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Isolation Distribution and Screening of Phosphate Solubilizing Bacteria from Different Crop Fields

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Abstract: **The distribution pattern of PSB was assessed in cultivated soils. PSB isolates were used for the production of IAA, measurement of pH and titrable acidity and germination of seeds. Nine strains namely PSB1, PSB2, PSB3, PSB4, PSB5, PSB6, PSB7, PSB8 and PSB9 were identified. Testing of these strains for phosphate solubilization revealed that among these, three were able to perform well (PSB1, PSB2, and PSB5) and among these three strains PSB 1 was found to be superior in forming halo zone of phosphate solubilization followed by PSB 5. The population level of PSB were highest in rhizosphere soil of Chilly (Capsicum) followed by Solanum, Radish and lowest in the rhizosphere soil of Banana. Further all isolates were able to secrete IAA and acid phosphatase under *invitro* condition. All the PSB strains were able to grow well in the pH range between 4.5-6.7. The seeds that were inoculated with PSB strains showed faster germination and linear growth.**

Keywords: **phosphate solubilizing bacteria (PSB), screening, distribution pattern, IAA.**

1. INTRODUCTION

Phosphorous is essential for growth and productivity of plants. Phosphate solubilizing bacteria (PSB) solubilize insoluble phosphate and make it available to the plants (Bhattacharyya and Jain, 2000). Phosphate solubilizing bacteria also produce amino acids, vitamins and growth promoting substances (Zimmer *et al.*, 1988) which promotes plant growth. Phosphate solubilizing bacteria (PSB) are being used as biofertilizer since 1950s (Kudashev, 1956; Krasilinikov, 1957). Population of Phosphate solubilizing bacteria depends on different soil properties (physical and chemical properties, organic matter, and Phosphorus content) and cultural activities (Kim *et al.*, 1998). The Phosphate solubilizing bacteria dissolve the soil Phosphorus through production of low molecular weight organic acids mainly gluconic and keto gluconic acids (Deubel *et al.*, 2000), in addition to lowering the pH of rhizosphere. The present study was undertaken to isolate and to assess the distribution pattern of Phosphate solubilizing bacteria from different soils. These isolates were further identified and screened for the production of phytohormones (IAA), phosphorus estimation, measurement of pH, titrable acidity and assess the feasibility of using these strains for germination of seeds.

2. MATERIALS AND METHODS

Isolation, screening and identification of PSB:

Rhizosphere soils were collected from the area near Kanchipuram and Chengalpattu. The soil samples were obtained from different field crops such as Banana, Brinjal, Chilly, Corm, Groundnut, Lady's finger, Mango, Radish and Solanum. These soils were air dried and used for isolation and identification of Phosphate solubilising bacteria. The Pikovskaya's (Glucose - 10.0g, Tricalcium phosphate -5.0g, Ammonium sulphate - 0.5g, Magnesium sulphate- 0.1g, Yeast extract - 0.5 g, Sodium Chloride - 0.2 g, Potassium Chloride - 0.2 g, Manganese Sulphate - 0.002 g, Ferrous Sulphate- 0.002g, Agar- 20.0 g , Distilled water- 1000 mL) medium was used for isolation and maintenance of PSB. PSB were isolated by serial dilution and pour plate method. PSB colonies were visually identified by the formation of clear zone around the bacterial colony. Isolated strains were streaked onto Pikovskaya's agar medium for screening. The colony diameter of PSB colony was measured by using metric scale. The isolated bacteria were identified by gram staining, motility and biochemical test using the key provided by Bergy's Manual of Determinative Bacteriology.

Distribution pattern of PSB:

In order to study the distribution pattern of PSB, 200-300 grams of soil sample were collected from the rhizosphere of different crop soils, such as Banana, Brinjal, Chilly, Corm, Groundnut, Lady's finger, Mango, Radish and Solanum. The number of PSB colonies was expressed in term of CFU on soil dry weight basis.

