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Effect of Carbon and Nitrogen Source on Phosphate Solubilization and Impact of Phosphate Solubilizing Bacteria, Rock Phosphate and Organic Waste Treatments on Maize Plants

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Abstract Phosphate solubilizing bacteria *Bacillus subtillis, Bacillus circulans, Pantoea dispersa* and *Pseudomonas syringae* isolated from Rhizospheres soil of coal mines landfills of Chhattisgarh, India were studied for improvement of maize crop with and without RP and crop residue during field study under agroclimatic regions. The experiment included twenty four treatments (soil; Soil + RP; Soil + RP+ RS; Soil + RP+ SB; Soil + PSB 2; Soil + PSB 5; Soil + PSB 8; Soil+ PSB 9; Soil + BC; Soil + RP + PSB 2; Soil + RP + PSB 5; Soil + RP + PSB 8; Soil + RP + PSB 9; Soil + RP + BC; Soil +RP + RS + PSB 2; Soil + RP + RS + PSB 5; Soil + RP + RS +PSB 8; Soil + RP + RS + PSB 9; Soil + RP + RS + BC; Soil +RP+ SB +PSB 2; Soil+ RP+ SB +PSB 5; Soil +RP+ SB +PSB 8; Soil+ RP+ SB+ PSB 9 and Soil+ RP+ SB + BC). The application of these bacterial strains as bio inoculants showed significant effect on maize crop yield and soil fertility. Maize crop yield was increased to 18.2% with inoculation, 16.9% with rock phosphate and 22.3% when inoculation was done with rock phosphate and agricultural waste. The present study suggest that *Bacillus subtillis, Bacillus circulans, Pantoea dispersa* and *Pseudomonas syringae* along with RP fertilization play an important role in plant growth promotion and improvement of soil fertility.

Keywords: *phosphate solubilizing bacteria, rock phosphate, biofertilizer, bioinoculants, Maize*

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1. Introduction

Phosphorus is the major essential macronutrient of plants. In India most tropical soils are acidic in nature, rich in iron and deficient insoluble forms of phosphorus (P) and its deficiency is a severe constraint to crop production. Regular application of chemical fertilizers escalated soil problems such as structural degradation and adversely affecting the soil microbial population [1]. Naturally occurring rock phosphate (RP) in mines have been recognized as an alternative source of P. In India, almost 260 million tons of rock phosphate deposits in mines they are cheap and used as a good P fertilizer for crop production. This can be applied directly to the soil with varying agronomic efficiency depending on the type of soil and crop. PSB have the ability to convert insoluble low grade RP into soluble forms available for plant growth [2].

Organic farming is a decent technique for cultivating which basically targets the land and raising crops yield in such a way as to keep the soil fertility and good health by use of organic wastes. Along with beneficial microorganisms (bio-fertilizers) to release nutrients to crops from increased sustainable production in eco-friendly pollution free environment [3]. Crop residues consisting of cellulose and hemicellulose contain 53-75% carbohydrate. Cellulose is a polymer of glucose and hemicellulose consists of xylose, arabinose, glucose, galactose and mannose [4]. Phosphate solubilizing bacteria are known to utilize carbohydrates of crop residues. Therefore, phosphate solubilizing bacteria with multiple functional traits such as plant growth promotion and crop protection are preferred to enhance mineralization and decomposition of crop residues [5]. Inoculation of phosphate solubilizing bacteria showed improvement in growth and yield of different crop [6]. In present study was to evaluate the efficiency of PSBs strains alone and along with RP and organic waste on crop yield and soil fertility of maize crop.

2. Materials and Methods

2.1. Bacterial strains

Phosphate solubilizing bacterial strains PSB 2 (*Bacillus subtillis)*, PSB 5 (*Bacillus circulans)*, PSB 8 (*Pantoea dispersa*) and PSB 9 (*Pseudomonas syringae*) isolated from Rhizospheres soil of coal mines landfills of Chhattisgarh, India were used in this study. This isolation was selected based on their phosphate solubilizing activities and their efficiency to improve the soil fertility.

