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Solubilization of Inorganic Rock Phosphate by Rhizobacteria of *Allium hookeri* Thwaites and Influence of Carbon and Nitrogen Sources Amendments

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Phosphate solubilizing bacterial strains (*Arthrobacter luteolus* S4C7, *Klebsiella pneumoniae* S4C9, *K. pneumoniae* S4C10, *Enterobacter asburiae* S5C7, *K. pneumoniae* S6C1 and *K. quasipneumoniae* S6C2) were isolated from the rhizospheric soil of *Allium hookeri* Thwaites. All the isolates were proved to be positive for rock phosphate (RP) solubilization at different concentration (0.5%, 1.0% and 1.5%). *K. pneumoniae* S4C10 was found to be most efficient as 81.6 μg/ml of soluble phosphate when amended with 1% of RP, followed by *K. quasipneumoniae* S6C2 where soluble phosphate release was 76.8 μg/ml in 0.5% RP amended medium. Also, maximum solubilization was noted to correlate with decrease in p^H of the medium. Strain *A. luteolus* S4C7 liberated small amount of P as compared to other strains. The process of phosphate solubilization was optimized for different carbon sources. Fructose was preferred as best carbon source by *K. pneumoniae* S4C10 with 85.6 μg/ml of solubilized P in NBRIP broth medium. However, after fructose, glucose also proved to be best carbon source by *K. quasipneumoniae* S6C2 (83.2 μg/ml) and *K. pneumoniae* S6C1 (78.4 μg/ml) in the NBRIP medium. Among different nitrogen sources, di-ammonium sulphate was found to be best for phosphate solubilization by the strain *K. pneumoniae* S6C1 (151.2 μg/ml).

Keywords: Phosphate solubilizing bacteria; rock phosphate; carbon; nitrogen.

Phosphorus (P) is an essential plant nutrient and plays a key role in plant growth and development, energy transport, signal transduction, macromolecular biosynthesis, photosynthesis, respiration, nutrient uptake, and nitrogen fixation[1]. It is the world's most second largest nutritional supplement for crops after nitrogen. Although, most agricultural soil have large amount of inorganic and organic P, these are immobilized and mostly become unavailable. Hence, very limited concentration of P is available to plants due to P deficient in soil[2]. P deficiency decreased agricultural productivity on more than 2 billion hectares worldwide and therefore, agronomic treatments to improve P availability in the soil and to increase P utilization in agriculture are of special importance[3]. But, the concentration of soluble P in soil solution is in the range of 100- 400 g P/ha[4]. Chemical fertilizers are extensively used in traditional agriculture, but their use increases the production costs and environmental risks[5]. Excessive use of chemical fertilizers leads to the harmful damage to soil structure, composition, microflora and other properties of soil. They can cause environment hazard and expensive as well[6,7].

Natural rock phosphates are the raw

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materials for production of phosphate fertilizers in sustainable agriculture system through microbial solubilization. Rock phosphate could play a significant role as cheaper P source to plants. However, it cannot be used in field because most of P in RP is present as non-exchangeable form, which is not directly available to plant uptake. One approach for solubilization in field condition is the application of rock phosphate as phosphate fertilizer along with activity of soil microorganisms (PSB) can be effective[8].

There are several genera of bacteria reported as Phosphate solubilizing bacteria (PSB) involved in the conversion of insoluble phosphate to soluble e.g. *Pseudomonas, Mycobacterium, Micrococcus, Bacillus, Flavobacterium, Rhizobium, Mesorhizobium, Sinorhizobium, Klebsiella, Arthrobacter, Enterobacter, Erwinia,* etc. and have been reported to modify P nutrition and increase its solubilization in soil through many process, such as decrease the p^H of the soil by producing organic and mineral acids, phytohormones, chelation and siderophores production which promote P solubilization in soil[9,10,11,12].

