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ROLE OF PHOSPHATE SOLUBILIZING BACTERIA (PSB) IN ENHANCING P AVAILABILITY AND PROMOTING COTTON GROWTH

M. A. Qureshi, Z. A. Ahmad*, N. Akhtar, A. Iqbal, F. Mujeeb, and M. A. Shakir

Soil Bacteriology Section, Ayub Agricultural Research Institute, Faisalabad.
*Institute of Soil Chemistry and Environmental Sciences, AARI, Faisalabad.
Corresponding author e-mail: qureshifsd@gmail.com

ABSTRACT

Plant growth promoting rhizobacteria (PGPR) mediate the soil processes such as decomposition, nutrient mobilization, mineralization, solubilization, nitrogen fixation and growth hormone production. Microorganisms having the phosphate solubilizing capacity can convert the insoluble phosphates into soluble forms through the production of organic acids. Inoculation of seed with P-solubilizing microorganisms is a promising technique which may alleviate the deficiency of phosphorus. This bioavailability of soil inorganic phosphorus in the rhizosphere varies considerably with plant species and nutritional status of soil. A field experiment was conducted at Fiber Crops Section, Ayub Agricultural Research Institute (AARI) Faisalabad to assess the ability of P-solubilizing rhizobacteria to enhance the growth and yield of cotton. Isolation and screening of P-solubilizer on Pikovskaya's medium was carried out at the Soil Bacteriology Section Faisalabad. The trial was conducted on clay loam soil with pH 8.3, EC 2.8 dSm^{-1}, N 0.040 %, organic matter 0.75% and available P 10.3 mg kg^{-1} with three fertilizer levels viz. 120-30, 120-60, 120-90 kg NP ha^{-1} with and without P-solubilizer (*Bacillus* sp.) inoculum. Results revealed that bacterial inoculum produced significantly higher seed cotton yield 1630 as compared to 1511 kg ha^{-1}. The highest seed cotton yield was observed at highest fertilizer level i.e. 1733 kg ha^{-1} with inoculum. The physical parameters like plant height, number of bolls per plant and boll weight and soil available P determined at regular intervals (30, 60, 90, days after sowing) was also found higher in the inoculated treatments. More exploration of this area of research should be carried out in different ecologies to compensate the mineral fertilizers

Keywords: Phosphate solubilizing bacteria (PSB), Available P, *Bacillus*, Phosphorus, cotton.

INTRODUCTION

Rhizosphere is the soil zone surrounding the plant roots while rhizoplane is directly in contact with the roots (Kennedy, 2005). Plant roots exuded the organic contents in the rhizosphere and subsequently increased the microbial activity and termed as "rhizosphere effect" by Hiltner (1904). Microorganism's exerted beneficial effect on plant growth and development through different means are termed as Plant Growth Promoting Rhizobacteria (PGPR) (Vessey, 2003).

PGPR influenced the plant growth by direct or indirect modes (Kloepper, 1993; Kapulnik, 1996; Lazarovits and Nowak, 1997). Direct modes are production of growth stimulators, improvement in plant nutrient status (release of phosphates and micronutrients from insoluble sources), lowering of the ethylene level in plant, and induction of systemic resistance. Indirect modes of action are production of antibiotics, production of biocontrol agents and degradation of xenobiotics (Jacobsen, 1997, Somers *et al.,* 2004).

Phosphorus, the second major plant nutrient is an integral part of plants generally deficient in soils (Batjes, 1997) due to its speedy fixation. Phosphate anions ($H_2PO_4^-$, HPO_4^{2-}) are extremely reactive and form metal complexes with Ca in calcareous soils (Lindsay *et al.,* 1989) and Fe^{3+} and Al^{3+} (Norrish and Rosser, 1983) in acidic soils. These metal ion complexes precipitated the 80% of added P fertilizer (Stevenson, 1986; Goldstein, 1986).

Phosphate solubilizing microorganisms (PSM) have attracted the researchers to exploit their potential to utilize phosphate reserves in semi arid regions and to enhance the crop yields (Goldstein *et al.,* 1993; Fasim *et al.,* 2002; Khan *et al.,* 2006). Phosphate solubilizing microorganisms have established their role for optimum growth of plants under nutrient imbalance conditions. (Glick 1995; Iguala *et al.,* 2001; Wu *et al.,* 2005).

Phosphate solubilizers are economical, eco-friendly and have greater agronomic utility to compensate the expensive inorganic sources of P fertilizers.

