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Factors Related to the Occurrence of Phosphate Solubilizing Bacteria and Their Isolation in Vertisols

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Abstract: The aim of present research is to isolate phosphate solubilizing bacteria and study their relationship with physical, chemical and biological properties of the soil. Phosphate Solubilizing Bacteria (PSB) were enumerated in 66 soil samples collected from eleven districts of Northern Karnataka. It revealed the presence of PSB in 31 soil samples and indicated a strong positive correlation to exist between total bacterial populations and the population of PSB. The organic carbon and available N content of soils also showed a significant positive correlation while soil pH, available P and total P showed a positive but non-significant correlation with that of PSB population. Thirty one PSB containing soil samples were grouped based on their similarity in bacterial population composition using cluster procedure of SAS and population of total and phosphate solubilizing bacteria as similarity measures. Five soil classes were created and subjected to ANOVA to find any significant effect of soil characteristics. Cation exchange capacity, exchangeable Ca (both in cmol kg^{-1}) and clay content showed significant differences between different soil unsupervised formed groups. A total of 55 PSB were isolated from the rhizosphere soil samples collected from different crops grown in vertisols of Northern Karnataka. These isolated PSB strains will be identified and efficient strains will be selected and used for further studies.

Key words: Vertisols, phosphate solubilizing bacteria, Pearson correlation coefficient, hierarchical cluster analysis, rhizosphere

INTRODUCTION

Vertisols are important both agriculturally and ecologically. They are extensively cultivated and their productivity makes a significant contribution to the national economy of India. According to estimates by the National Bureau of Soil Survey and Land Use Planning, India has about 72 million hectares of true vertisols and related black soils constituting nearly 22% of the country's geographical area. Vertisols and related soils are subject to a wide range of climatic conditions with annual rainfalls ranging from 500 to 1500 mm. Their depth ranges from less than 50 to more than 100 cm. The soils have high clay contents and a high water holding capacity. They are highly prone to erosion, with an estimated soil loss of 6 to 80 t ha^{-1} annum under present agricultural practices. The pH in vertisols is above 7.0 and majority of the mineral P is present in the form of poorly soluble calcium mineral phosphates due to their buffering capacity (Ae *et al.*, 1991).

Phosphorus is one of the plant macronutrients which play an important role in plant metabolism ultimately reflecting on the crop yields. Phosphorus is also involved in controlling key enzyme-reactions and in the regulation of metabolic pathways (Theodorou and Plaxton, 1993). It is estimated that about 98% of Indian soils contain insufficient amounts of available P to support maximum plant

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growth (Hasan, 1996). Plants utilize fewer amounts of phosphatic fertilizers that are applied and the remaining portion is rapidly converted into insoluble complexes in the soil (Vassilev and Vassileva, 2003). In soil more than 80% of P becomes immobile and unavailable for plant uptake because of adsorption, precipitation, or conversion to organic form (Holford, 1997).

The management of P in vertisols is complicated owing to their higher content of expanding clay, free calcium carbonate and high pH. Even though vertisols contain appreciable amounts of total P, it is not available to the crops in adequate amounts mainly due to its insoluble nature. This calls for P application in amounts high enough to compensate for crop removal and fixation by soils. The added P fertilizer will again lead to more accumulation of insoluble phosphates. Hence, there is a need to find ways to harvest these insoluble phosphates and increase the use efficiency of P fertilizers by the crops. In vertisols, because of the problem of P fixation, there is no proper response to applied phosphatic fertilizers even at higher doses. This difference has been explained by various hypotheses, notably high fixation of fertilizer P and poor production of soil available P by Olsen's test. A survey of available P status in Indian soils covering 372 districts has indicated that 45% of these soils are low in available P, 50% are medium and only 5% are high in available P (Tandon, 1987).

Soil microorganisms play an important role in making P available to plants by mineralizing organic P in soil and by solubilizing precipitated phosphates; the latter are called Phosphate Solubilizing Bacteria (PSB) and have been isolated from many soils (Pal, 1998; Chung *et al.*, 2005; Chen *et al.*, 2006). Inoculation of these microorganisms improved growth and increased the yield and P uptake in a variety of crop plants (Jisha and Alagawadi, 1996; Defreitas *et al.*, 1997; Kumar *et al.*, 2001; Zaida *et al.*, 2003; Hameeda *et al.*, 2006). Phosphate solubilizing microorganisms isolated elsewhere have not been very consistent in their performance everywhere owing to their poor adaptability to the changing soil and agroclimatic conditions (Alagawadi *et al.*, 1992). There is a wide gap in understanding the relationship between various soil properties and the P solubilizer population or their activities especially in vertisols. Thus, there is a need to study the mineral phosphate solubilizers and develop regional specific strains better suited for use in vertisols that would go a long way in increasing phosphorus use efficiency in these soils. In the present investigation an attempt was made in collecting rhizosphere and non rhizosphere soil samples from vertisols of Northern Karnataka. Phosphate solubilizing bacteria will be isolated and their relationship with physical, chemical and biological properties of the soils will be assessed. The study was conducted in the Department of Agricultural Microbiology, UAS, Dharwad, India.