Allium hookeri Thwaites is a member of family *Alliaceae* subgenus *Amerallium*, known to content its flavour content and therapeutic properties[13,14,15]. In this context, this study was designed to evaluate the P solubilization of insoluble RP of different concentration by the indigenous phosphate solubilizing bacteria from the rhizosphere of *A. hookeri* Th. and utilization of different carbon sources by PSB strains.

MATERIALS AND METHODS

Bacterial strains

A. luteolus (S4C7), *Enterobacter asburiae* (S5C7), *Klebsiella pneumoniae* (S4C9, S4C10, S6C1) and *Klebsiella quasipneumoniae* (S6C2) isolated from the rhizospheric soil of *Allium hookeri* Thwaites growing as wild herbs in Manipur, India at latitude of 23°83´N - 25°68´N longitude of 93°03´E - 94°78´E, were used in this study and identified through a comparison of the 16S rDNA sequences with GeneBank accession number KX603401 (S4C7), KX603398 (S4C9), KX603397 (S4C10), KX603399 (S5C7), KX603402 (S6C1) and KX603400 (S6C2). These PSB strains showed solubilizing ability of tricalcium phosphate by forming clear halo zone around the colony[16]. All these phosphate solubilizing bacterial species were maintained in Pikovskaya agar. All the cultures were revived and sub-cultured before use in the present study.

Quantitative estimation of RP solubilization under in vitro conditions

Strains of 24- 48 hours old cultures grown in NBRIP (National Botanical Research Institute of Phosphate) broth shaken (128 rpm) at 30±1°C were used for quantitative estimation of RP. Rock phosphate was supplemented at different concentration (0.5%, 1.0% and 1.5% to 100 ml of NBRIP broth) as a phosphorus source instead of TCP. Quantitative estimation of phosphorus in supernatant was measured by vanado-molybdate-yellow color method in NBRIP broth [10g $C_6H_{12}O_6$, 0.1g $(NH_4)_2SO_4$, 0.25g $MgSO_4.7H_2O$, 0.2g KCl, 5.0g $MgCl_2.6H_2O$, 5g $Ca_3(PO_4)_2$ in 1L distilled water][17]. 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 colour 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 ìg/mL over control. The p^H of culture supernatants was measured in each case.

Effect of carbon sources on solubilization of TCP in NBRIP broth

To study the effect of carbon sources on the growth and phosphate solubilizing activity of bacterial isolates, glucose was replaced with an equal amount (10 g/L) of sucrose, maltose, fructose, galactose or mannitol added to the NBRIP broth. The flasks were incubated at 30°C shaken at 120 rpm for 24- 48 hours. The media was analysed for soluble P and p^H reduction.

Effect of nitrogen sources on solubilization of TCP in NBRIP broth

To study the effect of nitrogen sources on the growth and phosphate solubilizing activity of bacterial isolates, $(NH_4)_2S0_4$ was replaced with an equal amount of (10g/L) of Sodium nitrate ($NaNO_3$) and urea added to the NBRIP broth. The flasks were kept on the shaker incubator at 120 rpm for 24-48 hours. The media was analysed for soluble P and p^H reduction by centrifugation the sample at 10000 rpm for 20 minutes and to 0.5 ml of

supernatent, add 2.5 ml of Barton's reagent, volume was made to 50 ml with de-ionized water. The absorbance of the resultant colour was read after 10 min at 430 nm in UV/Visible Spectrophotometer.

RESULTS AND DISCUSSION

Rock phosphates have been considered as a valuable alternative source for soluble P fertilizers especially for acid soil. But, unfortunately direct application of RP into the soils under alkaline condition is not always agronomically effective due to its low reactivity[18]. Research efforts are in progress to manipulate these rocks and convert them to value-added product by using chemico-physical means partially acidulating RP by using phosphate-solubilizing microorganisms[19]. In this present study, the PSB strains has the ability to solubilize the inorganic phosphate with indication of showing clear halo zone around the colony in Pikovskaya agar plate. Phosphate solubilization index (PSI) was calculated by the formula as described by Sharma *et al*.[20] (Fig.1).

