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Impact of phosphate solubilizing bacteria on growth and yield of maize

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Abstract

Soil microorganisms are supportive in the transformation of soil phosphorus (P) and are thus an important component of the soil P cycle. These are effective in releasing P both from inorganic and organic pools of total soil P through their respective solubilizing and mineralizing abilities. To evaluate this, five promising strains of PGPR [PS-01 (Burkholderai sp.), PS-12 (Bacillus sp.), PS-32 (Pseudomonas sp.), PS-41 (Flavobacterium sp.) and PS-51 (Pseudomonas sp.)] capable of solubilization of both organic and inorganic phosphorus as investigated under in vitro conditions were evaluated in a pot trial for their rhizosphere phosphatase activity and mineralization potential of organic P in soil, plant growth and yield at different farmyard manure (FYM) levels i.e. 0, 8 and 16 Mg ha^{-1}. These bacterial strains were also monitored for other attributes like chitinase activities and root colonization ability in addition to phosphatase activity, auxin production and ACC-deaminase activity. In response to inoculation with these selected rhizobacteria, significant increases in plant height, root length, shoot dry weight, root dry weight and grain yield were observed which were up to 16, 11, 42, 29 and 33%, respectively, over uninoculated control in the presence of FYM at 16 Mg ha^{-1}. Similarly, there were significant increases in the rhizosphere phosphatase activity, mineralization of organic P and soil available P which were 189, 185 and 62% higher than uninoculated control in the presence of FYM, respectively. The study demonstrated that the use of PGPR having multifaceted beneficial traits would be highly effective for improving growth and yield of crops.

Key words: Phosphorus, phosphate solubilising/mineralizing bacteria, phosphatase enzyme, farmyard manure

Introduction

Phosphorus plays a significant role in plant growth and metabolism by supplying energy needed for metabolic processes (Lal, 2002) and is considered obligatory for the synthesis of nucleic acid molecules (DNA and RNA). It has been reported that nearly 80 to 90% soils from arid and semiarid regions of the world are deficient in available phosphorus (Memon *et al.*, 1992). Moreover, in soil, the main problem with P for plant uptake is its availability in very minute quantity. The availability of P is affected by soil chemical properties as well as human management activities. Phosphorus containing chemical fertilizers after their application to agricultural soils either get fixed or precipitated in soils.

Phosphorus exists in soil as organic and inorganic forms. Most of the total P in soils is present in organic forms (Speir and Ross, 1978) as phospholipids, nucleotides and inositol phosphate (Turner *et al*., 2002). Soil organic phosphorus (SOP) thus plays a major role in P nutrition of crops especially in high P-fixing calcareous soils (Tarafdar and Claasson, 1988). The SOP can contribute substantially to total phosphorus (TP), ranging from 20 to 80% in most mineral soils and can supply a significant portion of plant-available P (Sharpley, 1985). As plant cannot take up P as organic form directly, therefore, it must be first transformed into inorganic form after being mineralized and catalysed by different soil enzyme processes (Sarapatka, 2003). Soil phosphatases help in hydrolysis of soil organic phosphorus which convert it into inorganic forms (HPO_4^- and $H_2PO_4^-$) before it can be utilized and taken up by plant roots from the soil solution (He *et al.,* 2004). This reaction is catalyzed by phosphatase enzymes present in soil, microorganisms, plant roots and also in extracellular forms in soil. Phosphatase-catalysed reactions are involved in the hydrolysis of both esters and anhydrides of H_3PO_4 (Tabatabai, 1994). Phosphatases are classified as acid and alkaline phosphatases because their maximum activities can occur at low (pH 6.5) and high (pH 11) pH ranges, respectively. Acid phosphatases are produced by both microorganisms as well as higher plants but alkaline phosphatases are mainly produced by microorganisms (Tabatabai and Bremner, 1969). The release of orthophosphate ions (HPO_4^{--} and $H_2PO_4^-$) from soil organic P is effectively mediated by both plant and microbial phosphatases, with some indication that microbial phosphatases show greater effectiveness in releasing P (Tarafdar *et al*., 2001).

