hookeri* Th., is a wild plant that grows in countries including Greece, Yunnan, Southern China, Bhutan, Sri Lanka and India (Hooker 1892). Besides having medicinal properties, it is used for seasoning ethnic cuisines, while its leaves and fleshy roots are also consumed as vegetables and also it has good marketability in areas of its common occurrences (Singh et al. 2003).

With the emphasis on the isolation and characterization, (Rodriguez and Fraga 1999; Harris et al. 2006; Perez et al. 2007) PSB are isolated and characterized for their ability to solubilize unavailable reduced phosphorus (P) to available forms. Such transformations increase P availability and promote plant growth (Rodriguez and Fraga 1999; Whitelaw 2000; Rudresh et al. 2005; Harris et al. 2006). However, to date, investigations on isolation, characterization and the
positive effects of PSB strains on *A. hookeri* Th. is not reported in the literature. In present study, application of efficient PSB, isolated from *A. hookeri* rhizosphere, has been described for integrated nutrient management with tricalcium phosphate and examine their effect on growth of host plants. Further, effect on availability of soluble phosphate in soil and uptake of P by plants was also estimated with bacterial augmentations and TCP amendments.

**Materials and methods**

**Plant species**

*A. hookeri* Th. (*Alliaceae* family) is a non-bulbous liliaceous plants and growing as a wild herb in a wide range of soils. The leaves are thick evergreen, linear with prominent mid-ribs, basal leaves membranous and shorter than the sub-trigeneric scape. Mainly, the freshly leaves and roots of this species are consumed as vegetables. Therefore, it has good marketability in areas of its common occurrence. Because of its therapeutic properties, it is used in excessive body temperature and vertigo and reducing blood pressure, stomach ulcer, hypertension, coronary heart diseases, etc. Due to its many benefits, it is used by the ethnic people of North East India as one of the important plants (Kala 2005; Pandey et al. 2008).

**Screening and Isolation of phosphate solubilizing bacteria**

Rhizospheric soils were collected from the *A. hookeri* Th. from different parts of Manipur, India. Manipur lies in the Eastern Himalayan region of India at latitude of 23°83′N–25°68′N and longitude of 93°03′E–94°78′E. The soil samples were collected using sterilized forceps and kept in the sterilized plastic bags. 1.0 g of soil sample were suspended in 9.0 ml of phosphate buffer saline with pH 7.2. Suitable dilution of soil samples was plated on yeast extract mannitol agar (YEMA) medium, and incubated at 30°C for 3 days. Bacterial colonies appearing on the medium were isolated and sub-cultured for further analysis. PSB strains were selected on the basis of clear zone of phosphate solubilization around the colony on Pikovskaya’s (PVK) agar having composition – glucose (10 g L⁻¹), (NH₄)₂SO₄ (0.1 g L⁻¹), MgSO₄·7H₂O (0.25 g L⁻¹), KCl (0.2 g L⁻¹), MgCl₂·6H₂O (5.0 g L⁻¹), Ca₃(PO₄)₂ (5.0 g L⁻¹) (Jackson 1973) in sterile conditions at 30°C. Amount of phosphorus in culture supernatant was measured by vanadomolybdate-yellow color method. To a 0.5 ml aliquot of the supernatant, 2.5 ml Barton’s reagent was added and volume was made to 50 ml with de-ionized water. The absorbance of the resultant color was read after 10 min at 430 nm in UV/Visible Spectrophotometer. The total soluble phosphorus was calculated comparing with the standard curve. The values of soluble phosphate liberated were expressed as μg/mL over control. The pH of culture supernatants was also measured for each sample.

**Indole-3-acetic acid assay**

IAA produced by the cultures was estimated by growing the isolates in YEM broth amended with 0.2% of L-tryptophan. The cultures were incubated in a shaker at 120 rpm at 30°C and harvested the cultures by centrifugation at 8000 rpm for 15 min. The production of IAA was observed after every 24 h interval. 1 ml of supernatant was mixed with 2 ml of Salkowski reagent (50 ml, 35% perchloric acid with 1 ml, 0.5 M ferric chloride [FeCl₃]) (Gordon and Weber 1951). The optimal density (OD) was measured at 530 nm and the amount of IAA produced was calculated by comparing with IAA standard curve.

**Siderophore production**

Siderophore was determined on Chrome-azurol S (CAS) with the formation of orange to yellow medium following the incubation at 28°C for 48 h (Schwyn and Neilands 1987). Quantitative estimation of siderophores was performed by CAS-shuttle assay (Payne 1994). 1 ml of culture supernatant was mixed with 1 ml of CAS reagent, and absorbance was measured at 630 nm against a reference consisting of 1 ml of uninoculated broth and 1 ml of CAS reagent. Siderophore content in the aliquot was calculated in percentage siderophore units (SU) by using the formula

\[
SU(\%) = \left(\frac{Ar - As}{Ar} \times 100\right)
\]

where \(Ar\) = absorbance of references at 630 nm (uninoculated broth + CAS reagent) and \(As\) = absorbance of sample at 630 nm (culture supernatant + CAS reagent).

In order to determine the threshold level of iron for siderophore production, iron content in succinic medium – (SM)
Nitrogenase activity

Nitrogenase activity of the isolates was determined in nitrogen free medium (Burk's medium) by the acetylene reduction assay (ARA) (Hardy et al. 1968). Pure cultures of all the isolates were inoculated in the Burk's medium and were grown for 48 h at 30°C on a rotatory shaker at 120 rpm. The vials were inoculated with each isolates (OD$_{600}$ of 1) and incubated at 30°C until exponential phase. Following the incubation, the gas phase of each vial was replaced with acetylene (10% v/v) and again incubated at 30°C for 6 h. Ethylene production was measured using a gas chromatography (Ceres 800 plus, Thermoscientific) using flame ionization detector (GC-FID) and a Porapak T stainless steel column. After the completion of the ARA, the free-living cultures, the cells were collected and broken by sonication. Protein concentration in the resulting mixture of the suspension was also determined (Lowry et al. 1951).

