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# Phosphate Solubilizing *Gluconacetobacter* sp., *Burkholderia* sp. and their Potential Interaction with Cowpea (*Vigna unguiculata* (L.) Walp.)

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**Abstract:** Eighty-one potential phosphate solubilizing bacteria isolated from rhizosphere soil were screened for their Mineral Phosphate Solubilizing (MPS) ability on Pikovskaya and National Botanical Research Institute's Phosphate (NBRIP) medium. The majority of the isolates exhibited a strong ability to solubilize hydroxyapatite in both liquid and solid media. The solubilization in liquid medium corresponded with a decrease in the pH of the medium. Two bacterial strains exhibiting high solubilization of Tricalcium Phosphate (TCP) in Pikovskaya liquid cultures were identified as *Gluconacetobacter* sp. and *Burkholderia* sp. on the basis of phenotypic features, whole cell Fatty Acid Methyl Ester (FAME) profiles, 16S rDNA typing and carbon Substrate Utilization (SU) using Biolog GN2 plates. Seed inoculation of cowpea by these novel phosphate solubilizers improved nodulation, root and shoot biomass, straw and grain yield and phosphorus and nitrogen uptake of the crop. The dehydrogenase, phosphatase and the available P contents of the soil were stimulated by the inoculation with the phosphate solubilizing bacteria. Among the bacterial strains best effect on yield was obtained with *Burkholderia* sp.

**Key words:** Phosphate solubilization, *Gluconacetobacter* sp., *Burkholderia* sp., FAME profiles, 16S rDNA typing

## INTRODUCTION

Phosphorus (P) is one of the major plant nutrients, lack of which limits plant growth. Most agricultural soil contain large reserves of total P, commonly in the range of 200-5,000 mg P $kg^{-1}$ with an average of 600 mg P $kg^{-1}$ and a part of P accumulates depends on regular application of chemical fertilizer (Fernandez *et al*., 2007). Both P fixation and precipitation occur in soil because of the large reactivity of phosphate ions with numerous soil constituents (Alikhani *et al*., 2006). Interest has been focused on the inoculation of phosphate solubilizing microorganisms (PSM) into the soil so as to increase the availability of native fixed P and to reduce the use of fertilizers (Illmer and Schinner, 1992). Many Phosphate Solubilizing Bacteria (PSB) belonging to the *Pseudomonas*, *Bacillus*, *Rhizobium*, *Agrobacterium*, *Achromobacter*, *Micrococcus*, *Aerobacter*, *Enterobacter*, *Flavobacterium* and *Erwinia* genera have been isolated from soil (Rodriguez and Fraga, 1999; Gulati *et al*., 2007). These bacteria can grow in media containing calcium phosphate complexes as the sole source of P, solubilze a large proportion of P, assimilate it and release in higher amounts. Phosphate solubilizing microorganisms brings about mobilization of insoluble phosphates in the soil and increase plant growth under conditions of poor phosphorus availability (Tripura *et al*., 2007). Soil enzyme activities indicate the potential of the soil to support biochemical processes essential for the maintenance of fertility of soil. The determination of enzyme activities in conjunction with soil respiration and composition of the soil microflora provide the most reliable index of microbial activity (Singh and Rai, 2004). The phosphate

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solubilization ability of microorganisms has been employed for improving soil microbial activities and crop yield in agriculture and horticulture (Kapoor *et al*., 1989; Rodriguez and Fraga, 1999). Soil of Kerala are highly P fixing and the P solubilizing organisms have significant role in solubilizing the insoluble P in the soil and hence P nutrition of plants. This is much relevant when the P is applied as insoluble form such as rock phosphate. Efficient P solubilizers have been obtained from the soil of Kerala through extensive isolation programmes (Sivaprasad and Meenakumari, 2005). Keeping in view of the role played by these organisms an attempt was made to isolate and screen new and efficient phosphate solubilizing bacteria from the rhizosphere of crop plants and to characterize these organisms with the help of molecular tools. The selected strains were further utilized for the plant growth promotion using cowpea (*Vigna unguiculata* (L.) Walp.) as test crop under green house conditions.

