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Distribution of phosphate solubilizing microorganisms in acidic soils of Manipur

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Twenty five (25) rhizospheric soils from agricultural areas of different districts of Manipur were sampled in the spring of 1998 to enumerate phosphate solubilizing microorganisms (PSM). Phosphate solubilizing and total bacterial and fungal populations were determined by serial dilution and plate count technique. The result revealed that 92% of the samples were inhabited with indigenous PSM. The total number varied and ranged from 154×10^7 to 389×10^7 and that of PSM from 2×10^7 to 33 $\times 10^7$ cells g^{-1} soil which indicated the percentage of PSM was 0.919 to 11.7% to 11.17% of total population.

Several fungi, yeast and bacteria were isolated from which eleven (11) strains were selected for detail study. Most of the isolates were found to be gram negative predominately of *Pseudomonas* strains and not a single strain of phosphate solubilizing *Bacillus* strain was obtained. Fungi were found superior to bacteria in solubilizing insoluble tricalcium phosphate {$(Ca_3 (PO_4)_2$} both in solid and liquid medium. *Aspergillus niger* showed nearly whole plate clearance whereas yeast and bacterial showed a clear zone of 1.8 cm to 3 cm. diameter. The most efficient strains of bacteria were identified as *Pseudomonas striata.* All the microorganisms acidified the liquid medium containing $Ca_3 (PO_4)_2$ and in all the cases there was decrease in pH of the medium.

Key words : Phosphate solubilizing microorganisms, rhizospheric soils, tricalcium phosphate.

INTRODUCTION

Phosphate solubilizing microorganisms (PSM) are found in majority of soils (Chhonker and Taraeder, 1984 ; Vankateshwarlu *et. al.*, 1984). The population of PSM is higher in soils under moist climates than in dry ones (Subba Rao, 1982) and generally their population is low in arid and semiarid regions, possibly due to low levels of organic matter and high temperature regime (Gupta *et. al.*, 1986). It is known that phosphorus is second to nitrogen as mineral required by both plants and microorganisms. Only a small percentage of the total phosphorus in soil is in a form available to plants, and an even smaller fraction is in the soil solution (Kucey, 1983). The remainder excluding the organically bound phosphorus is in chemical forms that are at best, only very slightly soluble (Stewart *et. al.*, 1980) Phosphorus in soils is emmobilized or becomes less soluble (Stewart *et. al.*, 1980). Phosphorus in soils is emmobilized or becomes less soluble, either by adsorption, chemical precipitation or both. Moreover, the North East Indian soils are acidic with the tendency to fix the natural or supplied phosphorus as iron or aluminium orthophosphate. In the light of the presence of phosphate solubilizing microorganisms and its important role through the release of fixed phosphorus, an investigation was made to study the abundance of phosphate solublizing microorganisms in the acidic soils of Manipur.

MATERIALS AND METHODS

Twenty five (25) rhizospheric soil samples from agricultural areas of different districts of Manipur were collected in the spring of 1998. The location, plant covers, soil pH, number of phosphate

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solublizing microorganisms and percentage of phosphate solubilizing microorganisms were noted. Composite soil samples from the rhizospheric of each site were thoroughly mixed, the 1 kg lot was used for enumeration of phosphate solubilizing microogranisms while the rest of the soil was air dried, sieved (2 mm. dim. sieve) and then used for determination of physical and chemical properties.

The phosphate solubilizing microorganisms were enumerated in 25 soil samples by empolying enrichment technique (Gaind and Gaur, 1971) in Pikovaskaya's medium. One gram of soil from each of the samples collected were added to 10 ml Sterile Pikovaskaya's broth. Three successive enrichment for three weeks were carried out by transferring aliquots from the previous medium to 100 ml fresh sterile Pikovaskaya's broth.

From the 3rd enriched flask serial dilutions were prepared by a standard method. From each dilution 0.1 ml were plated on sterial petriplates. Twenty (20) ml of molten sterile Pikovaskaya's medium was poured in the plates and after thorough mixing and solidification the plates were incubated at 28 ± 2°C for 4-5 days. The colonies showing clear zones around them were considered as indication of phosphate solubilization.

The phosphate solubilizing efficacy was determined by measuring the zone of solubilization. The pure culture isolates were inoculated at the centre of sterile Pikovaskaya's medium containing plates. After 4 to 5 days in incubation the isolates which shows larger zone of solubilization were selected for further study. The phosphate solubilzing efficacy of the isolates in liquid medium were determined by standard methods (Koining and Johnson, 1942). Finally the isolates having higher capacity were selected and preserved for further study. Morphlogical, physiological and biochemical characteristics were determined following the Bergey's Mannual, 1984 and The Prokaryotes Vol. I and II.