Estimation of IAA produced by PSB:

Three day old cultures of phosphate solubilizing bacteria were transferred to Pikovskaya's broth containing L-Tryptophan as a substrate for the production of IAA. The cultures were incubated at 37˚C on an orbital incubator with gentle agitation (100rpm). After three days culture filtrates were used to estimate IAA content according to the procedure given by Bric *et al*. (1991).

Estimation of phosphorus content:

The efficiency of Phosphorus solubilization by PSB strains was estimated by the method of Olsen *et al.* (1954). The broth culture was centrifuged at 10,000 rpm for 10 min and the clear supernatant was used for the estimation of available phosphorus.

Measurement of pH and titrable acidity:

A change in the medium due to the growth of phosphate solubilising bacteria was measured with a pH meter after three days of incubation. In order to study the titrable acidity of culture medium, three day old culture filtrates were centrifuged at 1000 rpm for 10 minutes. Five ml of supernatant was added with a few drops of phenolphthalein indicator and titrated against 0.01N NaOH.

Effects of PSB on germination of seeds:

Different seeds were inoculated in water containing phosphate solubilizing bacteria and incubated for eight days. After incubation period the plants are grown in pots under environmental conditions native to plant's habitat.

3. RESULTS AND DISCUSSION

Isolation, screening and identification of PSB:

Totally nine Phosphate solubilizing strains were isolated from soil samples collected from different field crops. The Phosphate solubilizing strain exhibited halo zones due to the presence of Phosphatase enzyme. The colonies which produce halo zones were obtained, which were further purified by quadrant streaking. The Pikovaskaya's medium was used in the present study because it acts as specific isolation medium for Phosphate solubilizing microorganism isolation due to the presence of Tricalcium phosphate which is known for halozone formation showing the capability of phosphate solubilization.

The efficient nine strains namely PSB1, PSB2, PSB3, PSB4, PSB5, PSB6, PSB7, PSB8 and PSB9 were selected based on the potential phosphate solubilization. Testing of these strains for phosphate solubilization revealed that among these, three showed good halozone formation (PSB1, PSB2, and PSB5) and among these three strains PSB 1was found to be superior in forming halo zone of phosphate solubilization followed by PSB 5.

Based on the biochemical tests, the Phosphate solubilizing bacterial strains were identified as generic level. The results of various biochemical tests for five isolates were summarized. Based on biochemical analysis the isolated colonies were identified as *Alcaligenes, Alteromonas, Bacillus, Pseudomonas* and *Flexibacterium*. It may be concluded that among the nine strains of Phosphate solubilizing bacteria, strains like PSB1, PSB2, and PSB5 are more promising than other strains. These strains may be more effective and perform better under field conditions in the view of enhancing plant metabolism and soil health. The biochemical characterization of the isolates PSB1, PSB4, PSB7, PSB8 and PSB 9 were more close to *Alcaligenes* sp.

Distribution pattern of PSB:

The distribution pattern of PSB in the rhizosphere soils of field crops are presented in Table 1. The number of PSB colonies was expressed in term of CFU on soil dry weight basis. It was obsevered that population level of PSB vary among the soil samples, likely because of the availability of root exudates in the rhizosphere.

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Table.1 Distribution pattern of PSB.

S.No	Soil sample	Strains	Colony count of PSB(cfu g^{-1})
1.	Banana	PSB7	1×10^{1} 0×10^{2} 0×10^{3} 2×10^{4} 0×10^{5}
2.	Brinjal	PSB2	0×10^{1} 2×10^{2} 4×10^{3} 0×10^{4} 0×10^{5}
3.	Chilly	PSB8	19×10^{1} 9×10^{2} 19×10^{3} 14×10^{4} 15×10^{5}
4.	Corm	PSB9	2×10^{1} 0×10^{2} 0×10^{3} 2×10^{4} 1×10^{5}
5.	Ground nut	PSB4	0×10^{1} 9×10^{2} 6×10^{3} 2×10^{4} 1×10^{5}
6.	Lady's finger	PSB6	4×10^{1} 7×10^{2} 5×10^{3} 0×10^{4} 0×10^{5}
7.	Mango	PSB1	7×10^{1} 5×10^{2} 1×10^{3} 0×10^{4} 0×10^{5}
8.	Radish	PSB5	14×10^{1} 3×10^{2} 4×10^{3} 5×10^{4} 5×10^{5}
9.	Solanum	PSB3	8×10^{1} 3×10^{2} 10×10^{3} 11×10^{4} 6×10^{5}