2.2. Effect of Carbon and Nitrogen Sources on PSBs

The effect of different carbon sources on the growth and phosphate solubilizing activity of bacterial isolates, glucose was replaced with an equal amount (10 gl^{-1}) of mannitol or xylose or galactose or arabinose sterilized separately and added to the Pikovskaya's broth [C source, 10.0 g; $(NH)_2SO_4$, 0.5 g; NaCl, 0.2 g; $MgSO_4.7H_2O$, 0.1 g; KCl, 0.2 g; Yeast extract, 0.5 g; $MnSO_4$, 0.1 mg; $FeSO_4.7H_2O$, 0.1 mg; tricalcium phosphate (TCP), 5.0 g (equivalent to 2.3 g P_2O_5); water, 1000 ml; pH 7.0±0.2]. Nitrogen sources were evaluated similarly by replacing ammonium sulfate with 0.5 gl^{-1}ammonium nitrate or ammonium chloride or ammonium bicarbonate or urea. The flasks were incubated at 37 °C under shaking for fifteen days. The media was analyzed for soluble P and pH reduction. The combination of carbon and nitrogen source in which the organism showed best solubilization was then used to test the solubilization of 5.0 g RPl^{-1} PVK (equivalent to 1.6g $P_2O_5l^{-1}$) by that particular organism.

2.3. Solubilization of Rock Phosphate (RP) in the Presence of Crop Residues

2.3.1. Preparation of Crop Residues

Two crop residue materials rice, viz., straw, Sugarcane bagasse and mixture of rice straw and sugarcane bagasse were soaked in water for two days and washed thoroughly to remove solid soil particles. It was over dried at 55°C and grinded with cross beater 0.5 mesh size.

2.3.2. Preparation of Water Extracted Rice Straw and Sugarcane Bagasse

About 250 g grinded rice straw and Sugarcane bagasse was dispensed into two liter conical flask containing 1000 ml of 1% NaOH and autoclaved at 121°C for 30 minutes. The supernatant was decanted and the residue was thoroughly washed with distilled water until coloring compounds were removed. The materials were dried at 60°C to constant weight.

2.4. Inoculums Formulation for Field Application with Respect to Phosphate Solubilization Efficiency

To check the effect of selected isolates on crop production and soil fertility, field experiments of maize crop were conducted at shanti nagar, bilaspur, Chhattisgarh, India. Field trials were conducted in a completely randomized block design. Each plot size was 3m × 3m ($9m^2$). The experiment contained 24 treatments consisted of: (Control: soil; Soil + Rock phosphate (RP); Soil + RP+ RS (rice straw); Soil + RP+SB (sugarcane bagasse)), PSBs was added to treatment (Soil + PSB 2; Soil+ PSB 5; Soil + PSB 8; Soil+ PSB 9; Soil + bacterial consortium (BC)), rock phosphate was added to treatment (Soil + RP + PSB 2; Soil + RP + PSB 5; Soil + RP + PSB 8; Soil + RP + PSB 9; Soil + RP + BC), the same amount of rice straw and sugarcane bagasse residue was added to treatment (Soil +RP + RS + PSB 2; Soil + RP + RS + PSB 5; Soil + RP + RS +PSB 8; Soil + RP + RS + PSB 9; Soil + RP + RS + BC), (Soil +RP+ SB +PSB 2; Soil+ RP+ SB +PSB 5; Soil +RP+ SB +PSB 8; Soil+ RP+ SB+ PSB 9; Soil+ RP+ SB + BC). During field study, maize was cultivated in the rainy season. Rock phosphate was amended in respective plots at the rate of 59 kg P_2O_5 ha^{-1} before seeding only once during maize cropping. Inoculums were added as a seed treatment. At the time of seeding, 100 g vermiculite having size of bacterial inoculums per maize seed was $7.5\text{-}8.5\times10^5$ cfu per seed. The plot were irrigated once before the sowing to ensure proper germination of seed and then regularly during crop growth as per agronomic practices. The maize crop was harvested after three months and various growth and yield parameters were measured. From plot, five randomly selected plants were uprooted and checked for shoot height, shoot weight, root weight, soil pH and total P.