Detection of organic acid

The analysis of organic acids produced by PSB cultures was studied. Briefly, the late log phase cultures of bacteria were filtered through 0.2 µm Whatmann No.1 filter membrane and 20 µl of filtrates were injected to HPLC (Model: Perkin-Elmer Series 200, USA) equipped with UV/Vis Detector. The mobile phase consisted of 0.1% phosphoric acid at a flow rate of 1 ml/min. Retention time of each signal was recorded at a wavelength of 210 nm and compared the peaks with standard organic acids. Organic acid standards including acetic acid, citric acid, gluconic acid, lactic acid, malic acid, propionic acid, succinic acid and oxalic acid were run in parallel.

Plant growth experiment

Each PSB isolate was grown separately in YEM broth at 30°C in a rotatory shaker at 120 rpm. The cultures were centrifuged at 6000 rpm for 15 min. The pellets thus obtained were re-inoculated in a rotatory shaker at 120 rpm. The cultures were centrifuged each 3 days by adjusting the pH to 7. The supernatant was collected and filtered through 0.2 µm Whatmann No.1 filter membrane and the resulting mixture of the suspensions was also determined.

Phosphorus use efficiency

The relative efficiency of phosphorus use (REP%) was calculated as the ratio between the plant DM (dry mass) under low Pi (Phosphate) and DM (dry mass) under high Pi, as described (Ozturk et al. 2005):

$$\text{REP} = \frac{\text{DM low Pi}}{\text{DM high Pi}} \times 100$$

The agronomic P use efficiency (APE, g DM g$^{-1}$ Pi) was obtained by expression adapted from Oliveira et al. (1987).

$$\text{APE} = \frac{\text{DM high Pi} - \text{DM low Pi}}{\text{difference in the total available P between high Pi and low Pi treatments}}$$

Statistical analysis

Statistical analysis was conducted by using Analysis of Variance (ANOVA) statistical package for social sciences (SPSS) software, version 21 followed by comparison of multiple treatment levels with the control, using the poshoc at $P \leq 0.05$ and Tukey's test.

Results

Isolation and identification of PSB from the rhizosphere soil

A total of 97 bacterial strains were isolated from rhizosphere of A. hookeri, and six PSB (S4C7, S4C9, S4C10, S5C7, S6C1, S6C2) were selected bacterial suspension of (OD$_{600}$ of 1). The plantlets were inoculated with each isolates (OD$_{600}$ of 1) and incubated for 48 h at 30°C on a rotatory shaker at 120 rpm. The vials were inoculated with each isolates (OD$_{600}$ of 1) and incubated at 30°C until exponential phase. Following the incubation, the gas phase of each vial was replaced with acetylene (10% v/v) and again incubated at 30°C for 6 h. Ethylene production was measured using a gas chromatography (Ceres 800 plus, Thermoscientific) using flame ionization detector (GC-FID) and a Porapak T stainless steel column. After the completion of the ARA, the free-living cultures, the cells were collected and broken by sonication. Protein concentration in the resulting mixture of the suspension was also determined (Lowry et al. 1951).
and S6C2) were selected on basis of better phosphate solubilization activity on Pikovskaya’s (PVK) agar plate amended with TCP. The morphological, physiological and biochemical characteristics of the bacterial isolates are given in Table 1. The selected six PSB showed optimum growth at pH 7.0, 35°C, and 0.5% salt concentration. The result of the BLAST search of the 16S rRNA gene sequences indicated S4C7 and S5C7 are closely related to Arthrobacter luteolus and Enterobacter asburiae respectively; S4C9, S4C10, S6C1 to Klebsiella pneumoniae and S6C2 to Klebsiella quasipneumoniae (Table 2). Based on the neighbor joining phylogenetic tree constructed with the 16S rRNA similarity (%), the nearest taxa of PSB isolates were identified as Arthrobacter luteolus LNR3 for S4C7 and S6C2 to Klebsiella pneumoniae and S6C2 to Klebsiella quasipneumoniae (S4C7, S4C9, S4C10, S5C7, S6C1 to Klebsiella pneumoniae S-49 for S4C9, Klebsiella pneumoniae F3 for S4C10, Enterobacter asburiae M16 for S5C7, Klebsiella pneumoniae SR-143 for S6C1 and Klebsiella quasipneumoniae 07A044 T for S6C2 (Figure 1). Though the isolate S6C2 (K. quasipneumoniae) belongs to the genus Klebsiella, it clustered closely with Enterobacter.

Phosphate solubilization

The P-solubilizing ability of selected isolates was estimated quantitatively. The maximum amount of available phosphate varied from 124.8 to 266.4 µg/mL in TCP amended NBRIP broth for selected isolates. The maximum amount of P was recorded after 96 h of incubation which was 266.4 µg/mL by S4C7 with decreased in pH to 3.77 (Figure 2). Amount of soluble phosphate with S4C9 was 236.8 µg/mL, while with S6C1, it was 228.4 µg/mL. In all isolates, it was invariably observed that P-solubilization was maximum when pH of the culture filtrate was minimum. The initial pH of medium was 6.8 that reduced below 5 within 24 h for all isolates except A. luteolus S4C7. For S4C9, S4C10, S5C7, S6C1, S6C2, the P solubilization activity gradually decreased with incubation, with successively increase in the pH, however, in A. luteolus S4C7, increase in phosphate solubilization was recorded with decreased in pH.

IAA production

IAA was estimated in medium supplemented with different concentration of L-tryptophan (0.0–1.0%) (Figure 3). The maximum amount of indole acetic acid produced by isolates was in the range of 52.9–186.65 µg/mL after 24 h of incubation. Production of IAA was induced by presence of L-tryptophan as IAA was not released in absence of L-tryptophan in all the isolates. A. luteolus S4C7 released highest amount of IAA (186.65 µg/mL, 0.8% L-tryptophan, 96 h) followed by S4C10 (84.056 µg/mL, 0.4% L-tryptophan, 96 h) >S6C1 (76.126 µg/mL, 1.0% L-tryptophan, 96 h) >S5C7 (73.644 µg/mL, 0.4% L-tryptophan, 120 h) >S4C9 (64.051 µg/mL, 1.0% L-tryptophan, 120 h) >S6C2 (52.190 µg/mL, 0.6% L-tryptophan, 96 h) after the maximum incubation of 72 h in the different concentration of L-tryptophan.