$$PSI = \frac{\text{Solubilization diameter}}{\text{Colony diameter}} \times 100$$

It is well known that many microorganisms isolated from the soil are able to dissolve different kinds of rock phosphate in liquid culture[21,22,23].The PSB isolates were able to solubilize insoluble RP in different concentration 0.5%, 1.0% and 1.5% in NBRIP broth and the maximum amount of P was recorded 24 hours with 81.6µg/ml at 1% of RP and 79.2µg/ml at 0.5% of RP by *K. pneumoniae* S4C10 (Table. 1), followed by *K. quasipneumoniae* S6C2 released solubilized P of 76.8% at 0.5% of RP (Table.2), then *K. pneumonia*e S6C1 (72.8 µg/ml of P at 0.5%) (Table.3)> *E. asburiae* S5C7 (56.2 µg/ml of P at 1.5%) (Table.4)> *K. pneumoniae* S4C9 (44.8 µg/ml of P at 0.5%) (Table.5)> *A. luteolus* S4C7 (33.6 µg/ml of P at1%) (Table. 6) compared with uninoculated broth served as control. The p^H reduction also observed corresponds to the respective increase in soluble P concentration. Kaur and Reddy[24] showed the isolates *Pantoea cypripedii* PSB-3 and *Pseudomonas plecoglossicida* PSB-5 are efficient for mineralizing inorganic phosphate with the accompaniment of decrease in the p^H of the culture. A significant relationship between quantities of phosphate solubilized and drop in p^H of culture filtrate in RP amended medium[25]. The combined of PSB (MRS 18 and MRS 34) and RP produced significant of amount of solubilized P release capacity (P mineralization) with reduction of p^H in the broth. It also substantially increased microbial abundance in rhizosphere compared to all individual treatments (T_0- T_8) confirmed the stimulating effect of PSB on increasing microbial population in the rhizosphere that improved P solubilization and enhanced plant growth promotion[26]. The solubilization of inorganic phosphate by PSB was accompanied by a significant drop in p^H in the liquid medium and it is correlated that the decrease in p^H with the high phosphate solubilization[27]. Reddy *et al*[28]., investigated that the PSMs *Aspergillus niger* and *Aspergillus tubingensis* capable of solubilizing of poorly soluble rock phosphates when grown in the presence of 2% of rock phosphate and can provide an efficient large scale biosolubilization of rock phosphates intented for P fertilizers. The arbuscular mycorrhizal fungi *Glomus intraradices* utilized more soluble phosphorus from soil mineral phosphate than non-inoculated plants. With the amendment of RP, the inoculated *G. intraradices* significantly stimulated plant growth and P content and affects the microbial activity in hyphosphere of *Acacia holosericea* plant[9]. The selected PSB strains (genera *Burkholderia* and *Bacillus*) isolated from the rhizosphere of maize are able to solubilize RP (Araxá and Itafós phosphate) extracted from the RP mine, Brazil with the reduction of p^H which suggests that the acidification of the culture medium can be one of the mechanisms involved in the solubilization of P[29].

Effect of carbon sources on phosphate solubilization by bacterial isolates

The bacterial isolates were evaluated in the presence of five carbon sources by replacing glucose with sucrose, maltose, fructose, galactose and mannitol. The form available carbon greatly affected the growth as well as the phosphate solubilization and was more active in presence of hexoses and pentoses or disaccharides[30]. The role of carbon source is important in phosphate solubilization was affected by the carbon source[31]. All the six strains demonstrated diverse levels of phosphate solubilization activity in the presence of various carbon sources. Among the six phosphate

