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Isolation, screening and characterization of phosphate solubilizing bacteria from various rhizospheric soils

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Abstract
The use of phosphate solubilizing bacteria (PSB) as biofertilizers has concurrently increased phosphorous uptake in plants and improved yields in several crop species. A laboratory study was conducted to isolate, identify and characterize the phosphate solubilizing bacteria (PSB) from various rhizospheric soils from District Kulgam. Based on the highest solubilization zone production in the solid Pikovskaya's medium, 7 isolates (PSBK-10, PSBK-11, PSBK-12, PSBK-13, PSBK-14, PSBK-15 and PSBK-16) were selected and used for further studies. The selected PSB strains were screened *in vitro*. Among these PSB isolates, 4 strains belong to genera *Bacillus sp*, 2 to *Pseudomonas* and 1 to *Micrococcus.*

Keywords: Phosphorus solubilizing bacteria (PSB), biofertilizers, Pikovskaya's medium, *Bacillus sp*, *Pseudomonas sp*, *Micrococcus sp.*

1. Introduction
Phosphorus (P) is one of the most essential plant nutrients which profoundly affect the overall growth of plants (Wang *et al*., 2009) [33] by influencing the various key metabolic processes such as cell division, photosynthesis, respiration, energy storage and transfer, cell enlargement and some other processes in the plants. It is applied to the soil in the form of Chemical fertilizers. Its availability to the plant utilization is limited. As inorganic phosphate, it is immobilized rapidly and becomes unavailable to plants (Akhtar *et al.*, 2010) [1]. Among the alternative P sources, the most important is locally available rock phosphate (Khan *et al.*, 2009) [16]. Majority of the soils throughout the world are P deficient (Muhammad, 2012) [19]. The concentration of Bioavailable P in soil is very low getting to the level of 1.0 mg kg–1 (Goldstein, 1994) [12]. Plants absorb P as phosphate anions (HPO^{4-}) or H_2PO^{4-}) from soil (Rodriguez and Fraga, 1999) [27].
Phosphate anions are highly reactive in the soil and their precipitation is soil pH dependent. In acidic soils phosphate anions get precipitated with free oxides and hydroxides of iron and aluminium, but in alkaline soils calcium is the main element involved in P fixation (I gual *et al*., 2001)[13]. Indian soils are characterized by poor and medium status with respect to available P (Baby, 2002, Li *et al*., 2003., Ramanathan *et al*., 2004) [2, 17, 25]. A number of bacterial species, mostly those associated with the plant rhizosphere, are able to exert a beneficial effect upon plant growth. This group of bacteria has been termed as 'plant growth promoting rhizobacteria (PGPR) and among them are strains from genera such as *Bacillus*, *Pseudomonas*, *Azotobacter*, *Micrococcus*, *Serratia*, *Azospirillum*, *Enterobacter*, *Rhizobium*, *Erwinia*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter* and *Flavobacterium* (Rodríguez *et al.*, 1999) [28]. Many of these microorganisms act as phosphate solubilizing agents and convert the insoluble phosphorus into soluble form HPO_4^{2-} and $H2PO^{4-}$ by production of acids, acidification, chelation, and polymeric substance formation (Delvasto *et al.*, 2008) [7]. These microorganisms thus reduce the dependence on chemical fertilizers and hence are being regarded as an alternative to the conventional chemical fertilizers.
Several workers have reported the isolation of various phosphate solubilizing bacteria from rhizospheres of a variety of plants. Islam *et al.*, (2006) [14] isolated and purified a total of 30 bacterial strains from the rhizoplane of rice by repeated streak culture on PVK medium. The phosphate solubilization index of the rice isolates varied from 1.2 to 6.7. The isolate *Acinetobacter* sp. BR-25 exhibited the highest phosphate solubilization index (6.7) followed by BR-15 (4.8) when calcium phosphate was used as P source. Maheshwar *et al.*, (2012) [18] collected the soil sample from different rhizosphere soil of groundnut and reported the

presence of phosphate solubilizing microorganisms. The isolated bacterial strains of *Bacillus subtilis* and *Bacillus cereus* exhibited maximum phosphate solubilization and were found to be active in solubilization of tricalcium phosphate under *in-vitro* condition.

