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The Influence of Physicochemical Parameters on Phosphate Solubilization and the Biocontrol Traits of *Pseudomonas aeruginosa* FP6 in Phosphate-Deficient Conditions

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Abstract

Phosphorous deficiency is a major constraint to crop production due to the rapid binding of the applied phosphorous into fixed forms making it not available to the plants. The aim of this study is to isolate phosphate solubilizing bacteria, and assess their effect on the growth of cowpea plant. When tested for its phosphate solubilizing potential, *Pseudomonas aeruginosa* FP6, a known biological control agent against several phytopathogens, showed 52.38 % solubilizing efficacy. *P. aeruginosa* FP6 showed optimum phosphate solubilization with glucose (181.75 µg/ml) and ammonium sulphate (184.75µg/ml). The presence of soluble phosphates, with different concentrations of KH_2PO_4 supplemented in Pikovskaya agar media (PVK), suppressed tri-calcium phosphate (TCP) solubilization activity by FP6. When grown on Tris buffered phosphate medium *P. aeruginosa* FP6 showed reduction in biocontrol traits except hydrogen cyanide (HCN) production. In pot experiments *P. aeruginosa* FP6 inoculation with TCP soil amendment (100 mg/ kg) showed significant increase in biometric parameters suggesting that the application of *P. aeruginosa* FP6 along with the right dose of a phosphate fertilizer could be considered as a sustainable substitute to a higher dose of a phosphorus fertilizer for the cowpea cultivation.

Keywords: Phosphate solubilization, *P. aeruginosa* FP6, Cowpea, TCP

1. Introduction

Phosphorus (P) is one the most essential elements for plant growth after nitrogen. Phosphorus plays a significant role in several physiological and biochemical plant activities like photosynthesis, the metabolic process of energy transfer, signal transduction, macromolecular biosynthesis, and respiration chain reactions (Khan *et al.*, 2010). One of the advantages of feeding the plants with phosphorus is to create deeper and more abundant roots. Phosphorus causes early ripening in plants, decreasing grain moisture, improving crop quality and it is a sensitive nutrient to soil pH (Soleimanzadeh, 2010).

Despite its wide distribution, phosphorus is one of the least available and the least mobile mineral nutrients for plants in the soil (Mahdi *et al.*, 2012). Many soils have a high reserve of total phosphorus accounting for about 0.05% of soil content on average; however, only 0.1% of the total phosphorus is available to plants (Sharma *et al.*, 2011). Therefore, phosphatic chemical fertilizers that contain large amounts of soluble phosphorus have been applied to the agricultural fields to maximize the production (Shen *et al.*, 2011). This soluble phosphorus in phosphatic fertilizers is easily and rapidly precipitated to insoluble forms with cations such as Ca^{2+}, Fe^{3+}, Al^{3+}, Co^{2+} or Zn^{2+}, and is adsorbed to calcium carbonate, aluminium oxide, iron oxide, and aluminium silicate depending on the particular properties of the soil (Mittal *et al.*, 2008). This transformation decreases the efficiency of soluble phosphorus to be taken up by the plants, and decreases the effectiveness of the fertilizer resulting in the application of increasing amounts of phosphatic fertilizers in the agricultural fields. This unmanaged use of phosphatic fertilizers has increased the agricultural costs and also instigated negative environmental impacts (Karpagam and Nagalakshmi, 2014). Recently, phosphate solubilizing microorganisms have attracted the attention of agriculturists as soil inoculums to improve the plant growth and yield (Nisha *et al.*, 2014).

Phosphate solubilizing microbes play fundamental roles in biogeochemical phosphorus cycling in the natural and agricultural ecosystems. Phosphate-solubilizing microbes can transform the insoluble phosphorus into soluble forms HPO_4^{2-} and $H_2PO_4^-$ by acidification, chelation, exchange reactions, and polymeric substances formation (Mahidi *et al.*, 2011). Therefore, the use of phosphate-solubilizing microbes in agricultural practices would not only offset the high cost of manufacturing phosphatic fertilizers, but would also mobilize insoluble phosphorus in the fertilizers and soils to which they are applied (Tarkka *et al.*, 2008). Bacteria belonging to

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Mesorhizobium, Rhizobium, Klebsiella, Acinetobacter, Enterobacter, Erwinia, Achrobacter, Micrococcus, Aerobacter and *Bacillus* have been reported as phosphate solubilizers, but strains belong to Pseudomonads are considered as efficient phosphate solubilizers due to their biofertilizing and biocontrol properties (Baudoin *et al.*, 2010). The application of phosphate-solubilizing microbes around the roots of plants in soils, and in fertilizers has been shown to release soluble phosphorus, promote plant growth, and protect plants from pathogen infection (Walpola and Yoon, 2012).