Siderophore production assay and influence of iron concentration

Siderophore production was confirmed by the development of an orange halo zone around the bacterial colonies on the CAS agar plate inoculated with PSB, observed after 24 h. All the PSB isolates produced siderophore, except A. luteolus S4C7. Siderophore release was further confirmed by quantitative CAS test where instant decolorization CAS reagent from blue to orange was observed. E. asburiae (S5C7) and the three K. pneumoniae -S4C9, S4C10, S6C1 and K. quasipneumoniae S6C2 released appreciable amount of siderophore within 24 h of incubation in YEM broth. Maximum siderophore was released by S5C7 after 72 h of incubation (89.772 units), followed by S4C9, S6C2, S6C1 and S4C10 (Figure 4A). Siderophore production was considerably affected by the presence of iron in medium. Initial increase in iron concentration induced siderophore production. Increase in iron concentration resulted in successive decrease of siderophore production by all PSB isolates. Maximum siderophore (86.172%) unit was observed with S5C7, at 1 µM concentration of iron. However, siderophore release decreased gradually corresponding to that of increase in concentration of iron (Figure 4B).

Nitrogenase activity and organic acid production

Among all six PSB isolates, nitrogen fixing ability was detected in four isolates of Klebsiella spp., while A. luteolus S4C7 and E. asburiae S5C7 did not exhibit nitrogenase activity as assessed by ARA, using GC-FID. K. quasipneumoniae S6C2 resulted in highest value for nitrogenase activity (28.638 nmol C₂H₄/µg/protien/hour), followed by K. pneumoniae S6C1 (19.15 nmol C₂H₄/µg/protien/hour), > S4C9 (19.08 nmol C₂H₄/µg/protien/hour), > S4C10 (13.17 nmol C₂H₄/µg/protien/hour), respectively. HPLC analysis confirmed the presence of organic acids in the broth culture in TCP amended liquid medium by all six PSB. The peak of strains S4C7 and S6C1 were identified as acetic acid; while S4C9, S4C10, S5C7 and S6C2 showed the peak for citric acid when compared with the retention time with the authentic standards.

Table 1. Physiological characteristics of PSB isolates.

| Biochemical               | S4C7 | S4C9 | S4C10 | S5C7 | S6C1 | S6C2 |
|---------------------------|------|------|-------|------|------|------|
| Catalase                  | +    | +    | +     | +    | +    | +    |
| Oxidase                   | -    | -    | +     | +    | -    | -    |
| Lactose fermentation      | LF   | LF   | LF    | LF   | LF   | LF   |
| Indole                    | +    | -    | -     | -    | -    | -    |
| Methyl red                | +    | +    | +     | +    | +    | +    |
| Voges-proskauer           | +    | +    | -     | +    | +    | +    |
| TSI                       | K/A  | K/A  | K/A   | K/A  | K/A  | K/A  |
| Simmon citrate            | +    | +    | +     | +    | +    | +    |
| Urease                    | +    | +    | +     | +    | +    | +    |
| Physiological characteristics* |      |      |       |      |      |      |
| Growth at different temperature* |      |      |       |      |      |      |
| 4°C                       | 0.049 | 0.090 | 0.087 | 0.061 | 0.081 | 0.085 |
| 6°C                       | 0.099 | 0.111 | 0.117 | 0.098 | 0.125 | 0.211 |
| 7°C                       | 0.313 | 0.471 | 0.437 | 0.398 | 0.445 | 0.465 |
| 8°C                       | 0.652 | 0.892 | 0.863 | 0.719 | 0.854 | 0.895 |
| 9°C                       | 0.609 | 0.855 | 0.817 | 0.701 | 0.812 | 0.819 |
| 6°C                       | 0.563 | 0.825 | 0.771 | 0.688 | 0.781 | 0.764 |
| Growth at different temperature|      |      |       |      |      |      |
| 30°C                      | 0.588 | 0.655 | 0.648 | 0.601 | 0.638 | 0.625 |
| 35°C                      | 0.547 | 0.628 | 0.611 | 0.595 | 0.605 | 0.588 |
| 40°C                      | 0.068 | 0.095 | 0.113 | 0.081 | 0.089 | 0.125 |
| Growth at different salt concentration |      |      |       |      |      |      |
| 0.5%                      | 0.902 | 1.068 | 1.112 | 0.923 | 1.091 | 1.079 |
| 2%                        | 0.877 | 0.977 | 0.898 | 0.892 | 0.915 | 0.865 |
| 5%                        | 0.481 | 0.568 | 0.511 | 0.503 | 0.499 | 0.485 |
| 10%                       | 0.099 | 0.123 | 0.131 | 0.106 | 0.120 | 0.127 |

*Values are given as average of three independent trials.
Effect of PSB on plant growth

With the inoculation of PSB, the growth of *A. hookeri* was enhanced significantly in unamended TCP soil. The best growth parameters were recorded with treatment of *K. pneumoniae* S6C1 where fresh shoot weight was 365.5% higher as compared to control, followed by *A. luteolus* S4C7 (227.58%). However, in fresh shoot length, the highest length were recorded with the treatment of *K. pneumoniae* S6C1 (32.1 cm), followed by *A. luteolus* S4C7 (31.5 cm).

Amendment of TCP did not affect the growth of *A. hookeri* Th. in comparison to un-amended control, as all the growth parameters were in similar range except moderate increase in root fresh weight. However, treatment of PSB with TCP enhanced the growth of *A. hookeri* Th. better than treatment with respective ‘PSB only’ in case of S5C7+TCP, and S4C10+TCP. There was 24.57% increase in shoot length with the treatment of S5C7+TCP as compared to S5C7 treatment alone. Similarly, with the treatment of S4C10+TCP, there

| PSB isolates | Length | Colony Morphology | Most closely related organism |
|--------------|--------|------------------|------------------------------|
| S4C7         | 1438   | C, E, R, Y, Cy   | *Arthrobacter luteolus* LNR3  |
| S4C9         | 1292   | C, E, R, OW, Cy  | *Klebsiella pneumoniae* S-49  |
| S4C10        | 1284   | C, E, R, OW, Cy  | *Klebsiella pneumoniae*       |
| S5C7         | 1438   | C, E, R, W, Cy   | *Enterobacter asburiae* L4311 |
| S6C1         | 1285   | C, E, R, OW, Cy  | *Klebsiella pneumoniae* S-49  |
| S6C2         | 1429   | C, E, R, OW, Cy  | *Klebsiella quasipneumoniae* O7A044T |

*Length: length of 16S rRNA gene sequenced. Colony Morphology: Colony morphology on YEMA medium with C: circular; E: entire; R: raised; Y/OW: yellow/off-white; Cy: creamy.*

Table 2. Colony morphologies and molecular characterization of PSB isolates.