It has been reported that soil microorganisms are helpful in releasing P from organic complexes of total soil P by mineralization (Abd-Alla, 1994; Bishop *et al.,* 1994).

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Most of the soils contain P in insoluble compounds which is unavailable to plants. Large quantities of chemical fertilizers are used to replenish soil nutrients, resulting in high costs and severe environmental contamination (Dai *et al.,* 2004). According to Jasinski (2006), the world reserves of phosphate rock (a raw material for phosphatic fertilizers) are becoming increasingly inadequate and it is estimated that they will be exhausted within 50-100 years. Moreover, the quality of phosphate rock is decreasing and cost is increasing (Cordell, 2008). In order to overcome these inefficiencies, microbial inoculants are now being explored worldwide for their potential to mobilize unavailable P, thereby, increasing the capacity of plant for uptake of P. Concurrent exudation of organic acids and phosphatases by phosphate solubilizing microorganisms could enhance P solubility, by releasing bound organic phosphates and its mineralization by escalating the rate of hydrolytic cleavage (Trolove *et al*., 2003). Organic amendments added to the soil in the forms of manure and plant residues are decomposed by microorganisms and help in the mineralization of organic P present in the added and native soil organic matter (Richardson and Simpson, 2011). So far, few efforts have been made to directly manipulate microbial populations which are able to increase organic phosphorus (Po) mineralization.

The rhizobacteria, associated with plant roots are beneficial to plants and are often referred to as plant growth promoting rhizobacteria (PGPR) (Vessey, 2003). They can have an effect on plant growth both directly or indirectly through different mechanisms of action (Mantelin and Touraine, 2004). The production of plant growth regulators or phytohormones is one of the direct mechanisms exhibited by PGPR in promoting plant growth (Glick, 1995). Several soil microorganisms are known to produce auxin in culture media and also in soil (Asghar *et al.*, 2000; Khalid *et al*., 2004). A number of phosphate solubilizing bacteria and fungi act as plant growth promoters because of their ability to release IAA (Souchie *et al*., 2007). A PGPR strain *Bacillu firmus* NARS1 positive for P solubilization, siderophore production and IAA *in vitro* elicited a strong growth promoting effect on inoculated plants (Mahejibin and Patel, 2007). Similarly, it is well established that ACC-deaminase producing bacteria can increase root elongation and seed germination by lowering plant ethylene levels (Arshad and Frankenberger, 2002; Nadeem *et al*., 2010). Bacterial strains having ACC-deaminase and phosphate solublizing activities were found to improve root length significantly (Hameeda *et al*., 2006). For improving growth of the soybean seedlings, the efficacy of IAA producing *Pseudomonas* is coupled with their ACC deaminase activities (Husen *et al.,* 2009).

Present attempt was made to appraise the potential of plant growth promotion of rhizobacteria with phosphate solubilization and mineralization ability coupled with ACC-deaminase activity and auxin production capability.

Materials and Methods

Preparation of inoculum

Rhizobacterial strains from the rhizosphere of maize were isolated using glucose peptone agar media (GPAM). Dilution plate technique was employed under aseptic conditions for the isolation of rhizobacteria. The inocula of the selected bacterial strains were prepared by growing them in 250 mL conical flasks containing general purpose media (Glucose, 0.75 g L^{-1}; ammonium sulphate, 0.25 g L^{-1}; potassium hydrogen phosphate, 0.25 g L^{-1}; peptone, 0.25 g L^{-1}; magnesium sulphate hepta-hydrate, 0.05 g L^{-1}). The inoculated flasks were incubated at 28 ± 1 °C for 72 hours in the orbital shaking incubator at 100 rev min^{-1}. Uniform optical density at 535 nm was achieved by dilution to maintain uniform cell density (10^8-10^9 colony forming units per mL) with a spectrophotometer. The suspension of the selected bacterial strains was used for seed inoculation.

