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Population dynamics and screening of phosphate-solubilizing bacteria isolated from tobacco (*Nicotiana tabacum*)-based cropping systems

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ABSTRACT

A study was conducted during 2005–08 to isolate phosphate-solubilizing bacteria (PSB), their distribution pattern and population density from the rhizosphere of different field crops in tobacco (*Nicotiana tabacum* L.) growing soils. PSB isolates were assessed for phosphate-solubilizing capacity, production of growth regulators, phosphatase activity, *p*H changes and titrable acidity. There was a significant difference on the population density. It is found to be higher in the rhizosphere soils of Bengalgram (15.3×10^5/g soil dry wt.), followed by redgram 14.1×10^5 and least in the soils of rice 7.05×10^5 and followed by maize 7.81×10^5. The distribution pattern of PSB in the rhizosphere soils showed that the population levels decreased with the distance of soil sampling from the plants. Among the 10 strains, MP07 was the best in solubilizing phosphate (41.06 ppm/ml of culture filtrate) while SOP 12 was the least (19.90). A wide variation in the phosphate-solubilizing efficiency (51 to 72%) by the PSB isolates was observed. Further, all the isolates were able to secrete phytohormones like indole acetic acid (IAA) and acid phosphatase under *in vitro* condition. The isolate BePO6 produced higher of amount of IAA (44.36 ppm), followed by the strain MaPO3 and least in RPO5 (32.14 ppm). phosphatase activity showed that The strain BePO6 isolated from Bengalgram soil showed highest activity of phosphotase (38.2 µmoles/g/hr), followed by the strain MaPO3 isolated from maize soil (34.01 µmoles/g/hr) with the least activity by the strain RP05 (16.02 µmoles/g/hr) isolated from rice soil.

Key words: *Nicotiana tabacum*, Phosphate-solubilizing bacteria, Rhizosphere

Phosphorus 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 (Subhashini and Padmaja 2010). Indian soils on an average contain 0.05% Phosphorus that constitutes 0.2% of plant dry weight. Even applied phosphorus combines with metal ions PSB are required for its release (Pathak and Shailesh 2010). PSB secrete organic acids and enzymes that act on insoluble phosphates and convert it into soluble form, thus, providing phosphorus to plants. PSB also produce amino acids, vitamins and growth-promoting substances (Wani *et al.* 2007, Singh *et al.* 2010), which promote plants growth. Increased growth and yield of oats, coffee, tea, banana, mustard, maize, rice, sorgham, barley, chickpea, soybean, groundnut, sugarbeet, cabbage and tomato to the extent of 10–20% have been reported by using of PSB (Saxena and Sharma 2003, Ponmurugan and Gopi 2006). The present study was undertaken to assess the population density and distribution pattern of PSB in tobacco growing soils from the rhizosphere of tobacco-based cropping systems. PSB isolates were also screened for their performance under *in-vitro* conditions.

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MATERIALS AND METHODS

Soil samples were drawn from the rhizosphere of different field crops such as tobacco, redgram, blackgram, mustard, maize, rice, sunhemp, bengalgram, soybean and sunflower grown in different agroclimatic zones of tobacco-growing soils. These soils were air dried and used for isolation of PSB. 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). Soil samples were analysed for available nutrients, besides soil reaction (*p*H) and total soluble salt content (E C) by adopting the methods of Muhr *et al.* (1965). Soil samples were collected for every half feet away and half feet depth from the plants to study the distribution pattern of PSB. The number of PSB colonies was expressed in terms of cfu on soil dry weight basis. Three-day-old cultures of PSB were transferred to Pikovskaya's broth containing L-Tryptophan as a substrate for the production of indole acetic acid (IAA) and gibberllic acid (GA). The cultures were incubated at 37ºC on an orbital incubator with gentle agitation (100 rpm). After four days, culture filtrates were used to estimate IAA and GA contents (Mahadevan and Sridhar 1996). The Phosphorus solubilization potential of PSB strains was tested *in vitro* by estimating available Phosphorus in the Pikovskaya's broth amended with known amount of tricalcium phosphate as a substrate. The flasks were inoculated with culture broth of cultures broth of cultures at OD 2 (A_{600}). Uninoculated flasks were used as control. The flasks were incubated at 30ºC for 7 days and centrifuged at 10 000 rpm. Phosphorus was determined in supernatant solution (Natarajan and Buvana 2000). Phosphorus solubilization efficiency (SE) was calculated as SE (%) = (Z-C)/C × 100 where Z is solubilization zones, C is colony diameter. Culture filtrates were centrifuged and subjected to estimate phosphatase activity using ß-Glycerophosphate (Tabatabai and Bremner 1969). Phosphatase activity was calculated by referring to a standard graph prepared with p-nitrophenol.