solubilizing bacterial strains, *K. pneumoniae* S4C10 showed most efficient strain with the maximum P released with fructose of 85.6 µg/ml in NBRIP broth medium (Table 7). Mardad *et al*[32]., reported that fructose was found to be best carbon sources of 112.46% and 104.25% of soluble phosphate liberated by *Enterobacter* sp. PSB6 and Bacterium DR172 PSB5 respectively in NBRIP broth medium as compared with the positive control *Acitenobacter sp*. and maximum drop in p^H also reported due to the organic acids secreted to the medium. Fructose has been identified as the best carbon source for *Rhodotorula minuta* NCIM 3359 and *Saccharomyces cerevisiae* ATCC 9896 in cultures[33]. After *K. pneumoniae* S4C10, the strain *K. quasipneumoniae* S6C2 showed PS activity in all the carbon sources test, but the efficiency varied with the carbon source. This strain showed maximum PS activity of 83.2 µg/ml in glucose containing NBRIP medium, followed by maltose and sucrose containing medium. Hameeda *et al*[34]., reported the bacterial isolates from the different composts, farm waste compost (FWC), rice straw compost (RSC), Gliricidia vermicompost

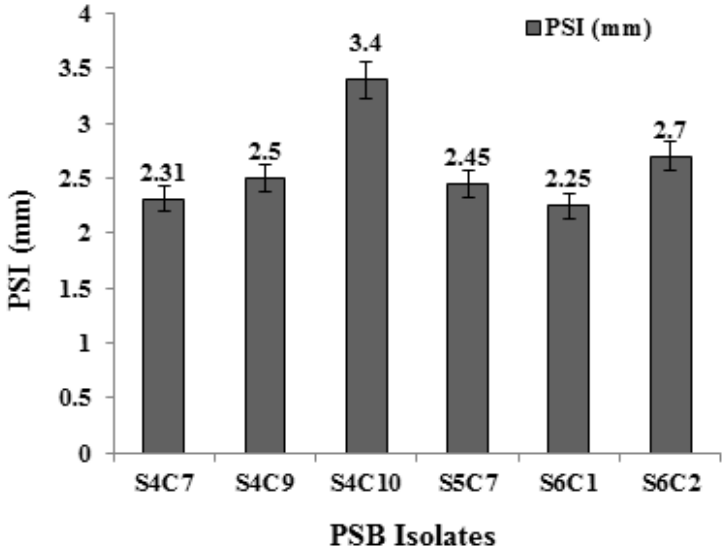

Fig. 1. Solubilization effciency of PSB isolates obtained from the rhizosphere of *A. hookeri* Thwaites plant

Table 1. Soluble P and p^H reduction by *K. pneumoniae* S4C10 in NBRIP broth having rock phosphate (equivalent to 100 mg P_2O_5/100 ml) as the sole phosphate

Time intervals	[a]Uninoculated broth	p^H	Concentration of RP [b]0.5%	p^H	[c]1.0%	p^H	[d]1.5%	p^H
24	28.8±3.0	5.89±0.1	52.00±2.0	5.87±0.5	68.00±2.0	5.8±0.2	49.5±4.0	5.92±0.3
48	23.4±2.0	5.85±0.4	79.2±4.0	5.72±0.4	81.6±1.0	5.61±2.0	64.8±2.0	5.73±0.5
72	15.2±5.0	6.19±0.3	44.8±4.0	5.96±0.3	32.8±2.0	6.08±0.4	30.4±2.0	6.12±0.5
96	20.8±4.0	6.10±0.5	50.4±2.0	5.91±0.4	33.6±2.0	6.11±0.5	34.4±5.0	6.14±0.4
120	17.6±2.0	6.13±0.3	47.2±1.0	5.88±0.1	31.2±1.0	6.05±2.0	31.2±2.0	6.12±1.0
144	15.2±5.0	6.23±0.5	32.8±1.0	6.08±0.5	19.2±1.0	6.24±1.0	24.0±4.0	6.44±0.5
168	26.4±4.0	6.00±0.1	53.6±2.0	5.83±0.2	36.00±3.5	6.01±0.3	40±1.0	6.37±0.3

Values are mean±SD, n=3.
a, b, c, d indicates the value of solubilized P in µg/ml