Studies revealed that inoculation of PSM's enhanced the crop yields by solubilizing the soil fixed and applied phosphates (Zaidi, 1999; Gull *et al.,* 2004). Species of the genus *Bacillus*, *Pseudomonas, Rhizobium, Aspergillus* and *Penicillium* are the potential P-solubilzers commonly present in the soil (Rodriguez and Fraga, 1999). Asea *et al.* (1988) reported that *Bacillus megaterium* is the most effective phosphate solubilizer.

PSM's produced the low molecular weight organic acids (gluconic, 2-ketogluconic, glyoxylic, citric, malic, lactic acids etc.) to solubilize the insoluble phosphates and lowering of pH in the cell surroundings

(Halder *et al.,* 1990; Illmer and Schinner, 1992; Goldstein, 1993; Kim *et al.,* 1997; Jones, 1998; Maliha *et al.,* 2004; Khan *et al.,* 2006). Organic acids acted like chelates by releasing their hydroxyl and carboxyl groups, chelate the phosphate bonded cations and solubilize the insoluble phosphates (Kpomblekou and Tabatabai, 1994). Besides organic acid production, the enzyme phosphatase has a role in P-solubilization (Al-Ghazali *et al.,* 1986). It has also been reported that siderophores, chelating compounds and mineral acids also responsible for P-solubilization (Gyaneshwar *et al.,* 1998; Wu *et al.,* 2005).

Present attempt was made to assess the effect of phosphate solubilizer i.e. *Bacillus* sp on the growth of cotton and flux of available P in soil under cotton crop.

MATERIALS AND METHODS

Isolation of P-solubilizer: Isolation of *Bacillus* sp was carried out by standard dilution plate technique. The rhizosphere soil of cotton growing in the permanent layout plot at Soil Bacteriology Section AARI, Faisalabad was used to prepare the serial dilutions. The serial dilutions of rhizosphere soil samples was subjected to heat shock at 80 ^{o}C for 30 minutes in an oven (Claus,1964) and inoculated the selective medium (Nautiyal, 1999). Plates carrying selective medium were incubated at 28 ± 2 ^{o}C for seven days. The growth of *Bacillus* was screened out thrice for purification on the selected medium to get a pure culture. Isolates were checked for their solubilization on Pikovskaya's medium (Pikovskaya, 1948). Isolates of *Bacillus* forming halos on the above mentioned medium were treated as P-solubilizers and maintained to check the extent of solubilization. After preliminary screening, standard methods [Gram (+), Catalase (+), urea hydrolysis (-) and citrate utilization (+)] as outlined in Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984) the pure culture was predicted as *Bacillus sp.*

Determination of Auxin biosynthesis: Isolates of *Bacillus* sp (four of each) named as (B_1, B_2, B_3, & B_4) were screened for their auxin biosynthesis potential. Isolates of *Bacillus* were maintained on the Pikovskaya's broth culture for 48-72 hours. Auxin biosynthesis potential was determined as Indole-3-acetic acid (IAA) equivalents using Salkowski's reagent (2 mL of 0.5M $FeCl_3$ + 98 mL of 35% $HClO_4$) as described by Sarwar *et al.* (1992). Isolates of *Bacillus* sp having the highest auxin biosynthesis potential were selected for the experimentation.

Determination of Phosphate Solubilization: The potential of *Bacillus* isolates (B_1, B_2, B_3 and B_4) for solubilization of insoluble phosphates were checked on the Pikovskaya's medium (Pikovskaya, 1948). Isolates having the potential to solubilize insoluble phosphates on the Pikovskaya's medium by forming the halos. The growth and solubilization diameter were determined after incubation at 28 ± 2 ^{o}C for seven days. On the bases of diameter of clearing halo zones, solubilization efficiency (SE) and solubilization index (SI) (Gaur, 1990; Nguyen *et al.,* 1992; Vazquez *et al.,* 2000) were calculated using the following formulas.

$$SE = \frac{\text{Solubilization diameter}}{\text{Growth diameter}} \times 100$$

$$SI = \frac{\text{colony diameter + halozone diameter}}{\text{colony diameter}}$$

Auxin biosynthesis potential of *Bacillus* sp was ranged from 2.04-2.78 ppm. Isolate of *Bacillus* sp (B_1) having the highest auxin biosynthesis and phosphate solubilization potential (Table 1) was selected for further experimentation.