MATERIALS AND METHODS

Sampling of Bulk Samples and Their Analyses

Sixty six bulk soil samples (2 kg) were collected from 0-15 cm depth from various sites in the eleven districts of Northern Karnataka. Colony forming units (cfu) of total bacteria and PSB were enumerated on Modified Sperber's agar as reported by Krishnaraj (1996). Soil samples were also analyzed for various physicochemical properties to explore the relationship between soil properties and PSB. There were four replicates for each bulk soil sample.

The clay content of soil was analyzed by the International pipette method of Piper (1966). Analyses of available N (Subbaiah and Asija, 1959), available P (Jackson, 1973), total P (Jackson, 1973), organic C (Jackson, 1973), pH (1:2.5 soil: water dilution using a pH meter), electrical conductivity (using conductivity bridge), exchangeable calcium and magnesium (Black, 1965), cation exchange capacity (Black, 1965) and calcium carbonate content (Piper, 1966) were done for the soil samples. The total bacterial population of soil was enumerated by the standard serial dilution plate count method using soil extract agar (Bunt and Rovira, 1955).

Isolation of PSB from the Rhizosphere

The rhizosphere soil samples of various crops grown in the vertisols at various locations of Northern Karnataka were collected and analyzed for PSB population by following method

of Pikovskaya (1948). The bacterial colonies showing zone of solubilization on Pikovskaya's agar medium were purified, sub cultured and stored for further use.

Statistical Analysis

Pearson correlation coefficient between PSB and various soil properties including total bacteria were computed (Kleinbaum, 1988) using Correlation Proc of SAS software. Same set of analysis was also done for total soil bacteria (TB), total Bacteria on MSM (TBM) and PSB in relation to the soil properties. This data on bacterial population was subjected to hierarchical cluster analysis by SAS procedure (SAS, 1999), to identify the similarity of soils based on the bacterial population composition and group them. Different soil properties of unsupervised grouped soil classes were subjected to ANOVA and Duncan's multiple comparisons of means to find the soil characteristics with significant impacts on total and phosphate solubilizing bacterial populations.

RESULTS

Population of PSB in Relation to Physicochemical and Biological Properties of Vertisols

Soil samples from the eleven districts of Northern Karnataka were used for enumeration of total bacteria and PSB on Modified Sperber's agar medium. The population of total bacteria on Modified Sperber's medium ranged from 32×10^3 to 95×10^3 cfu g^{-1} soil whereas the population of bacteria showing zone of solubilization ranged from 1×10^3 to 18×10^3 cfu g^{-1} (Table 1). Out of the 66 soil samples used for enumeration, only 31 contained PSB. The per cent PSB ranged from 2.63 to 19.56.

Table 1: Population of phosphate solubilizing bacteria in the vertisols of Northern Karnataka

Place of sampling	Total bacteria on MSM (cfu $\times 10^3$ g^{-1} soil)	Population of bacteria showing zone of solubilization on MSM (cfu $\times 10^3$ g^{-1} soil)
Hukkeri	63	8
Rupihala	46	5
Arabhavi	33	1
Yaragatti	52	6
Hanchinal	47	3
Kundagol	80	15
Harapanahalli	95	18
Gangavathi I	40	2
Gangavathi II	36	1
Bannikoppa	39	2
Koppal	43	5
Binkadakatti	37	1
Athani	37	2
Kusugal	38	2
Kurahatti	92	18
Bagalkot	32	2
Beelagi	52	6
Mudhol	68	10
Sarwad	33	1
Devarahippargi	41	4
Basavanabagewadi	38	1
Yarnal	40	2
Hungund	54	7
Sindhanur	39	2
Raichur	42	2
Shahapur	48	5
Jevargi	36	1
Surpur	40	2
Lingasugur	44	2
Mudagal	43	4
Siruguppa	43	4

MSM: Modified Sperber's medium

The soil samples harbouring PSB were analyzed for various soil properties and total bacterial population (Table 2). The pH of these soils ranged from 7.6 to 9.1, electrical conductivity from 0.12 to 0.84 dS m⁻¹, available P from 3.11 to 6.88 kg ha⁻¹, total P from 114.7 to 614.4 kg ha⁻¹, organic C content from 0.13 to 0.99%, available N from 210 to 513 kg ha⁻¹, calcium carbonate content from 0.5 to 37.0%, exchangeable calcium from 32.1 to 52.2 cmol kg⁻¹, exchangeable magnesium from 3.6 to 9.9 cmol kg⁻¹, clay content from 42.1 to 63.5%, cation exchange capacity from 41.0 to 63.6 cmol kg⁻¹ and total bacterial population from 32.0 to 135.0×10⁶ cfu g⁻¹ soil (Table 2).