(GVC) and macrofauna, showed rock phosphate (RP) solubilization in buffered medium in plate culture. The strains *Enterobacter cloacae* EB27, *Serratia marcescens* EB67, *Serratia sp.* EB75, *Pseudomonas sp*. BW75 showed solubilized RP in RP broth medium. In the presence of different carbon sources, both strains showed a drop in p^H and solubilized RP, P released was maximum with glucose (1212 and 522 µmol) by *Serratia marcescens* EB67 and *Pseudomonas* sp. CDB35 respectively. Earlier, phosphate solubilizing bacteria NBRI0603, NBRI2601, NBRI3246 and NBRI4003 isolated from the rhizosphere of chickpea and alkaline soils shows diverse levels of phosphate solubilization activity under in vitro conditions in the presence of various carbon sources. Xylose, lactose, xylose and glucose reported to be the best carbon sources for phosphate solubilization by strains NBRI0603, NBRI2601, NBRI3246 and NBRI4003 respectively[35]. Sridevi *et al*[36]., revealed that the isolates *Rhizobium* species from *Crotalaria species* (*C. juncea*, *C. laburnifolia*, *C. retusa* and *C. verrucosa*) are able to solubilize tricalcium phosphate (TCP) and glucose was found to be best carbon source for P solubilization among the other carbon sources. *Rhizobium sp*. from *C. retusa* and *C. verrucosa* showed maximum solubilization at 3% concentration of glucose. Maximum decrease in p^H also revealed in glucose containing medium. Another *B. subtilis* and *B. cereus* also reported as efficient for phosphate solubilization in different carbon sources viz. Glucose, sucrose, lactose and mannitol. However, incorporation of glucose increased the rate of

Table 2. Soluble P and pH reduction by *K.quasipneumoniae* S6C2 in NBRIP broth having rock phosphate (equivalent to 100 mg P2O5/100 ml) as the sole phosphate

Time intervals	[a]Uninoculated broth	p^H	Concentration of RP [b]0.5%	p^H	[c]1.0%	p^H	[d]1.5%	p^H
24	13.6±4.0	6.24±1.0	57.6±2.0	5.81±0.1	61.2±2.0	5.83±0.1	45.6±4.0	6.09±2.0
48	16.8±3.0	6.2±1.0	76.8±4.0	5.54±0.3	70.4±2.0	5.62±2.0	52.8±2.0	5.88±0.1
72	25.6±4.0	6.03±0.3	45.2±4.0	6.09±0.6	32.8±2.0	6.06±2.0	20.8±4.0	6.22±1.0
96	23.2±4.0	6.05±0.4	49.2±1.0	6.03±0.5	37.6±3.4	6.02±0.3	23.2±4.0	6.24±0.2
120	32.4±5.0	6.06±0.2	44.8±4.0	6.11±0.5	34.4±2.0	6.05±2.0	16.8±5.0	6.32±0.4
144	16.0±3.0	6.25±0.5	29.6±4.0	6.28±0.5	24.0±4.0	6.22±0.5	13.6±4.0	6.27±0.7
168	20.0±3.0	6.12±0.5	49.6±3.0	6.06±0.5	40.0±1.0	6.01±0.3	26.0±3.0	6.08±0.4

Values are mean±SD, n=3.
a, b, c, d indicates the value of solubilized P in µg/ml.

Table 3. Soluble P and pH reduction by *K. pneumoniae* S6C1 in NBRIP broth having rock phosphate (equivalent to 100 mg P2O5/100 ml) as the sole phosphate