Pot experiment

On the basis of growth promoting activities of fifteen strains as observed in the jar experiment under axenic conditions (data not shown), five putative bacterial strains [PS-01 (*Burkholderai* sp.*)*, PS-12 (*Bacillus* sp.), PS-32 (*Pseudomonas* sp.), PS-41 (*Flavobacterium* sp.) and PS-51 (*Pseudomonas* sp.)] were selected for conducting a pot trial at different levels of manure [0, 8 and 16 Mg ha^{-1}]. Maize seeds (Pioneer-31-R-88) were inoculated by slurry method. Slurry was prepared by mixing peat with 10% sugar solution. Inoculum was prepared as described earlier and mixed in peat (inoculum to peat ratio, 1:1 v/w). In the case of uninoculated control, seeds were coated with slurry prepared by mixing sterilized (autoclaved) peat, sterilized sugar solution and sterilized broth without inoculation. Inoculated ten maize seeds were sown in each pot containing 12 kg soil and thinned to one plant after seven days of germination. The soil used for pot trial was sandy clay loam and initially with pH, 8.1; EC, 2.6 d Sm^{-1}; $CaCO_3$, 6.3%; organic matter, 0.67%; total nitrogen, 0.045%; available phosphorus, 8.11 mg kg^{-1} and extractable potassium, 148 mg kg^{-1}. Pots were placed in a wire house according to completely randomized design. Recommended doses of NPK fertilizers were applied at 300-150-125 kg ha^{-1} in each pot. The effectiveness of inoculation was assessed in the presence and absence of farmyard manure (FYM) in the form of da iry manure as an organic amendment. Plant growth parameters like plant height, root length, shoot and

root dry weights and grain yield were recorded at harvest stage. Rhizosphere phosphatase activity (alkaline) was evaluated as proposed by Eivazi and Tabatabai (1977) and the total and inorganic phosphates in soil were determined following the methodology of Mehta *et al*. (1954). Organic P was estimated by subtracting inorganic P from total soil P. The selected bacterial strains used in pot experiment were also characterized for their chitinase activity using modified procedure of Chernin *et al*. (1998) and root colonization assay as described by Simons *et al*. (1996). The strains were identified by Biolog® Identification System (Microlog ™ System Release 4.2, Hayward, CA, USA).

Statistical analysis

Data regarding growth and yield parameters were recorded after harvesting and analyzed statistically using two ways factorial set up (Steel *et al*., 1996). Means were compared by least significant difference test.

Results

Study revealed that plant height of maize increased significantly due to inoculation with all the selected bacterial strains at manure level 0 Mg ha^{-1} except with PS-41 (*Flavobacterium* sp.) (Table 2) and maximum increase in plant height was observed in response to inoculation with PS-51 (*Pseudomonas* sp.). Inoculation with PS-01 (*Burkholderai* sp.) caused respective increases in plant height at manure level 8 and 16 Mg ha^{-1} which were 14 and 16% higher compared to uninoculated control. The inoculation effect at manure level 8 and 16 Mg ha^{-1} was statistically at par with each other but was significant over respective control. In case of root length (Table 2), significant increase was recorded by inoculation with selected bacteria in the absence and presence of FYM compared to uninoculated control. Maximum increases in root length at manure level 8 and 16 Mg ha^{-1} were obtained by PS-01 (*Burkholderai* sp.) which were 9 and 11% higher compared to uninoculated control.

It is obvious from the data that all the strains were also effective in increasing shoot dry weight compared to respective uninoculated control both in the presence and absence of FYM. Maximum increase in shoot dry weight at manure level 8 Mg ha^{-1} was shown in response to inoculation with PS-51 (*Pseudomonas* sp.) that caused up to 34% higher shoot dry weight compared to respective uninoculated control while at manure level 16 Mg ha^{-1}, the strain PS-32 (*Pseudomonas* sp.) caused up to 42% more shoot dry weight over control. Data regarding inoculation effect on root dry weight showed that maximum increase in root dry weight at manure level 0 Mg ha^{-1} was obtained in response to inoculation with strain PS-32 (*Pseudomonas* sp.) which was 12% higher than respective uninoculated control and at manure level 8 Mg ha^{-1}, the strain PS-12 (*Bacillus* sp.) resulted in up to 14% higher root dry weight over control. The inoculation with bacterial strain PS-01 (*Burkholderia* sp.) caused maximum increase (29% over control) at farmyard manure level 16 Mg ha^{-1}.