## MATERIALS AND METHODS

### Isolation and Screening of Phosphate Solubilizing Bacteria

Soil samples were collected from the rhizosphere of different crop plants of Kerala, India. A total of 29 composite soil samples (pH 4.5-6.0), were used for the isolation of phosphate dissolving bacteria. Pikovskaya medium was used for the isolation, cultivation and maintenance of phosphate solubilizing bacteria (Gaur, 1990). One hundred milliliter of Pikovskaya and NBRIP broth (Nautiyal, 1999) containing 0.5% tricalcium phosphate (TCP) was inoculated with 500 µL bacterial culture (inoculum adjusted to ~ $5\times10^8$ cfu $mL^{-1}$). All the flasks were maintained at 30±2°C for 14 days with intermittent shaking twice a day. Uninoculated medium served as control and each experiment was done in triplicate set. The soluble $P_2O_5$ in the supernatant solution was determined by vanadomolybdo phosphoric yellow colour method (Jackson, 1973). The final pH of the medium was recorded using a digital pH meter (Spectronic 20-D). A standard phosphate solubilizing bacteria (*Pseudomonas striata*) obtained from division of microbiology, Indian Agricultural Research Institute, New Delhi was used as a reference strain.

### Phenotypic Characterization of Selected Isolates

The selected strains were subjected to cultural, morphological, biochemical and physiological characterization as mentioned in Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). The bacterial strains were further characterized by their metabolic fingerprints using BIOLOG GN2 plates. The plates perform 95 discrete tests simultaneously and the test results yield a characteristic pattern of Substrate Utilization (SU) called as metabolic fingerprint. The C sources include aminoacids, carbohydrates, carboxylic acids and polymers together with a number of miscellaneous compounds. Pure cultures grown on Biolog Universal Growth agar for 24 h were suspended in sterile saline (0.85% NaCl) to a final optical density ($OD_{600}$) of 0.28 for Gram negative bacteria using BIOLOG turbidometer. BIOLOG microtitre plates were inoculated with 150 µL of the bacterial suspensions per well and incubated at 28°C for 48 h. The plates were read automatically with the BIOLOG Microstation (MicroLog-3, 4.01B, BIOLOG Inc., Hayward CA) and results were determined. BIOLOG Microplate data were normalized by the Average Well Colour Development (AWCD) as described by Garland and Mills (1991). A positive response was taken to an $OD_{595}$ 25% greater than that of the control well. The SU patterns were investigated using AWCD as a covariable.

### Whole Cell Fatty Acid Methyl Ester (FAME) Analysis

The bacterial colonies were grown on tryptic soy agar, incubated at 28°C for 24 h and approximately 30 mg fresh weight of cells were harvested. Cellular fatty acids were extracted and derivatized to their FAME profiles according to the procedures described already (Poonguzhali *et al*., 2006). FAME analysis was performed by the Microbial Identification System (MIDI) TSBA50

method version 5 (Microbial ID Inc., Newark, DE, USA). All samples were analyzed with a Hewlett-Packard 6890 series Gas Chromatography system containing a HP-ultra 2 column [diphenyl-dimethyl-siloxane (5:95) co-polymer]. The FAME data were analyzed by Sherlock 4.50 MIDI TSBA50 5.00 library and the bacteria were identified based on a similarity (SIM) Index value.