Time intervals	[a]Uninoculated broth	p^H	Concentration of RP [b]0.5%	p^H	[c]1.0%	p^H	[d]1.5%	p^H
24	13.6±4.0	6.24±1.0	61.6±5.0	5.77±0.5	60.8±1.0	5.76±0.5	44.8±3.0	6.01±0.4
48	16.8±3.0	6.2±1.0	72.8±4.0	5.7±0.5	68.4±2.0	5.68±0.5	55.2±2.0	5.85±0.5
72	25.6±4.0	6.03±0.3	38.4±2.0	6.09±0.3	34.4±5.0	6.11±1.0	17.6±2.0	6.21±0.5
96	23.2±4.0	6.05±0.4	46.4±4.0	5.99±0.3	40.0±1.0	6.07±0.8	22.4±4.0	6.11±0.4
120	32.4±5.0	6.06±0.2	28.4±4.0	6.21±1.0	38.0±2.0	6.08±0.5	34.0±3.5	6.11±0.4
144	16.0±3.0	6.25±0.5	32.0±2.0	6.05±0.5	26.0±4.0	6.15±0.5	18.4±1.0	6.19±0.6
168	20.0±3.0	6.12±0.5	41.6±1.0	6.0±0.3	42.4±4.0	6.03±0.3	22.4±2.0	6.18±0.4

Values are mean±SD, n=3.
a, b, c, d indicates the value of solubilized P in µg/ml.

Table 4. Soluble P and pH reduction by *Enterobacter asburiae* S5C7 in NBRIP broth having rock phosphate (equivalent to 100 mg P2O5/100 ml) as the sole phosphate

Time intervals	Concentration of RP							
	[a]Uninoculated broth	p^H	[b]0.5%	p^H	[c]1.0%	p^H	[d]1.5%	p^H
24	13.6±4.0	6.24±1.0	44.8±2.0	5.89±0.1	45.6±2.0	5.81±0.4	52.0±2.0	5.87±0.3
48	16.8±3.0	6.2±1.0	51.2±4.0	5.88±0.3	55.2±3.0	5.95±0.3	56.8±2.0	5.83±0.3
72	25.6±4.0	6.03±0.3	17.2±1.0	6.18±0.4	19.2±3.0	6.24±0.3	23.2±1.0	6.05±0.4
96	23.2±4.0	6.05±0.4	20.8±3.0	6.12±0.5	25.2±4.0	6.04±0.5	29.6±4.0	6.1±0.5
120	32.4±5.0	6.06±0.2	16.8±5.0	6.25±0.5	22.4±4.0	6.11±1.0	23.2±3.6	6.05±0.2
144	16.0±3.0	6.25±0.5	24.8±4.0	6.09±0.2	28.8±2.0	6.02±0.4	30.4±2.0	6.12±0.3
168	20.0±3.0	6.12±0.5	19.2±4.0	6.22±0.5	23.2±4.0	6.09±0.4	26.4±4.0	6.22±0.4

Values are mean±SD, n=3.
a, b, c, d indicates the value of solubilized P in µg/ml.

Table 5. Soluble P and pH reduction by *K. pneumoniae* S4C9 in NBRIP broth having rock phosphate (equivalent to 100 mg P2O5/100 ml) as the sole phosphate

Time intervals	Concentration of RP							
	[a]Uninoculated broth	p^H	[b]0.5%	p^H	[c]1.0%	p^H	[d]1.5%	p^H
24	13.0±4.0	6.23±0.2	23.2±2.0	6.05±0.4	14.4±4.0	6.21±0.4	16.8±2.0	6.2±0.2
48	13.6±4.0	6.24±0.2	26.4±2.0	6.0±0.2	12.8±3.0	6.2±0.4	14.4±5.0	6.21±1.0
72	20.8±3.0	6.1±0.3	44.8±4.0	5.96±0.1	23.3±4.0	6.05±0.4	23.2±4.0	6.05±0.5
96	19.2±3.0	6.24±0.4	32.0±1.0	6.06±0.4	31.6±2.0	6.09±0.3	32.8±2.0	6.08±0.4
120	20.8±3.0	6.11±0.5	32.8±5.0	6.08±0.9	20.8±4.0	6.1±0.3	26.0±4.0	6.02±0.4
144	13.6±4.0	6.22±0.5	36.8±1.0	6.01±0.2	26.4±2.0	6.0±0.3	28.8±4.0	6.18±0.2
168	15.2±5.0	6.23±0.2	22.4±2.0	6.05±0.2	18.4±2.0	6.19±0.5	20.0±3.0	6.11±0.4

Values are mean±SD, n=3.
a, b, c, d indicates the value of solubilized P in µg/ml.