The data in Table 3 indicated that at manure level 8 and 16 Mg ha^{-1}, maximum increase in grain yield was obtained in response to inoculation with PS-12 (*Bacillus* sp.) which was 33% more than respective control. Again, the inoculation effect at manure level 8 and 16 Mg ha^{-1} was statistically at par with each other but was significant over respective control. Regarding rhizosphere soil phosphatase activity (Table 3), the strain PS-12 (*Bacillus* sp.) caused maximum increase in rhizosphere soil phosphatase activity at manure level 0 and 16 Mg ha^{-1} which were 58 and 189% higher, respectively, than uninoculated control. The effect of inoculation on mineralization of organic phosphorus in soil (Table 3) revealed that maximum increases in mineralization organic P at manure level 8 and 16 Mg ha^{-1} were 187 and 185%, respectively, due to inoculation with strain PS-12 (*Bacillus* sp.) compared to uninoculated control. Similarly, inoculation with selected bacterial strains also enhanced the capacity of soil in available P contents and maximum increase in soil available P at manure level 0 Mg ha^{-1} was observed in response to inoculation with PS-01(*Burkholderia* sp.) (11% over respective control). On the other hand, at higher manure level i.e. 8 and 16 Mg ha^{-1}, inoculation with PS-12 (*Bacillus* sp.) resulted in maximum increase in soil available P which were 38 and 62% higher than respective uninoculated control, respectively.

Regarding chitinase activity of the selected strains (Table 1), it was observed that out of five, three strains (PS-01, PS-12 and PS-32) were found to be positive for chitinase activity while other two strains (PS-41and PS-51) were negative for chitinase activity. Data regarding root colonization assay (Table 1) revealed that all the strains showed variable efficacy for colonizing root of maize. The strain PS-01 exhibited higher root colonization (7.74×105 cfu g^{-1}) followed by PS-12 (6.42×105 cfu g^{-1}). The selected bacterial strains were identified as PS-01, *Burkholderai* sp.; PS-12, *Bacillus* sp.; PS-32, *Pseudomonas* sp.; PS-41, *Flavobacterium* sp. and PS-51, *Pseudomonas* sp.

Discussion

The study demonstrates the effectiveness of plant growth promoting rhizobacteria containing phosphate

Table 1: *In vitro* traits of the selected bacterial strains used in pot trial

Bacterial Strain	P-Solubilization (µg mL^{-1})	Phosphatase activity (µg PNP g^{-1})		Auxin production (IAA equivalents) (µg mL^{-1})		ACC-deaminase activity (nmol α ketobutyrate g^{-1} biomass h^{-1})	Chitinase activity (qualitative)	Root colonization (cfu g^{-1})
		Acid	Alkaline	Without L-tryptophan	With L-tryptophan			
PS-01 (*Burkholderai* sp.)	682.88 ± 19.13	35.18 ± 1.5	23.12 ± 1.0	1.15 ± 0.2	21.11 ± 1.1	335 ± 9	+ve	7.84×10^5
PS-12 (*Bacillus* sp.)	661.47 ± 10.24	42.87 ± 1.5	27.66 ± 1.1	1.94 ± 0.3	24.80 ± 4.1	351 ± 4	+ve	6.92×10^5
PS-32 (*Pseudomonas* sp.)	523.24 ± 21.07	29.44 ± 1.6	14.21 ± 1.0	1.72 ± 0.3	25.13 ± 3.7	356 ± 13	+ve	5.12×10^4
PS-41 (*Flavobacterium* sp.)	619.60 ± 21.83	21.08 ± 1.2	15.64 ± 0.8	1.32 ± 0.2	30.56 ± 2.0	240 ± 4	-ve	4.27×10^4
PS-51 (*Pseudomonas* sp.)	733.68 ± 10.27	25.43 ± 1.7	21.14 ± 0.9	3.18 ± 0.6	36.63 ± 2.0	316 ± 7	-ve	5.42×10^4

Table 2: Effect of inoculation with selected bacterial strains on differed growth parameters of maize at different farmyard manure (Mg ha^{-1}) levels in a pot trial

