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Original Research Paper

Impact of *Pseudomonas putida* on Available Soil Phosphorus Dynamics and Crop Productivity under Lowland Rice Ecology

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ABSTRACT

The ability of phosphorus solubilizing bacteria (PSB) to solubilize soil phosphorus (P) in actual field condition is never beyond scientific contradiction. Further, its (PSB) dynamics under unique waterlogged, anaerobic rice ecosystem is a matter of interest. In this context, performance of *Pseudomonas putida* was tested as PSB in lowland rice soil. No significant impact of *P. putida* on soil available P status was found. The treatment of crop seedlings with PSB also showed no major response onto the vegetative as well as yield parameters of rice crop. Results inferred that, in presence of optimum amount of soil N and K, the addition of external P through phosphatic fertilizer and/or vermicompost was the controlling factor for soil P availability as well as productivity and quality of rice. Further, vermicompost boosted the soil total microbial population. The irrelevant impact of *P. putida* as PSB onto the soil available P status and rice productivity might be partially due to the anaerobic waterlogged rice environment, which did not support the proliferation and activity of these aerobic gram-negative bacteria. However, in comparison to the earlier findings, a further detailed study at field level is necessary to understand the dynamics of PSB in submerged rice soil.

INTRODUCTION

Presence of plant available phosphorus (P) in the soil is necessary for optimum and sustainable agricultural production (Matula 2011). Slow diffusion of P in soil and high fixation by mineral matrix (Shen et al. 2011) is the major limiting factor in this regard. For this, soil P is being paid more attention as a non-renewable resource with the increasing global population pressure and related food insecurity (Cordell et al. 2009, Gilbert 2009). Unregulated application of chemical phosphatic fertilizers for the past few decades resulted in severe environmental pollution (Salimpour et al. 2010) in exchange of no significant long-term improvement in the soil available P status and productivity. In this context, use of P solubilizing bacteria (PSB) to maintain a continuous P supply level in the rhizosphere zone is a globally accepted method (Postma et al. 2003, Welbaum et al. 2004, Suri et al. 2011). However, the performance of PSB in field condition is not beyond scientific contradiction (Khan et al. 2007).

The application of PSB as biofertilizer in soil results in acidic (H^+) environment (Igual et al. 2001) due to release of several organic acids like citric, oxalic, gluconic, lactic, succinic and propionic acid (Chen et al. 2006), which break down the long chain P-molecules increasing P solubility. Release of enzymes (such as phosphatase and phytase) and production of siderophore by PSB also increase the solubility of low soluble inorganic phosphate in soil (Pandy et al. 2006). The PSB can be utilized in combination with suitable doses of phosphatic fertilizers for better soil sustaina- bility and productivity (Shaharoona et al. 2008).

South and south-eastern Asia, including Indian sub-continent share noteworthy amount of global population (Sanderson 1995) and rice (*Oryza sativa*) is the major staple food in this part of the world (IRRI 1993). The average productivity of rice in this region is low (Zeigler & Puckridge 1995) and irrational fertilization strategy is one of the prime reasons behind this (Khosla et al. 2002). Considering 76% of global rice production under lowland condition (Fageria et al. 2011), application of PSB in waterlogged rice cultivation is a matter of concern associated with productivity as well as environmental issue. Although, number of researches were conducted worldwide on the application of PSB onto several crops (Chung et al. 2005, Jha et al. 2012), only a few have been done in the tropical and sub-tropical Indian context. Further, there are no noteworthy investigations about the impact of *Pseudomonas putida*, an aerobic, gram-negative bacterium, as PSB onto the physical and yield parameters of lowland submerged rice crop. Following the variation in the response of different plant species to P supply in soil (Bhadoria et al. 2004), the present study aims to find out the effect of *P. putida* as PSB with/without phosphatic fertilizers on growth and yield improvement of rice crop and onto the chemical and biological properties of soils in tropical/sub-tropical Indian continent.