Figure 1. Neighbor joining (kimura 2 model) phylogenetic dendogram based on partial 16S rRNA gene sequences. The tree includes six sequences of rhizobacterial (phosphate solubilizing bacteria) strain S4C7, S4C9, S4C10, S5C7, S6C1 and S6C2 determined in the present study (bold) and 24 sequences from NCBI Genbank database. The Genbank accession number of each taxa is in the parenthesis.
was increased in fresh shoot weight by 409.09% as compared to S4C10 treatment alone (Table 3, Figure 5).

**Effect of PSB on P content of soil, and plant tissues**

Inoculation with PSB individually increased the available P content of the soil. The results revealed the maximum available of P by the *K. quasipneumoniae* S6C2 treatment in the soil. Un-amended soil with the inoculated *K. quasipneumoniae* S6C2 had 0.056 g available P content 64.7%, followed by *A. luteolus* S4C7 at 0.053 g (55.882%), higher than the control, (Table 4). The TCP amended soil, *K. quasipneumoniae* S6C2 had 1.866 g of P available an increase of 261.6%, followed by *K. pneumoniae* S4C10 at 1.818 g (252.32%), compared to the TCP amended soil control.

Further, P uptake by plants was increased, as higher P content was recorded in the leaves and roots. The maximal increase in P uptake was shown by the shoots and roots inoculated with *K. quasipneumoniae* S6C2 (235.2% in shoots and 202.5% in roots, in un-amended soil; 403.9% in shoot and 294.9% in roots, in TCP amended soil, as compared to control) (Table 4). Inoculation with other PSB showed increase in P uptake in shoots and roots in both the TCP un-amended and amended soil, as compared to control (Table 4). Inoculation with other PSB showed increase in P uptake in shoots and roots in both the TCP un-amended and amended soil, when compared to the uninoculated PSB soil as control. Overall, the amount of uptake of P by the shoots in TCP amended soil is higher than the roots and un-amended TCP soil of shoots and roots of the plants.

**P-use efficiency**

The values of P-use efficiency were higher for TCP amended soil with PSB inoculation in *A. hookeri* Th. plants. It was observed that P use efficiency, as calculated by the ratio between the plant DM under low Pi and DM under high Pi (APE, g DM/g P), was 0.892 for control (without bacterial inoculation) and 1.848 with *K. quasipneumoniae* S6C2 treatment (Table 5), which corresponds to a 107.17% increase of P-use efficiency. *A. luteolus* S4C7 also resulted in 92.60% increase of P-use efficiency.

**Discussion**

*A. hookeri* Th. is a wild herb abundantly available in wide range soils found in North eastern region under Himalayan range of India. The leaves and its fibrous roots riched in carbohydrate, minerals, vitamins, are consumed as vegetables in soups and pickles. Because of its medicinal properties, and undefined agri-practices, need of an eco-friendly organic cultivation strategy was identified, and therefore role of PSB in its growth was studied. The rhizosphere soil of *A. hookeri* Th., which is used as condiment of intrinsic Manipuri cuisines, was selected for the isolation of PSB. Out of the 97 rhizobacteria that grew on the PVK medium plate, only 6 PSB isolates are selected based on their excellent ability of P solubilization on Pikovskaya agar plate medium. Based on 16S rRNA sequence analysis, the bacterial isolates were identified as *A. luteolus*, *K. pneumoniae*, *E. asburiae*, *K. quasipneumoniae*, Klebsiella sp. and Enterobacter sp. are recognized as phosphate solubilizing bacteria from the rhizosphere of crop plants as reported from Korea (Chung et al. 2005). The different strains of phosphate solubilizing bacteria *Proteus vulgaris*, *K. pneumoniae*, *E. aerogenes*, Burkholderia cepaciae, Citrobacter freundii, *A. lwoffi* and *Pseudomonas fluorescens* isolated from the rhizosphere of different plants has been reported as efficient for phosphate solubilization by forming halozones on agar plates of Pikovskaya growth medium (PVK) by solubilizing tricalcium phosphate of the medium (Sadiq et al. 2013). The plant growth-promoting P-solubilizing bacteria such as *Bacillus circulans* has been investigated as the potential strain from the rhizospheric
region of apple plant, Himachal Pradesh, India (Mehta et al. 2015). In several reports, the efficient PSB Bacillus spp. able to convert insoluble forms of phosphorus to accessible forms and found to mobilize P efficiently in the sunflower and improved the plant’s activities (Ekin 2010). Similarly, Elkoca et al. (2007) reported phosphate solubilizing bacteria Rhizobium, B. subtilis and B. megaterium as the efficient strains for phosphate solubilization. Several strains PGPR genera Acetobacter, Acinetobacter, Methylococcus, Bacillus, Micrococcus, Planococcus from the rose plant rhizosphere had the capacity to solubilize phosphate when the solid medium was supplemented with tricalcium phosphate. (El-Deeb et al. 2012). Enterobacter sp., isolated from nodules of Arachis hypogea L. was reported to solubilize tricalcium phosphate in unbuffered or buffered medium (Anzuay et al. 2013). In fact, these bacteria had been reported as biofertilizers for other plants such as Arthrobacter sp. for Zea mays L. (Arruda et al. 2013) and Glycine max L. (Kloepper et al. 1992); Enterobacter sp. for Phaseolus radiates (Zhao et al. 2011) and Arachis hypogaea L. (Anzuay et al. 2013) and Klebsiella sp. for Brassica campestris (Ahemad and Khan 2011) and Oryza sativa (Chaiharn and Lumyong 2011). Chen et al. (2006) reported phosphate solubilization ability of Arthrobacter sp. isolated from sub-tropical soil. However, reports on K. quasipneumoniae as PSB are scanty in literature. Only recently, K. quasipneumoniae AG5-3 has been reported for their ability to solubilize and fix the free nitrogen from the acid sulphate soil rhizosphere of rice grown of Mekong Delta, Vietnam (Xuan et al. 2016). In this work, A. luteolus, K. pneumoniae, E. asburiae, and K. quasipneumoniae are being reported as PSB from rhizosphere of A. hookeri Th, which has not been explored previously for this purpose.