Table 6. Soluble P and pH reduction by *Arthrobacter luteolus* S4C7 in NBRIP broth having rock phosphate (equivalent to 100 mg P2O5/100 ml) as the sole phosphate

Time intervals	Concentration of RP							
	[a]Uninoculated broth	p^H	[b]0.5%	p^H	[c]1.0%	p^H	[d]1.5%	p^H
24	13.0±4.0	6.23±0.2	13.6±5.0	6.25±0.2	13.2±5.0	6.24±0.2	13.6±5.0	6.24±0.2
48	13.6±4.0	6.24±0.2	16.8±5.0	6.19±0.4	31.2±2.0	6.01±0.2	21.6±2.0	6.06±0.2
72	20.8±3.0	6.1±0.3	25.6±4.0	6.03±0.3	21.6±2.0	6.06±0.4	25.6±4.0	6.03±0.1
96	19.2±3.0	6.24±0.4	23.2±4.0	6.05±0.4	14.4±5.0	6.2±0.3	23.2±4.0	6.05±0.3
120	20.8±3.0	6.11±0.5	32.4±2.0	6.0±0.1	25.6±4.6	6.04±0.3	33.6±2.0	6.0±0.4
144	13.6±4.0	6.22±0.5	16.0±5.0	6.24±0.2	16.0±5.0	6.25±0.4	23.2±4.0	6.05±0.4
168	15.2±5.0	6.23±0.2	20.0±2.0	6.12±0.2	25.6±4.0	6.03±0.4	16.8±5.0	6.2±0.2

Values are mean±SD, n=3.
a, b, c, d indicates the value of solubilized P in µg/ml.

Table 7. Effect of different carbon sources of P solubilization

Strains	Carbon sources											
	Glucose	p^H	Sucrose	p^H	Maltose	p^H	Fructose	p^H	Galactose	p^H	Mannitol	p^H
S4C7	13.6±4.0	6.23±0.7	18.8±5.0	6.13±0.1	29.6±4.0	6.12±0.3	36.8±3.5	6.09±0.3	10.4±5.0	6.88±0.4	6.8±5.0	6.69±0.5
S4C9	54.4±2.0	5.81±0.3	56.8±2.0	5.79±0.2	48.8±3.0	5.88±0.3	58.4±1.0	5.79±0.4	55.2±2.0	5.80±0.1	47.2±3.0	5.91±0.4
S4C10	63.2±2.0	5.72±0.3	57.6±2.0	5.80±0.3	70.4±1.0	5.73±0.5	85.6±1.0	5.65±0.4	73.6±2.0	5.86±0.2	58.4±4.0	5.79±0.5
S5C7	55.4±2.0	5.83±0.4	28.8±3.0	6.08±0.2	32.4±3.0	6.08±0.5	45.6±5.0	5.89±0.2	62.8±4.0	5.75±0.2	46.0±4.0	5.92±0.3
S6C1	78.4±2.0	5.69±0.4	70.4±1.0	5.81±0.4	72.0±2.0	5.65±2.0	81.6±2.0	5.70±0.3	63.2±4.0	5.72±0.2	67.2±1.0	5.87±0.4
S6C2	83.2±2.0	5.65±0.3	74.4±3.0	5.85±0.4	78.4±3.0	5.54±0.3	63.2±2.0	5.72±0.2	73.2±4.0	5.63±0.3	73.2±1.0	5.83±0.5

Values are mean±SD, n=3

solubilization of phosphate[37].

Effect of nitrogen sources on phosphate solubilization by bacterial isolates

All the strains behave differently in the presence of different nitrogen sources. The efficiency of three different nitrogen sources on PS activity was studied. Phospho-solubilization is related to the excretion of protons (H^+) that accompanies the breathing on the assimilation of NH_4^+ [38]. Bacterial cultures increased their solubilzation when ammonium was added[39,40].