RESULTS AND DISCUSSION

Population density of PSB

The population density of PSB with respect to different crop soils are presented in Table1. 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 Bengalgram (15.3×10^5/g soil dry wt.) followed by redgram 14.1×10^5 and least in the soils of rice 7.05×10^5. This variation in the population of PSB might be attributed to many soil factors such as soil nutrients, *p*H, moisture contents, organic matter and some soil enzymes activities (Table 1). The results thus throw light on the existence of microbial solubilizing of Phosphorus in rhizosphere soils of different field crops. Ponmurugan and Gopi (2006b) carried out an investigation on microbial dynamics in the rhizosphere and reported that there was a significant difference on the population level of PSB in different food and forage crops. Further, they have observed that there is wide variation in the capacity to solubilise phosphorus by PSB isolates.

Phosphate-solubilizing efficiency

All the strains of PSB solubilized inorganic phosphate contents effectively in the medium (Table 2). Among the 10 strains, MP07 was found as the best in solubilizing phosphate (41.06 ppm/mL of culture filtrate) while S0P 12 was the least (19.90). Phosphate-solubilizing efficiency ranged between 51 and 72%. The results showed wide range of variations in P-solubilizing efficiency as reported by Ponmurugan and Gopi (2006b). There was no correlation drawn between P-solubilization efficiency on solid and liquid medium as noticed earlier also by Srivastav *et al.* 2004. The results on the phosphatase activity showed that the strain BePO6 isolated from bengalgram soil had higher activity (38.21 µmoles/g/hr), followed by the strain MaP03 (34.01 µmoles/g/h) isolated from maize soil (Table 2). The enzymes activity was least in SOP12, followed by SuP14 and BP15. However, there was a positive correlation between phosphate- solubilizing capacity and phosphatase activity.

Production of growth-promoting substances

Data showed that there was reduction in *p*H of the medium but an increase in titrable acidity which might be due to secretion of organic acids by PSB. The results on the production of growth-promoting substances indicated that all the isolates of PSB were able to produce phytohormone such as IAA (Table 2). The isolate BeP06 produced higher of amount of IAA (44.36 ppm), followed by the strain MaPO3 and least in RP05 (32.14 ppm). Present study indicated that PSB strains from various tobacco based field crops release maximum quantity of IAA in the presence of a physiological precursor, tryptophan in a culture medium (Table 2). This is

Table 1 Properties of rhizosphere soils collected from different field crops

Field crop	Soil *p*H	Soil EC	Avail. N (ppm)	Avail. P (ppm)	Ex. K (ppm)	Organic carbon (%)	Pop. density×10^5/g (soil dry wt.)	Strain
Tobacco	8.1	0.04	92	14.59	206	0.58	13.30	TP09
Redgram	7.7	0.03	88	13.12	198	0.52	14.10	RP02
Blackgram	7.9	0.04	84	12.56	186	0.51	12.41	BP15
Mustard	7.8	0.03	90	12.92	194	0.49	11.25	MP07
Maize	7.9	0.04	89	15.21	201	0.45	07.81	MaPO3
Sunhemp	7.6	0.03	88	12.54	176	0.49	11.45	SP06
Rice	7.7	0.03	88	13.48	180	0.56	07.05	RP05
Bengalgram	7.9	0.05	82	12.24	191	0.54	15.30	BeP06
Soybean	8.2	0.05	80	11.86	172	0.41	09.84	SoP12
Sunflower	8.0	0.05	86	13.11	168	0.50	08.91	SuP14
S Em±	0.13	0.01	1.02	0.02	1.46	0.01	0.49	
CD (P = 0.05)	NS	NS	3.03	0.05	4.34	0.03	1.48	
CV%	2.84	24.92	2.04	0.24	1.35	3.89	7.6	

Table 2 *In vitro* phosphorous-solubilizing capacity and phosphatase acitivity of PSB strains