2. Materials and Methods

2.1. Bacterial Strain

The phosphate producing bacterium was isolated from the rhizospheric soil of vegetable crops from in and around Bangalore, India. The potential isolate was identified as *P. aeruginosa* by 16S rDNA sequence analysis. The nucleotide sequence of the 16S rRNA of *P. aeruginosa* FP6 has been deposited in the GenBank database under the accession number JN861778 (Sasirekha *et al.*, 2013).

2.2. Phosphate Solubilisation in Different Media

Quantitative estimation of inorganic phosphate solubilization was done in six different types of liquid media reported in literature. Compositions of different media are given in Table 1 (Halder *et al.*, 1991; Kim *et al.*, 1997; Vassileva *et al.*, 1998; Nautiyal, 1999; Pikovskaya, 1948). The 250 mL flasks containing 50 mL of media inoculated with 500 µL bacterial culture (10^8 CFU/mL) was incubated at 28°C upto 7 days. Uninoculated media under a similar set of conditions was used as the control. An aliquot of 5 mL was collected every day and cells were removed by centrifugation at 7,500 x g for 10 min. The phosphorus content in culture filtrates was estimated by Fiske and subbarow method (Fiske and Subbarow, 1925). Phosphate solubilizing activity was expressed in terms of tricalcium phosphate (TCP) solubilization, which in turn represents µg/mL of available orthophosphate as calibrated from the standard curve of KH_2PO_4 (0-100 µg/mL). The pH variation in different media during the growth of *P. aeruginosa* FP6 isolate was also observed. The rest of the experiment was performed using Pikovskaya medium (PVK) with 0.5 % TCP.

A rock phosphate sample (RP-140) having a P_2O_5 (phosphorous pentoxide) content of about 18.8% was used. Quantitative estimation of the phosphate solubilization activity was carried out in a PVK medium amended with 0.5% (w/v) rock phosphate with the other conditions being the same as for TCP solubilization for duration of seven days.

Table 1. Composition of different media used in this study.

Media Components (g/L)	Medium1 (AYG; Halder *et al.*, 1991)	Medium2 (Kim *et al*., 1997)	Medium3 (Vassilev *et al.*, 1998)	Medium 4 (PVK; Pikovskaya, 1948)	Medium 5 (NBRIY; Nautiyal, 1999)	Medium 6 (NBRIP; Nautiyal, 1999)
Glucose	20	10	100	10	10	10
$(NH_4)_2SO_4$	1	-	-	0.5	0.5	0.1
$MgSO_4.7H_2O$	0.5	0.4	0.2	0.1	0.1	0.25
Yeast Extract	0.2	0.5	-	0.5	-	-
KCl	-	-	-	0.2	0.2	0.2
NaCl	-	1	-	0.2	0.2	-
$FeCl_3$	Trace	-	-	-	-	-
$FeSO_4.7H_2O$				0.002	0.002	-
$MnSO_4.H_2O$	Trace	-	-	0.002	0.002	-
$MgCl_2.6H_2O$	-	-	-	-	-	5.0
$CaCl_2$	-	0.2	-	-	-	-
NH_4NO_3	-	1.5	0.5	-	-	-
$ZnSO_4$	-	-	0.004	-	-	-
$Ca_3(PO_4)_5$	-	-	-	5.0	5.0	5.0
pH	6.8	7	5	7	7	7

2.3. Effect of Carbon and Nitrogen Sources on P Solubilisation

The effect of different carbon sources on the P solubilization was done with the addition of 1% of respective sugars (sucrose, maltose, fructose, xylose, galactose) in place of the glucose in the PVK medium. Similarly for determining the effect of different nitrogen sources, ammonium sulphate $(NH_4)_2SO_4$ in PVK medium was replaced by 0.5% of different nitrogen salts such as casein, urea, potassium nitrate (KNO_3), sodium nitrate ($NaNO_3$), ammonium chloride (NH_4Cl) and sodium nitrite ($NaNO_2$).

