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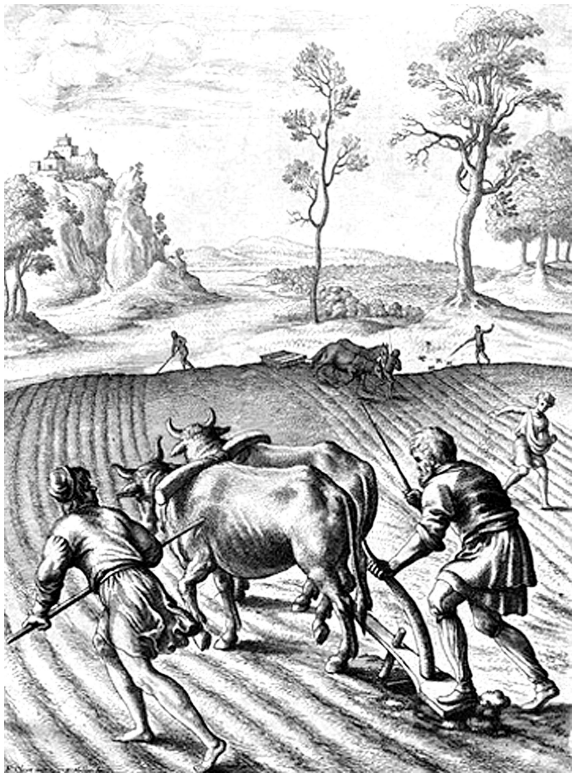

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Distribution Pattern and Screening of Phosphate Solubilizing Bacteria Isolated from Different Food and Forage Crops

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Abstract: The distribution pattern and population density of Phosphate Solubilizing Bacteria (PSB) was assessed in cultivated soils. PSB isolates were assessed for phosphate solubilizing capacity, production of growth regulators, phosphatase activity, pH changes and titrable acidity. The population levels of PSB were highest in the rhizosphere soil of Groundnut and lowest in the rhizosphere of Ragi, Sorghum and Maize. It could be observed from the data that the distribution pattern of PSB in the rhizosphere soils showed that the population levels decreased with the distance of soil sampling from the plants. A wide variation in the capacity to solubilize phosphorous by the PSB isolates was observed. Further, all the isolates were able to secrete phytohormones like gibberelic acid (GA_3) and indole acetic acid (IAA) and acid phosphatase under *in vitro* condition.

Key words: Phosphobacteria, screening, rhizosphere, phosphorous, distribution pattern

INTRODUCTION

Phosphorous is an essential nutrient for plants, but is often not available due to its fixation in soil. Phosphate Solubilizing Bacteria (PSB) solubilize insoluble phosphate and make it available to the plants (Bhattacharyya and Jain, 2000). Indian soils on an average contain 0.05% phosphorous that constitutes 0.2% of plant dry weight. Even applied phosphorous combines with metal ions PSB are required for its release (Bagyaraj and Varma, 1995, Schachtman *et al*., 1998). PSB secrete organic acids and enzymes that act on insoluble phosphates and convert it into soluble form, thus, providing phosphorous to plants. PSB also produce amino acids, vitamins and growth promoting substances (Gonzalez *et al*., 1983; Zimmer *et al*., 1988), which promote plants growth. Increased growth and yield of Oats, Coffee, Tea, Banana, Musturd, Maize, Rice, Sorgham, Barley, Chickpea, Soyabean, Groundnut, Sugarbeet, Cabbage and Tomato to the extent of 10-20% have been reported by using of PSB (Saxena and Sharma, 2003; Saifudheen and Ponmurugan, 2003; Ponmurugan and Gopi, 2006). The present study was undertaken to assess the population density and distribution pattern of PSB in rhizosphere soils of different field crops. PSB isolates were also screened for their performance under *in vitro* conditions.

MATERIALS AND METHODS

The present study was conducted at School of Biotechnology, K.S.Rangasamy College of Technology, Tiruchengode, Namakkal District of Tamilnadu, India, for a period of three year (2003 to 2006).