**16S rDNA Typing**

DNA extraction was carried out as previously described by Rivas *et al*. ( 2001). Amplification of 16S rDNA was performed using 8f-(5 AGAGTTTGATCCTGGCTCAG 3) and 1492r (5 TACGGATACCTTGTTAGCACTT 3) primers. The total volume of PCR reaction mixture was 50 μL, comprising 200 μM dNTPs, 50 μM each primer, 1x PCR buffer, 3 U Taq DNA polymerase and 100 ng genomic DNA. The thermocycling procedure involved an initial denaturation at 94°C for 4 min, followed by 35 cycles at 94°C for 1 min, 52°C for 1 min, 72°C for 2 min and a final extension at 72°C for 8 min. The gel purified 16S rDNA was ligated to PCR-TRAP cloning vector and transformed in *E. coli*. The sequence of the insert was determined using the automated DNA sequencing service provided at Institute of Microbial Technology (IMTECH), Chandigarh, India. The sequences were analyzed using the gapped BLASTn (www. ncbi.nlm.nig.gov) search algorithm and aligned to their nearest neighbours.

**Pot Culture Experiment**

Loamy soil (pH: 6.1, Electrical conductivity: $0.1\times10^3$ mhos $cm^{-1}$, Organic carbon: 1.3%, Available phosphorus: 8.8 kg $ha^{-1}$ and Available potassium: 11 kg $ha^{-1}$) collected from the agricultural fields of Kottayam district, Kerala, India was used to evaluate the effect of inoculation of selected strains of phosphate solubilizing bacteria on yield and uptake of nutrients by cowpea. The surface soil collected from the top 15 cm layer was dried in shade, passed through 2 mm sieve and filled in earthern pots (180×160 mm) at the rate of 5 kg $pot^{-1}$. Fertilizers at the rate, urea: 32.5 kg $ha^{-1}$, muriate of potash: 21.38 kg $ha^{-1}$ and FYM@: 250 kg $ha^{-1}$ were weighed separately for each pot and added to soil as basal dose before sowing. The seeds were mixed with *Rhizobium japonicum* culture and rice water for two hours, dried in shade and used for sowing. Two milliliter of the 3 day old culture (inoculum adjusted to ~ $5\times10^8$ cfu $mL^{-1}$) of phosphate solubilizing bacteria were poured over the seeds. The treatments include, (1): Control (no inoculation and no fertilizer application), (2): Superphosphate (30 kg $P_2O_5$ $ha^{-1}$), (3): Rock phosphate (60 kg $P_2O_5$ $ha^{-1}$), (4): *Gluconacetobacter* sp., (5): *Burkholderia* sp., (6): *Pseudomonas striata*, (7): *Gluconacetobacter* sp.+RP, (8): *Burkholderia* sp. +RP and (9): *Pseudomonas striata* +RP. The experiments were conducted in a randomized block design with three replications under green house conditions (natural illumination, temperature 28-30°C and humidity 80%). When plants were established, total number of plants in each pot was thinned to three. The plants were watered regularly to maintain optimum moisture regime. Rhizosphere soil were collected from each pot at 30 days interval and used for enzyme assays and available soil P. Crops were harvested at maturity.

Soil dehydrogenase activity was measured colorimetrically using Triphenyl Tetrazolium Chloride (TTC). The enzyme activity was expressed in terms of formazan produced and the quantity of formazan was calculated from a standard graph using triphenyl formazan in methanol (Casida *et al*., 1964). Similarly soil phosphatase activity was measured following the method of Tabatabai and Bremner (1969) by using *p*-nitrophenyl phosphate as the substrate. Available P content of soil was tested after each 30 days by Bray's method (Jackson, 1973).

The plants were subjected to the following observations for plant growth parameters; dry matter yield, number of seeds, dry weight of seeds, number of pods, dry weight of pods and nodule weight. Samples of leaf, shoot and pods of all treatments were dried and powdered separately for the purpose of chemical analysis. Estimation of nitrogen in plant samples were done by Kjeldahl digestion method

and total nitrogen in the samples was determined colorimetrically using Technicon Nitrogen Autoanalyser (Tecator Kjeltec 1030 Auto Analyser, Sweden). For total phosphorus estimation in plant materials, the plant matter was digested with triacid mixture on a hot plate and the volume of the digested material was made up to 100 mL with distilled water. The contents of the flasks were filtered to remove the silica. Phosphorus was estimated by taking a suitable aliquot filtrate by the vanadomolybdate phosphoric yellow colour method (Jackson, 1973).