Pearson Correlation coefficient of these soil properties with PSB population showed a positive significant (p<0.01) correlation between total bacterial population and that of PSB population (r = 0.80**) (Table 3). The organic carbon content of the soils also showed a significant (p<0.01) positive correlation (r = 0.40*) with that of PSB population. Available N had a significant positive correlation (r = 0.40, p<0.05) with PSB population whereas CaCO₃ showed a negative correlation (r = -0.31) in the border line significance region (0.05<p<0.10). The soil pH, available P and total P showed a positive but non-significant correlation with that of PSB population. Electrical conductivity, calcium carbonate content, exchangeable calcium, exchangeable magnesium, clay content and cation exchange capacity showed negative but non-significant correlation with that of PSB population.

The correlation effect between the soil factors was also studied. It revealed that exchangeable calcium showed a highly significant positive correlation with exchangeable magnesium, clay content and cation exchange capacity (r = 0.53, 0.86 and 0.95 with p<0.01, respectively) while it had a border line significant negative correlation with available P (r = -0.33, p<0.07) (Table 3). Similarly exchangeable magnesium was highly significantly correlated with clay content (r = 0.78, p<0.01) and

Table 2: Physicochemical and biological properties of vertisols used for enumeration of Phosphate Solubilizing Bacteria (PSB)

Soil sample	pH	EC (dS m ⁻¹)	Available P (kg ha ⁻¹)	Total P (kg ha ⁻¹)	Organic C (%)	Available N (kg ha ⁻¹)
Hukkeri	8.2	0.15	4.80	602.1	0.36	461
Rupihala	8.2	0.24	4.83	560.8	0.58	365
Arabhavi	7.9	0.80	3.75	430.1	0.78	440
Yaragatti	9.1	0.49	3.15	430.1	0.63	233
Hanchinal	9.0	0.35	3.69	114.9	0.42	236
Kundagol	7.6	0.13	5.20	565.1	0.59	513
Harapanahalli	8.7	0.21	3.68	551.6	0.93	448
Gangavathi I	8.2	0.84	4.50	601.8	0.53	371
Gangavathi II	8.8	0.33	4.42	487.4	0.29	348
Bannikoppa	8.9	0.26	5.11	372.7	0.65	501
Koppal	8.5	0.21	5.02	258.0	0.39	356
Binkadakatti	8.6	0.14	5.11	429.3	0.34	372
Athani	8.3	0.51	6.88	573.4	0.35	325
Kusugal	8.2	0.30	4.62	258.0	0.49	311
Kuruhatti	8.9	0.76	5.01	315.4	0.83	448
Bagalkot	8.3	0.36	3.11	458.8	0.71	365
Beelagi	8.3	0.16	4.28	572.4	0.93	318
Mudhol	7.9	0.12	4.07	430.1	0.53	216
Saarwad	9.0	0.24	4.23	390.2	0.21	359
Devarahip paragi	8.4	0.31	6.18	411.3	0.13	360
Basana bagewadi	8.7	0.28	3.78	344.1	0.15	336
Yamal	8.8	0.25	5.22	614.4	0.35	210
Hungund	8.9	0.29	3.60	421.3	0.73	463
Sindhanur	8.2	0.30	4.85	372.6	0.99	318
Raichur	8.9	0.31	3.61	114.7	0.16	328
Shahapur	9.1	0.29	3.93	320.1	0.21	311
Jevargi	8.1	0.17	3.35	117.3	0.36	270
Surpur	8.5	0.18	3.87	516.1	0.79	339
Lingasugur	8.1	0.13	3.47	315.4	0.61	310
Mudagal	8.7	0.23	5.06	372.7	0.58	318
Siraguppa	8.9	0.21	4.40	258.0	0.29	362