2.4. Effect of Soluble Phosphate Source on P Solubilisation

The effect of soluble P on the phosphate solubilization ability of the *P. aeruginosa* FP6 was carried out in a PVK medium amended with KH_2PO_4 (10 - 150 mM) for a duration of seven days. The broth was inoculated with a FP6 isolate and incubated at 28°C.

2.5. Effect of Tris on P Solubilization

Sterile Tris buffer (pH, 8.0) at 0, 20, 30, 40, 60, 60, 70 and 80 mM concentrations (Sambrook *et al.,* 1989) was added to the PVK broth. 100 µL of the overnight broth culture of the isolate was inoculated and incubated for 24 h at 37°C. The effect of P solubilization in buffered condition in the presence of Tris buffer in the media was assessed by noting the change in pH, and by estimating the amount of P solubilized. Control media was without Tris buffer.

2.6. Multiple Biocontrol Traits of P. aeruginosa FP6 under Buffered Condition

The effect of buffering on phosphate solubilization and other biocontrol traits of FP6 was determined in 100M Tris-HCl (pH-8.0) buffered minimal medium containing (g/L) glucose-10; tricalcium phosphate [$Ca_3(PO_4)_2$]-5; ammonium sulphate [$(NH_4)_2SO_4$]- 0.5; sodium chloride [NaCl]- 0.2; magnesium sulphate [$MgSO_4.7H_2O$]- 0.1; potassium chloride [KCl]- 0.2; yeast extract- 0.5; manganese sulphate [$MnSO_4.H_2O$]- 0.002; ferrous sulphate [$FeSO_4.7H_2O$]-0.002. The Culture filtrate was screened for its phosphate solubilization ability along with its antagonistic activity, siderophore production (Schwyn and Neilands, 1987), HCN production (Kremer and Souissi, 2001) and IAA production (Bric *et al.,* 1991). The same was compared with non- buffered condition (PVK media).

2.7. Effect of P. aeruginosa FP6 on Biometric Parameters

2.7.1. Inoculum Preparation for Pot Experiment

FP6 was grown in King's B broth for forty-eight hours under shaking condition (150 rpm) at room temperature (28±2°C). Cell pellet was collected by centrifugation and suspended in distilled water to give a final concentration of 10^8 CFU/ mL. The seeds of cowpea, (C-152), were used throughout the study They were surface-sterilized in 70% ethanol for two minutes and in 2% sodium hypochlorite for five minutes, followed by washing (ten times) in sterile distilled water. Surface sterilized cowpea seeds were soaked in aqueous solution containing *P. aeruginosa* FP6 (10^8 CFU/mL) and were left for one hour to allow the bacteria to bind the seeds. Control seeds were soaked in sterile distilled water.

2.7.2. Pot Experiments

A mixture of soil: sand (1:1) was autoclaved at 121°C for fifteen minutes. The autoclaved soil: sand was supplemented with three different levels of TCP and two different levels of single super-phosphate (SSP) as insoluble (bound) and soluble phosphate sources, in six different combinations (Table 3). A total of twelve different treatments, including six combinations of phosphate sources (TCP and SSP) and two levels of inoculum treatment (control and test) with three replications of each, were used for conducting the pot studies on the plant growth enhancement of cowpea. The seedlings were watered daily to maintain the moisture at approximately 60% water holding capacity of the soil. The values were expressed as their mean. All experiments were carried out in triplicate. The effects of promoting the bacterial-growth treatments were assessed by measuring the biometric parameters.

2.7.3. Control and Test Pots

Control pots were supplemented with three different levels of tricalcium phosphate and two different level of single super-phosphate. Surface sterilized seeds were placed in control pots. Test pots contained surface-sterilized cowpea seeds coated with *P. aeruginosa* FP6.

2.8. Statistical Analysis

Each data presented was the mean of three replicates. All data were subjected to one way analysis of variance and the mean difference was compared with the least significant difference (LSD). Comparison with $p< 0.05$ was considered significantly different. The analysis of variance was performed using SPSS (version 18) statistical package and mean comparison were carried out using Duncan's multiple range test.

3. Results

FP6 isolate showed a distinct zone of clearance around the colony on PVK medium after five days of incubation due to solubilization of inorganic phosphate. Maximum solubilization efficiency and an index of 52.38 % and 2.26 were observed on PVK media. The results of the present study demonstrated *P. aeruginosa* FP6 to be a potent phosphate solubilizer.

