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Efficacy of phosphate solubilizing microorganisms in utilizing native phosphorus in an alkaline alluvial soil of North India

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ABSTRACT

Utilization of phosphate solubilizing microorganisms (PSMs) to solubilize the fixed P pool in soil is a promising method considering the shrinking availability of raw material (rock phosphate) of phosphatic fertilizer production in one hand and global food security on the other. An incubation study was carried out for 90 days to assess the ability of the two PSMs, namely *Enterobacter* sp. and *Aspergillus niger* in mediating the release of P from fixed pool in an alluvial soil (pH= 8.30) of IARI farm, New Delhi. On an average, both the microorganisms significantly increased the release of P into solution from fixed P pool of soil. However, *Enterobacter* sp. treated soil showed better P release (0.52 mg/kg) than that of *Aspergillus niger* treated soil (0.44 mg/kg) over uninoculated control (0.42 mg/kg). *Enterobacter* sp. was capable in mediating P release into soil solution during the whole period of incubation. Soil treated with *Enterobacter* sp. showed almost similar level of solution P concentration from 2nd week to 6th week of incubation and after that, there was a decrease. Whereas, *A. niger* was able to mediate P release into soil solution only up to 28 days of incubation, after that solution P concentration of treated soil became statistically at par with untreated control. Thus, *Enterobacter* sp. performed better than *A. niger* in utilizing native P in alluvial soil.

Key words: Alluvial soil, Fixed pool, Phosphate solubilizing microorganism (PSM), P release pattern, Solubilization

Phosphorus (P) is popularly known as the 'key of life'. For optimum crop yield, an adequate supply of P is a pre-requisite. Although most of the soils have sufficiently high total P stock, soil solution concentration of P is usually very low. It is estimated that on > 40% of the cultivable lands of the world, P deficiency limits the crop productivity (Balemi and Negisho 2012). Therefore, in intensive cultivation, huge quantity of water soluble phosphatic fertilizers is used to meet the crop demand. But, more than 80% of applied fertilizer-P are converted into plant unavailable forms within a short time either due to sorption, precipitation or due to microbial immobilization leading to very low P-use efficiency (Roberts and Johnston 2015). Precipitation in the form of insoluble P compounds may occur by reaction with Al^{3+} and Fe^{3+} in acidic soils and with Ca^{2+} in calcareous soils (Roberts and Johnston 2015). The high P fixation ability of soils coupled with low P use efficiency (PUE) of most crops tends to accumulate the surplus P input from fertilizers in fixed pool of soil. Such fixation or build-up of P ultimately leads to the wastage of costly P fertilizers. So, a more sustainable P management approach in agriculture has become indispensable. In this context, it has been suggested that the total amount of accumulated P in agricultural soils worldwide could adequately supply P to crops needed for optimum yields for about 100 years if it can be made available to crops (Khan *et al.* 2007). But the irony is that the accumulated P in soil is not easily available to plants for absorption. In this context, it is worthwhile to mention that phosphate solubilzing microorganisms (PSMs), through various mechanisms of solubilization and mineralisation are able to convert inorganic and organic soil P to plant available forms, respectively (Mohammadi 2012). The use of PSM for enhancing P availability for plants is an eco-friendly method as well as inexpensive too (Owen *et al.* 2015). Phosphate solubilizing microorganisms are an integral part of the soil P cycle and are important for the transfer of P between different fractions of soil P. Due to application of PSM, the fixed or relatively unavailable fraction of P may be distributed to comparatively more labile fractions, which

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can be taken up by the plants.

Information on the release pattern of P into soil solution due to microbial solubilization in alluvial soil is limited. Therefore, the present investigation was carried out to assess the release of P from fixed pool as mediated by phosphate solubilizing microorganisms (PSMs) in an alluvial soil of New Delhi, India.