Table 2: Continued

Soil sample	CaCO ₃ (%)	Exchangeable Ca (c mol kg ⁻¹)	Exchangeable Mg (c mol kg ⁻¹)	Clay content (%)	CEC (c mol kg ⁻¹)	Population of total bacteria (cfu×10 ⁶ g ⁻¹ soil)
Hukkeri	19.0	44.7	3.6	50.0	52.6	124
Rupihala	15.0	32.1	4.0	43.8	41.4	120
Arabhavi	19.0	52.2	5.8	59.1	62.6	101
Yaragatti	11.0	44.4	3.7	52.5	54.6	125
Hanchinal	37.0	47.8	5.8	52.6	58.6	117
Kundagol	14.5	45.0	5.6	53.9	55.4	132
Harapanahalli	10.0	39.1	4.4	48.5	46.0	135
Gangavathi I	4.5	40.4	5.5	45.8	48.8	107
Gangavathi II	16.0	40.0	4.7	46.6	49.5	99
Bannikoppa	14.5	34.2	4.2	45.6	42.6	106
Koppal	26.5	38.4	5.4	43.5	47.5	118
Binkadakatti	23.0	40.6	5.5	52.2	50.2	79
Athani	10.0	35.4	4.4	43.2	44.0	112
Kusugal	7.0	36.7	4.2	44.2	45.6	111
Kuruhatti	0.5	34.8	6.1	44.1	43.4	133
Bagalkot	15.0	50.0	8.9	61.8	63.6	108
Beelagi	10.0	40.0	6.7	52.5	50.6	124
Mudhol	1.5	47.8	8.1	61.4	60.1	129
Saarwad	16.0	44.4	9.2	58.8	58.2	105
Devarahip paragi	21.0	46.3	8.5	61.2	59.2	118
Basanabagewadi	2.5	49.2	9.9	63.5	62.6	103
Yarnal	27.5	32.8	6.1	42.1	41.0	109
Hungund	14.0	42.8	6.3	51.0	52.8	126
Sindhaur	17.5	34.4	5.7	47.2	46.0	111
Raichur	8.0	40.1	9.2	56.3	55.5	115
Shahapur	20.5	36.9	5.7	48.2	47.0	116
Jevargi	25.5	36.9	7.0	50.5	49.4	105
Surpur	16.0	40.1	6.6	52.3	51.2	113
Lingasugur	2.5	40.4	7.1	55.5	57.0	114
Mudagal	16.0	48.0	9.7	58.2	61.2	117
Siraguppa	14.0	50.1	8.8	60.3	62.5	119

Table 3: Pearson correlation coefficient of soil properties in relation to population of phosphate solubilizing bacteria

	pH	EC	AP	TP	OC	AN	CaCO ₃	Ca	Mg	Clay	CEC	TB	PSB
pH	1.00	0.06	-0.16	-0.30	-0.26	-0.13	0.17	-0.08	0.10	-0.08	-0.08	-0.04	-0.07
EC	0.00	0.76	0.40	0.10	0.16	0.48	0.37	0.67	0.59	0.68	0.67	0.81	0.71
AP	0.06	1.00	0.04	0.07	0.17	0.13	-0.21	0.04	-0.16	-0.17	-0.06	-0.07	0.00
TP	0.76	0.00	0.81	0.71	0.37	0.48	0.25	0.85	0.38	0.36	0.76	0.72	1.00
OC	-0.16	0.04	1.00	0.34	-0.20	0.18	0.09	-0.33	-0.21	-0.37	-0.39	-0.04	0.05
AN	0.40	0.81	0.00	0.06	0.28	0.32	0.63	0.07	0.27	0.04	0.03	0.82	0.81
CaCO ₃	-0.30	0.07	0.34	1.00	0.30	0.26	-0.17	-0.11	-0.35	-0.21	-0.25	0.11	0.22
Ca	0.10	0.71	0.06	0.00	0.10	0.15	0.35	0.57	0.05	0.27	0.18	0.55	0.23
Mg	-0.26	0.17	-0.20	0.30	1.00	0.26	-0.23	-0.14	-0.29	-0.19	-0.20	0.36	0.40
Clay	0.16	0.37	0.28	0.10	0.00	0.16	0.22	0.45	0.11	0.32	0.29	0.05	0.03
CEC	-0.13	0.13	0.18	0.26	0.26	1.00	-0.15	0.03	-0.21	-0.07	-0.08	0.14	0.40
TB	0.48	0.48	0.32	0.15	0.16	0.00	0.41	0.87	0.25	0.72	0.67	0.46	0.03
PSB	0.17	-0.21	0.09	-0.17	-0.23	-0.15	1.00	0.00	-0.14	-0.14	-0.05	-0.27	-0.29
	0.37	0.25	0.63	0.35	0.22	0.41	0.00	0.98	0.44	0.45	0.79	0.14	0.11
	-0.08	0.04	-0.33	-0.11	-0.14	0.03	0.00	1.00	0.53	0.86	0.95	0.01	-0.05
	0.67	0.85	0.07	0.57	0.45	0.87	0.98	0.00	0.002	<0.0001	<0.0001	0.96	0.81
	0.10	-0.16	-0.21	-0.35	-0.29	-0.21	-0.14	0.53	1.00	0.78	0.70	-0.09	-0.21
	0.59	0.38	0.27	0.05	0.11	0.25	0.44	0.00	0.00	<0.0001	<0.0001	0.61	0.25
	-0.08	-0.17	-0.37	-0.21	-0.19	-0.07	-0.14	0.86	0.78	1.00	0.94	-0.05	-0.12
	0.68	0.36	0.04	0.27	0.32	0.72	0.45	<0.0001	<0.0001	0.00	<0.0001	0.80	0.51
	-0.08	-0.06	-0.39	-0.25	-0.20	-0.08	-0.05	0.95	0.70	0.94	1.00	-0.04	-0.16
	0.67	0.76	0.03	0.18	0.29	0.67	0.79	<0.0001	<0.0001	<0.0001	0.00	0.84	0.40
	-0.04	-0.07	-0.04	0.11	0.36	0.14	-0.27	0.01	-0.09	-0.05	-0.04	1.00	0.80
	0.81	0.72	0.82	0.55	0.05	0.46	0.14	0.96	0.61	0.80	0.84	0.00	<0.0001
	-0.07	0.00	0.05	0.22	0.40	0.40	-0.29	-0.05	-0.21	-0.12	-0.16	0.80	1.00
	0.71	1.00	0.81	0.23	0.03	0.03	0.11	0.81	0.25	0.51	0.40	<0.0001	0.00