Phosphate solubilizers play a vital role in soil P dynamics and availability of phosphate to plants (Richardson, 2001) [26]. Many soil microorganisms particularly those present in rhizosphere of plants, are able to solubilize fixed form of P to soluble form and makes it available to plants (Dave and Patel, 2003., Dubey *et al*., 1997., Narayanasamy *et al*., 1981, Sashidhar, B., and Podile, A.R. 2010 [6, 8, 21, 29]. The principal mechanism of p solubilization is the production of organic acids and phosphatase enzymes which play key role in phosphatase solubilization in the soil (Surange *et al*., 1995., Duttan and Evans, 1996., Nahas, 1996., Shankar *et al*., 2013) [32, 9, 20, 30].

The present paper discusses the isolation and Characterization of phosphate solubilizing from various rhizospheric soils.

Material and Methods

Different strains of phosphate solubilizing bacteria (PSB) were isolated from various rhizospheric soils. It was a laboratory scale study and whole of the work was undertaken in the laboratories of division of Plant Pathology, SKUAST-Kashmir.

Sample preparation

In order to isolate PSB, 10 mixed soil samples were collected from rhizospheric soils of Apple, walnut, peach, almond, cherry, brinjal, from different locations of kulgam, Jammu and Kashmir. The samples were collected at a depth of 10-35 cm. The tools used for soil sampling were surface sterilized using 70% ethanol and soil samples were placed in sterile bags, transported to laboratory, stored at 4 ^{0}C and finally processed.

Isolation and screening of efficient PSB strains

The isolation of phosphorus solubilizing bacteria (PSB) from rhizospheric soils was done by following the serial dilution technique. For isolation, soil samples were serially diluted from 10^{-3} to 10^{-6} and inoculated on modified Pikovskayas's agar medium (Pikovskayas's, 1948) [23]. which consists of glucose, 10.0 g; Ca3(PO4)2, 5.0 g; (NH4)2SO4, 0.5 g; NaCl,0.2 g; MgSO4·7H2O, 0.1 g; KCl, 0.2 g; MnSO4·H2O, 0.002 g; FeSO4·7H2O, 0.002 g; yeast extract, 0.5 g; agar, 18.0 g for solid medium; distilled water, 1000 ml., pH was adjusted to 7 before sterilization, followed by pour plate technique and the 48 h incubation at 30^{0}C, discrete colonies showing halo zones were picked up, sub cultured in Nutrient agar slants and then preserved

Characterization of PSB Strains

Morphological Characters

Suspension of each purified culture was prepared and poured on plates having solid media by spread plate method. The inoculate plates were incubated at 25^{0}C till the appearance of colonies. Morphological characters of colonies like size, shape, color and elevation were measured as (Goenadi *et al*., 2000) [11].

Microscope Characters

The isolated strains were heat fixed called as smear. Crystal violet was flooded for one minute and washed gently by tape water. Then the smears were exposed to Gram's iodine for one minute and washed and drained carefully. 95% alcohol was applied for 30 seconds and washed. Finally the smears were washed and drained with 0.25% safranin for 30 seconds and examined under microscope. It focused on shape and size of bacteria. Pink colored bacteria were named as Gram negative while purple colored were named as Gram positive

Estimation of phosphate solubilization efficiency

For testing the P solubilizing capability of PSB strains, each PSB culture was poured on

Pikovskayas's agar plate containing insoluble tricalcium Phosphate as P source. The plates having culture were incubated for 4 days at 28 ^{o}C. Solubilization index was measured by Edi-Premono formula (Edi- Premono *et al*., 1996) [10].

$$PSI = \frac{Colony\ Diameter + Halo\ Zone\ Diameter}{Colony\ Diameter}$$

Biochemical tests

The most efficient PSB strains were characterized by biochemical tests (Smibert and Krieg, 1994) [31].