MATERIALS AND METHODS

Collection and characterization of the experimental soil

One bulk surface soil sample (0-15cm) was obtained from IARI farm, New Delhi (Inceptisol, pH= 8.30) and was air-dried and ground to pass through a 2-mm sieve. Soil texture was determined by hydrometer method (Bouyoucos 1962), while pH of the soil sample was measured in 1:2 soil: water suspension as per Jackson (1973). The electrical conductivity (EC) was measured in the supernatant liquid of the same extracts used for pH determination (Jackson 1973). Ammonium acetate method of Jackson (1973) was used to determine the cation exchange capacity (CEC) of the soil sample. Organic carbon content in the soil was determined by wet oxidation method as described by Walkley and Black (1934), while available nitrogen in soil was determined by alkaline permanganate method (Subbaiah and Asija 1956). Available P of the soil sample was determined by ascorbic acid blue colour method at 730 nm after extraction with 0.5*M* $NaHCO_3$ solution (Watanabe and Olsen 1965), while available potassium was extracted by 1*N* ammonium acetate solution of pH 7.0 (Hanway and Heidel 1952). Free calcium carbonate ($CaCO_3$) of the soil samples were determined by rapid titration method given by Richards (1954). Some important physical and chemical properties of the experimental soil are presented in Table 1. The soil belonged to the textural class of sandy clay loam. The soil was low in organic carbon and available N, while high in available K content. In this soil, 1.54% free $CaCO_3$ was present, which implies that the soil was not calcareous. In this soil, though available P content (22.5 kg ha^{-1}) was medium in range but the soil had sufficiently high amount of total P (1348 kg ha^{-1}), which indicated that most of the P in this soil might be present in the fixed pool. Thus, the soil selected for the present investigation based on pH, available P and total P content was appropriate as the objective of the investigation was to solubilize the fixed or build-up P in an alkaline soil using PSMs.

Maintenance of microbial population in pure culture

Pure culture of phosphate solubilizing bacterium (PSB), *Enterobacter* sp. and phosphate solubilizing fungus (PSF), *Aspergillus niger* was maintained in the respective media and stored at 4°C for further use. The composition of Pikovskaya medium used for PSB was as follows: Yeast extract: 0.50 g/L, Dextrose: 10.0 g/L, Calcium phosphate: 5.00 g/L, Ammonium sulphate: 0.50 g/L, Potassium chloride: 0.20 g/L, Magnesium sulphate: 0.10 g/L, Agar: 15.0 g/L and pinch of Manganese sulphate and Ferrous sulphate.

Table 1 Physical and chemical properties of the experimental soil

Mechanical composition	
Sand (%)	54.1
Silt (%)	21.6
Clay (%)	24.3
Textural class	Sandy clay loam
pH	8.30
EC (dS m^{-1})	0.15
Organic carbon (%)	0.40
CEC [cmol (p^+)/kg]	10.1
Free $CaCO_3$ (%)	1.54
Macro nutrients	
Available N (kg/ha)	196
Available P (kg/ha)	22.5
Available K (kg/ha)	344
Total P (kg/ha)	1348

For preparation of potato dextrose agar medium used for PSF, 200g of sliced potatoes were boiled in 1 l of distilled water for 1 hr. After that, the content was filtered and 20 g D-glucose and 15 g agar was added to it. Then the volume was made up to 1 l and the content was steamed until agar was dissolved. The dilution plate technique (Waksman and Fred 1922) was followed for enumeration of the isolates in soil periodically. Petri plates were kept at 28 °C in an incubator and cell count was performed after 2 days for the fungus treated soil and after 4 days for the bacterium treated soil.

Incubation study

An incubation study was conducted in the laboratory at ambient temperature (average temperature was 25±5 ºC throughout the incubation period) to assess the release of P from soil as influenced by PSMs. For this purpose, one PSB (*Enterobacter* sp.), one PSF (*Aspergillus niger*) and one uninoculated control was used. A series of 50 g processed soil samples were taken in 500 mL wide mouth plastic reagent bottles with lids closed. Fifteen mL of liquid pure culture of either PSB or PSF was added to each bottle as per the treatment combinations. Fifteen mL of double-distilled water was added to maintain saturation moisture content inside the bottle. In case of control bottles, simply 30 mL double-distilled water was added to the soil. Each treatment combination was replicated thrice in a completely randomized design. Four to five fine perforations were made in the lid of each bottle to prevent the development of anaerobic condition inside the bottle. Constant moisture content of the soil within the bottle was maintained by periodic determination of water loss from each bottle and subsequent replenishment by water addition. Samplings were done at 7, 14, 21, 28, 35, 42, 49, 56 and 90 days after incubation (DAI). Whole amount of soil in each bottle was extracted by 0.01*M* $CaCl_2$ at a soil: solution ratio of

1:2 with 2 hr shaking and P concentration was measured by ascorbic acid blue colour method as mentioned earlier (Watanabe and Olsen 1965). The P in the extract was considered as water soluble P present in soil solution. Separate bottles were kept for enumeration of PSMs in soil and soil was drawn periodically on 14, 42 and 90 days of incubation for this purpose.

Statistical analyses

Analysis of variance method was followed to study the impact of PSMs on P release pattern in soil as per completely randomized design (Snedecor and Cochran 1967).