Statistical analysis was performed by Analysis of Variance (Two way and One way ANOVA). As post test the means were compared by Tukey multiple comparison test.

## RESULTS

In the present investigation, 81 potential phosphate solubilizing bacteria were isolated by plating enriched rhizosphere soil samples on to Pikovskaya agar medium. All the isolates were screened for their phosphate solubilizing potential in Pikovskaya and NBRIP broth. The bacterial isolates showed significant differences in their phosphate solubilizing potential, their extend of solubilization ranged between 11.38-73.97 mg/100 mL of $P_2O_5$ in liquid medium. The P solubilizing ability of the isolates were evidently visible on plates of Pikovskaya agar, where it produced a clear halo zone. A significant decline in the pH of medium was observed during the solubilization of tricalcium phosphate. Among the isolated strains, two bacterial isolates (PSB 12 and 73) showed promising results and selected for further analysis.

PSB 12 was isolated from the rhizosphere soil of rubber and PSB 73 was from vanilla rhizosphere. PSB 12 was able to solubilize 73.97 mg/100 mL of $P_2O_5$ in liquid medium and reduced the pH of the medium to 3.6 after 14 days of incubation, while PSB 73 solubilized 68.95 mg/100 mL of $P_2O_5$ and reduced the pH to 4.3, respectively. The phosphate solubilizing ability of both the strains were found to be superior to that of standard *Pseudomonas striata* (50.12 mg/100 mL, pH 4.5).

### Identification of Selected Strains

The selected phosphate solubilizing bacterial isolates showed differences in their morphological, biochemical and physiological characteristics. Both strains were Gram negative, aerobic, motile, nonsporing, catalase positive rods. PSB 73 found to produce a brownish pigment. Both the strains were able to utilize glucose, mannitol, mannose and arabinose as their C source. But assimilation of inositol and galactose was only detected in PSB 73. Out of 95 C-source used in Biolog identification system, PSB 12 was able to utilize 19 substrates while PSB 73 assimilated 40 substrates as their sole source of carbon. On the basis of above cultural, morphological, biochemical and physiological characteristics the PSB 12 and PSB 73 were identified as *Gluconacetobacter* sp. (MTCC 8368) and *Burkholderia* sp. (MTCC 8369), respectively.

FAME analysis showed subtle differences in the composition of cell wall fatty acids of two isolates. Both isolates exhibited a specific fatty acid composition, making it a microbial fingerprint. The MIDI system identified PSB 12 as *Acetobacter liquefaciens* (SIM = 0.837) and PSB 73 as *Burkholderia gladioli* (SIM = 0.361). The 16S rDNA genes of both strains were amplified by PCR reaction and products were resolved in 2% agarose gel. Partial 16S rDNA sequences (830 base pairs) of PSB 12 and complete sequence (1388 letters) of PSB 73 were obtained after sequence analysis. The results of BLAST search of 16S rDNA sequences of the PSB 12 compared with the available 16S rDNA sequences in the Gene bank database indicated that *Gluconacetobacter* sp. was the closest related species. The sequence of PSB 73 showed maximum similarity to *Burkholderia* sp.

### Effect of PSB Isolates on Soil Enzymatic Activities

The dehydrogenase activities of the cowpea growing soils were checked at every 30 days intervals till harvest (Table 1). Maximum activity was shown by the soil inoculated with *Pseudomonas striata* +RP at 60 days after sowing (95.36 µg TPF $g^{-1}$ soil/day) where as the least