Identification of bacterial strain

Different tests like Biochemical, Morphological and Physiological of the selected phosphate solubilizing bacterial isolates were performed for identification, as per methods defined in Bergey's Manual of Determinative Bacteriology (J.G. Holt, *et al*., 1994) [15]. The isolates belong to genus *Bacillus*, *Pseudomonas*, *Micrococcu*s. PSBK 10, 11 to genus *Pseudomonas*, PSBK12 to *Micrococcus*, PSBK 13, 14, 15, 16 to *Bacillus.*

Statistical analysis

Statistical comparison of different PSB strains for P solubilization efficiency, PSI was undertaken.

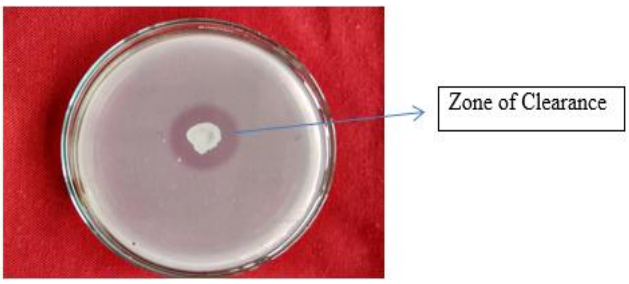

Fig 1: P Solubilization on PVK Medium.

Results and discussion

Phosphorus is an important limiting factor in agriculture production and microbial activation seems to be an effective way to solve the solidified phosphorus in the soil. Microorganisms capable of producing a zone of clearance due to the solubilization of inorganic phosphorus (Das, 1989) [5] and were routinely screened in the laboratory by a plate assay method using Pikovskayas's agar medium (pikoviskayas 1948) [23] or tricalcium phosphate medium (Nautiyal, 1999) [22]. The primary screening protocol used for the identification of PSB strains usually depends on the use of tricalcium phosphate as a sole source of P in indicator plates. we decided to use tricalcium phosphate as source of P for screening of PSB isolated from rhizospheric soils.

Results indicates that significant clear halo zone formation

around bacterial colonies on Pikovskayas's agar with tricalcium phosphate as P source, which was in agreement with reports of (Chung *et al*., 2005, Barroso and Mahas, 2005) [4, 3]. According to morphological and biochemical characterization the microorganisms showing zone of clearance belongs to the genera *pseudomonas sp*, *Micrococcus sp*, *Bacillus sp* (Table 1 to 3).

Table 1: Morphological Characters of Phosphorus solubilizing Bacteria.

Rhizospheric soil	Isolates	Colony Characteristics	Morphological Characters		
			Cell shape	Gram reaction	Spore
Apple	PSBK10	Whitish, entire, flat, translucent, large.	Small rods	-	-
Apple	PSBK11	White gummy, small, entire.	Small rods	-	-
Walnut	PSBK12	White, circular, raised, small, Opaque, large	Minute cocci	+	-
Peach	PSBK13	White, entire, flat, small, Opaque,	Long rods	+	+
Cherry	PSBK14	Creamy, irregular, flat, opaque, large	Small rods	+	+
Almond	PSBK15	Whitish, irregular, umbonate, opaque, large	Small rods	+	+
Brinjal	PSBK16	Whitish gummy, circular, less raised, opaque, small	Long rods	+	+

Table 3: Zone of Clearance and phosphorus solubilization index of 7 isolates

PSB isolates	Diameter of zone of Clearance(cm)	Colony Diameter (cm)	Phosphorus solubilization index(PSI)
PSBK10	0.70	0.40	2.75
PSBK11	0.50	0.30	2.67
PSBK12	0.60	0.30	3.00
PSBK13	0.60	0.30	3.00
PSBK14	0.70	0.40	2.75
PSBK15	0.90	0.40	3.25
PSBK16	0.90	0.40	3.25