Note: Highly significant (p<0.01), Significant at (p<0.05), Borderline significance (0.05<p<0.10), EC: Electrical Conductivity; AP: Available Phosphorus; TP: Total Phosphorus; OC: Organic Carbon; AN: Available Nitrogen; CaCO₃: Calcium Carbonate content; Ca: Exchangeable Calcium; Mg: Exchangeable Magnesium; Clay: Clay content; CEC: Cation Exchange Capacity; TB: Total Bacteria; PSB: Phosphate Solubilizing Bacteria

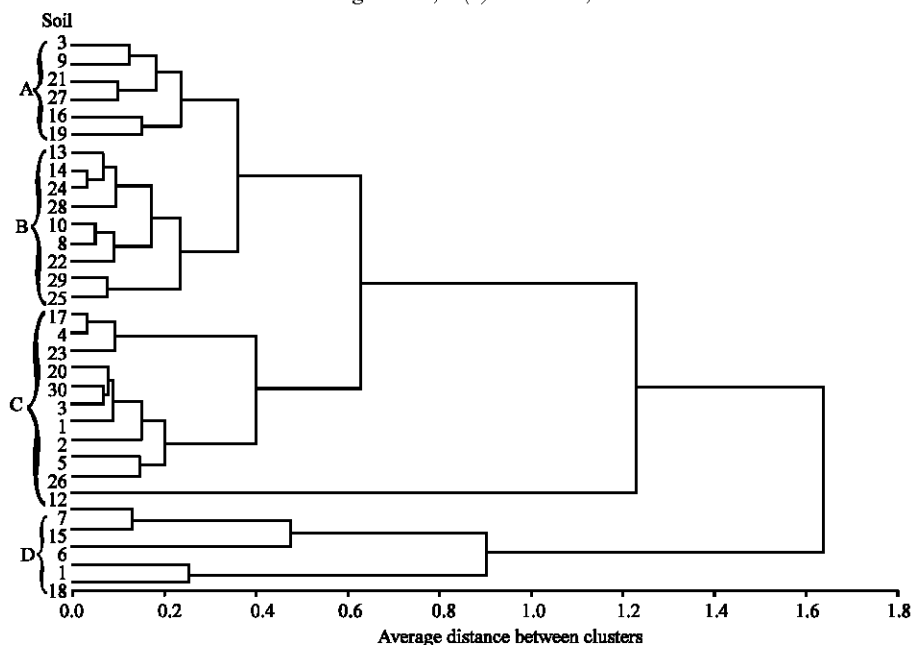


Fig. 1: Grouping of 31 soil samples based on bacterial population composition. Dendrogram was generated based on Hierarchical cluster analysis of total soil bacterial colonies, total bacteria on Modified Sperber's media and population of phosphate solubilizing bacteria. The scale represents the Euclidean distance of soil samples as an index of their similarity