Enumeration, isolation and identification of PSB: Rhizosphere soil samples were collected from different agroclimatic zones of Namakkal district. The soil samples approximately 200-300 g were obtained from different field crops such as Brinjal, Chilly, Cotton, Green grain, Groundnut, Maize, Paddy, Ragi, Sorghum and Turmeric. These soils were air dried and used for isolation of PSB and the analysis of physicochemical properties. The Pikovskaya (10 g glucose, 5 g tricalcium phosphate, 0.5 g ammonium sulphate, 0.2 g potassium sulphate, 0.1 g magnesium sulphate, 0.5 g yeast extract, trace amount of manganese sulphate and ferrous sulphate, 20 g agar, 1000 mL distilled water) medium was used for isolation, enumeration and maintenance of PSB. Aliquots of serial diluted soil samples were spread on the above agar medium containing suspended insoluble phosphate compound (tricalcium phosphate). Bacterial colonies causing clear phosphate solubilizing halozones by a turbid white background were selected and purified for

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further study. The colony diameter of PSB colony (halo zones) was measured by using metric scale. PSB isolates were identified based on the morphological tests such as motility, cell shape and size and biochemical tests such as glucose fermentation, urea hydrolysis, nitrate reduction, citrate utilization, indole production, Voges-Proskauer and methyl red (Kannan, 2002).

Experiments on the distribution pattern of PSB: The physicochemical properties of soil such as pH, Ec, the total organic carbon (Walkley and Black, 1934), total nitrogen (AOAC, 1990), exchangeable potassium (Page *et al*., 1982) and available phosphorus (Jackson, 1973) were determined.

Experiments on the distribution pattern of PSB: In order to study the distribution pattern of PSB, 200-300 gram of soil samples were collected for every half feet away from the plants of Brinjal, Groundnut, Ragi and Sorghum. However, distribution pattern of PSB with reference to various depths in soils towards the plant root system was also studied, for which, soil samples were collected for every half feet depth from the plants. The number of PSB colonies was expressed in terms of cfu on soil dry weight basis.

Estimation of growth regulators produced by PSB: Three day old cultures of PSB were transferred to Pikovskaya's broth containing L-Tryptophan as a substrate for the production of IAA and GA_3. The cultures were incubated at 37°C on an orbital incubator with gentle agitation (100 rpm). After three days, culture filtrates were used to estimate IAA and GA_3 contents according to the procedure given by Tien *et al*. (1979) and Mahadevan and Sridhar (1996) respectively.

Estimation of phosphorous contents: The phosphorous solubilization potential of PSB strains was tested *in vitro* by estimating available phosphorous in the Pikovskaya's broth amended with known amount of tricalcium phosphate as a substrate. The flasks were inoculated with culture broth of cultures at OD 2(A_{600}). Uninoculated flasks were used as control. The flasks were incubated at 30°C for 7 days and centrifuged at 15,000 rpm. Phosphorous was determined in supernatant following the procedure of Natarajan and Buvana (2000). Phosphorous solubilization on solid medium was measured in terms of Solubilization Efficiency (SE) as SE (%) = (Z-C)/C × 100 where Z is solubilization zone, C is colony diameter.

Determination of phosphatase activity: For phosphate solubilization, PSB produce phosphatase enzyme. In an attempt to study the phosphatase activity in response to phosphorous enrichment, experiments were done using β-Glycerophosphate as the phosphorous source. Culture filtrates were centrifuged and subjected to estimate phosphatase activity following the procedure of Tabatabai and Bremner (1969). The phosphatase activity was calculated by referring to a standard graph prepared with p-nitrophenol. Enzyme activity was expressed as μg of p-nitrophenol released/mg cell protein in 24 h.

Measurement of pH and titrable acidity: A change in pH of the medium due to the growth of PSB was measured with a pH meter after three days of incubation. In order to study the titrable acidity of culture medium, three days old culture filtrates were centrifuged at 1000 rpm for 10 min. Five milliliter of supernatant was added with few drops of phenolphthalein indicator and titrated against 0.01 N NaOH. The titrable acidity was expressed as mL of 0.01 N NaOH consumed per 5.0 mL of culture filtrate.