3. Results and Discussion

3.1. Effect of carbon and nitrogen sources on solubilization of TCP by bacterial isolates

The bacterial isolates showed different solubilizing patterns in different carbon sources. The results showed that Bacillus subtillis (PSB 2) showed highest solubilization in presence of glucose followed by mannitol and presence of galactose the amount of soluble P and pH reduction was found to be minimum. *Bacillus circulans* (PSB 5) showed best solubilization and maximum pH reduction in presence of glucose followed by galactose as a C source and minimum in mannitol. *Pantoea dispersa* (PSB 8) showed highest P solubilization in presence of glucose followed by xylose as a C source and minimum solubilization in galactose. The maximum solubilization as well as pH reduction in case of *Pseudomonas syringae* (PSB 9) was in the medium having glucose followed by xylose as a C source and minimum in Galactose. When P soublilization by bacterial isolates with different N sources was tested. The results was found that all four isolates *Bacillus subtillis* (PSB 2), *Bacillus circulans* (PSB 5), *Pantoea dispersa* (PSB 8) and *Pseudomonas syringae* showed maximum P solubilization in media having urea. And pH reduction in other N sources was more than in Urea. *Bacillus circulans* (PSB 5) and *Pseudomonas syringae* (PSB 9) showed minimum P solubilization in media having ammonium chloride. In the case of *Bacillus subtillis* (PSB 2) showed minimum P solubilization in media having ammonium sulphate and Pantoea dispersa (PSB 8) showed minimum P solubilization in media having ammonium bicarbonate. Dave and Patel (2003) experimentally proved that N sources such as ammonium sulphate and ammonium nitrate were best for the solubilization of rock phosphate [7]. PSB strains were utilized the carbon and nitrogen sources but preferential is varied from strain to strain. This will greatly affect the P solubilization by PSBs [8]. The form of available carbon and nitrogen sources greatly affects the growth as well as the phosphate solubilization was more active in the presence of glucose as a carbon source and urea as nitrogen source [9]. Similar results were also reported by Mohan and Menon (2015) [10].

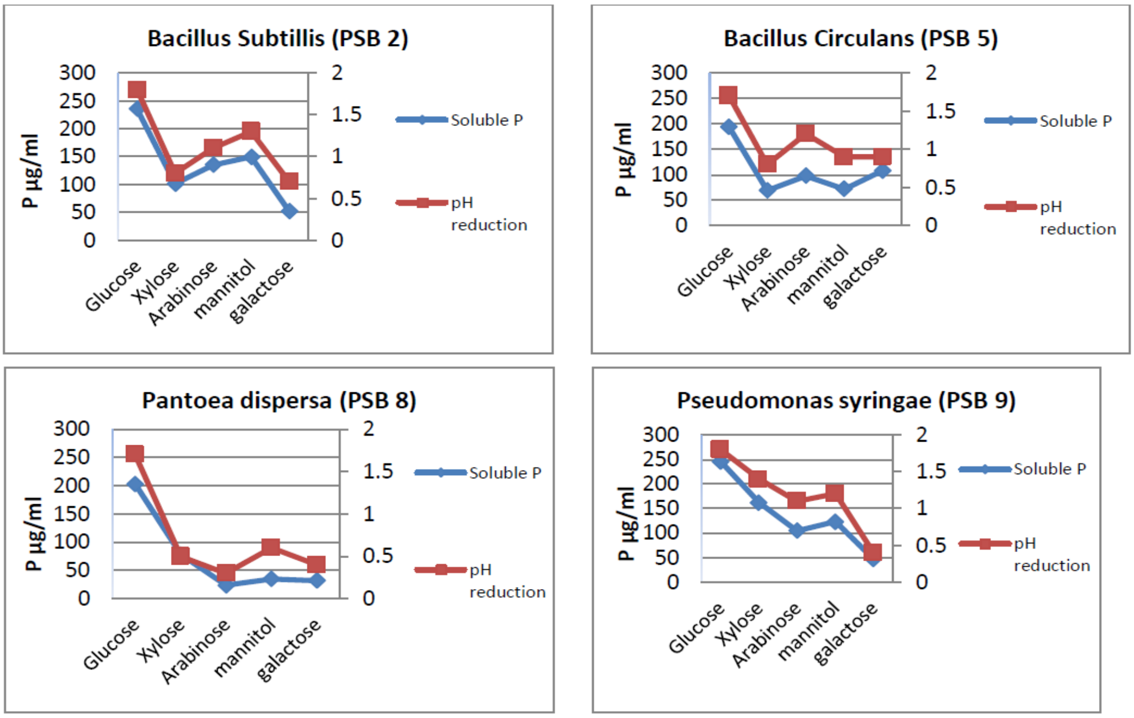

Figure 1. Effect of Carbon sources on phosphate solubilization solubilization by *Bacillus subtillis*, *Bacillus circulans*, *Pantoea dispersa* and *Pseudomonas syringae*