cation exchange capacity ($r = 0.70$, $p < 0.01$) and the interaction between clay content and cation exchange capacity had a highly significant positive correlation ($r = 0.94$, $p < 0.01$). Both cation exchange capacity and clay content had highly significant negative correlation with available P ($r = -0.39$ and -0.37 , $p < 0.05$, respectively). The total bacterial counts and PSB population were positively correlated with organic C ($r = 0.36$ and 0.40 , $p < 0.05$), available N ($r = 0.35$ and 0.40 , $p < 0.05$) and negative correlation were observed between total bacterial population on MSM but at border line significance ($r = -0.31$, $p < 0.09$). A positive correlation at border line level of significance ($r = 0.30$, $p < 0.10$) was observed between total P and organic carbon. Exchangeable magnesium had a significant negative correlation with total P ($r = -0.35$, $p < 0.05$) and pH and available P showed negative/positive correlation with total P at border line significance level ($r = -0.30$ and $+0.34$, respectively at $0.05 < p < 0.10$).

Soil samples were classified into four groups with the use of total soil bacterial colonies, total bacteria on Modified Sperber's medium (MSM) ($\text{cfu} \times 10^3 \text{g}^{-1}$ soil) and PSB on MSM ($\text{cfu} \times 10^3 \text{g}^{-1}$ soil) in a Hierarchical Cluster Analysis (HCA) which generated a cladogram (Fig. 1). The soils from Arabhavi, Gangavathi II, Bagalkot, Saarwad, Basavanabagewadi and Jevargi were classified under group A while soils from Gangavathi I, Bannikoppa, Athani, Kusugal, Yarnal, Sindhanur, Raichur, Surpur and Lingasugur were classified under group B by cluster analysis. Similarly, the soils from Rupihala, Yaragatti, Hanchinal, Koppal, Binkadakatti, Beelagi, Devarahipparagi, Hungund, Shahapur, Mudagal and Siraguppa were assigned to group C while the soils from Hukkeri, Kundagol, Harapanahalli, Kuruhatti and Mudhol were assigned to group D (Fig. 1).

Analysis of variance of soil properties on unsupervised generated soil groups with bacterial population similarities showed that electrical conductivity, cation exchange capacity and clay content were significant ($p < 0.05$) and border line significant ($p < 0.07$) between different soil groups, respectively (Fig. 2). The soils from group A had higher electrical conductivity, cation exchange capacity and clay content when compared with other groups.

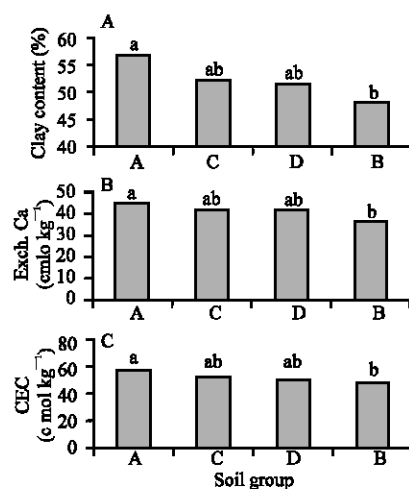


Fig. 2: Duncan's Multiple Range Test for clay content (%) (A), exchangeable Ca (B) and CEC © (both in cmol kg⁻¹) ($p < 0.05$) between different soil classes. Bars with the same letter are not different at 5% level

Isolation of PSB from Rhizosphere Soils

PSB were isolated from the rhizosphere of sorghum, maize, bajra, paddy, wheat, navane, cotton, chilli, tomato, sunflower, redgram, greengram, cowpea, soybean, groundnut, castor, coriander and some weeds. A total of 55 bacterial isolates showing clear zones on Pikovskaya's medium were obtained from the 181 rhizosphere samples. Out of the 55 isolates, 13 were from Belgaum district, ten from Koppal district, eight each from Davanagere and Dharwad districts, six from Raichur district, three from Bagalkot district and two each from Bijapur, Gadag and Gulbarga districts and one from Bellary district. These isolates were purified and maintained for further use.