Inoculum preparation: Inoculum (B_1 isolate) was prepared in the selective medium (Nautiyal, 1999). The medium was inoculated in 500 mL conical flask containing 150 mL broth and incubated at 28 ± 2 ^{o}C under shaking at 100-150 rpm for three days to give an optical density of 0.5. Peat was sterilized at 121 ^{o}C and 15 psi pressure for one hour and inoculated with broth culture *Bacillus* sp (100 mL per kg of peat). Peat based inoculum was incubated at 28 ± 2 ^{o}C by adding 10% sugar solution for 3-4 days to increase the population of respective microbe up to 10^8 CFU mL^{-1}. *Bacillus* inoculation having @10^8 MPN bacterial cells per gram of peat were applied to cotton seed as seed coating.

Field Experiment: Study was conducted to assess the influence of P-solubilizer (*Bacillus* sp) on the growth and yield of cotton in field conditions. The pre-sowing soil samples were collected, air dried, thoroughly mixed, sieved and analyzed for various physico-chemical characteristics. The soil was medium textured having pH 8.3, EC 2.8 dS m^{-1}, N 0.040 %, organic matter 0.75% and available P 10.3 mg kg^{-1} at research area of Fiber Crops Section, AARI, Faisalabad. There were six treatments having three P levels i.e. 30, 60 and 90 kg ha^{-1} as SSP were tested with and without P-solubilizer inoculation while uniform dose of N i.e. 120 kg ha^{-1} as urea was applied. All P was applied as basal to all the treatments and N in two splits (half at sowing and remaining half after one month of germination) and study was laid out in randomized complete block design (RCBD).

Data regarding seed cotton yield, plant height, boll weight and boll number per plant were recorded. Samples for available P were collected at 30, 60 and 90 days of sowing. Nitrogen was determined according to Kjeldhal method (Bremner and Mulvany, 1982) while phosphorus by modified Olsen method (Olsen and Sommers, 1982). Data were subjected to statistical analysis by following RCBD using standard procedures (Steel *et al.,* 1997). The differences among treatment

means were checked by applying the Duncan's multiple range tests (Duncan, 1955).

RESULTS

Phosphate solubilizing *Bacillus* sp significantly enhanced the seed cotton yield and plant height (Table 2). The highest seed cotton yield was produced with *Bacillus* inoculation (1733.3 kg ha^{-1}) at 90 kg P ha^{-1}. Bacterial inoculation produced higher seed cotton yield at all P levels compared to their respective control. Percent increase in seed cotton yield with inoculation was 8.08, 7.93 and 7.57% at 30, 60 and 90 kg P ha^{-1}, respectively. Similarly, bacterial inoculation produced significantly higher plant height than their respective controls. Application of different P levels also has a pronounced effect on the seed cotton yield.

Bacterial inoculation also has significant influence on the number of boll $plant^{-1}$and boll weight (Table 3). Highest number of boll $plant^{-1}$ i.e. 37 was observed with inoculation at 90 kg P ha^{-1} which was statistically at par with 60 kg P ha^{-1}. Percent increase in number of boll $plant^{-1}$ and boll weight with inoculation was 17.24, 12.50 and 15.63% and 4.56, 5.29 and 5.22% at 30, 60 and 90 kg P ha^{-1}, respectively. Inoculation influenced the boll weight positively at all P levels.

Data regarding NP content in cotton leaves was presented in (Table 4). Inoculation with *Bacillus* sp produced highest N-content (1.707%) at 90 kg P ha^{-1}. Higher N and P-content in cotton leaves was observed with inoculated treatments as compared to un-inoculated ones. Percent increase in cotton leaves P-content with *Bacillus* sp inoculation was 7.05, 9.20 and 7.78 % at 30, 60 and 90 kg P ha^{-1}, respectively as compared to respective un-inoculated ones.

Data regarding available P status in the soil was depicted in the (Figures1, 2 and 3). Available P was determined after 30, 60 and 90 days of sowing. At all P levels available P was higher as compared to their respective controls especially after 30 days of sowing. Highest available P (19.1, 15.2 and 15.7 mg kg^{-1} soil) with *Bacillus* sp was observed after 30, 60 and 90 days of sowing at highest P fertilizer level i.e. 90 kg P ha^{-1}. All the three observations taken at 30, 60 and 90 days of sowing revealed that inoculated treatments have higher level of available P than the un-inoculated respective control. Percent increase in available P after 30, 60 and 90 days of sowing was (2.58, 3.53 and 3.24%), (6.96, 0.68 and 1.33%) and (2.40, 4.14 and 6.80%) at (30, 60 and 90 kg P ha^{-1}), respectively.

Table 1. Some important traits of isolates tested during the examination.