MATERIALS AND METHODS

Research farm of Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur (22°19'22' N Latitude and 87°19'65' E Longitude), West Bengal, India (Fig. 1) was selected to perform the experiment.

Experimental design: The experiment was designed to represent the impact of PSB (with or without phosphatic fertilizers) in comparison to only phosphatic fertilizers or phosphatic fertilizers with vermicompost onto the yield and agronomic parameters of rice and soil properties. In this regard, different treatments with three field replications were used and later the soil samples and plant parts under different treatments were analysed in the laboratory by standard methods.

Field experiment: A field experiment was carried out during June to October, 2012. Semi-dwarf, cross-breed, medium duration (110-120 d) transplanted rice cultivar IR-36 (seed rate 40 kg ha^{-1}) was selected for the experiment (row to row spacing 20 × 20 cm and plant to plant spacing 20 × 20 cm). The experiment was laid out in a split-plot design where treatments with PSB were allowed in the main plot and other fertilizer treatments in the subplots. Single super phosphate (SSP) (50 kg P ha^{-1}) was applied to the experimental plots (6×4 m^2) by broadcasting just before transplanting. Apart from chemical fertilizer, 2.5 t ha^{-1} vermicompost was also used as a source of soil P. For the application of PSB, *P. putida* has been mixed-up with 2 L of nutrient agar solution (composition: yeast extract 2.0 g, beef extract 1.0 g, peptone 5.0 g, NaCl 5.0 g and agar 15.0 g in 1.0 L distilled water). The roots of the rice seedlings were dipped for 1 h in the solution before transplanting at PSB treated plots. Six different field treatments (T) were followed *viz.*

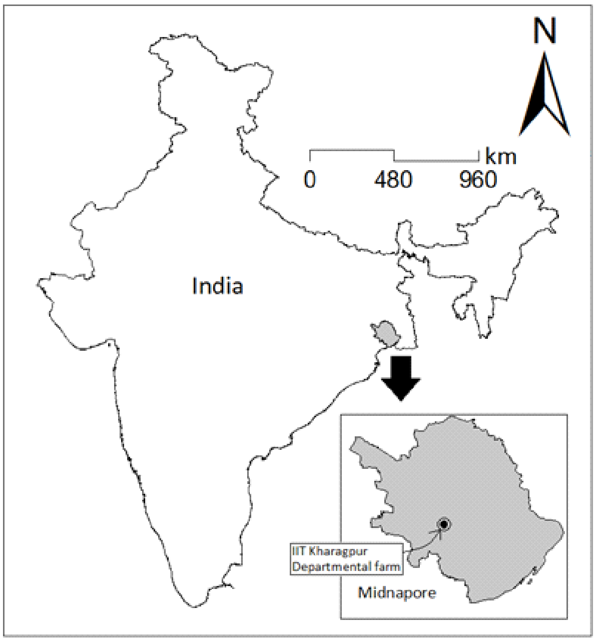

Fig. 1: Location of the experimental field in the map of India.

T1 No application of phosphatic fertilizers, vermicompost and PSB - P_0

T2 50 kg P ha^{-1} through only phosphatic fertilizers without vermicompost and PSB (PF_{50}) - P_{50}

T3 25 kg P ha^{-1} through phosphatic fertilizers and application of vermicompost but no PSB - P_{25} + VM

T4 No use of phosphatic fertilizers and vermicompost but use of PSB - P_0 + PSB

T5 50 kg P ha^{-1} through phosphatic fertilizers and use of PSB but no vermicompost - P_{50} + PSB

T6 25 kg P ha^{-1} through phosphatic fertilizers, application of vermicompost and PSB - P_{25} + VM + PSB

Nitrogen (N) and potassium (K) were also applied to all the treatment plots by broadcasting @ of 100 kg N ha^{-1} (as urea) and 60 kg K ha^{-1} (as muriate of potash).