The analysis of media formulation for phosphate solubilization by *P. aeruginosa* FP6 showed maximum phosphate solubilization in medium 6 (NBRIP) containing 1% glucose and 0.01% $(NH_4)_2SO_4$ (412.5 µg/mL), with a drop in pH from 7.2 to 4.9 on the third day followed by medium 5 (NBRIY) 330 µg/mL (pH 7.2 to 4.7) and medium 4 (PVK) 270 µg/mL with a decrease in pH from 7.2 to 5 (Figure. 1) while pH being stable in control. Low levels of P solubilization were observed in other phosphate media. FP6 also solubilized rock phosphate (202.5 µg /mL) by seven days of incubation. Results showed negative correlation (r^2 = -0.159, p = 0.39) between decrease in pH and phosphate solubilization in PVK medium.

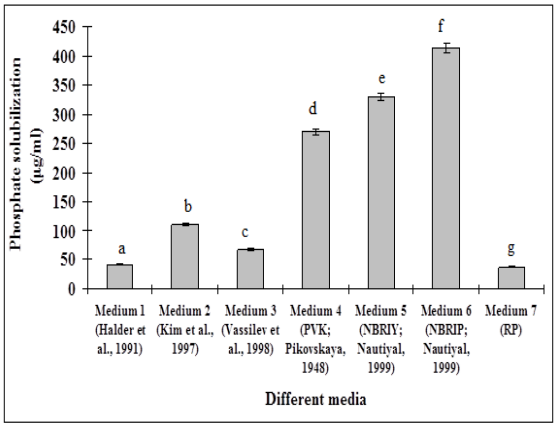

Figure 1. Effect of different media on phosphate solubilization. Different letters above the bars indicate significant difference between different media, according to LSD ($p< 0.05$).

Considering the amount of glucose used in the medium, and the corresponding efficacy of P solubilization, the PVK medium proved to be the most cost effective without compromising the solubilization. Therefore, PVK medium was used for further studies.

3.1. Effect of Carbon and Nitrogen Sources on P Solubilization

The Phosphate solubilization activity of FP6 when evaluated in the presence of five different carbon and seven nitrogen sources by replacing glucose and $(NH_4)_2SO_4$ of the PVK medium showed maximum phosphate solubilization with a decrease in pH in the medium containing glucose (pH 4.85; 181.75 µg/mL) after twenty-four hours of incubation followed by fructose (pH 5.83; 168.25 µg/mL), and xylose (pH 5.64; 57.75 µg/mL) (Figure. 2), whereas sucrose and maltose showed negligible amount of phosphate solubilization with no decrease in pH. A significant negative correlation (r^2 = -0.77, p = 0.04) was observed between phosphate solubilization and the reduction in pH.

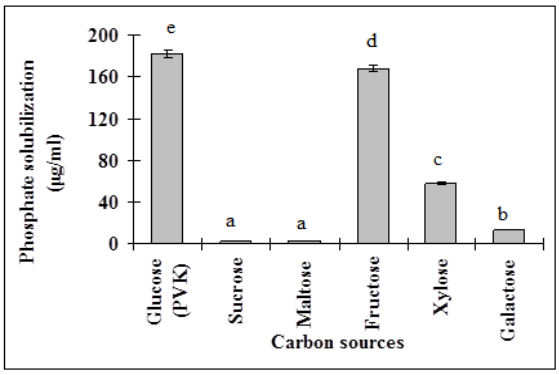

Figure 2. Effect of different carbon sources on phosphate solubilization. Different letters above the bars indicate significant differences between carbon sources, according to LSD ($p< 0.05$).

Nitrogen salts having either ammonium or nitrate groups were used as nitrogen source. Nitrogen salts with ammonium group- ammonium sulphate and ammonium chloride were found to be the best in reducing the medium pH to 4.87 and 5.22, respectively with simultaneous solubilization of 184.75 µg/mL and 169 µg/mL of P (Figure 3) after twenty-four hours of incubation. Other nitrogen sources in the form of urea, nitrate and nitrite failed to support growth of FP6. Though casein showed a pH drop to 4.52, it showed only marginal P solubilization (44.25 µg/mL). Correlation between phosphate solubilization and acidification was found to be statistically significant (r^2 = -0.74, p = 0.038).