Table 1: Effect of PSB inoculation on soil dehydrogenase, phosphatase and available phosphorus level of potted soil

| | DAS | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Dehydrogenase activity (μg TPF $g^{-1}$ soil/day) | | | Phosphatase activity (μg PNP $g^{-1}$ soil) | | | Available phosphorus level (kg $ha^{-1}$) | | |
| Treatments | 30 | 60 | 90 | 30 | 60 | 90 | 30 | 60 | 90 |
| Control | 10.58 | 11.93 | 10.45 | 6.14 | 7.40 | 6.5 | 9.53 | 10.34 | 10.23 |
| RP | 22.96 | 26.32 | 25.35 | 7.16 | 10.21 | 9.61 | 10.18 | 11.94 | 10.60 |
| SP | 33.87 | 35.31 | 34.56 | 9.49 | 13.56 | 11.38 | 20.70 | 21.00 | 18.32 |
| *Gluconacetobacter* sp. | 51.88 | 54.48 | 52.73 | 11.96 | 17.69 | 12.56 | 14.00 | 20.79 | 14.06 |
| *Burkholderia* sp. | 42.20 | 45.63 | 35.89 | 14.27 | 18.44 | 16.82 | 15.88 | 21.28 | 16.04 |
| *Pseudomonas striata* | 52.72 | 64.27 | 60.29 | 16.80 | 21.20 | 17.73 | 16.50 | 21.22 | 17.62 |
| *Gluconacetobacter* sp.+RP | 57.22 | 62.74 | 61.94 | 13.83 | 19.47 | 13.90 | 17.40 | 21.64 | 15.67 |
| *Burkholderia* sp.+RP | 70.85 | 77.88 | 74.85 | 15.56 | 20.65 | 18.02 | 20.98 | 25.93 | 19.79 |
| *Pseudomonas striata* +RP | 88.42 | 95.36 | 87.99 | 16.8 | 23.17 | 20.41 | 21.65 | 26.13 | 19.58 |
| Mean | 47.85 | 52.65 | 49.33 | 12.44 | 16.86 | 14.10 | 16.31 | 20.03 | 15.76 |
| Source | CD (5%) | | | CD (5%) | | | CD (5%) | | |
| Duration | 1.66 | | | 0.33 | | | 0.63 | | |
| Treatments | 2.87 | | | 0.58 | | | 1.08 | | |
| Interaction | 4.98 | | | 1.01 | | | 1.88 | | |

activity was shown by the control at 90 days after sowing (10.45 μg TPF $g^{-1}$ soil/day). Compared to the first month dehydrogenase activity of all soil increased in the second month and again decreased at the third month.

Phosphatase activity of the cow pea growing soil were noted after each 30 days (Table 1). Soil samples inoculated with *Burkholderia* sp. with or without RP showed more phosphatase activity than soil sample inoculated with *Gluconacetobacter* sp. At 30 days of incubation *Pseudomonas striata* with and without RP showed same activity (16.80 μg PNP $g^{-1}$ soil) immediately followed by *Burkholderia* sp. +RP. At 60 days and 90 days also activity of standard culture was only slightly higher than *Burkholderia* sp. Here also phosphatase activity was found to decrease with the maturation of the crop.

**Available P Content in Soil**

The available $P_2O_5$ content of the soil was increased due to PSB inoculation as compared to the uninoculated controls (Table 1). The higher accumulation of available P in the inoculated series indicated that introduced microorganisms establish themselves and proliferated. At 30 days after sowing the maximum available P content (21.65 kg $ha^{-1}$) was encountered in *Pseudomonas striata* +RP treated soil while in control it was 9.53 kg $ha^{-1}$. After 60 days also the highest available P content (26.13 kg $ha^{-1}$) was found with the same treatment, while it was decreased to 19.58 kg $ha^{-1}$ at the time of harvest.

**Yield Parameters**

The activity of PSB exerted a considerable influence on cowpea as shown by increased dry matter yield, dry weight of seeds, number of pods and nodule weight (Table 2). Maximum dry matter yield was shown by the treatments inoculated with *Pseudomonas striata* and RP (32.60 g). Number of seeds and dry weight of seeds are highest to *Burkholderia* sp.+RP (8.66 and 0.069 g). Number of pods was same for *Burkholderia* sp. +RP and *Pseudomonas striata* +RP (8.33). Dry weight of pods is maximum to *Pseudomonas striata* +RP (0.266 g). Maximum nodule weight was shown by the treatments inoculated with *Gluconacetobacter* sp.+RP (2.43 g).