Soil sampling and analysis: Composite surface soil samples (0-20 cm) were collected from each plot at initial (0) and 20, 40, 60, 80 days after transplanting (DAT) by soil auger. Before soil analyses, the soil samples were air-dried, sieved by 2 mm sieves and visible plant residues and stones were removed. The < 2 mm soil sub-samples were then grounded to powder form for different physical and chemical analysis. Core sampler had been used to collect soil subsamples for determination of bulk density (Blake & Hartge 1986). The pH_w (soil: water:: 2: 5) and electrical conductivity (EC) (dS m^{-1}) (soil: water:: 1: 5) of the soil samples were assessed using a pH meter (Systronics, Model 1100) and conductivity-meter (Systronics, Model 304) respectively. Separate field-moist soil samples from each field-plot were stored at 4°C for soil microbial analysis.

Determination of soil available P: Following the slightly acidic nature of the soils, Bray and Kurtz 1 (0.03 N NH_4F, 0.025 N HCl) extracting solution was used to determine soil

Table 1: Effect of different treatments on vegetative parameters of rice plant (mean values).

Treatment	Tillers per plant				Stem dry weight (g hill^{-1})			
	Days after transplanting							
20	40	60	80	20	40	60	80	
T1 (PF$_0$)	7.2^{CD}	10.0^{B}	9.8^{B}	9.3^{B}	1.02^{C}	9.21^{D}	8.48^{E}	8.12^{D}
T2 (PF$_{50}$)	7.7^{B}	11.9^{A}	11.8^{A}	10.6^{A}	1.31^{A}	11.49^{B}	11.21^{B}	11.09^{AB}
T3 (PF$_{25}$VM)	8.7^{A}	12.0^{A}	11.6^{A}	10.0^{AB}	1.18^{ABC}	10.92^{BC}	10.68^{BC}	10.64^{B}
T4 (PF$_0$PSB)	6.8^{D}	10.2^{B}	10.2^{B}	9.9^{AB}	1.23^{AB}	9.41^{D}	9.67^{D}	8.93^{C}
T5 (PF$_{50}$PSB)	7.6^{BC}	12.4^{A}	12.0^{A}	10.6^{A}	1.27^{AB}	10.51^{C}	10.36^{C}	10.36^{B}
T6 (PF$_{25}$VM PSB)	8.3^{A}	12.1^{A}	11.4^{A}	10.5^{A}	1.10^{BC}	12.26^{A}	12.01^{A}	11.62^{A}

Different superscripted capital letters within treatment are significantly different at $P = 0.05$ according to Duncan's Test for separation of means.

Table 2: Impact of different treatments on yield parameters of rice (mean values).

Treatment	Panicles m^{-2}	Grain yield (t ha^{-1})	Filled grains panicle^{-1}	Unfilled grains panicle^{-1}
T1 (PF$_0$)	218.8^{B}	4.7^{B}	92^{B}	47^{A}
T2 (PF$_{50}$)	256.3^{A}	5.2^{A}	125^{A}	19^{B}
T3 (PF$_{25}$VM)	265.3^{A}	5.0^{A}	124^{A}	20^{B}
T4 (PF$_0$PSB)	221.1^{B}	4.6^{B}	95^{B}	47^{A}
T5 (PF$_{50}$ PSB)	255.0^{A}	5.2^{A}	128^{A}	21^{B}
T6 (PF$_{25}$VM PSB)	262.5^{A}	5.1^{A}	127^{A}	19^{B}

Different superscripted capital letters within treatment are significantly different at $P = 0.05$ according to Duncan's Test for separation of means.

available P (two laboratory replications). The extracted P from soil samples was analysed colorimetrically in 660 nm wavelength with a double beam spectrophotometer (Systronics, Model 2202) (Baruah & Barthakur 1999).

Soil microbial count: Field moist soil samples, stored at 4°C were used for soil microbial analysis. Soil microbial count was done following Pour Plate method, which represents count of only the living cells. For estimation of the population of only *Pseudomonas* genera within total microbial count, *Pseudomonas* Agar Base Media has been used.