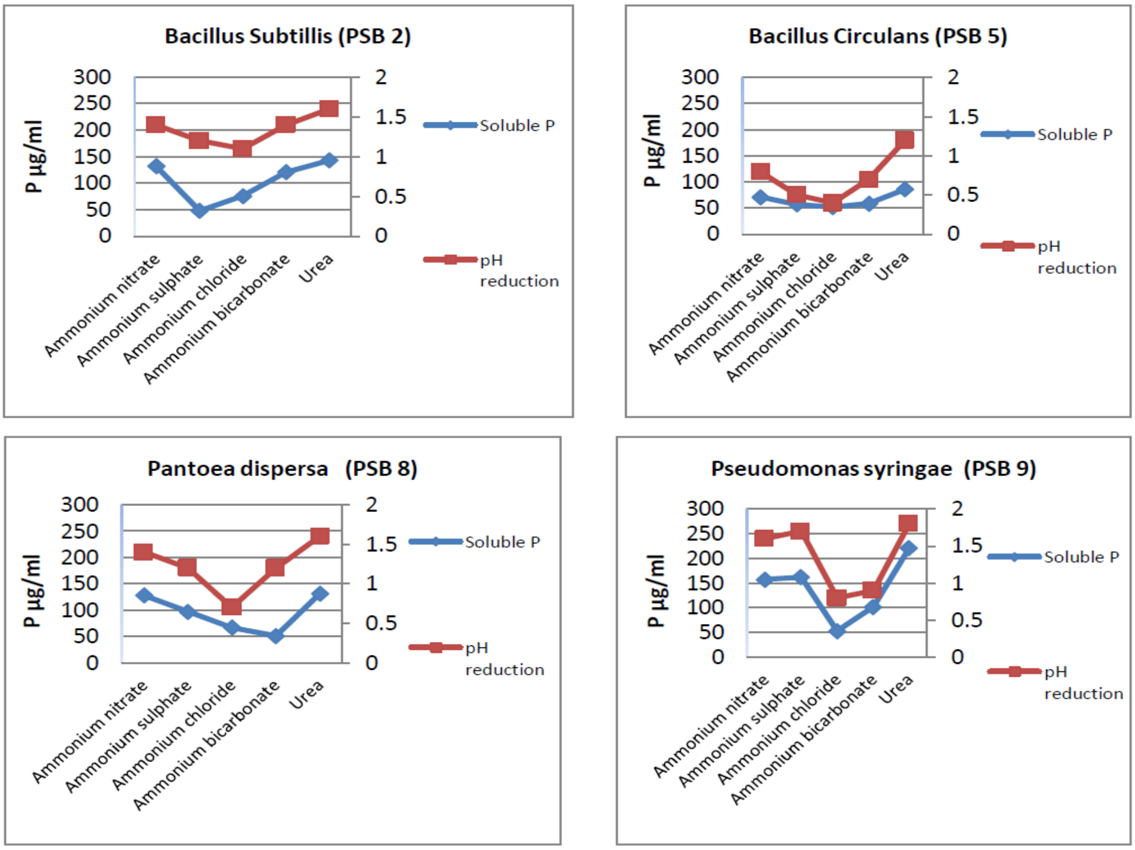

Figure 2. Effect of nitrogen source on phosphate solubilization by *Bacillus subtillis, Bacillus circulans, Pantoea dispersa* and *Pseudomonas syringae*

3.2. Solubilization of RP Using Best C and N Sources

The solubilization of RP (1% P_2O_5) was tested using best combination of C and N source for a particular isolate. The solubilization increased drastically as compared to PKV media (used during screening) when glucose and urea were used in media for all four isolates named *Bacillus subtillis, Bacillus circulans, Pantoea dispersa* and *Pseudomonas syringae* respectively as C and N sources (Table 1). The solubilization was same as observed during screening. There was no significant difference in the pH reduction by all the four isolates. The pH of the culture medium turned to acidic was indicated that o\production of organic acid by PSBs, which facilitate the solubilization of phosphate [11]. The maximum reduction of pH was recorded with *Pseudomonas syringae*. A fall in pH accompanied phosphate solubilization due to the production of organic acid, there is correlation between acidic pH and soluble phosphate. Medium pH tended to decrease in all cases of growth [12].