Isolates	IAA equivalents (ppm)	Solubilization Efficiency (SE)	Solubilization Index (SI)
B_1	2.78	275.0	3.8
B_2	2.52	240.0	3.4
B_3	2.68	233.3	3.3
B_4	2.04	260.0	3.6

Table 2. Inoculation effect on seed cotton yield and plant height of cotton.

Treatments	See cotton Yield (kg ha^{-1})		Plant Height (cm)	
kg P ha^{-1}	Un-inoculated	Inoculated	Un-inoculated	Inoculated
30	1377.7 e*	1489.0 d	152.5 e	156.5 cd
60	1544.3 cd	1666.7 ab	154.5 de	158.7 ab
90	1611.3 bc	1733.3 a	157.2 bc	160.2 a
LSD	107.25		2.067	

*Means sharing the same letter(s) in a column do not differ significantly at $p<0.05$ according to Duncan's Multiple Range Test

Table 3. Inoculation effect on number of boll and boll weight of cotton.

Treatments	No. of bolls $plant^{-1}$		Boll weight (g)	
kg P ha^{-1}	Un-inoculated	Inoculated	Un-inoculated	Inoculated
30	29 c*	34 ab	3.51 d	3.67 bc
60	32 bc	36 a	3.59 cd	3.78 ab
90	32 bc	37 a	3.64 cd	3.83 a
LSD	3.499		0.134	

*Means sharing the same letter(s) in a column do not differ significantly at $p<0.05$ according to Duncan's Multiple Range Test

Table 4. Inoculation effect on NP content in cotton leaves.

Treatments	N-content (%)		P-content (%)	
kg P ha^{-1}	Un-inoculated	Inoculated	Un-inoculated	Inoculated
30	1.627 c*	1.660 b	0.227 d	0.243 cd
60	1.660 b	1.683 ab	0.250 c	0.273 ab
90	1.670 b	1.707 a	0.257 bc	0.277 a
LSD	0.024		0.017	

*Means sharing the same letter(s) in a column do not differ significantly at $p<0.05$ according to Duncan's Multiple Range Test.

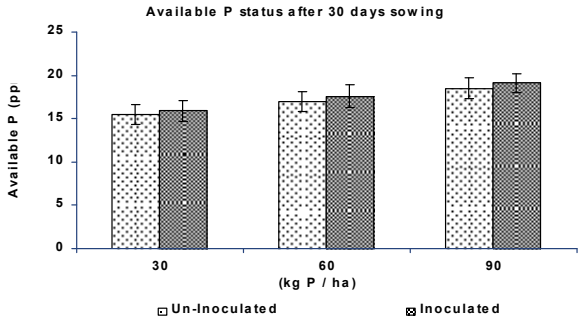

Figure 1. Available P status after 30 days of sowing.

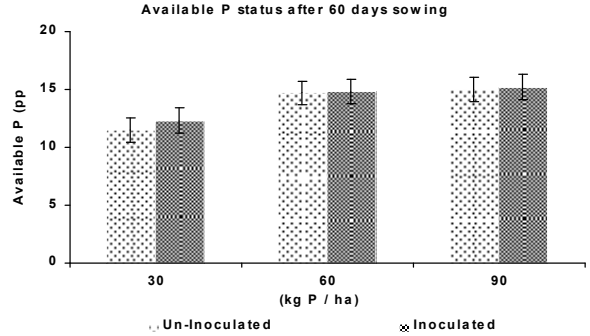

Figure 2. Available P status after 60 days of sowing.

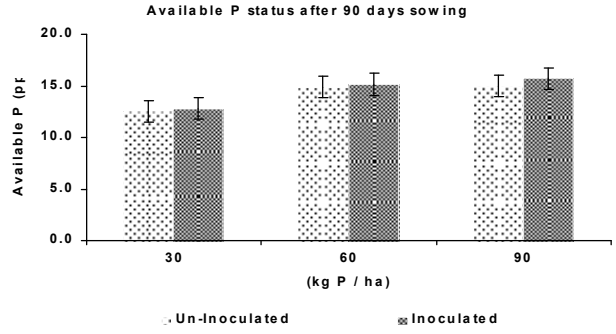

Figure 3. Available P status after 90 days of sowing

DISCUSSION

Bacillus sp was isolated by preparing the serial dilutions from the rhizosphere of cotton and characterized for their auxin biosynthesis and phosphate solubilization potential. Four isolates of *Bacillus* sp used in this study have auxin biosynthesis expressed as IAA equivalents and phosphate solubilization on Pikovskaya's medium (Illmer and Schinner. 1992; Rodriguez and Fraga, 1999; El-Komy, 2005) (Table 1). Literature revealed the microbial production of auxins and in plant growth promotion has been described by many researchers (Sarwar *et al.,* 1992; Gull *et al.,* 2004; Martins *et al.,* 2004).