RESULTS AND DISCUSSION

The population density of PSB with respect to different crop soils are presented in Table 1. It is generally observed that there was a significant difference on the population density. It is found to be higher in the rhizosphere soils of Groundnut (14.9×10^5/g soil dry wt.) Followed by cotton and least in the soils of Ragi and sorghum followed by maize. This variation in the population of PSB might be attributed to many soil factors such as soil nutrients, pH, moisture contents, organic matter and some soil enzyme activities (Table 2). The results thus throw light on the existence of microbial solubilizing of phosphorous in rhizosphere soils of different field crops. Baby *et al*. (2001) carried out an investigation on microbial dynamics in the rhizosphere of

Table 1: Population density of Phosphate Solubilizing Bacteria (PSB) in rhizosphere soils of different field crops

Field crops	Population density ($\times 10^5$/g soil dry wt)	Designation of strain
Brinjal	12.34	BP07
Chilly	10.42	CP01
Cotton	13.41	CP22
Green gram	08.08	GP11
Groundnut	14.90	GP12
Maize	08.61	MP01
Paddy	10.08	PP14
Ragi	07.33	RP07
Sorghum	07.66	SP33
Turmeric	11.56	TP03

Table 2: Properties of rhizosphere soils collected from different field crops

Name of field crops	Soil pH	Soil EC	Total nitrogen (%)	Available phosphorous (ppm)	Exchangeable potassium (ppm)	Total organic carbon (%)
Brinjal	7.4	0.02	1.87	12.74	149.34	12.37
Chilly	7.6	0.03	1.94	12.38	132.33	11.32
Cotton	8.2	0.05	1.87	12.34	144.35	15.38
Green gram	7.8	0.03	2.47	13.48	152.38	13.47
Groundnut	7.9	0.04	2.98	15.38	154.38	14.08
Maize	8.3	0.05	2.55	11.88	148.38	11.32
Paddy	7.6	0.03	2.14	13.38	152.34	13.56
Ragi	7.9	0.04	2.37	11.37	150.38	12.87
Sorghum	8.3	0.05	2.38	13.44	152.71	13.44
Turmeric	8.6	0.06	2.38	12.14	168.32	16.38

tea plants reported that there was a significant difference on the population level of PSB in different clones/seedlings of tea. Further, they were reported that the population of nitrogen fixing *Azospirillum* and PSB were higher in young tea fields than older fields.

The phosphate solubilizing efficiency of isolated strains of PSB indicated that all the strains were solubilized inorganic phosphate contents effectively in the medium (Table 3). Among the ten strains, GP12 was found as the best in solubilizing phosphate (44.08 ppm mL^{-1} of culture filtrate) while GP11 was the least (24.88). It was observed that the phosphate solubilizing efficiency ranged between 44 and 75%. The results showed a wide range of variations in P-solubilization efficiency. Similar results have been reported by many investigators (Kapoor *et al*., 1989; Singh and Kapoor, 1994). There was no correlation drawn between P-solubilization efficiency on solid and liquid medium as also noticed earlier (Srivastav *et al*., 2004).

The results on the phosphatase activity showed that the strain GP12 that was isolated from Groundnut soil had higher activity (36.87 μmoles/g/h) followed by the strain SP33 isolated from Sorghum soil (Table 3). The enzyme activity was very least in GP11 followed by CP22, BP07 and PP14. However, there was a positive correlation between phosphate solubilizing capacity and phosphatase activity. This might be due to availability of higher amount of phosphorous in the medium and the ability of the strains (Barik and Purushothaman, 1998). There is increasing evidence that PSB improve plant growth due to biosynthesis of plant growth substances rather than their action to release available phosphorous.

From the Table 4 observed that there was reduction in pH of the medium but an increase in titrable acidity. This might be due to secretion of organic acids by PSB (Lal, 2002). The results on the production of growth promoting substances indicated that all the isolates of PSB were able to produce phytohormones such as IAA and GA_3 (Table 4). However, it could be seen that all the isolated strains were produced more IAA compared to GA_3. The isolate CP01 produced higher of amount of IAA (45.31 ppm) followed by the strain RP07 while isolate GP11

Table 3: *In vitro* phosphorous solubilizing capacity and phosphatase activity of PSB strains

Name of strain	Available Phosphorous (ppm mL^{-1} of culture filtrate)	Phosphatase activity (μ moles/g/h)	Phosphate solubilization Efficiency (%)
BP07	29.41	17.55	52
CP01	30.44	21.31	75
CP22	28.08	16.23	62
GP11	24.88	14.74	62
GP12	44.08	36.87	68
MP01	35.56	24.57	52
PP14	29.41	19.08	44
RP07	40.69	30.34	68
SP33	42.38	32.38	68
TP03	34.47	22.76	62