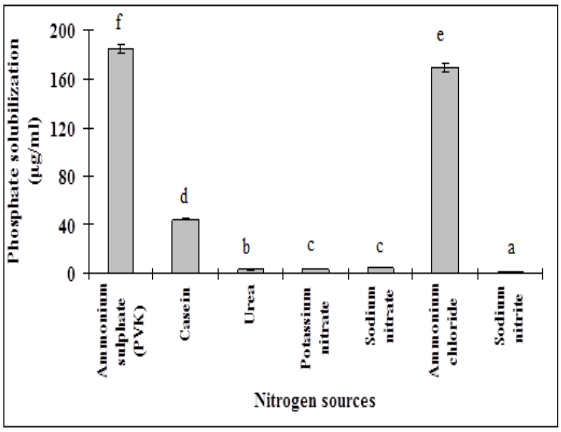

Figure 3. Effect of different nitrogen sources on phosphate solubilization. Different letters above the bars indicate significant differences between nitrogen sources, according to LSD ($p< 0.05$).

3.2. Effect of KH_2PO_4 (free Soluble Phosphate) and Tris on Phosphate Solubilization

The presence of free soluble phosphate in the form of KH_2PO_4 in the medium inhibited phosphate solubilization by FP6 (Figure. 4).The solubilization of phosphate exhibited a negative correlation with a reduction in pH ($r = -0.29$, $r \leq 0.480$).

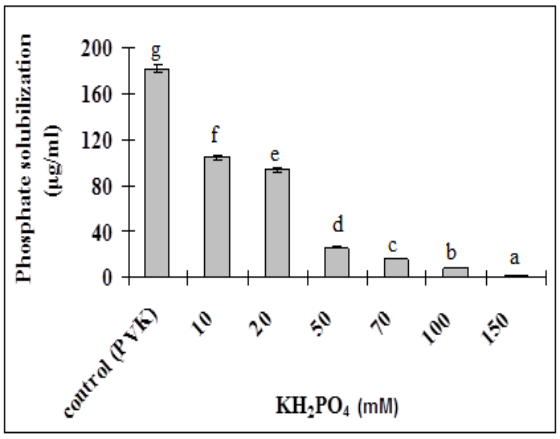

Figure 4. Effect of KH_2PO_4 on phosphate solubilization. Different letters above the bars indicate significant differences between KH_2PO_4 concentrations, according to LSD ($p< 0.05$).

Since the results of the present study showed a drop in pH corresponding to phosphate solubilization, the ability of the strain to solubilize phosphate in different buffering conditions was carried out. Phosphate solubilization was observed up to 80mM Tris, and optimum phosphate solubilization was seen in the presence of 30mM Tris (82.5 µg/mL) with concomitant drop in pH to 4.6. Additions of Tris from 40mM onwards resulted in a drastic decrease in phosphate solubilization with no absolute drop in pH (Figure 5). A statistically significant (r^2 = -0.91, p = 0.0017) negative correlation was developed between the solubilization of phosphate and the decline in pH.

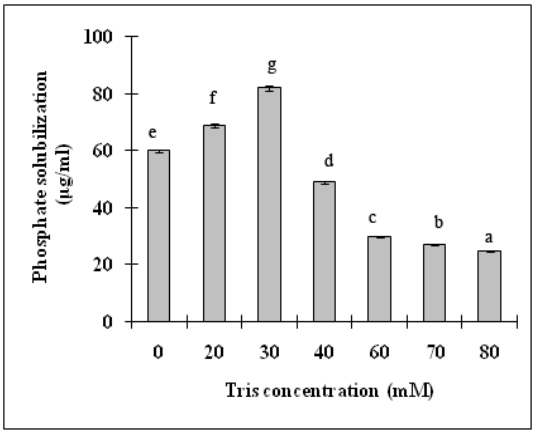

Figure 5. Effect of different concentration of Tris on P solubilization. Different letters above the bars indicate significant differences between Tris concentrations, according to LSD ($p< 0.05$).