It is known that PSB populations are largely found in agricultural soils (Yahya and Al-Azawi 1989), but A. hookeri Th. is generally not grown in agricultural soils for large scale harvesting. The amount of soluble P released was different in

Figure 3. Quantitative estimation of indole acetic acid (IAA) produced by PSB isolates (A. S4C7, B. S4C9, C. S4C10, D. S5C7, E. S6C1 and F. S6C2) at different L-tryptophan (L-tryp) concentrations. Standard deviation is showed as bars.
Table 3. Effect of PSB and TCP treatments on growth of Allium hookeri Th. estimated with pot assay (120 DAI).

| Treatments  | Length (cm) | Fresh weight (g) | Dry weight (g) | No. of leaves |
|-------------|-------------|------------------|----------------|--------------|
|             | Shoot       | Root             | Shoot          | Root         | Shoot      | Root      | Shoot      | Root      |             |
| S (control) | 23.16 ± 1.0a | 14.8 ± 2.7a      | 0.58 ± 0.3a    | 0.9 ± 0.4a   | 0.11 ± 0.06a | 0.1 ± 0.1a | 3.3 ± 0.57a |           |
| S+ S4C7     | 31.3 ± 1.1b  | 15.0 ± 1.0a      | 1.98 ± 1.1b    | 3.3 ± 0.5b   | 0.7 ± 0.18b  | 0.6 ± 0.1b | 7.6 ± 1.5b  |           |
| S+ S4C9     | 23.3 ± 3.8a  | 17.1 ± 3.5b      | 0.94 ± 0.5c    | 2.5 ± 1.0c   | 0.27 ± 0.07a | 0.5 ± 0.3b | 5.0 ± 0.0c  |           |
| S+ S4C10    | 25.4 ± 1.7c  | 14.5 ± 1.6a      | 0.55 ± 0.1a    | 3.8 ± 0.2b   | 0.5 ± 0.08a  | 0.8 ± 0.04c | 6.0 ± 1.0d  |           |
| S+ S5C7     | 29.3 ± 1.5d  | 20.4 ± 2.4c      | 1.0 ± 0.6c     | 5.2 ± 0.7d   | 0.54 ± 0.13a | 0.9 ± 0.14c | 5.6 ± 0.57c |           |
| S+ S6C1     | 32.1 ± 2.1e  | 16.4 ± 4.0b      | 2.7 ± 1.5d     | 3.4 ± 0.9b   | 0.64 ± 0.2c  | 0.7 ± 0.23c | 6.1 ± 1.1c  |           |
| S+ S6C2     | 28.2 ± 2.1d  | 20.6 ± 4.0c      | 1.2 ± 0.7c     | 5.7 ± 1.3d   | 0.65 ± 0.28c | 1.1 ± 0.19c | 6.3 ± 0.57d |           |
| S+ TCP+ S4C7| 25.9 ± 1.5m  | 15.7 ± 3.3m      | 0.5 ± 0.3m     | 3.0 ± 1.6m   | 0.24 ± 0.07m | 0.4 ± 0.25m | 4.0 ± 1.0m  |           |
| S+ TCP+ S4C10| 35.0 ± 2.6n | 18.5 ± 2.2n      | 1.8 ± 0.9n     | 6.1 ± 1.0n   | 0.86 ± 0.13n | 1.3 ± 0.33n | 6.6 ± 0.57n |           |
| S+ TCP+ S5C7| 30.8 ± 1.9o  | 16.1 ± 2.0m      | 0.9 ± 0.1m     | 3.8 ± 0.4m   | 0.69 ± 0.05o | 0.7 ± 0.06o | 6.6 ± 0.5n  |           |
| S+ TCP+ S6C1| 32.2 ± 3.4p  | 16.2 ± 1.2m      | 2.8 ± 1.6p     | 3.8 ± 1.3m   | 0.68 ± 0.2o  | 0.8 ± 0.25o | 7.6 ± 1.1o  |           |
| S+ TCP+ S6C2| 36.5 ± 1.7q  | 20.0 ± 0.5m      | 1.1 ± 0.6o     | 4.9 ± 0.5o   | 0.67 ± 0.07o | 0.8 ± 0.09o | 6.3 ± 0.5m  |           |
| S+ TCP+ S6C1| 31.0 ± 1.1r  | 16.8 ± 1.3m      | 2.8 ± 0.4m     | 3.4 ± 0.5m   | 0.6 ± 0.07o  | 0.7 ± 0.03o | 6.6 ± 0.5n  |           |
| S+ TCP+ S6C2| 30.9 ± 3.4o  | 20.7 ± 2.0o      | 1.5 ± 0.8n     | 6.05 ± 0.8n  | 0.66 ± 0.13o | 1.0 ± 0.12p | 5.3 ± 1.5n  |           |

*S- unamended soil.

Values are means of three replicates, Mean values (mean±S.D) sharing the same letter do not differ significantly by Tukey at $P \leq 0.05$.

TCP- Tricalcium phosphate.
supernatants. Earlier, Trivedi and Sa (2008) reported the similar results of production of organic acids identified as gluconic acid and 2-ketogluconic acid by phosphate solubilizing bacteria *Pseudomonas corrugata* (NRRL B-30409). However, information on type of organic acid produced by *K. quasipneumoniae* in NBRIP broth was not available in literature.

IAA is essential for the growth and development of plants. Organisms such as 80% of bacteria isolated from the rhizosphere are able to make physiologically active IAA that may

### Figure 5.
Effects of PSB isolates on growth promotion of *A. hookeri* Th. A and C, 1. Control (Soil), 2. Soil+S4C7, 3. Soil+S4C9, 4. Soil+S4C10, 5. Soil+S5C7, 6. Soil+S6C1, 7. Soil+S6C2) in TCP-unamended soil. B and D, 1. Control (Soil+TCP), 2. Soil+TCP+S4C7, 3. Soil+TCP+S4C9, 4. Soil+TCP+S4C10, 5. Soil+TCP+S5C7, 6. Soil+TCP+S5C1, 7. Soil+TCP+S6C2 TCP amended soil.