Table 1. Best combination of C and N source utilized by *Bacillus subtillis, Bacillus circulans, Pantoea dispersa* and *Pseudomonas syringae.*

C and N sources	Bacterial isolates	pH reduction	Soluble P (µg/ml)
Glucose + urea	*Bacillus subtillis*	1.3 ± 0.3	59.2±4.8
Glucose + urea	*Bacillus circulans*	1.7±0.2	76.4±2.1
Glucose + urea	*Pantoea dispersa*	1.4 ±0.2	66.3±3.4
Glucose + urea	*Pseudomonas syringae*	2.1±0.4	82.6±1.5

3.3. Field Application of Phosphate Solubilizing Bacteria in Organic Farming with Rock Phosphate

Organic farming is an eco-friendly system of farming which helps to maintain health of soil in terms of soil biological fertility and productivity. Crop production in organic farming mainly depends on nutrients released as a result of mineralization processes in soils [13]. An active soil microflora and a considerable pool of accessible nutrients are, therefore, important priorities in organic farming. Organic farming avoids the input of synthetic chemicals and their consequences. The build- up of a large and active soil microbial biomass is, therefore, critically important for sustaining the productivity of soils in organic farming system. A field experiment was conducted in which *Bacillus subtillis, Bacillus circulans, Pantoea dispersa* and *Pseudomonas syringae* isolates were inoculated alone or along with rock phosphate. Maize was selected as test crop for field experiments.

3.4. Field Study with Maize Crop

A field experiment of maize was conducted during study in an organic farming. The shoot height, shoot and root dry biomass and maize crop yield was significantly increased due to inoculation and inoculation along with rock phosphate compared to un-inoculation control soil (Table 2). Total phosphate in plant tissues (grains, shoots and roots) was increased significantly in inoculated and rock phosphate treatments compared to control soil. It was observed that plant growth parameters, maize crop yield, total phosphate uptake was significantly increased in all the inoculums, rock phosphate soil treatments, but the result were more pronounced when inoculation was done along with rock phosphate and agricultural waste (rice straw and sugarcane bagasse) than individual inoculation and control. Bacterial inoculation slightly decreased the soil pH in all the treatments compared to control in all field trials (Table 3). Maize crop yield was increased to 18.2% with inoculation, 16.9% with rock phosphate and 22.3% when inoculation was done with rock phosphate and agricultural waste (rice straw and sugarcane bagasse).

Table 2. Treatment consisted of: Growth parameters and phosphate uptake in maize plants as affected by rock phosphate and different Inoculums

Treatments	Shoot hight (cm)	Shoot dry weight (g)	Root dry weight (g)
Soil	211±3.2	29±1.2	9.7±0.11
Soil + PSB 2	214±3.4	31±1.9	9.8±0.14
Soil+ PSB 5	217±2.1	34±2.1	10.2±0.97
Soil + PSB 8	217±3.5	32±1.5	10.4±0.55
Soil+ PSB 9	220±3.2	36±2.3	10.7±0.63
Soil + bacterial consortium (BC)	223±4.2	41±2.1	11.3±0.94
Soil + Rock phosphate (RP)	219±3.0	32±2.2	10.3±0.41
Soil + RP + PSB 2	227±4.2	44±2.6	10.8±0.12
Soil + RP + PSB 5	230±3.4	54±2.7	11.9±0.72
Soil + RP + PSB 8	229±3.3	49±2.9	11.1±0.45
Soil + RP + PSB 9	238±4.1	54±2.2	12.7±0.11
Soil + RP + bacterial consortium (BC)	258±3.6	61±2.5	13.8±0.43
Soil + RP+ RS (rice straw)	224±4.5	41±2.3	10.1±0.63
Soil +RP + RS + PSB 2	237±4.9	49±1.2	11.2±0.38
Soil + RP + RS + PSB 5	247±5.2	56±1.5	13.2±0.56
Soil + RP + RS +PSB 8	242±4.7	52±2.3	12.6±0.12
Soil + RP + RS + PSB 9	253±5.3	64±1.7	13.5±0.23
Soil + RP + RS + bacterial consortium (BC)	261±5.4	67±2.6	14.4±0.85
Soil + RP+SB (sugarcane bagasse)	228±4.7	44±2.5	10.6±0.11
Soil +RP+ SB +PSB 2	231±5.4	51±1.2	11.9±0.44
Soil+ RP+ SB +PSB 5	244±5.2	59±2.6	13.7±0.57
Soil +RP+ SB +PSB 8	238±5.7	54±2.6	13.2±0.32
Soil+ RP+ SB+ PSB 9	249±5.4	67±1.8	14.3±0.98
Soil+ RP+ SB + bacterial consortium (BC)	264±5.9	70±2.2	15.6±0.90