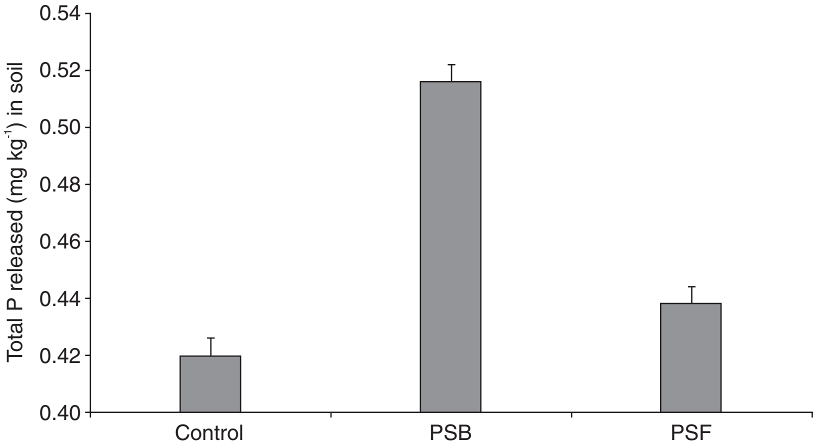

Fig 1 Impact of phosphate solubilizing microorganisms on total phosphorus released (mg/kg) in soil. Error bars denote the least significant difference (LSD, P=0.05) between the two treatments.

RESULTS AND DISCUSSION

Changes in population of P solubilizers in soil during incubation

In the experimental soil, population of PSB was 2 × 10^5 cfu/g dry soil and that of PSF was 6 × 10^2 cfu/g dry soil. Periodical changes in PSB and PSF population of the soil treated with the respective microbes is presented in Table 2. It is evident from the data that, there was a gradual decrease in the population of both the microbes in soil as the incubation period proceeded. However, number of bacterial isolates was always much higher than fungal isolates, which is quite common in soils of alkaline pH. The PSF population reduced drastically beyond 42 days of incubation.

Effect of phosphate solubilizing microorganisms on release of P into soil solution

An incubation study was conducted to assess the impact of PSMs on the release of P from soil and the results are presented in Table 3. Both PSB and PSF significantly increased the release of P into solution from fixed P pool of soil. However, PSB treated soil showed better P release (0.52 mg/kg) than PSF treated soil (0.44 mg/kg) over uninoculated control (0.42 mg/kg). Significant positive effect of PSM application on 0.01*M* $CaCl_2$ extractable P in soil as obtained in the present investigation is in agreement with the findings of few other researchers (Illmer *et al.* 1995; Wang *et al.* 2014). Phosphate solubilizing microorganisms have a capacity to solubilize insoluble or complexed inorganic P through the process of acidification, chelation and exchange reactions (Singh and Reddy 2011; Wang *et al.* 2014). These organisms release a number of metabolites like several low molecular weight organic acids into the soil solution. Hydroxyl and carboxyl groups of such organic acids chelate cations like Al^{3+}, Fe^{3+} or Ca^{2+} and thereby release P associated with these cations to the soil solution (Illmer *et al.* 1995). Moreover, due to production of these organic acids, soil pH is reduced and this reduced pH helps in the solubilization of Ca-phosphates (Mohammadi 2012). This is of great importance, particularly in the case of alkaline or calcareous soil, where most of the P is present in the form of Ca bound P. Also, these organic anions (ligands) compete with phosphate for adsorption sites in the soil reducing P fixation (Nahas 1996; Mohammadi 2012). Solubilization may also occur due to the release of H^+ by the PSMs, thereby lowering the pH of surrounding soil. Release of H^+ may occur either because of NH_4^+ assimilation or due to the activity of ATPase enzyme, which translocates H^+ to outer solution from cytoplasm (Illmer and Schinner 1992).