### Table 4. Uptake and available – P content in shoots, roots of *A. hookeri* Th. and soil through PSB and TCP treatments (120 DAI).

| Treatments         | Shoot (mg/g) | Root (mg/g) | Available P content in soil (mg/g) | Soil pH |
|--------------------|--------------|-------------|-----------------------------------|---------|
| **PSB**            |              |             |                                   |         |
| S (control)        | 0.71 ± 0.02a | 0.39 ± 0.005a | 0.034 ± 0.003a                    | 6.2 ± 0.11a |
| S+ S4C7            | 0.83 ± 0.033a | 0.57 ± 0.074a | 0.053 ± 0.001a                    | 6.4 ± 0.15b |
| S+ S4C9            | 1.87 ± 0.14c | 0.49 ± 0.050a | 0.048 ± 0.003a                    | 6.5 ± 0.20c |
| S+ S4C10           | 2.27 ± 0.08b | 0.53 ± 0.025a | 0.039 ± 0.001a                    | 6.5 ± 0.10c |
| S+ S5C7            | 2.38 ± 0.60b | 1.18 ± 0.160b | 0.045 ± 0.003a                    | 6.6 ± 0.15d |
| S+ S6C1            | 1.60 ± 0.63c | 0.94 ± 0.360a | 0.045 ± 0.002a                    | 6.6 ± 0.15d |
| S+ S6C2            | 2.32 ± 0.47b | 1.17 ± 0.190b | 0.056 ± 0.003a                    | 6.4 ± 0.11b |
| S+ TCP (control)   | 1.27 ± 0.25m | 0.59 ± 0.180m | 0.516 ± 0.029m                    | 6.7 ± 0.10m |
| S+ TCP+ S4C7       | 3.60 ± 0.31n | 2.30 ± 0.078n | 1.250 ± 0.266n                    | 6.5 ± 0.05n |
| S+ TCP+ S4C9       | 5.19 ± 0.17p | 1.76 ± 0.086n | 1.166 ± 0.060n                    | 6.8 ± 0.05o |
| S+ TCP+ S4C10      | 3.31 ± 0.50m | 1.80 ± 0.1560 | 1.818 ± 0.062o                    | 6.6 ± 0.05p |
| S+ TCP+ S5C7       | 6.40 ± 0.09q | 2.33 ± 0.230n | 1.390 ± 0.128p                    | 6.5 ± 0.05n |
| S+ TCP+ S6C1       | 4.20 ± 0.30o | 2.22 ± 0.320n | 1.766 ± 0.125d                    | 6.6 ± 0.05p |
| S+ TCP+ S6C2       | 5.30 ± 0.34p | 2.07 ± 0.560n | 1.866 ± 0.085o                    | 6.6 ± 0.05p |

*Superscript letters signify statistical significance at *P* ≤ 0.05.

1 S- unamended soil.
2 Values are means of three replicates. Mean values (mean±S.D) sharing the same letter do not differ significantly by Tukey at *P* ≤ 0.05.
3 TCP- Tricalcium phosphate.
have pronounced effects on plant growth and development. The results showed that the isolate *A. luteolus* S4C7 produced IAA in range of 52.9–186.65 µg/mL supplemented with different amount of L-tryptophan. Dastager et al. (2010) reported a plant growth promoting, phosphate solubilizing bacteria, NII 0909 *Micrococcus* sp. isolated from the Western Ghats forest soil produced IAA of 109 µg/mL. The attribute of IAA synthesis is considered essential for selecting favorable microorganisms as there have been reports suggesting that the phosphate solubilizing bacteria producing IAA at the range of 28.2 µg/mL have reflective effects to enhance the growth of *Aloe barbadensis* Miller (Gupta et al. 2012). Earlier, Ahmad et al. (2008) investigated the rhizobacteria like Azotobacter, fluorescent *Pseudomonas*, *Mesorhizobium* and *Bacillus* produced IAA at 22.02 µg/mL supplemented with different concentration of L-tryptophan and exhibited multiple plant growth promoting (PGP) traits on soil-plant system as effective PGPR.

Another important trait of PGPR, that may indirectly influence the plant growth, is the production of siderophores. They bind to the available form of iron Fe³⁺ in the rhizosphere, thus making it unavailable to the phytopathogens and protecting the plant health. In the present study, *E. asburiae* S5C7 and three *K. pneumoniae* – S4C9, S4C10, S6C1 and *K. quasipneumoniae* S6C2 were able to produce siderophore. *A. luteolus* S4C7 was unable to produce siderophore. Though in earlier reports, *A. luteolus* isolated from rare earth environment of Chavara, Kerala, India, was found to produce catechol-type siderophore (Emmanuel et al. 2012). The results showed that *E. asburiae* (S5C7) produced 89.77 unit of siderophore in YEM broth after 72 h of incubation and further followed by PSB *K. pneumoniae* -S4C9, S4C10, S6C1 and *K. quasipneumoniae* S6C2. Previously, *Pseudomonas fluorescens* NCIM 5096 and *Pseudomonas putida* NCIM 2847 has been reported to produce 87% and 83% units of siderophore (Sayed et al. 2005). The influence of different iron concentration on siderophore production unit was conducted in succinic acid medium (SM). The most suitable iron concentration for siderophore production was 1 µM for all the isolates with the highest record of 86.172 unit of siderophore by *E. asburiae* S5C7 in SM. It was also reported that *P. fluorescens* NCIM 5096 produced 72% unit of siderophore at 1 µM of iron concentration. Earlier, Pandey et al. (2005) reported that the production of siderophore was induced by iron in *P. aeruginosa* GRC1 and was found maximum at 0.2 µM of iron concentration. Also, siderophore release was reported to decrease gradually with corresponding increase in iron concentration, which is similar to results obtained with *E. asburiae* S5C7, *K. pneumoniae* -S4C9, S4C10, S6C1 and *K. quasipneumoniae* S6C2 in present work. Siderophore releasing rhizobacteria have not been reported earlier from *A. hookeri* Th.