DISCUSSION

In an attempt to understand the possible relationship between soil properties and the predominance of PSB bacteria, the different soil samples showing the presence of PSB were analyzed for physical, chemical and microbiological properties. The correlation coefficient of soil characteristics with that of population of PSB was worked out and is given in Table 3. Among the various properties, population of total bacteria was found to have a positive and highly significant correlation (0.80**) with that of PSB population. Similarly, organic carbon content (0.40*) and available N (0.40*) of soil also showed significant positive correlation with that of PSB population. In the present study in most cases we observed that the soils with higher organic matter content showed more number of PSBs compared to others. It may be possible that PSB present in those soils are heterotrophic bacteria. Although no reports are available on the exact relationship between the total bacterial population and that of PSB, it is possible that the organic carbon in soil support the growth of both general bacteria as well as PSB as observed in the present study. While a positive and significant correlation between the phosphate solubilizing microorganisms and organic matter content of the soil is reported by Venkateswarlu *et al.* (1984), organic matter content of the soils is known to significantly influence the population of PSB (Gupta *et al.*, 1986). Available P is negatively correlated with cation exchange capacity ($r = -0.39^{**}$), clay content ($r = -0.37^{**}$) and exchangeable calcium ($r = -0.33^{**}$) and cation exchange capacity is in turn positively correlated with exchangeable calcium ($r = +0.95^{**}$),

exchangeable magnesium ($r = +0.70^{**}$) and clay content ($r = +0.94^{**}$). Irrespective of internal network of interactions between different soil properties namely clay content, cation exchange capacity, exchangeable calcium and magnesium all of them have a direct negative impact on soil available P. Available P has a positive correlation with total P which in turn has a positive correlation on organic carbon and indirectly with total and PSB populations.

Amongst the other soil properties, electrical conductivity, available P and total P showed positive but non-significant correlation with the population of PSB whereas the pH, calcium carbonate, exchangeable calcium and magnesium, clay content and cation exchange capacity of the soils showed non-significant negative correlation with that of PSB population. Similar results of insignificant relationships were reported to exist between population of PSB and total or available P (Kucey, 1983) and that between population of PSB and electrical conductivity, available P, cation exchange capacity, soil moisture and pH (Yahya and Al-Azawi, 1989) which are known.

A total of 181 rhizosphere soil samples of various crop plants and weeds grown on vertisols of Northern Karnataka were collected and used for isolation of PSB on Pikovskaya's agar medium. About 55 PSB with a characteristic phenotype of clearing zone around the colony were selected, purified and stored for further use. Among the 55 PSB strains, 13 were isolated from the rhizosphere samples of Belgaum district, ten from Koppal district, eight each from Dharwad and Davanagere districts, six from Raichur district, three from Bagalkot and two each from Bijapur, Gadag and Gulbarga districts and one from Bellary district. Majority of the soil samples collected from Bellary, Belgaum, Koppal and Raichur districts were from irrigated areas, those of Dharwad district were from transitional belt and the samples of Bagalkot, Bijapur, Gadag and Gulbarga were from rainfed areas, indicating the wider distribution and occurrence of PSB in the rhizosphere of irrigated crops than in dry land crops. The occurrence of PSB in the rhizosphere of various crop plants have been reported earlier by various workers (Illmer and Schinner, 1992; Thakkar *et al.*, 1993; Dave and Patel, 1999; Vazquez *et al.*, 2000; Chung *et al.*, 2005; Chen *et al.*, 2006). Further studies related to the identification of the PSB isolates, their efficiency in solubilizing various inorganic phosphates and production of growth promoting substances will be considered.

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REFERENCES

- Ae, N., J. Arihara and K. Okada, 1991. Phosphorus Response of Chickpea and Evaluation of Phosphorus Availability in Indian Alfisols and Vertisols. In: Phosphorus Nutrition of Grain Legumes. Johansen, C., K.K. Lee and K.L. Sahrawat, (Eds.), ICRISAT, India, pp: 33-41.
- Alagawadi, A.R., M.N. Sheelavantar, R.B. Patil and S.V. Patil, 1992. India should take the best advantage of biofertilizers. In: Some Aspects of Agriculture and Rural Development. ISARD Publication, Dharwad, pp: 93-113.
- Black, C.A., 1965. Methods of Soil Analysis, Chemical and Microbial Properties. American Society of Agronomy, Madison, WI, pp: 1569.
- Bunt, J.S. and A.D. Rovira, 1955. Microbiological studies of subantarctic soils. *J. Soil Sci.*, 6: 119-128.
- Chen, Y.P., P.D. Rekha, A.B. Arun, F.T. Shen, W.A. Lai and C.C. Young, 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Applied Soil Ecol.*, 34: 33-41.