Strain	Available phosphorous (ppm/ml of culture filtrate)	Phoshpatase activity (µmoles/g/hr)	Phosphate solubilization efficiency (%)	*p*H of the medium*	Titrable acidity of the medium **	Growth-promoting substance (ppm) *IAA
TP09	28.68	33.45	69	5.8	2.6	40.28
RP02	32.06	32.11	66	5.7	2.6	35.21
BP15	35.68	21.22	58	5.3	3.9	40.51
MP07	41.06	26.55	61	5.0	4.0	38.91
MaPO3	28.91	34.01	69	5.2	3.5	43.01
SP06	35.16	28.62	60	5.7	2.7	35.25
RP05	32.19	16.20	52	5.6	3.1	32.14
BeP06	33.22	38.21	72	5.4	3.9	44.36
SoP12	19.90	18.11	51	5.4	3.6	36.11
SuP14	24.86	20.16	54	5.6	3.0	35.30
S Em±	1.07	0.17	0.95	0.12	0.09	0.19
CD (P = 0.05)	3.18	0.52	2.82	0.36	0.26	0.58
CV%	6.01	1.13	2.69	3.87	4.58	0.89

*Initial *p*H was 6.9, ** Titrable acidity expressed as mL of 0.01 N NaOH consumed per50 ml of culture filtrate. Titrable acidity of control (uninoculated control) was 2.0.

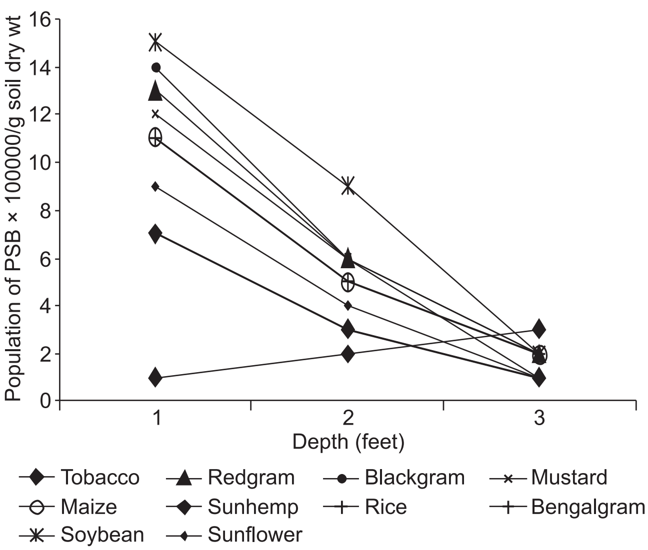

Fig 1 Distribution pattern of phosphate-solubilizing bacteria representing depth from the plants

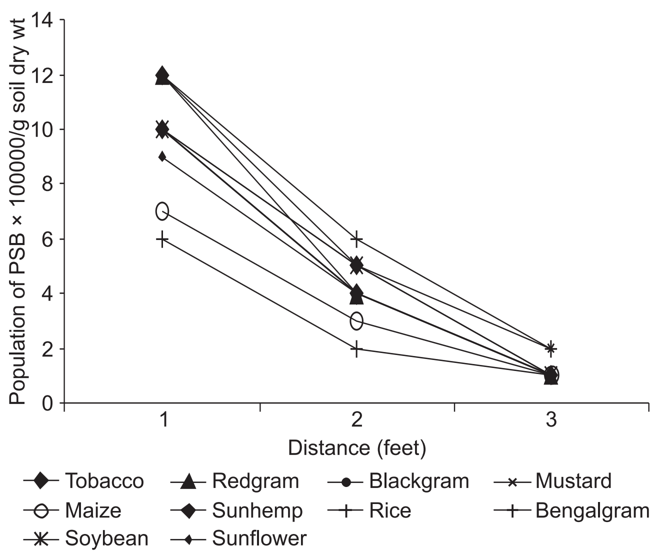

Fig 2 Distribution pattern of phosphate-solubilizing bacteria representing distance from the plants

supported by the finding of Vijila (2000), who reported that the production of IAA varies greatly among different crops and is also influenced by culture conditions, growth stage and availability of substances.

Distribution pattern of PSB

The distribution patterns of PSB in the rhizosphere soils of field crops are presented in Figs 1, 2. It could 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 due to the availability of root exudates in the rhizosphere (Saifudheen and Ponmurugan 2003). The same was also reported by Ponmurugan and Gopi (2006a), that there was a significant difference on the population level of PSB in various distances of rhizosphere from the plants.

It may be concluded that among the 10 isolated strains of PSB collected from tobacco-based cropping system, the strains BePo[6], MaPo[3] and TPo[9] are more promising than other strains. These strains may be more effective and perform better in enhancing plant metabolism and soil health.

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