**N and P Uptake**

The data in Table 3 show that the application of microbial cultures with or without RP substantially increased the nitrogen and phosphorus uptake by plants. At the time of harvest

Table 2: Effect of PSB inoculation on different yield parameters of cowpea

| Treatments | Dry matter yield (g) | No. of seeds | Dry weight of seeds (g) | No. of pods | Dry weight of pods (g) | Nodule weight (g) |
|---|---|---|---|---|---|---|
| Control | 3.60 | 5.00 | 0.032 | 2.000 | 0.116 | 0.220 |
| RP | 4.66 | 5.00 | 0.034 | 2.330 | 0.121 | 0.530 |
| SP | 4.66 | 5.00 | 0.034 | 2.330 | 0.093 | 0.330 |
| *Gluconacetobacter* sp. | 21.16 | 6.66 | 0.057 | 4.660 | 0.173 | 2.030 |
| *Burkholderia* sp. | 30.66 | 8.33 | 0.061 | 6.330 | 0.200 | 1.830 |
| *Pseudomonas striata* | 31.66 | 7.66 | 0.060 | 6.000 | 0.186 | 1.560 |
| *Gluconacetobacter* sp.+RP | 21.68 | 6.66 | 0.058 | 6.330 | 0.220 | 2.430 |
| *Burkholderia* sp.+RP | 31.50 | 8.66 | 0.069 | 8.330 | 0.230 | 2.100 |
| *Pseudomonas striata* +RP | 32.60 | 8.56 | 0.068 | 8.330 | 0.266 | 1.800 |
| Mean | 20.24 | 6.83 | 0.048 | 5.180 | 0.178 | 1.420 |
| CD (5%) | 1.55 | 1.02 | 0.045 | 1.191 | 0.196 | 0.445 |

Table 3: Nitrogen and phosphorus uptake by cowpea as influenced by PSB inoculation

| | Leaves | | Shoot | | Pods | |
|---|---|---|---|---|---|---|
| Treatments | N uptake | P uptake | N uptake | P uptake | N uptake | P uptake |
| | (mg pot$^{-1}$) | | | | | |
| Control | 36.160 | 4.07 | 51.620 | 1.440 | 0.802 | 0.020 |
| RP | 49.800 | 5.13 | 64.060 | 2.190 | 1.267 | 0.060 |
| SP | 56.000 | 8.29 | 78.690 | 3.150 | 1.630 | 0.060 |
| *Gluconacetobacter* sp. | 97.630 | 10.22 | 103.640 | 6.720 | 8.032 | 0.310 |
| *Burkholderia* sp. | 338.950 | 20.87 | 241.710 | 9.300 | 8.634 | 0.500 |
| *Pseudomonas striata* | 313.920 | 21.63 | 176.430 | 14.920 | 9.125 | 0.580 |
| *Gluconacetobacter* sp.+RP | 105.580 | 15.76 | 116.270 | 8.040 | 5.859 | 0.500 |
| *Burkholderia* sp.+RP | 521.320 | 31.20 | 274.920 | 11.350 | 12.306 | 0.800 |
| *Pseudomonas striata* +RP | 345.170 | 27.03 | 247.620 | 14.920 | 11.645 | 0.620 |
| Mean | 207.170 | 16.03 | 150.550 | 8.000 | 6.580 | 0.380 |
| CD (5%) | 1.295 | 0.49 | 1.753 | 0.328 | 0.060 | 0.133 |

highest N and P uptake was shown by *Burkholderia* sp.+RP treatment except in case of P uptake of shoot and least value was shown by control.