Table 2 Population of phosphate solubilizing microorganisms (cfu/g dry soil) in inoculated soil during incubation period

Days of incubation (D)	PSB ($\times 10^5$)	PSF ($\times 10^4$)
14	86.0	52.0
42	26.0	0.90
90	9.00	0.08

Table 3 Effect of phosphate solubilizing microorganisms on release of phosphorus (mg/kg) from soil with time

Days of incubation (D)	PSM (M)			Mean
	Control	PSB	PSF	
7	0.41	0.46	0.43	0.43
14	0.42	0.57	0.47	0.48
21	0.41	0.56	0.46	0.48
28	0.42	0.57	0.46	0.48
35	0.44	0.56	0.45	0.48
42	0.43	0.55	0.44	0.48
49	0.44	0.49	0.44	0.46
56	0.40	0.46	0.40	0.42
90	0.40	0.42	0.40	0.40
Mean	0.42	0.52	0.44	
LSD (P= 0.05)	D =0.02	M =0.01	D × M =0.03	

Release pattern of phosphorus in soil as mediated by phosphate solubilizing micro-organisms

In PSB treated soil, the release of P into soil solution was highest initially but the magnitude of increase decreased gradually with increasing days of incubation (Fig 2). A sharp increase in the release of P into soil solution on 14th day of incubation (0.57 mg/kg) was observed. After that, almost similar solution-P concentration was maintained up to 6th week of incubation, beyond which the solution-P concentration of PSB treated soil decreased gradually. Such type of P release pattern may be due to the increase in the availability of soluble P in the soil solution by gradual solubilization of fixed P pool by PSB. After 14 days of incubation, soluble P already present in the soil solution might have inhibited further solubilization of insoluble phosphates (Narsian *et al.* 1995). Illmer and Schinner (1992) suggested that an organo-P compound was formed, induced by the different organic metabolites that were released into the solution by the microbial culture and that reduced the amount of P in solution subsequently. The PSB was capable in mediating P release into soil solution during the whole period of incubation. Even at 90 days of incubation, PSB treated soil showed significantly higher level of solution P concentration (0.42 mg/kg) than untreated control (0.40 mg/kg). The population of bacteria is highest among all the microorganisms and they have very high multiplication rate also, which might be a reason of the better solubilization activity of *Enterobacter* sp. In case of PSF treated soil, highest amount of solution P concentration was recorded at 14th days of incubation (0.47 mg/kg), which decreased further with time (Fig 2). However, the PSF was able to mediate P release into soil solution only up to 28 days of incubation, beyond which solution P concentration of treated soil became statistically at par with that of untreated control. The PSF, *Aspergillus niger*, is capable of producing a large amount of organic acids (predominantly citrate) and this fungus is an efficient solubilizer of $AlPO_4$ (Illmer *et al.* 1995). The relatively poorer performance of *Aspergillus niger* in this soil may be due to the fact that initially the activity of PSF was higher, which decreased gradually with time. Khan *et al.* (2010) also stated that generally a rapidly declining trend in the density of artificially introduced PSF population was found upon incubation in soils. Another reason may be the high pH (pH= 8.30) of the experimental soil, which might have suppressed the activity of PSF.

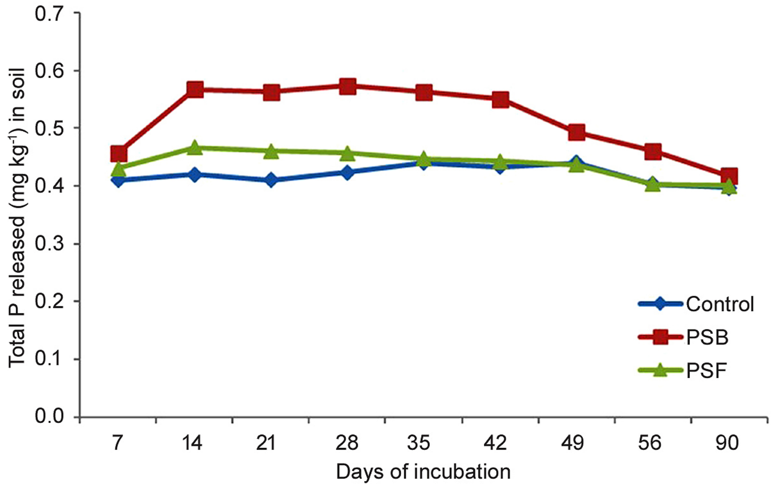

Fig 2 Release pattern of phosphorus (mg/kg) in soil as mediated by phosphate solubilizing microorganisms.

Conclusions

The present study demonstrated that the PSB, *Enterobacter* sp. was more effective than the PSF, *Aspergillus niger* in mediating P release from fixed P pool of soil, both in terms of amount as well as duration of P release. Thus, *Enterobacter* sp. performed better than *Aspergillus niger* in utilizing native P in alluvial soil, which was alkaline in pH. These results have an importance towards sustainable and economic P management, one of the prime issues for global food security considering the shrinking availability of raw materials for phosphatic fertilizer production. However, further field experiment is needed to test the efficacy of this microbial strain in mediating P release in soil and supporting crop growth thereby.

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