In this study, inoculation of P-solubilizer (*Bacillus* sp) was evaluated for the growth promotion of cotton and flux of available P in soil at different P levels viz. 30, 60 and 90 kg ha^{-1}. Results demonstrated that phosphate solubilizing *Bacillus* sp enhanced the yield components of cotton compared to un-inoculated control (Rodriguez *et al.,* 1996). PGPR having the potential of phosphate solubilization enhanced the growth hormone production, availability of phosphorus and rate of nitrogen fixation (Ponmurugan and Gopi, 2006). *Bacillus* sp inoculation enhanced the seed cotton yield, plant height, NP contents in cotton leaves and available P status of soil. Our findings are in accordance with the work of many researchers who confirmed the microbial influence on growth of crops (Antoun *et al.,* 1998; Zahir *et al.,* 2005; El-Komy, 2005; Khalid *et al.,* 2006; Ponmurugan and Gopi, 2006).

Bacillus sp inoculation influenced the growth of cotton at each level of P fertilizer might be attributed to rhizosphere colonization, better nutrient availability and biosynthesis of growth hormones as reported by many researchers (Patten and Glick 2002; Kamilova *et al.,* 2006; Rai, 2006; Idris *et al.,* 2007). *Bacillus* sp synthesized growth hormones that might be the most probable mean to promote plant growth. Microbe having the potential of synthesizing plant hormones might be responsible for expansion of root surface area and enhanced plant-microbe interaction resulted in more nutrient uptake. (Antoun *et al.,* 1998; Biswas *et al.,* 2000; Zahran, 2001; Gyaneshwar *et al.,* 2002; Yuming *et al.,* 2003). Phosphate solubilizers besides P-solubilization also produced phytohormones and thus increased the growth of plant (Arshad and Frankenberger, 1998).

Bacillus sp inoculation enhanced N and P contents in cotton leaves might be due to increase nutrient concentration in the root zones. Phospahte solubilizer increased P-content in cotton, dissolved organophosphates and N-content in biomass (Chaykovskaya *et al.,* 2001). Among the phosphate

solubilizers *Bacillus* sp solubilized phosphorus and increased growth and yield of cotton (Gyaneshwar *et al.,* 2002; El-Komy, 2005). Egamberdiyeva *et al.* (2004) reported that phosphate solubilizer combined with phosphorit significantly increased P-content in cotton. Shah *et al.* (2001) also endorsed our results that bacterial inoculation having high phosphate solubilization extent (SI and SE) and phosphorus application enhanced nutrient uptake efficiency. Results can also be verified with Egamberdeyiva et al. (2004) who observed that *Bacillus meliloti* significantly increased phosphors content of cotton plant as compared to uninoculated control. Phosphate solubilizing bacteria having potential of phosphate solubilization produced more available P by the production of organic acids which act like chelates and solubilized insoluble phosphorus (Zaidi *et al.,* 2004; Khan *et al.,* 2006). Results clearly demonstrated that with the increase of P fertilizer level, available P was also increased. Since P fertilizer was added as basal, therefore maximum available P was observed after 30 days of sowing. Inoculated treatments have higher available P after 30, 60 and 90 days of sowing. Phosphorus acquisition of cotton plant mainly depends on the excretion of phosphatase and exploration of roots to labile pool of phosphorus in surface and subsurface soil (Khan *et al.,* 2006; Wang *et al.,* 2008). Higher root exudation and proton release in the rhizosphere of cotton was not observed in P-deficient soils. (Wang *et al.,* 2008). It means that cotton lack the ability to use sparingly soluble P sources like Ca-phosphate and to change its rhizosphere chemistry to mobilize non-labile inorganic P sources (Egamberdiyeva *et al.,* 2004).

Our findings are confirmed by the results of Egamberdiyeva *et al.,* (2004) who observed the positive influence of P-solubilizer inoculation on soil available P and observed that phosphate solubilizer increased the available P in soil at different growth stages (tillering, flowering and maturity) of cotton. Dorahy *et al.* (2008) reported that cotton seedlings derive most of its P from top 10 cm layer to the applied fertilizer and at a later growth stage (36 days after sowing), cotton uptake showed significant portion (more than 90% of total) from the soil P pools.

Study clearly demonstrated phosphate solubilising bacteria can play an essential role in growth and yield of cotton and increased more NP content in plants and enhanced the available P in soil.

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