The PSB strains utilized different nitrogen sources tested viz. Sodium nitrate, di-ammonium sulphate and urea. Di-ammonium sulphate supported maximum phosphorus solubilization (151.2 µg/ml and 144.0 µg/ml) by the strains *K. pneumoniae* S6C1 and *E. asburiae* S5C7 respectively with concomitant decrease in p^H (Table 8). While sodium nitrate is utilized by the strains *K. pneumoniae* S4C9 (66.4 µg/ml) and *K. quasipneumoniae* (50.4 µg/ml) for solubilization of P. *B. subtilis* showed the ability of phosphate solubilization activity in different nitrogen sources with insoluble form of phospahte sources like tricalcium phospate and rock phosphate. *B. subtilis* utilized ammonium sulphate at highest level among other nitrogen sources both in TCP and RP supplemented medium where the p^H drifted from the neutral to acidic with all carbon source in presence of TCP[41]. Prabhavati and Mallaiah[42] supported our result where the strain *Rhizobium* HGR19 isolated from the root nodules showed maximum PS activity containing ammonium sulphate in the medium. Kumar and Ram[43] reported The rhizobial strains (*Sinorhizobium sp*. MRR101, *Agrobacterium tumifaciens* MRR102, *Rhizobium sp*. 103, *Sinorrhizobium kostiense* MRR104, *Agrobacterium tumefaciens* MRR105, *Rhizobium sp*. MRR106) isolated from the root nodules of *Vigna tribolata* plants utilized different nitrogen sources for the solubilization of phosphorus. *Sinorrhizobium kostiense* MRR104 and *Rhizobium sp*. MRR106 reported to be high phosphate solubilization in ammonium sulphate containing in PVK medium. Reduction in p^H also reported in the strains when nitrogen sources are used.

Nahas[44] investigated most of the phosphate solubilizing microorganisms are heterotrophs and depends on carbon and energy sources that can be found in the rhizosphere or by recycling crop

Table 8. Effect of different nitrogen source of P solubilization

Strains	Sodium nitrate	p^H	Di-ammonium sulphate	p^H	Urea	p^H
S4C7	46.4±3.0	5.79±0.2	129.6±3.0	5.31±0.1	6.4±4.0	6.8±0.5
S4C9	66.4±4.0	5.68±0.3	128.0±2.0	5.35±0.2	9.6±4.0	6.78±0.4
S4C10	36.0±2.0	6.08±0.5	140.8±2.0	5.25±0.3	7.2±5.0	6.82±0.3
S5C7	26.4±4.0	6.17±0.5	144.0±4.0	5.22±0.1	54.4±3.0	5.72±0.2
S6C1	42.4±4.0	5.81±0.2	151.2±1.0	5.07±0.2	10.4±4.0	6.83±0.5
S6C2	50.4±5.0	5.75±0.2	120.0±1.0	5.35±0.2	7.2±5.0	6.85±0.3

Values are mean±SD, n=3

residues. In addition, nitrogen sources may be considered as control factors as they influence microorganisms growth and consequently their solubilization capacity. Qualitative analysis of the phosphate solubilized by various groups correlated well with grouping based upon quantitative analysis of bacteria isolated from soil and effect of carbon and nitrogen sources[45].

CONCLUSIONS

This study has revealed that, most of the isolates released more soluble P in all different concentration of rock phosphate (RP) and phosphate solubilizing bacteria were efficient in P solubilization with the decrease of p^H in the medium. *K. pneumoniae* S4C10 and *K. quasipneumoniae* S6C2 are the most potential strains among the other PSB strain. Also, addition of fructose or glucose in the NBRIP medium by *K. pneumoniae* S4C10 and *K. quasipneumoniae* S6C2 showed maximum P release. The contribution of these strains, individually or in combination, can increase the P nutrition, growth and yield of the plants.

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