3.3. Multiple Biocontrol Traits of P. aeruginosa FP6 under Phosphate Deficient Condition (Buffered Condition)

In the environment, the plant growth is limited by the availability of P despite the abundance of PSBs in the rhizosphere due to the high buffering capacity of soils and the reduced/ loss of P solubilizing efficiency of bacteria under buffered conditions. *P. aeruginosa* FP6 demonstrated P solubilization ability under buffered conditions (upto 80mM Tris). Therefore in the present study we explored biocontrol and PGPR abilities of this strain under phosphate deficient conditions. When grown on Tris-buffered (100mM) PVK media (P deficient), *P. aeruginosa* FP6 showed three fold reductions in IAA, and siderophore production as compared to unbuffered PVK medium (P sufficient). However, a threefold increase in the HCN production was observed in a P-deficient medium (Table 2). There was a concurrence of P-solubilization phenotype, and multiple biocontrol traits in *P. aeruginosa* FP6 under P-deficient conditions, and only the HCN production was found to be independent of the P solubilization trait.

Table 2. Biocontrol traits in PVK medium and Tris buffered PVK medium.

PGPR traits	PVK medium	Tris buffered PVK medium
Phosphate solubilization	310 µg/mL	100 µg/mL
IAA	6 µg/mL	2µg/mL
Siderophore	5 µM	2 µM
HCN (O.D)	0.05	0.15

3.4. Biometric Parameters in the Cowpea Plant

The effect of P on plant growth performance of the cow pea in pot conditions was studied. Compared to the control, a significant increase in all the growth parameters was observed with increasing the concentration of phosphate, but significant difference was not observed, except in shoot weight when treated with 100 and 165 mg/ kg of TCP and superphosphate. Significant increase in shoot length and fresh shoot weight was observed in the combined application of phosphate source (TCP and superphosphate) and *P. aeruginosa* compared to *P. aeruginosa* control indicating the phosphate solubilizing efficacy of the strain (Table 3). However, the combined application of TCP (100 mg/ kg) with *P. aeruginosa* FP6 showed significant a stimulatory effect in biometric parameters compared to control. This increase can be attributed to the absorbance of more P from the soil and its accumulation, resulting in increased shoot length (27.88 cm), root length (10.5 cm), fresh shoot weight (0.51g), and fresh root weight (0.07g). This indicates a positive influence of the combined inoculation regarding the nutrient availability and plant growth.

Table 3. Influence of *P. aeruginosa* FP6 inoculation on the growth of cowpea in the presence of different phosphorus sources

Phosphate treatment (Soil)	Treatment	Shoot length (cm)	Root length (cm)	Fresh weight	
				Shoot weight (g)	Root weight (g)
TCP_0 SSP_0	Control	16.81^{a}	6.69^{a}	0.29^{a}	0.02^{a}
	P. aeruginosa FP6 (test)	21.13^{b}	$8.19^{a,b}$	$0.37^{a,b,c}$	0.02^{a}
TCP_0 SSP_1	Control	20.43^{b}	8^{a}	$0.33^{a,b}$	0.05^{a}
	P. aeruginosa FP6 (test)	$26.31^{c,d,e}$	$9^{a,b}$	$0.42^{b,c,d}$	0.08^{a}
TCP_1 SSP_0	Control	21.13^{b}	$6.59^{a,b}$	$0.33^{a,b}$	0.04^{a}
	P. aeruginosa FP6 (test)	$27.88^{d,e}$	10.5^{b}	$0.51^{d,e}$	0.07^{a}
TCP_1 SSP_1	Control	$23.39^{b,c,d}$	$7.65^{a,b}$	$0.40^{a,b,c,d}$	0.04^{a}
	P. aeruginosa FP6 (test)	$26.88^{d,c}$	$9.54^{a,b}$	$0.49^{c,d}$	0.05^{a}
TCP_2 SSP_0	Control	$24.31^{b,c,d,e}$	$8.6^{a,b}$	$0.37^{a,b,c}$	0.03^{a}
	P. aeruginosa FP6 (test)	$24.4^{b,c,d,e}$	$8.68^{a,b}$	$0.42^{b,c,d}$	0.04^{a}
TCP_2 SSP_1	Control	$22.75^{b,c}$	$7.13^{a,b}$	$0.43^{b,c,d}$	0.02^{a}
	P. aeruginosa FP6 (test)	$25.4^{c,d,e}$	$8.3^{a,b}$	0.55^{e}	0.039^{a}
F value		91.62	115	101.83	6.94
CD @ 0.05 %		3.40	1.17	0.07	0.02

TCP (tri-calcium phosphate), SSP (single superphosphate); Subscripts 0, 1 and 2 for TCP indicate concentrations of 0, 100 and 200 mg/ kg soil respectively. Subscripts 0 and 1 for SSP indicate concentrations of 0 and 165 mg/ kg soil respectively. Values represent mean. Mean values followed by the same letter are not significantly different at the 0.05 level of confidence.