Analysis of plant parameters: Number of tillers per plant were recorded at 20 d interval from transplanting to harvesting period (20, 40, 60 and 80 DAT). Plant samples were also collected at these time intervals and oven dried (70°C) to estimate the stem dry weight (g hill^{-1}).

After harvesting, the above-ground plant samples were washed to get rid of surface contamination and then divided into shoots (leaf sheath + stem), leaves, panicles followed by determination of parameters like number of panicles per m^2, number of filled and unfilled/chaffy grains per panicle. To estimate grain yield, harvested grain sample weight was recorded from a unit area (1 m^2) of each plot followed by oven drying (70°C).

RESULTS AND DISCUSSION

The climate of the area belongs to sub-humid and sub-tropical and the topography was plain. Soils were non-saline in nature, moderate to slightly acidic in reaction, lateritic and sandy loam in texture. Under all the treatments, pH$_w$ range of the soils varied from 5.2 - 6.4, which indicated the partial probability of P fixation in soils as Fe/ Al-phosphate (Hsu & Jackson 1960, Bhadoria et al. 2002) or with sesquioxides (Udo & Uzu 1972).

Impact of *Pseudomonas putida* treatment onto soil parameters: Soil samples collected during transplanting showed more or less similar P status (Fig. 2) under all treatments. At 20 DAT, soil available P was maximum under treatment 2 (P$_{50}$) and treatment 5 (P$_{50}$ + PSB). At the later stage of crop growth (40, 60 and 80 DAT) also, maximum level of plant available P was observed in soils under these 2 treatments. During this whole time period, the minimum available P was in soils with no addition of external P (T1 and T4) with/without PSB treatment.

Microbiological analysis and bacterial count, conducted on field-moist soil samples stored at 4°C, inferred that the total microbial as well as *Pseudomonas* population was similar in all the soils under six treatments during transplanting (Fig. 3). Soil samples collected at 20 d intervals after transplanting (20, 40 and 60 DAT) showed comparatively higher soil total microbial population under treatment 3 (P$_{25}$ + VM) and 6 (P$_{25}$ + VM + PSB). There was no significant trend of increase of *Pseudomonas* population with PSB treatments.

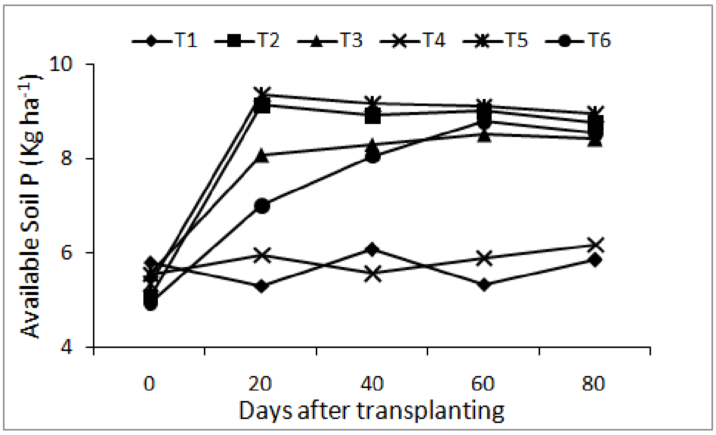

Fig. 2: Impact of different treatments on soil available phosphorus.