The ability to reduce acetylene is an indirect measure of N₂-fixation, it is specific for monitoring functional nitrogen-ase activity and is indicative of N₂-fixing potential. Park et al. (2005) reported the surveyed the rhizosphere soil of agriculturally important crops widely cultivated in Cheongiu province, Korea for the presence of nitrogen fixing and plant growth promoting bacteria grown on Burk’s N−free medium, from rhizosphere of different crops. The ARA of different isolates PM-3, PM-6, PM-7, PM9 and PM-23 were reported to be 23.74, 15.52, 19.85, 20.19 and 14.10 nmol C₂H₄/µg/protein/hour respectively. In the present study, PSB isolate *K. quasipneumoniae* S6C2 was found to have maximum 28.638 nmol C₂H₄/µg/protein/hour in Burk’s N−free medium among the genus *K. pneumoniae* -S4C9, S4C10 and S6C1, accessed by acetylene reduction assay using GC-FID technique. The other isolates – *A. luteolus* and *E. asburiae* were not able to fix N₂. Venieraki et al. (2011) isolated *Azospirillum brasilensis* from the rhizosphere of Mesicalli, Kopaida, Vietia, with the ability of fixation of N₂ of 9.9 nmol C₂H₄/µg/protein/hour.

The inoculation of PSB isolates – *A. luteolus* S4C7, *E. asburiae* S5C7, *K. pneumoniae* - S4C9, S4C10, S6C1 and *K. quasipneumoniae* S6C2 enhanced the growth of *A. hookeri* Th. plants in TCP amended, as well as un-amended soil, putatively due to their plant growth promoting attributes, as detected in *in-vitro* experiments. The data presented in Table 3 and Figure 5 clearly suggests substantial (365.5%) increase in fresh shoot weight of *A. hookeri* Th. by treatment of PSB *K. pneumoniae* S6C1 in TCP un-amended soil. It was followed by treatment with *A. luteolus* S4C7 (227.58%). The fresh shoot length, was recorded highest with the treatment of *K. pneumoniae* S6C1 (32.1 cm), followed by *A. luteolus* S4C7 (31.5 cm). In a similar study, P-solubilizer *P. synxantha* was found to increase shoot length by 36.9 cm, and root length by 11.9 cm, of Aloe vera plant (Gupta et al. 2012). Yu et al. (2011) also reported that treatment with PSB – *P. chlororaphis*, *P. fluorocens*, *B. cereus* increased the height of walnut plant by 32.18, 32.06, 29.7 in un-amended TCP soil. The plant heights of tomato increased treated with Pantoea agglomerans (PSB-1) and Burkholderia anthina (PSB-2) by 134.01 and 139.67 cm respectively (Walpola and Yoon 2013). In this work, treatment of PSB, along with TCP enhanced the growth of *A. hookeri* Th. better than treatment with respective PSB alone. There was 24.57% increase in shoot length with the treatment of S5C7+TCP as compared to S5C7 treatment alone as evident from data given in Table 3. Similarly, with the treatment of S4C10+TCP, there was increased in fresh shoot weight by 409.09% as compared to S4C10 treatment alone. This is in accordance to earlier reports where treatment of PSB with TCP amendment in soil showed increased in all possible growth parameters of host plants (Yu et al. 2011; Gupta et al. 2012; Walpola and Yoon 2013). Kumari et al. (2008) have explained that the influence of PSB in TCP added soil could be due to its simple structure and the absence of any free carbonates when compared with the crystalline lattice structure of the other form of insoluble phosphates.

The inoculation of PSB provided increased the amount of available P in soil. The highest amount of available P in TCP un-amended soil was 56 mg/kg with the treatment with *K. quasipneumoniae* S6C2, which was 64.7% higher than

| Treatments | Dry mass (g/plant) | REP (%) | APE (g DM/g Pi) |
|------------|-------------------|---------|----------------|
|            | Low Pi | High Pi | Low Pi | High Pi |
| Control    | 0.21   | 0.64   | 32.81  | 0.892   |
| S4C7       | 1.3    | 2.16   | 60.18  | 1.718   |
| S4C9       | 0.77   | 2.16   | 35.64  | 1.243   |
| S4C10      | 1.3    | 1.48   | 87.83  | 1.101   |
| S5C7       | 1.44   | 1.47   | 94.08  | 1.133   |
| S5C7       | 1.44   | 1.47   | 94.08  | 1.133   |
| S6C1       | 1.34   | 1.48   | 90.54  | 1.081   |
| S6C2       | 1.75   | 1.86   | 97.95  | 1.985   |

REP was calculated as (DMLow Pi/DMHigh Pi) × 100. APE was calculated how (DMHigh Pi − DMLow Pi)/difference in the total available P between high Pi and Low Pi treatments. The data are averages from three repetitions.
control. A. luteolus S4C7 also resulted in improving the soluble P content by 55.882% (53 mg/kg) in TCP un-amended soil (Table 4). In previous report, treatment with the PSB from the calcareous cinnamon soil of China increased significantly the availability of P in reclaimed soil in coal mining subsidence region by 35.11% (Shi et al. 2017). Similar study also reported that inoculation with PSB- Erwinia tasmaniensis TP08, Pseudomonas aeruginosa TP16 and Pseudomonas aeruginosa TP373 increased the availability of P in the soil of Oryza sativa L. plant by 10%, 1.5% and 10% respectively (Durah et al. 2011). In contrast, the PGPR strains Bacillus megaterium M3, Bacillus subtilis OSU-142, Paenibacillus polymyxa RC05 and Azospirillum brasilense Sp245 gave no significant increase in the availability of P (without PGPR application was higher than that of the treatments with PGPR treatment) to the soil of wheat plant (Turan et al. 2012), while in present study, the availability of soluble P increased by application of PSB along with TCP in A. hookeri rhizosphere. Earlier, Yu et al. (2011) reported that the amount of available P by treatment of P. chlororaphis, P. fluorescens and B. cereus was 32.72%, 24.08% and 9.68% higher respectively in TCP un-amended soil as compared to control. However, in this work, treatment with TCP amended soil plus K. quasipneumoniae S6C2 resulted in 1866 mg/kg of available P, which was 261.6% higher as compared to TCP amended soil without bacterial treatment. Shi et al. (2017) reported that treatment with PSB+TCP significantly enhanced the availability of P (28.5%) in the soil. Similarly, Qureshi et al. (2012) reported treatment with phosphate solubilizing bacteria Bacillus sp. significantly enhanced the available P in the soil of cotton plant by 6.80%. It was observed that, K. pneumoniae S4C10 plus TCP resulted in 1818 mg/kg of available P that was 252.32% higher, compared to the TCP amended soil. This was in accordance to earlier report with Stevia rebaudiana Bertoni plant treated with TCP and PSB, where Burkholderia gladioli 10216 caused 332.02% increase of soluble P, and Enterobacter aerogenes resulted in 269.1% available P (Mamta et al. 2010).