4. Discussion

Phosphorus is one of the most essential elements for the growth of plants, yet it is not a renewable resource, and its future use in agriculture will be impacted by its declining availability and increased cost (Hameeda *et al.*, 2008). P gets precipitated with calcium, iron and

aluminium and becomes unavailable to plants. Theoretical estimates have suggested that the accumulated P in the soil is sufficient to sustain crop yields worldwide for about 100 years (Khan *et al.,* 2010). Phosphatic biofertilizers in the form of microorganisms can help increase the availability of accumulated phosphates for plant growth by solubilization.

Several species of *Pseudomonas* fall within the promising category of growth-promoting rhizobacteria, and a number of them have been studied for their phosphate solubilising activity (Sharma *et al.,* 2011).

The lack of correlation between phosphate solubilization and pH indicated that solubilization of $Ca_3(PO_4)_2$ was not predominantly due to organic acid release, but alternatively, can also be attributed to other mechanisms such as the release of protons accompanying respiration or NH_4^+ assimilation.

Depending on the composition of the bacterial medium and the final pH of the cultured medium, bacterial P solubilization reported in the literature ranges from 31.5 mg/L to 898 mg/L (Ma *et al.,* 2009; Oliveira *et al.,* 2009). The present study is in agreement with the above mentioned reports.

The slow rate of rock phosphate solubilization could be attributed to its structural complexity and particle size, as well as the nature and quantity of the organic acid secreted by the microorganisms, which suggests the good adaptation of this strain to its ecological niche. Furthermore, the inherent ability of a strain to solubilize these natural forms of insoluble rock P can possibly be improved by optimizing the growth parameters.

The results of the present study on the carbon source effect are in agreement with those of Son *et al.* (2006) and Pallavi and Gupta (2013) who found glucose to be the best carbon source for phosphate solubilization in *Pseudomonas* species. The role of carbon sources is important in phosphate solubilization because the nature of the produced acid is affected by the carbon source. Glucose and fructose are the most frequent and abundant sugars detected in plant exudates that might possibly affect the microbial population which solubilize insoluble phosphates. The increased phosphate solubilization with glucose may be attributed to the greater availability of the energy source for the growth of the organism and for the acid production.

The present study results on the effect of nitrogen source correlates with earlier reports (Sharma *et al.,* 2013; Karunai Selvi and Ravindran, 2012). The Phosphate solubilization in ammonium salts, as the nitrogen source, was substantially higher than with other nitrogen sources, which suggested a P solubilization by the production of organic acids through NH_4^+/H^+ exchange mechanisms. Relwani *et al*. (2008) have stated that the nitrate increased the solubilization of several phosphates. The nitrate uptake by the cell stimulates acid secretion to compensate the cellular ionic potential. Such a stimulus was not observed in the present study.

The inhibition of phosphate solubilization in the presence of soluble phosphate indicates that the phosphate solubilization ability is a stress-induced response. Similar results have also been reported by Srividya *et al.* (2009). The buffering capacity of the medium reduced the effectiveness of FP6 strain in releasing P from TCP. This could be attributed to the resistance in pH change by the buffering activity of Tris. A similar observation has been reported by Joseph and Jisha (2009).

Joseph and Jisha (2009) reported a loss of the P solubilization in phosphate solubilizing bacteria due to buffering. In contrast to the above-mentioned studies, FP6 isolate showed a considerable amount of phosphate solubilization, and other PGPR ability in buffered condition indicating that FP6 can perform better in acidic soils.

Walpola and Yoon (2013) linked the highest growth performance and P uptake of the mung bean to co-inoculating PSB strains and adding TCP. Several studies have shown increase in plant growth and P uptake due to the addition of phosphate-solubilising bacteria (Velineni and Brahmaprakash 2011; Hameeda *et al.,* 2008). Malviya *et al.* (2011) showed a significant increase in dry matter and the groundnut yield in phosphate solubilizing fungi inoculated soil compared to control soil.

5. Conclusion

The current study highlights the phosphate solubilising ability of *P. aeruginosa* FP6. The use of this strain as bioinoculant could be a sustainable practice to enhance the soil fertility and plant growth. The successful use of phosphate solubilising microorganisms along with indigenously available cheaper sources such as the low-grade rock phosphate (RP) can be economical.

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