Phosphate solubilizing bacterial population density was significantly improved due to inoculation, rock phosphate and agricultural waste (rice straw and sugarcane bagasse) along with bacterial inoculation compared to control treatments. population density of phosphate solubilizing bacteria was tested in rhizospheric soil of each plot after harvesting of the crop and it was 1.7×10^6 cfu/g in control treatment, $1.0\text{-}1.4\times10^8$ cfu/g in inoculums treatments, 3.2×10^6 cfu/g in rock phosphate alone and $1.6\text{-}2.1\times10^8$ cfu/g in inoculums along with rock phosphate, $2.2\text{-}2.7\times10^8$ cfu/g in inoculums along with rock phosphate and agricultural waste (rice straw and sugarcane bagasse).

there was a significant increase in population density of phosphate solubilizing bacteria in inoculums treatments along with rock phosphate and agricultural waste (rice straw and sugarcane bagasse) compared to other treatments. Field study of maize crop in organic farming showed a significant improvement in crop yield, total phosphate uptake and soil fertility. Khalimi *et al*. (2012) reported same results of increased yield of several crops [14].

Table 3. Soil characteristics of maize plants as affected by rock phosphate and different inoculums

Treatments	pH	Total P in (mg/kg)	Available P (mg/kg)
Soil	8.23±0.02	198±4	1.32±0.08
Soil + PSB 2	8.11±0.07	208±2	1.56±0.09
Soil+ PSB 5	8.02±0.03	217±7	2.01±0.05
Soil + PSB 8	8.02±0.05	205±6	1.89±0.02
Soil+ PSB 9	7.98±0.04	217±4	2.09±0.05
Soil + bacterial consortium (BC)	7.94±0.09	219±5	2.12±0.11
Soil + Rock phosphate (RP)	8.09±0.04	298±9	4.87±0.13
Soil + RP + PSB 2	8.03±0.05	374±5	5.11±0.10
Soil + RP + PSB 5	7.96±0.02	411±11	5.91±0.17
Soil + RP + PSB 8	7.98±0.07	404±7	5.45±0.11
Soil + RP + PSB 9	7.89±0.03	421±13	6.11±0.19
Soil + RP + bacterial consortium (BC)	7.66±0.02	445±9	6.45±0.22
Soil + RP+ RS (rice straw)	7.91±0.03	343±8	5.02±0.07
Soil +RP + RS + PSB 2	7.89±0.07	371±7	6.94±0.11
Soil + RP + RS + PSB 5	7.17±0.05	411±4	7.98±0.09
Soil + RP + RS +PSB 8	7.35±0.04	398±12	7.45±0.11
Soil + RP + RS + PSB 9	7.12±0.09	481±15	8.12±0.15
Soil + RP + RS + bacterial consortium (BC)	6.92±0.04	497±17	8.49±0.34
Soil + RP+SB (sugarcane bagasse)	7.97±0.03	358±9	5.16±0.12
Soil +RP+ SB +PSB 2	7.91±0.02	387±7	6.31±0.09
Soil+ RP+ SB +PSB 5	7.66±0.02	467±9	7.78±0.14
Soil +RP+ SB +PSB 8	7.78±0.07	439±9	7.04±0.17
Soil+ RP+ SB+ PSB 9	7.21±0.04	486±11	8.02±0.08
Soil+ RP+ SB + bacterial consortium (BC)	6.98±0.08	502±13	8.58±0.18

In the present study. Significant increase was found in biometric parameter (shoot height, shoot and root dry biomass) of maize plants with PSBs. The effect was more pronounced when PSBs inoculation was done along with RP. PSBs inoculation along with RP and organic waste showed a stimulatory effect on P uptake of plants. This might be due to better utilization of P from the pool of soil nutrient by the action of P solubilizing bacteria [15].

4. Conclusion

PSBs and organic waste effective in solubilizing RP by decreasing soil pH in treatment and increasing the organic matter into soil. Results of present study revealed that *Bacillus subtillis*, *Bacillus circulans*, *Pantoea dispersa* and *Pseudomonas syringae* significantly increase the yield and total P uptake in maize crop and improved the soil fertility during field study but the effect were more pronounced and significant when RP was supplemented along with organic waste as fertilizer.

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