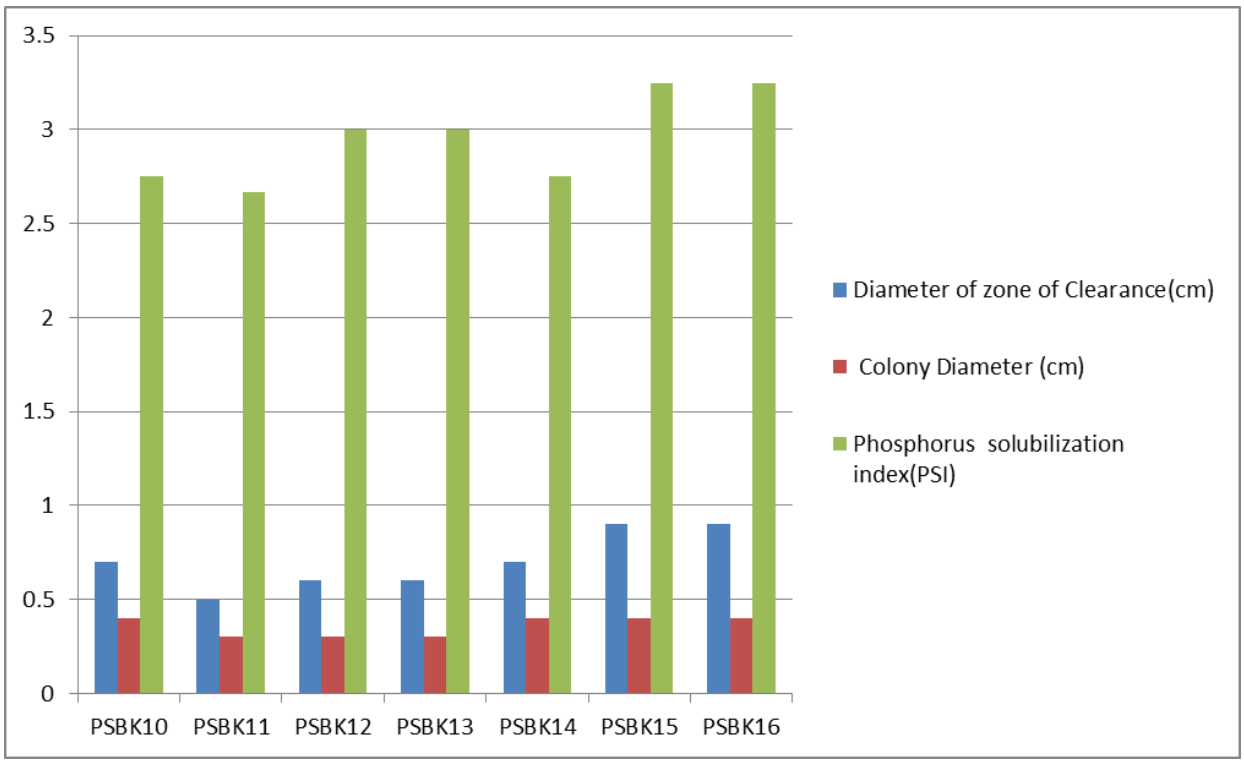

Fig 2: Graphical representation of solubilization index.

Table 2: Biochemical Characterization of 7 PSB isolates.

Isolates PSB	C	U	O	D	MR	VP	S.H	C.D	CA	A.P	G.P	H2S	G.L	NaCl	S	M	G	CI	Probable Genus
PSBK10	+	-	+	-	-	-	+	+	+	+	-	-	+	-	+	+	+	+	*Pseudomonas*
PSBK11	+	-	+	-	-	-	+	+	-	+	-	-	+	-	-	+	-	-	*Pseudomonas*
PSBK12	+	+	+	-	-	-	+	+	+	-	-	-	-	-	+	+	+	-	*Micrococcus*
PSBK13	+	+	-	-	+	+	+	+	+	-	-	-	+	-	+	-	+	+	*Bacillus*
PSBK14	+	+	-	-	+	+	+	+	+	-	-	-	+	-	-	+	+	+	*Bacillus*
PSBK15	+	+	-	-	+	+	+	+	+	-	-	-	+	-	+	-	-	+	*Bacillus*
PSBK16	+	+	-	-	+	+	+	+	+	-	-	-	+	-	+	+	+	+	*Bacillus*

