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Isolation and characterization of phosphorus solubilizing bacteria (PSB) from rhizospheric soils of apple (*Malus Domestica*)

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Abstract
Phosphate solubilizing bacteria (PSB) can promote the dissolution of insoluble phosphorus (P) in soil, enhancing the availability of soluble P. Thus, their application can reduce the consumption of fertilizer and aid in sustainable agricultural development. An attempt was made to isolate and screen potential phosphate solubilizing bacteria (PSB) which are able to solubilize phosphate and can be used as biofertilizers in future. Apple (*Malus domestica*) rhizospheric soil samples were collected from the different villages of Anantnag, Kashmir, India. we have isolated twenty five different bacterial strains, out of 25 strains seven strains were selected which showed highest Zone of Clearance on modified Pikovskayas's solid medium. All the Seven isolates (PSB1, PSB2, PSB3, PSB4, PSB5, PSB6, and PSB7) were morphologically and biochemically Characterized.

Keywords: phosphorus solubilizing bacteria (PSB), rhizospheric soil, malus domestica, biofertilizer, pikoviskayas solid medium

Introduction
P is the essential macronutrient for plant growth and development. It accounts 0.2% of plant dry weight, limits the growth of plants and crop yield [20, 32]. It promotes early root formation, plant growth and improves quality of vegetables, fruits and is vital to seed formation [8]. It is required for various metabolic processes, for example synthesis of biomolecules, photosynthesis, cell division, signalling and enzyme activation regulation by phosphorylation of serine, histidine, aspartate, threonine or tyrosine residues [27]. P is one of the least available nutrients in the soil, only 0.1% availability to that of its total pool present in the agricultural soils [15]. Approximately 70-90% of available P of chemical fertilizers precipitates soon after their application [11, 21, 4]. Therefore, over use of chemical fertilizers is further posing burden on the land and causes nutrient imbalance which is the point of environmental consideration [31]. Synthetic chemicals can negatively affect the environment, human and animal health [1, 2]. Plants absorb P as phosphate anions (HPO4-) or H2PO4-) from soil [30]. 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 [13]. Indian soils are characterized by poor and medium status with respect to available P [28].

Soil microorganisms have an important influence on soil fertility and plant growth [23]. Phosphorus solubilizing bacteria (PSB) were already proposed as a viable solution to resolve the problems of precipitation of soluble superphosphate fertilizers [34, 18, 12]. There are various types of soil microbes which can solubilize this fixed form of P and make available to plants [14, 19, 29]. 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 [7, 9, 24]. Despite these phosphorus compounds being abundant in agricultural soils, the majority of them occurs in an insoluble form [22]. These bacteria make available the soluble phosphates to the plants, and in return gain root borne carbon compounds, mainly sugars and organic acids, necessary for bacterial growth [17]. Bacteria form the largest group which bring about mineralization of organic phosphorus compounds and are known as phosphate solubilizing bacteria [3]. As observed in many experiments, the principal mechanism is the production of mineral dissolving compounds such as organic acids, siderophores, protons, hydroxyl ions and CO2 [32].

The research work was carried out to isolate and screen phosphate solubilizing bacteria from rhizospheric soils of apple from different apple orchards of Anantnag, Kashmir, India.

The present paper discusses the isolation and Characterization of Phosphorus solubilizing bacteria (PSB).

Material and Methods

Soil Sampling and collection

In order to isolate PSB, 10 Apple rhizospheric soil samples were collected from different villages of Anantnag, Kashmir, India. The samples were collected at a depth of 10-25 cm. All the tools used in 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 processed within a week.

Isolation of PSB strain

The rhizospheric soil samples of apple were serially diluted and inoculated on modified Pikovskayas's agar medium [26]. The bacteria were isolated and screened on Pikovskayas's medium which consists of: Glucose 10 g, Magnesium sulphate 0.1g, Ferrous sulphate trace, Manganese sulphate trace, Tricalcium phosphate as P source, agar agar 15 g, Distilled water 1L, pH was adjusted to 7 before sterilization, followed by pour plate technique and the 48h incubation at 30 ^{0}C, discrete colonies showing halo zones were picked up, sub cultured in Nutrient agar slants and then preserved.

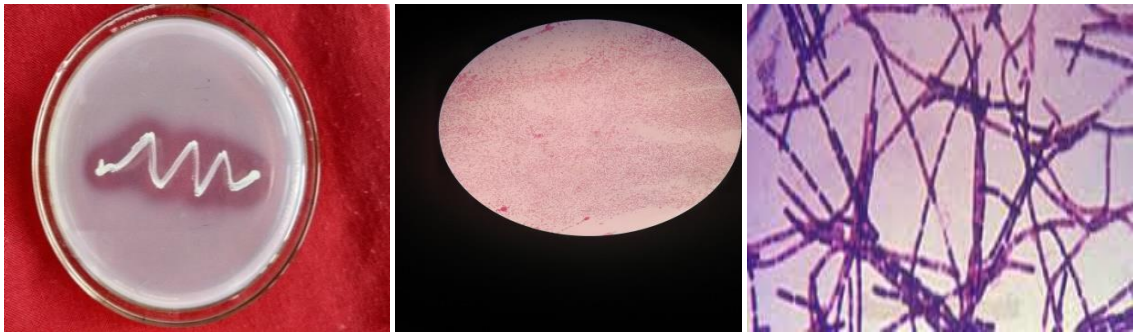
Estimation of phosphate solubilization efficiency

The solubilization efficiency of PSB to solubilize tri calcium phosphate on pikoviskayas agar medium was determined in terms of solubilization index (PSI).PSI was calculated by measuring the colony diameter and halo zone diameter, using the fallowing formula of Edi premono et al., 1996) [10].