Treatment	Plant height (cm)			Root length (cm)			Shoot dry weight (g plant^{-1})			Root dry weight (g plant^{-1})		
	0	8	16	0	8	16	0	8	16	0	8	16
Uninoculated control	125.7 g	127.5 fg	131.0 e-g	97.7 h	101.1 gh	103.2 f-h	51.1 i	54.1 hi	58.4 gh	52.5 g	56.7 fg	60.4 d-f
PS-01 (*Burkholderai* sp.)	135.6 de	145.7 a-c	152.4 a	107.4 c-f	110.2 a-e	115.0 a	67.4 d-f	68.1 d-f	78.0 ab	58.3 ef	63.7 b-d	77.4 a
PS-12 (*Bacillus* sp.)	135.3 de	144.3 bc	147.3 ab	106.6 d-g	109.0 b-f	113.1 ab	63.2 e-g	72.6 b-d	77.5 ab	57.8 ef	64.6 b-d	68.2 b
PS-32 (*Pseudomonas* sp.)	134.1 d-f	137.3 de	140.1 cd	107.9 c-f	107.3 c-f	112.2 a-d	67.8 d-f	71.2 b-d	83.2 a	58.7 ef	60.8 d-f	65.9 bc
PS-41 (*Flavobacterium* sp.)	130.3 e-g	137.0 de	139.1 cd	105.5 e-g	108.2 c-f	110.6 a-e	61.9 fg	63.4 e-g	69.7 c-e	56.1 fg	60.7 d-f	62.1 c-e
PS-51 (*Pseudomonas* sp.)	136.3 de	138.9 cd	140.0 cd	108.4 c-f	109.1 a-e	113.1 a-c	62.4 fg	73.3 b-d	75.8 bc	56.7 fg	62.1 c-e	66.7 bc
LSD	**6.979**			**5.8697**			**7.2858**			**4.7291**		

Table 3: Effects of inoculation with selected bacterial strains on maize yield, soil phosphatase activity, P mineralization and soil available P at different farmyard manure (Mg ha^{-1}) levels in a pot trial

Treatment	Grain yield (g plant^{-1})			Rhizosphere soil phosphate activity (µg PNP g-1dwt soil-h^{-1})			Mineralization of organic P in soil (mg kg^{-1})			Soil available phosphorus (mg kg^{-1})		
	0	**8**	**16**	**0**	**8**	**16**	**0**	**8**	**16**	**0**	**8**	**16**
Uninoculated control	34.46 f	38.81 de	40.65 d	106.3 i	122.3 hi	132.1 h	13.6 i	14.2 i	17.5 hi	9.54 j	9.76 ij	10.08 h-j
PS-01 (*Burkholderai* sp.)	42.03 d	50.35 ab	50.41 a	162.4 g	270.8 c	366.1 a	28.7 f	38.1 c-e	43.3 b	10.56 gh	12.82 e	14.99 b
PS-12 (*Bacillus* sp.)	36.37 ef	51.67 a	53.87 a	168.2 g	252.5 d	381.3 a	28.6 f	40.8 b-e	49.9 a	10.54 gh	13.47 d	16.30 a
PS-32 (*Pseudomonas* sp.)	42.41 cd	46.13 bc	46.34 b	157.5 g	233.5 e	288.0 b	25.8 fg	36.8 e	42.1 bc	10.43 gh	12.09 f	14.56 bc
PS-41 (*Flavobacterium* sp.)	34.90 f	39.42 de	40.78 d	116.6 hi	214.4 f	234.8 e	21.5 gh	36.6 e	41.6 b-d	10.11 h-j	10.95 g	13.05 de
PS-51 (*Pseudomonas* sp.)	35.98 ef	41.03 d	41.83 d	121.7 hi	214.8 f	303.0 b	25.1 fg	37.3 de	42.9 b	10.22 hi	11.84 f	14. 17 c
LSD	**3.8058**			**15.982**			**4.3947**			**0.646**		