C = Catalase test, U = Urea Hydrolysis, O = oxidase, Dn= Denitrification test, MR = Methyl red test, V.P = V.P test, SH = Starch Hydrolysis test, C.D = Cellulose degradation, Ca = Casein test, A.P = Acid production, G.P = Gas production, H2S = H_2S production test, G. l = Gelatine hydrolysis, Ci = citrate test, S = sucrose, M = Mannital, G = glycerol, NaCl = 70% NaCl.

Conclusion

Apple, walnut, peach, cherry, almond and brinjal rhizospheric soil presented a diverse population of PSBs. All the screened isolates PSBK10 to PSBK16 were more efficient in P solubilization. Therefore, these isolates can be used in the production of biofertilizer in order to improve growth of some agricultural crops in P-deficient soils, constituting an interesting alternative to the application of P fertilizers, reducing costs and improving crop yields.

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References

1. Akhtar N, Iqbal A, Qureshi MA, Khan KH. Effect of phosphate solubilizing bacteria on the phosphorus availability and yield of cotton (*Gossypium hirsutum*). Journal of Scientific Research. 2010; 40(1):15-24.
2. Baby UI. Biofertilizers in tea. Planters' Chronicle. 2002; 98:395-396.
3. Barroso CB, Nahas E. The status of soil Phosphate fractions and the ability of fungi to dissolve hardly Soluble Phosphates. Appl. Soil Ecol. 2005; 29:73-83.
4. Chung H, Park M, Madhaiyan M, Seshadri S, Song J, Cho H *et al*. Isolation and Characterization of Phosphate Solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil Biol. Biochem. 2005; 37:1970-1974.
5. Das AC. Utilization of insoluble Phosphates by soil fungi. J Indian Soc Soil Sci. 1989; 58:1208-1211
6. Dave A, Patel HH. Impact of different carbon and nitrogen sources on phosphate solubilization by *Pseudomonas fluorescens*. Indian Journal of Microbiology. 2003; 43:33-36.
7. Delvasto P, Valverde A, Ballester A, Muñoz JA, González F, Blázquez ML *et al*. Diversity and activity of phosphate bioleaching bacteria from a high-phosphorus iron ore. Hydrometallurgy. 2008; 92:124-129.
8. Dubey SK, Balasundaram VR, Pant LM, Jayasheela N, Kawale BR, Mishra B. Effect of phosphate dissolving bacteria applied with rock phosphate on nodulation and yield of rain fed soybean under different agriculture climatic conditions. Journal of Indian Society for Soil Science. 1997; 45:503-505.
9. Dutton VM, Evans CS. Oxalate production by fungi: its role in pathogenicity and ecology in the soil environment. Can. J Microbiol. 1996; 42:88-895.
10. Edi-Premono M, Moawad A, Vleck PLG. Effect of phosphate solubilizing *Pseudomonas putida*on the growth of maize and its survival in the rhizosphere. Indonasian. Journal of Crop Science. 1996; 11:13-23.
11. Goenadi DH, Siswanto, Sugiarto Y. Bio activation of poorly soluble phosphate rock with Phosphorus solubilizing fungus. Soil Science Society of America Journal. 2000; 64:927-932.
12. Goldstein AH. Involvement of the quinoprotein glucose dehydrogenises in the Solubilization of exogenous phosphates by gram negative bacteria, 1994. In: A. Torriani.
13. Igual JM, Valverde A, Cervantes E, Velázquez E. Phosphate solubilizing bacteria as inoculants for agriculture: use of updated molecular techniques in their study. Agronomie. 2001; 21:561-568.