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

Identification of bacterial strain

Different tests like Morphological, Biochemical and Physiological tests of the selected phosphate solubilizing bacterial isolates were performed for identification, as per methods defined in Bergey's Manual of Determinative Bacteriology [16].



P solubilisation | Gram negative | Gram positive

Fig 1: Photos of petri plates and staining

Results and Discussion

Screening and identifying PSB isolates.

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 [6] and were routinely screened in the laboratory by a plate assay method using pikoviskayas agar medium [26] or tricalcium phosphate medium, [25]. 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. However, the available P fraction in soils is found to be adsorbed to the surface of soil minerals or occurs as poorly soluble precipitates of Ca3 (PO4)2, AlPO4 or FePo4 [29]. We decided to use tricalcium phosphate as source of P for screening of PSB isolated from rhizospheric soils of apple. Results indicates that significant clear Halo zone formation around bacterial colonies on pikoviskayas agar with tricalcium phosphate as P source, which was in agreement with reports of [5, 33]. According to morphological and Biochemical Characterization the microorganisms showing zone of clearance belongs to the bacterial genera and varoius tests were performed like, Catalase test, Urea Hydrolysis, oxidase, Denitrification test, Methyl red test, V.P test, Starch Hydrolysis test, Casein test, Acid production, Gas production, H_2S production test, Gelatine hydrolysis, citrate test, sucrose, Mannital, Glucose, 7% NaCl

In the present study, 7 isolates of PSB were isolated by plating soil dilutions on Pikovskayas's agar which showed the development of Sharp Phosphate Solubilization zones, ranging from 1 to 3.14 (PSI). The morphological and biochemical characterization of the 7 isolates is given as:

Table 1: Morphological Characters of 7 PSB.

Isolates PSB	Pigmentation	Margin	Gram reaction	Slightly raised	Highly raised	Transp-arent	Opaque	Consiste-ncy	Spore
PSB1	Whitish	Entire	+	+	-	+	-	viscous	+
PSB2	White	Entire	+	+	-	-	+	viscous	+
PSB3	Creamy	Elevated	-	+	-	-	+	viscous	-
PSB4	Whitish	Entire	+	+	-	-	+	viscous	+
PSB5	Creamy	Elevated	-	+	-	-	+	viscous	-
PSB6	Whitish	Entire	+	+	-	-	+	viscous	-
PSB7	White	Elevated	-	+	-	-	+	viscous	-

Table 2: Biochemical Characterization of 7 PSB isolates.

Isolate. (PSB)	C	U	O	Dn	M.R	VP	ST	Ca	A.P	G.P	H_2S	Gel	Cit	SU	M	G	NaCl
PSB1	+	+	+	-	+	-	-	+	+	-	-	+	+	+	+	-	-
PSB2	+	+	+	-	+	-	+	+	+	+	-	+	-	+	-	-	-
PSB3	+	+	+	-	+	-	+	-	+	-	-	-	+	-	+	-	+
PSB4	+	+	+	-	+	-	+	+	+	-	-	-	+	+	+	-	-
PSB5	+	+	+	-	+	-	-	-	+	+	-	+	+	+	+	+	-
PSB6	+	+	+	-	+	-	+	-	+	-	-	-	+	+	-	-	+
PSB7	-	+	+	-	+	-	+	-	+	+	-	-	-	+	+	-	-

C = Catalase test, U = Urea Hydrolysis, O = oxidase, Dn= Denitrification test, MR = Methyl red test, V.P = V.P test, St = Starch Hydrolysis test, Ca = Casein test, A.P = Acid production, G.P = Gas production, H2S = H_2S production test, Gel = Gelatine hydrolysis, Cit = citrate test, SU = sucrose, M = Mannital, G = glucose, NaCl = 70% NaCl.

Table 3: Zone of Clearance and Phosphorus Solubilization Index of 7 isolates.

PSB isolates	Diameter of zone of Clearance(cm)	Diameter of colony(cm)	Phosphorus Solubilization index (PSI)
PSB1 *(Bacillus)*	1.00	0.70	2.43
PSB2 *(Bacillus)*	1.20	0.80	2.5
PSB3*(Pseudomonas)*	1.40	0.80	2.75
PSB4 *(Bacillus)*	1.50	0.70	3.14
PSB5*(Pseudomonas)*	1.00	0.60	2.6
PSB6 *(Micrococcus)*	0.70	0.50	1.9
PSB7*(Pseudomonas)*	0.90	0.50	2.3

Conclusion

Apple rhizospheric soil presented a diverse population of *Bacillus*, *Pseudomonas* and *Micrococcus* as Phosphate solubilizers. All the screened isolates PSB1 to PSB7 were more efficient in P solubilization. Therefore these isolates can be used in the production of biofertilizers in order to improve growth of agricultural crops in P deficient soils, constituting an alternative to the application of P fertilizers.

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