solublizing and/or phosphatase producing ability along with their ACC-deaminase and auxin production traits for increasing the growth and yield of maize under potted conditions. All the strains significantly increased the shoot and root length, fresh and dry weights as compared to uninoculated control. The inoculation with microorganisms having phosphate solublizing ability concurrently improved plant P uptake and crop growth. This increase in growth may be attributed to auxin production (Gyaneshwar *et al*., 2002; Fankem *et al.,* 2008), ACC-deaminase activity (Zafarul- Hye *et al*., 2007; Naik *et al*., 2008), production of organic acids (Fankem *et al*., 2008) or phosphatases (Abd-Alla, 1994; Chabot *et al*., 1996) to solubilize/mineralize P, thereby increasing phosphate nutrition of inoculated plants. Similarly, Kapri and Tewari (2010) also got increased shoot /root length, fresh and dry weight of shoot/root of chickpea due to inoculation with phosphate solubilizing and phosphatase producing *Trichoderma* sp. Similarly, Linu *et al*. (2009) found that *Burkholderia* sp. gave better results in improving growth of cowpea and this strain had been previously evaluated by Pandey *et al*. (2005) to have phosphate solubilization, auxin production, ACC deaminase activity and also nitrogen fixing ability.

In our pot experiment, all the strains increased growth and yield parameters of maize in comparison with control treatments. The performance of the selected strains in increasing different growth, yield parameters and P contents of soil with increasing levels of organic matter showed that these strains might have mineralized the applied manure by releasing phosphatase enzyme (Chabot *et al.,* 1996: El-Tarabily *et al*., 2008). Statistical analysis revealed that a positive correlation was found between grain yield and bacterial *in vitro* plant growth promoting traits of phosphatase activity (r= 58), ACC-deaminase activity (r= 44) and auxin production (r = 23). It is likely that the increase in soil available P might have resulted due to greater microbial activities in response to inoculation with selected strains which dissolved the fixed forms of soil phosphorus (inorganic P) or they might have mineralized organic matter to release organic P (Richardson and Simpson, 2011). Afzal and Bano (2005) also demonstrated that phosphate solublizing microorganisms in combination with phosphatic fertilizer and organic manure significantly enhanced seed phosphorus content, tillers per m^2, grain and biological yield of wheat.

The mechanism involved in plant growth promotion in the present study by the selected bacterial strains may also be related to their auxin production and ACC-deaminase besides phosphate solubilization. However, the increased rhizosphere phosphatase activity in response to inoculation with selected bacterial strains revealed that these microbes might have secreted phosphatase enzyme to dissolve P

present in the organic matter applied in the form of farmyard manure. Chabot *et al*. (1996) reported an increase in dry matter of lettuce and maize due to inoculation with phosphate solubilizing *Rhizobium leguminosarum* biovar *phaseoli* capable of solublizing both organic and inorganic phosphorus sources when tested *in vitro*. Microbial production of organic acids and acid phosphatase has important role in mineralizing organic P present in soil (Cherr *et al*., 2006; Wilhelm *et al.,* 2007).

Our study demonstrates that multifaceted bacteria could be more effective PGPR to improve crop growth and yield. Correlation analysis revealed that relationship existed among the bacterial traits shown in Table 1 and their relationship with yield of maize. Similarly, Minaxi *et al.* (2012) isolated a rhizobacterial strain RM-2 identified as *Bacillus* sp. having many useful traits like P solubilization, ACC deaminase activity, indole acetic acid production, antifungal activity and ammonia production and it had positively affected the growth and nutrient uptake of cowpea plants. As the inoculation effect at manure level 8 and 16 Mg ha^{-1} was statistically at par with each other in most of the growth and yield parameters, therefore, we recommend the FYM level 8 Mg ha^{-1} in combination with inoculation to make this technology more economical and cost effective.

The study demonstrates that the use of PGPR with P solubilization, auxin production and ACC deaminase activity traits could be highly effective for improving growth and yield of maize crop. It is concluded from the experimentation that phosphate solublizing bacteria enhance the growth through simultaneous exudation of organic acids (by decreasing pH) and/or through releasing phosphatases and ACC-deaminase. It can be emphasized that during screening and selection strategies, the selection of bacteria with multiple traits is an attracting approach.

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