***In vitro* screening of bacterial isolates for IAA production:**

All the nine Phosphate solubilizing bacteria strains produced pink colour when qualitative assay was done indicating the production of IAA in their culture filtrates. In the present study IAA production by Phosphate solubilizing bacteria was investigated and found that all the Phosphate solubilizing bacteria had the ability to produce IAA. Quantification experiment revealed that *Alcaligenes* sp. has produced relatively higher amounts of IAA followed by *Bacillus* sp (Table 2).

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Phosphorus content:

The results on the phosphorous content showed that the strain *Alteromonas* had higher activity (13 µg/mL) followed by the strain *Flexibacterium* (Table 2). In this study among the nine strains *Alteromonas* sp. was found as the best in solubilising phosphate while *Alcaligenes* sp. was the least. Similar reports on Phosphorous contents have been reported by many investigators (Kapoor *et al.*, 1989; Singh and Kapoor, 1994).

pH and titrable acidity:

From the Table 2 it was observed that there was a reduction in the pH of the medium but an increase in titrable acidity. This might be due to secretion of organic acids by Phosphate solubilizing bacteria (Lal, 2002). In the present study all the nine strains of Phosphate solubilizing bacteria were able to grow well in the pH 4.5 to 6.7.

Table.2 Production of IAA, phosphorus content, pH and titrable acidity.

S.No	Strain	IAA (µg/mL)	Phosphorous (µg/mL)	pH	Titrable acidity
1.	*Flexibacterium*	7.0	11.0	5.8	1.9
2.	*Alteromonas*	6.0	13.0	6.6	2.1
3.	*Alcaligenes*	20.0	4.0	6.7	3.2
4.	*Pseudomonas*	7.0	6.0	4.4	4.5
5.	*Bacillus*	12.0	8.0	4.5	3.4
6.	*Alcaligenes*	6.0	7.0	4.7	3.8
7.	*Alcaligenes*	8.0	10.0	5.1	2.3
8.	*Alcaligenes*	10.0	4.0	4.7	4.9
9.	*Alcaligenes*	8.0	5.0	6.5	2.0

Effects of PSB on germination of seeds:

The seeds that were inoculated with Phosphate solubilizing bacteria strains showed faster germination and linear growth when compared to control (Table 3). The strain PSB1 promoted faster germination and linear growth of seeds while PSB4 showed the least.

Table.3 Effect of PSB on germination of seeds.

S.no	Strain	Ragi			Fenugreek			Mustard		
		Length of (cm)		No.of	Length of (cm)		No.of	Length of (cm)		No.of
		Root	Coleoptile	Germinated Seeds	Root	Coleoptile	Germinated Seeds	Root	Coleoptile	Germinated Seeds
1.	Control	2.1	1.5	15	0.9	3.4	12	0.9	1.5	11
2.	*Flexibacterium*	2.8	1.6	13	2.2	4.6	13	1.3	2.2	12
3.	*Alteromonas*	2.6	0.9	12	1.7	4.3	15	1.0	1.2	14
4.	*Alcaligenes*	6.3	2.4	19	3.0	7.0	20	2.2	4.3	19
5.	*Pseudomonas*	2.5	1.3	11	2.1	6.8	12	1.8	2.8	13
6.	*Bacillus*	-	-	-	2.1	4.8	11	0.9	1.8	11
7.	*Alcaligenes*	1.7	1.9	14	1.7	4.4	13	1.3	2.1	15
8.	*Alcaligenes*	4.9	2.1	12	2.6	4.9	10	0.9	0.8	11
9.	*Alcaligenes*	1.0	1.4	3	-	-	-	1.7	4.1	16
10.	*Alcaligenes*	4.0	1.7	10	2.0	6.2	14	1.1	1.9	14

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