The importance of phosphate solubilizing microorganisms is also considered in terms of P immobilized in the biomass of host plants (Demetz and Insam 1999). The stimulatory effect of the inoculation of PSB was evident on P uptake by A. hookeri Th. plants, which might be due to the better availability of soluble P. Maximal P content in shoots and roots was shown by in treatments with K. quasipneumoniae (S6C2) in un-amended TCP soil, where P content was respectively 235.2% and 202.5% higher than control. Earlier, Kaur and Reddy (2013) reported the efficiency of Pantoaea cyanipiedii PSB- 3 to enhance the total P uptake in shoots by 20.0% and in roots by 22.22%, in maize plant. Another PSB, Pseudomonas plecoglossicida PSB- 5 was reported to result in 29.92% and 41.73% higher P in shoot and root tissues respectively in maize plant. The P uptake was even better in bacterial treatments with TCP amended soil. In fact, the P content in shoot and root tissues were 403.9% and 294.9% higher, respectively, with E. asburiae S5C7 + TCP treatment. In earlier report, inoculation with bioinoculants of PSB (Bacillus polymyxa), showed the enhancement of nutrient (P) uptake by 31.61% by the plant pigeon pea (Cajanus cajan L.) over the control (Singh and Singh 2011). The inoculation with the P-solubilizing bacteria Pseudomonas putida showed significantly increased P content of the plant Stevia rebaudiana Bertoni when comparison to the control (Vafadar et al. 2014). Similar work has been reported in maize plant that the inoculation with Enterobacter radicicantus and Pseudomonas fluorescens increased the P uptake by maize plant by 1.86% and 1.49% respectively (Krey et al. 2013). Duarah et al. (2011) investigated phosphate solubilizing bacteria Erwinia tasmaniensis TP08, Pseudomonas aeruginosa TP16 and Pseudomonas aeruginosa TP373 among the plant growth promoting rhizobacteria (PGPR) used as biofertilizers for plant growth and nutrient (P) use efficiency on yard-long bean (Vigna unguiculata) plant by 65.21%, 86.95% and 30.43% respectively. Some workers have reported similar observations in other plants with TCP (Mamta et al. 2010), such as, Walpola and Yoon (2013) reported that shoot and root had respectively 26.02% and 155.05% higher P with the treatment of Pantoea agglomerans in TCP amended soil. Gupta et al. (2012) also observed the similar results in Aloe vera plant, where 52.41% higher P was recorded in plant treated with TCP + Enterobacter hormaechei. The PGPR (Bacillus subtilis HJ3) associated with the maize (Zea mays L.) from the region of Himalayan region showed the maximum total P contents both in shoot and root by 488.57% (Zahid et al. 2015). Also, Turan et al. (2012) demonstrated a similar connection among the inoculation of PGPR’s Bacillus subtilis OSU142 and Bacillus megaterium M3 has growth stimulatory effect that reflected by higher P concentrations in most plant organs- leaves (30% and 139% respectively) and roots (140% and 300% respectively). Similarly, treatment with rhizobacterial strain S7 from rhizospheric region of field grown runner bean (Phaseolus coccineus L.) significantly increased the grain yields with 15.03% and yield increase can be attributed with the nutrient (P) uptake by the plants and other plant activities (Stefán et al. 2013). Mäder et al.(2011) found that mutualistic root microorganisms such as plant growth promoting rhizobacteria (PGPR) Pseudomonas jessenii R62 and Pseudomonas syxanthana R81 enhanced the P concentration of the plant by 45.32%, in this way, improves the plants fitness and growth responses to plants like Wheat (Triticum aestivum L.), with the inoculation of PGPR. The bacteria, by increasing the volume and root development, increased the plant’s access to nutrients and water, thereby attracting the plant nutrients. Finally, the plants nutrients’ uptake increased the plant shoot growth (Davoodi-fard et al. 2012).

Further, agronomic P-use efficiency and relative efficiency of phosphorus (APE and REP) was recorded maximum with the application of K. quasipneumoniae S6C2 with the proportionate amount of TCP (107.17%). Mäder et al. (2011) reported that the inoculation with Pseudomonas strains (P. jessenii and P. syxanthana) increased the P-use efficiency of 95% which means efficiently taken up from the soils of the wheat plants. Kumar and Chandra (2008) obtained enhanced P uptake of 38% in lentil crops inoculated with Pseudomonas diminuta. Neto et al. (2016) reported that agronomic P-use efficiency varied among coffee cultivars, E16Shoa, E22Sidamo, Lémon and Acaiá cultivars were classified as the most efficient and responsive to Pi supply. Enhancement of APE and REP with the application of PSB in TCP amended soil provide evidences that these PSB, including K. quasipneumoniae S6C2 and A. luteolus S4C7 have potential for commercialization and may be utilized in systematic cultivation of A. hookeri Th.
Conclusions

The results of present study provide substantial evidence that P-solubilizers (A. luteolus S4C7, K. pneumoniae – S4C9, S4C10, S6C1, E. asburiae SSC7, and K. quasi-pneumoniae S6C2) isolated from A. hookeri Th. rhizosphere, facilitate availability of free P in soil, when applied with TCP, and also enhance the growth of plant. The effect of inoculation of PSB with TCP was more pronounced to enhance the growth of host plant which indicated the possibility of integrated nutrient management in A. hookeri Th. The significant increase in the P content of PSB treated plants emphasizes the potential of an economically and eco-friendly means of achieving higher levels of phosphorus. Therefore, these P-solubilizers can be used as a plant biofertilizer and may be used to develop an economic and eco-friendly cultivation strategy for A. hookeri Th.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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