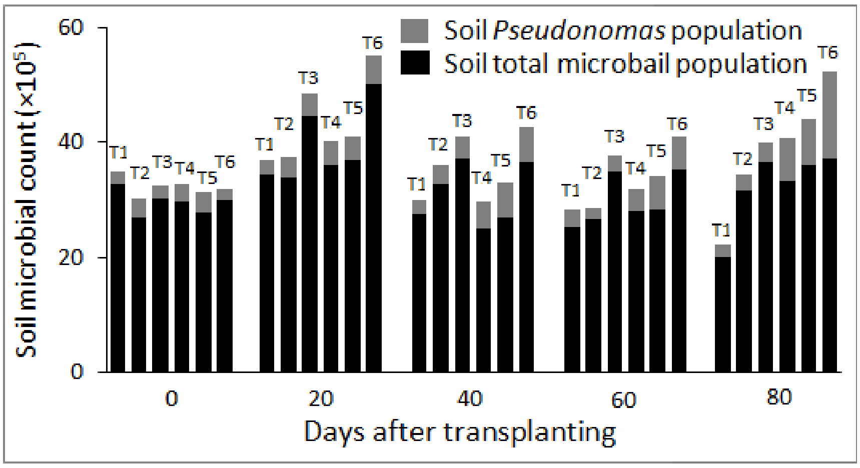

Fig. 3: Impact of different treatments on soil total microbial and *Pseudonomous* population.

However, soils treated with PSB (T4, T5 and T6) showed higher *Pseudomonas* population at 80 DAT.

Throughout the cropping period, minimum available P level in soils under treatment 1 (P_0) and 4 (P_0 + PSB) (Fig. 2) indicated the necessity of external P addition in rice cultivation. Observations of this research inferred an initial boost in the level of soil available P, followed by a slow decrease under treatment 2 (P_{50}) and 5 (P_{50} + PSB). Initial easy release of available P from inorganic phosphatic fertilizers followed by fixation in the soil matrix as Fe/Al-phosphate were the probable reason here (Bhadoria et al. 2002). Soils under treatment 3 (P_{25} + VM) and 6 (P_{25} + VM + PSB) revealed a comparative slow but consistent trend of increase in available soil P throughout the time-scale, which supported the findings of earlier researches i.e., inorganic phosphatic fertilizer in combination with organic manure resulted in higher plant available P in soils (Bhadoria et al. 2003, Wickram-atilake et al. 2011) in the long run.

As per the results, application of *P. putida* made no significant impact onto soil available P dynamics in rice ecology (Fig. 2). While comparing between treatment 1 and 4 (P_0 and P_0 + PSB respectively), treatment 2 and 5 (P_{50} and P_{50} + PSB respectively) as well as treatment 3 and 6 (P_{25} + VM and P_{25} + VM + PSB respectively), it was found that soil available P dynamics might have been determined by the chemistry of phosphatic fertilizer and vermicompost and not by the presence of PSB. One probable reason behind this was the submerged soil condition (aquic moisture regime) under lowland rice ecology, which might not support the growth of aerobic *P. putida* during the whole timeline as evident from Fig. 3. After the physical crop maturity, removal of water from rice field changed the soil environment to aerobic in later days. This might be the reason for higher *Pseudomonas* population at 80 DAT in soils (Fig. 3). However, it was apparent that the short stress-free activity of *P. putida* near the end of the growth period was not sufficient enough to change the available P dynamics in the soil (Fig. 2). During this aerobic period (around 80 DAT), higher *Pseudomonas* population was observed in soils under treatment 6 (P_{25} + VM + PSB) in comparison to treatment 5 (P_{50} + PSB). Availability of large quantity of decayed organic substrate as the food and energy source for gram-negative saprotrophic *P. putida* from vermicompost may be the possible reason behind this observation (Rao 2005).

Impact of *Pseudomonas putida* treatment onto vegetative and yield parameters of rice: A trend of increase of tiller number per plant from 20 to 40 DAT and slight decrease afterwards depicted the peak vegetative period of rice plant at around 40 DAT (Table 1). At this stage, highest tillers per plant were observed under treatment 5 (P_{50} + PSB). Plants under treatment 2 (P_{50}), 3 (P_{25} + VM) and 6 (P_{25} + VM + PSB) also showed good tillers per plant. Comparative low tillers per plant were observed where there was addition of no P in soils (T1 and T4). This trend remained almost analogous in 60 and 80 DAT. Similar to tillers per plant, stem dry weight of rice (g hill^{-1}) reached its highest at around 40 DAT. Results depicted highest stem dry weight (g hill^{-1}) under treatment 6 (P_{25} + VM + PSB) followed by treatment 2 (P_{50}) (T2) at 40, 60 and 80 DAT (Table 1). Plants grown with no added P (T1 and T4) showed the lowest stem dry weight.