14. Islam T, Deora A, Hashidoko Y, Rahman A, Ito T, Tahara S. 16S rRNA genes sequencing data as *Acinetobacter* sp. BR-12, *Klebsiella* sp. BR-15, *Acinetobacter* sp.BR-25, *Enterobacter* sp. BR-26, *Microbacterium* sp. BRS-1 and *Pseudomonas* sp. BRS-2 showed varying levels of phosphate solubilizing activity in both agar plate and broth assays. Z. Naturforsch. 2006; 62c:103-110.
15. Holt JG, Krieg NR, Sneath PHA, Staley JT. Williams S.T. In, Bergy's Manual of Determinative Bacteriology, (9th Ed.), Williams and Wilkins Pub, MD, USA, 1994.
16. Khan AA, Jilani G, Akhtar MS, Naqvi SS, Rasheed M. Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. J Agric. Biol. Sci. 2009; 1:48-58.
17. Li ST, Zhou JI, Uang HY, Chen XQ, Du CW. Characteristics of fixation and release of phosphorus in three soils. Acta Pedologzca Sznica. 2003; 40:908-914.
18. Maheswar N, Sathiyavani G. Quantitative analysis of organic acid produced by phosphate solubilizing Bacteria. Journal of Chemical and Pharmaceutical Research. 2012; 4(8):4007-4011.
19. Muhammadi K. Phosphorus solubilizing Bacteria, occurrence, mechanisms and their role in crop production. Journal of Research and Environment. 2012; 2(1):80-85.
20. Nahas E. Factors determining rock phosphate solubilization by microorganism isolated from soil. World Journal of Microbial Biotechnology. 1996; 12:18-23.
21. Narayanasamy G, Ghosh SK, Sarkar MC. Chemical and mineralogical composition of P-rock deposits occurring in India. Fertilizer News. 1981; 26:3-9.
22. Nautiyal CS. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Lett. 1999; 182:265-270
23. Pikovskaya RI. Mobilization of Phosphorus in soil in connection with vital activity of some microbial species. Microbiologiya. 1948; 17:362-370
24. Raghothama KG. Phosphate acquisition. Annual Review of Plant Physiology and Plant Molecular Biology. 1999; 50:665-6693.
25. Ramanathan S, Natarajan K, Stalin P. Effect of foliar nutrition on grain yield of rice fallow black gram. Madras Agriculture. 2004; 91:160-163.
26. Richardson AE. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Funct. Plant Biol. 2001; 28:897-906.
27. Rodriguez H, Fraga R. The use of phosphate solubilizing bacteria as inoculants simultaneously increases P uptake by the plant and crop yield. Biotechnol. Adv. 1999; 17:319-339
28. Rodríguez H, Fraga R. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances. 1999; 17:319-339.
29. Sashidhar B, Podile AR. Mineral phosphate solubilization by rhizosphere bacteria and scope for manipulation of the direct oxidation pathway involving glucose dehydrogenase. Journal of Applied Microbiology. 2010; 109:1-12.
30. Shankar T, Sivakumar T, Asha G, Sankaralingam S, Meenakshi Sundaram V. Effect of PSB on Growth and Development of Chilli and Maize Plants. World Appl. Sci. J. 2013; 26(5):610-617.
31. Smibert RM, Kreig NR. Phenotypic characterization. In: methods for general and molecular bacteriology. Washington DC: American Society of Microbiology. 1994; 12:607-654-49.
32. Surange S, Wollum AG, Kumar N, Nautiyal CS. Characterization of *Rhizobium* from root nodules of leguminous trees growing in alkaline soils. Can. J Microbiol. 1995; 43:891-894.
33. Wang X, Wang Y, Tian J, Lim BL, Yan X, Liao H. Overexpressing AtPAP15 enhances phosphorus efficiency in soybean. Plant Physiol. 2009; 151:233-240.