- Chung, H., M. Park, M. Madhaiyan, S. Seshadri, J. Song, H. Cho and T. Sa, 2005. Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. *Soil Biol. Biochem.*, 37: 1970-1974.
- Dave, A. and H.H. Patel, 1999. Inorganic phosphate solubilizing soil pseudomonads. *Indian J. Microbiol.*, 39: 161-164.
- Defreitas, J.R., M.R. Banerjee and J.J. Germida, 1997. Phosphate solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus* L.). *Biol. Fertil. Soils*, 24: 358-364.
- Gupta, R.D., K.K.R. Bharadwaj, B.C. Marwah and B.R. Tripathi, 1986. Occurrence of phosphate dissolving bacteria in some soils of North-West Himalayas under varying biosequence and climosequence. *J. Ind. Soci. Soil Sci.*, 34: 498-504.
- Hameeda, B., G. Harini, O.P. Rupela, S.P. Wani and G. Reddy, 2006. Growth promotion of maize by phosphate solubilizing bacteria isolated from composts and macrofauna. *Microbiol. Res.*, (In Press).
- Hasan, R., 1996. Phosphorus status of soils in India. *Better Crops International*, 10: 4-5.
- Holford, I.C.R., 1997. Soil phosphorus: Its measurement and its uptake by plants. *Aust. J. Soil Res.*, 35: 227-239.
- Illmer, P. and F. Schinner, 1992. Solubilization of inorganic phosphates by microorganisms isolated from forest soils. *Soil Biol. Biochem.*, 24: 389-395.
- Jackson, M.L., 1973. *Soil Chemical Analysis*. Prentice Hall of India Pvt. Ltd., New Delhi.
- Jisha, M.S. and A.R. Alagawadi, 1996. Nutrient uptake and yield of sorghum (*Sorghum Bicolor* L. Moench) inoculated with phosphate solubilizing bacteria and cellulolytic fungus in a cotton stalk amended vertisol. *Microbiol. Res.*, 151: 213-217.
- Kleinbaum, D.G., L.L. Kupper and K.E. Muller, 1988. *Applied Regression Analysis and Other Multivariable Methods*. PWS-Kent Publishing Company, Boston.
- Krishnaraj, P.U., 1996. Genetic characterization of mineral phosphate solubilization in *Pseudomonas* sp. Ph.D Thesis, Indian Institute of Agricultural Sciences, New Delhi, India.
- Kucey, R.M.N., 1983. Phosphate solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. *Can. J. Soil Sci.*, 63: 671-678.
- Kumar, V., R.K. Behl and N. Narula, 2001. Establishment of phosphate-solubilizing strains of *Azotobacter chroococcum* in the rhizosphere and their effect on wheat cultivars under greenhouse conditions. *Microbiol. Res.*, 156: 87-93.
- Pal, S.S., 1998. Interactions of an acid tolerant strain of phosphate solubilizing bacteria with a few acid tolerant crops. *Plant Soil*, 198: 169-177.
- Pikovskaya, R.I., 1948. Mobilization of phosphates in soil in connection with the vital activities of some microbial species. *Mikrobiologiya*, 17: 362-370.
- Piper, C.S., 1966. *Soil and Plant Analysis*. Hans Publishers, Bombay, pp: 362.
- SAS® Institute Inc., 1999. *SAS/STAT user's guide*, version 8. Cary, North Carolina.
- Subbaiah, B.V. and G.L. Asija, 1959. Rapid procedure for the estimation of available nitrogen in soils. *Curr. Sci.*, 25: 259-260.
- Tandon, H.L.S., 1987. Phosphorus research and agricultural production in India. *Fertilizer Development and Consultation Organization*. New Delhi, pp: 160.
- Thakkar, J., V. Narsian and H.H. Patel, 1993. Inorganic phosphate solubilization by certain soil bacteria. *Indian J. Expt. Biol.*, 31: 743-746.
- Theodorou, M.E. and W.C. Plaxton, 1993. Metabolic adaptations of plant respiration to nutritional phosphate deprivation. *Plant Physiol.*, 101: 339-344.

- Vassilev, N. and M. Vassileva, 2003. Biotechnological solubilization of rock phosphate on media containing agroindustrial wastes. *Appl. Microbiol. Biotechnol.*, 61: 435-440.
- Vazquez, P., G. Holguin, M.E. Puente, A. Lopez-Cortes and Y. Bashan, 2000. Phosphate solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biol. Fert. Soils*, 30: 460-468.
- Venkateswarlu, B., A.V. Rao and P. Raina, 1984. Evaluation of phosphorus solubilization by microorganisms isolated from aridisols. *J. Indian Soc. Soil Sci.*, 32: 273-277.
- Yahya, A.I. and S.K. Al-Azawi, 1989. Occurrence of phosphate solubilizing bacteria in some Iraqi soils. *Plant Soil*, 117: 135-141.
- Zaida, A., M.S. Khan and M.D. Amil, 2003. Interactive effect of rhizotrophic microorganisms on yield and nutrient uptake of chickpea (*Cicer arietinum* L.). *Eur. J. Agron.*, 19: 15-21.