## DISCUSSION

Phosphorus solubilizing microorganisms brings about mobilization of insoluble phosphates in the soil and increase plant growth under conditions of poor phosphorus availability. These microorganisms also hold the potential of ecological amelioration of P and there by improving growth and establishment of plants under low phosphorus availability. These beneficial bacteria enhance plant growth by improving soil nutrient status, secreting plant growth regulators and suppressing soil borne pathogens (Gulati *et al*., 2007). The genus *Burkholderia* have nitrogen fixing ability, secretes phytohormones, ACC deaminase, solubilizes phosphates and is antagonistic to phytopathogens (Pandey *et al*., 2005). Similarly, Loganathan and Nair (2003) reported a novel, salt tolerant, $N_2$-fixing and phosphate solubilizing *Gluconacetobacter* sp. Dehydrogenase is an intercellular enzyme related to the oxidative phosphorylation process, which is being used as an overall index of microbial activity (Kalam *et al*., 2004). Kamaleshkukhereja *et al*. (1991) noticed that the total microbial biomass and activity of dehydrogenase was significantly increased in plots that received 90 ha$^{-1}$ of farmyard manure continuously for over 20 years. Present data corroborate fully when dehydrogenase activity was seen to be stimulated in presence of microbial inoculants. The higher phosphatase activity recorded could be due to higher microbial activities recorded in terms of microbial biomass. The findings are in line with the observations made by Oberson *et al*. (1993). The increase in available P content may be due to the activities of introduced phosphate dissolving microflora, which might have dissolved the chemically fixed inorganic phosphate compounds or might have mineralized the organo-phosphatic compounds. Similarly, SP and RP treated soil also showed considerable amount of available P. The available P was decreased towards the maturation of crop. This might be attributed to greater uptake of this nutrient by the plant and chemical fixation of soluble P in soil (Dadhich *et al*., 2006). Seed and

soil inoculation with phosphobacteria was reported to improve the yield and nutrient uptake of various crops. The favourable effect of the inoculants on plant growth and nutrient uptake was due to the improved phosphate nutrition and production of growth promoting substances by PSB (Gaur, 1990). Increased dry matter yield of leguminous crops was reported by Bajpai (1965), Wani *et al*. (1979) and Khalafallah *et al*. (1982), who reported significant increase in dry matter of cowpea, gram and *Vicia faba*, respectively due to PSB inoculation. Highest yield parameters were shown by PSB inoculated and PSB and RP inoculated plants as compared to uninoculated control plants. PSB inoculation alone and in combination with chemical P-fertilizers was found effective and contributed significantly over control (Meshram *et al*., 2004). Increase in nodule number due to PSB inoculation is not only due to increased supply of phosphorus but also due to the direct effect of phosphorus on nodule bacteria. PSB stimulates the multiplication of rhizobia and was found conducive for the development of mobile forms which are essentially required to migrate through the soil towards the root system (White, 1953; Madhok, 1961). The supply of phosphorus to host plants also influences nodulation (Schreven, 1958). The availability of nutrients in soil in sufficient quantities for plant uptake greatly determines the growth and yield of crop plants. The higher yield of inoculated plants could be assigned to increased nutrient availability and uptake by plants. Increased nitrogen and phosphorus uptake in leaves, shoot and pods of cowpea was obtained as a result of PSB inoculation. Mohod *et al*. (1989) reported significant increase in nitrogen uptake of rice due to PSB inoculation. The phosphorus uptake of Bengal gram was more due to PSB inoculation (Gaur and Gaind, 1992). The application of rock phosphate along with phosphobacteria has also been reported as a possible substitute for super phosphate without any apparent reduction in crop yields (Gaur *et al*., 1980; Gyaneshwar *et al*., 2002). The use of a higher dose of rock phosphate coupled with efficient phosphate solubilizers helped in saving super phosphate. The results of the study clearly showed that microbial inoculation with novel PSB augment the efficiency of applied phosphorus source and thereby improving the yield and uptake of cowpea.

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