Comparative analysis of these outcomes inferred no significant impact of application of *P. putida* as PSB onto the vegetative growth of rice. However, addition of P by any external source (phosphatic fertilizer and/or vermicompost) made a noteworthy increase in tillers per plant as well as stem dry weight. It seems that proper application of N, P and K fertilizer in soils was enough to maintain a balanced vegetative growth dynamics in rice with or without the treatment of PSB.

Considering the yield parameters of rice, number of panicle m^{-2} was highest under treatment 3 (P_{25} + VM) followed by treatment 6 (P_{25} + VM + PSB). Rice crops under treatment 2 (P_{50}) and 5 (P_{50} + PSB) also showed relatively high panicles m^{-2}. Comparatively less panicles m^{-2} was observed in rice plants without any addition of external P (T1 and T4). Highest grain yield of rice was obtained under treatments 2 (P_{50}) and 5 (P_{50} + PSB). Plants grown under treatments 3 (P_{25} + VM) and 6 (P_{25} + VM + PSB) also showed good results. Similar to the trend of panicles m^{-2}, grain yield was low under treatments 1 (P_0) and 4 (P_0 + PSB). Comparison of results under treatments 2 and 5 as well as treatments 3 and 6 indicated no significant contribution of PSB treatment on rice seedlings. Analysis of filled and unfilled/chaffy grains panicle^{-1} inferred that addition of P from external source favoured grain filling in rice and significantly reduced the number of chaffy grains per panicle. However, these results also showed no significant positive impact of *P. putida* as PSB onto rice yield parameters.

Several earlier studies suggested the beneficial impacts of PSB onto crop productivity (Illmer & Schinner 1992, Sundara et al. 2002, Kaur & Reddy 2014). On the contrary, a study showed no notable relationship of PSB with the P uptake capacity of Soybean (Fernández et al. 2007). Maximum of these researches were conducted in upland non-waterlogged ecology, where the soil environment remains aerobic. Chemistry of lowland rice soils, submerged for a long part of a cropping season, is different (Sahrawat 2004, Hussain et al. 2012). Severe depletion in oxygen level (Fageria et al. 2011) makes this very ecology an unfavourable niche for soil aerobic micro-organisms like *P. putida*. However, earlier research in rice ecology depicted higher available P in soil as well as greater P uptake and dry weight of the plants with application of mixed culture of *Bacillus circulans* and *B. subtilis* (Banik & Dey 1981). Among these *B. subtilis* is a facultative anaerobe (Ye et al. 2000) which can sustain and grow in submerged soil unlike *P. putida*. No/less impact of aerobic *P. putida* on soil P dynamics and plant productivity, thus, might be explained by the anaerobic rice ecology. An augmentation in the population of *P. putida* in post-waterlogged dry field also supports this premise.

CONCLUSION

All over the world, use of PSB for higher crop production and quality is a common practice now-a-days. Nevertheless, it is a matter of scientific concern that actually how much significant contribution is made by PSB in crop yield improvement. So far researches while confirmed the certain efficiency of PSB to solubilize phosphatic compounds in laboratory conditions, the same is not been proved in field condition. There are several soil and management factors which actually decide the efficiency of PSB in soils. Furthermore, as discussed in the present study, the waterlogged anaerobic soil environment in rice ecology might be another issue which partially blends the impact of PSB.

Following the complex and paradoxical dynamics of PSB in soils, it can be said that neither the present research nor the earlier ones are enough to conclude about the capacity of PSB to solubilize P and increase productivity under lowland rice ecology. It only signifies that further series of detail researches are necessary to make a holistic understanding in this regard.

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