Table 4: *In vitro* production of growth promoting substances and phosphate solubilization efficiency of PSB strains

Name of strain	pH of the medium*	Titrable acidity of the medium**	Growth promoting substances (ppm)*	
			IAA	GA_3
BP07	5.8	2.5	34.03	13.95
CP01	5.7	2.5	45.31	15.09
CP22	5.8	2.4	36.51	13.33
GP11	5.9	2.6	40.93	16.85
GP12	5.0	3.9	34.39	14.05
MP01	5.5	2.7	35.31	12.89
PP14	5.8	2.5	40.41	10.05
RP07	5.4	3.3	43.09	14.83
SP33	5.2	3.5	41.45	12.47
TP03	5.6	2.8	38.53	13.27

*Initial pH was 6.8, ** Titrable acidity expressed as mL of 0.01 N NaOH consumed per 5.0 mL of culture filtrate. Titrable acidity of control (uninoculated control) was 1.8

produced higher amount of GA_3 (16.85 ppm) followed by CP01 (15.08 ppm). PSB isolated from rhizosphere soils are known to produce growth-regulating substances and some of them are capable of dissolving phosphate (Barea *et al*., 1978). Lal (2002) reported that PSB isolated from the soils of pearl millet produced IAA, GA3 and cytokinin like substances, which ultimately enhanced the plant metabolism. The PSB cultures release a maximum quantity of IAA in the presence of a physiological precursor, tryptophan in a culture medium. Production of IAA varies greatly among different crops and is also influenced by culture conditions, growth stage and availability of substrate(s) (Vijila, 2000).

The distribution patterns of PSB in the rhizosphere soils of field crops are presented in Fig. 1 and 2. It could

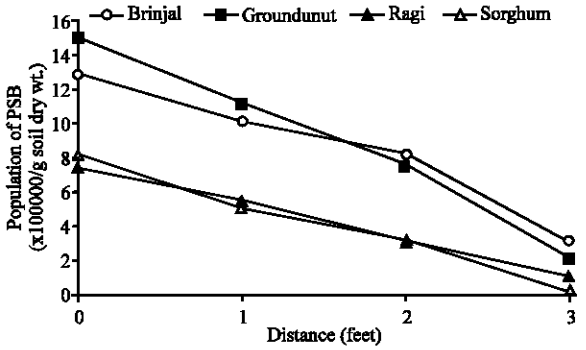

Fig. 1: Distribution pattern of phosphate solubilizing bacteria with reference to distance from the plants

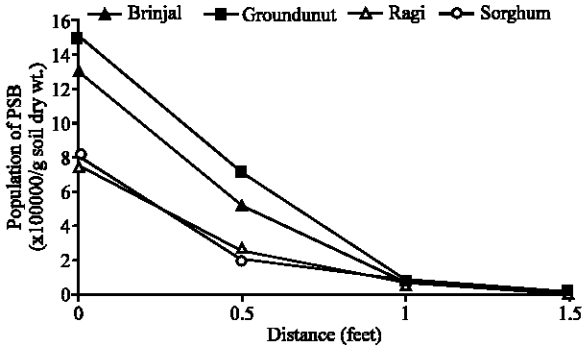

Fig. 2: Distribution pattern of phosphate solubilizing bacteria with reference to depth from the plants

be observed from the data that the population level was decreased when the distance as well as depth of soil sampling from the plants was increased, likely because of the close relationship between the plants and microbes. The close relationship between the plants and microbes is due to the availability of root exudates in the rhizosphere (Saifudheen and Ponmurugan, 2003). However, the soil conditions vary in the amount and type of nutrients, available moisture, degree of aeration, temperature and pH towards the root system of higher plants and soil microorganisms (Lal, 2002). Baby *et al.* (2001) carried out an investigation on distribution pattern of PSB in the rhizosphere of tea plants reported that there was a significant difference on the population level of PSB in the view of various distances of rhizosphere form the plants.

Identification of selected isolates based on Bergey's manual of determinative bacteriology (Sneath *et al.*, 1984) revealed that they were gram negative and were positive for glucose fermentation, urea hydrolysis, nitrate reduction and citrate utilization but negative for indole production, Voges-Proskauer and methyl red test. They were identified as *Pseudomonas* sp. based on biochemical tests. It may be concluded that among the ten isolated strains of PSB, strains like GP12, SP33 and RP07 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.

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