RESULTS AND DISCUSSION

Phosphate solubilizing, total bacterial and fungal population were determined by serial dilution and plate count technique. The revealed that 92% of the samples wre inhabited with indigenous PSM. The total number varied and ranged from 154×10^7 to 389×10^7 and that of phosphate solubilizing microorganims from 2×10^7 to 33×10^7 cells g^{-1} soil which indicates the precentage of PSM was 0.919 to 13.1 % of the population. The details are given in the Table 1. Altogether, 140 phosphate solublizing cultures were isolated, of which 28 strains belonged to *Pseudomonas* spp., 9 to actinomycetes, 6 to yeasts and 11 to fungi and the remaining were bacterial strains other than *Pseudomonas.*

Table 1 : The total fungal, bacterial, PSM and % of PSM obtained from the soil samples tested.

Plant cover	Location	Soil pH.	No. of orgs/g soil	No. of PSM/ml	PSM (%)
Paddy	Mantripukhri	6.0	300.3×10^7	31.63×10^7	10.53
Cabbage	Canchipur	5.5	366.35×10^7	32.53×10^7	8.8
Garlic	Kanglatongbi	6.1	389.6×10^7	31.67×10^7	8.1
Maize	Kanglatongbi	6.3	314.3×10^7	28.04×10^7	8.9
Chilli	Churachanpur	5.6	212.45×10^7	12.7×10^7	5.97
Citrus plant	Ukhrul	6.0	244.12×10^7	15.98×10^7	6.59
French bean	Morhe	6.0	257.95×10^7	11.928×10^7	4.62
Brinjal	Kangpokpi	5.7	271.1×10^7	17.48×10^7	6.44
Turmeric	Sawombung	5.5	155.73×10^7	12.5×10^7	8.16
Paddy	Pukhao	5.1	263.3×10^7	—	—
Pineapple	Gaurnagar	4.9	291.01×10^7	33.31×10^7	13.16
Greenpea	Top (river bank)	5.5	221.45×10^7	22.26×10^7	10.2
Allium	Top	6.0	154.55×10^7	15.11×10^7	9.84
Paddy	Yaran Lake (Top)	5.2	172.88×10^7	18.52×10^7	10.17
Grass	Moirang hill	5.5	155.89×10^7	16.8×10^7	10.8
Broad bean	Thanga island	6.0	268×10^7	—	—
Arum	Bisenpur	5.1	161.64×10^7	5.23×10^7	3.23
Supari	Jiribam	5.5	172.02×10^7	18.6×10^7	10.40
Coconut	Jiribam	5.7	299.63×10^7	16.65×10^7	5.56
Arhur	Thoubal	5.7	265.64×10^7	20.9	7.88
Banana	Tamenglong	5.5	275.02×10^7	2.53×10^7	0.919
Sugarcane	Kakching	6.2	225.49×10^7	13.6×10^7	6.03
Brinjal	Lilong	5.5	241.61×10^7	11.89×10^7	4.92
Ladiesfinger	Sekmaijing	5.6	177.8×10^7	18.00×10^7	10.1
Cauliflower	Konthoujam	5.7	228.8×10^7	14.16×10^7	6.19

By spot inoculation method the area of zone of solubilization in cm. were recorded. *Aspergillus niger* showed largest zone of solubilization, among bacteria *Pseudomonas striata, Pseudomonas aeruginosa, Beijerinkia mobilis* showed larger zone of solubilization showing 3.5 cm, 3.2 cm and 3.2 cm diameter respectively on the basis of their activity to solubilized insoluble phosphate on Pikovaskaya's agar plate containing 0.5% insoluble tricalcium phosphate. Eleven efficient phosphate solubliziing cultures as shown in Table 1 were slected for further studies.

Quantitative estimation of soluble phosphate in filtrates for 2 weeks showed that most of the strains other than *Arthrobacter* sp and *Corynabacterium* sp. solubilized more than 50% of the tricalcium phosphate added in the Pikovaskaya's medium, although the solubilization rate varied from strain to strain.

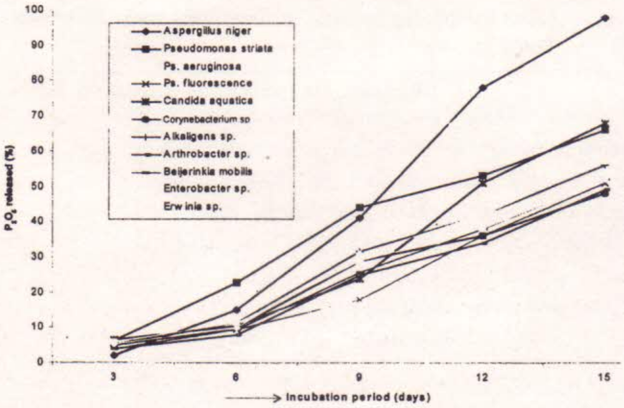

Fig. 1 : Solubilization of Tri-calcium phosphate at normal growth condition by selected isolates

The efficiency of these organisms in phosphate solubilization varied widely fungi being superior of bacteria (Table 2 and Fig. 1) *Aspergillus niger* showed the maximum solubilization by releasing 89 % of the phosphate added in the medium while *Pseudomonas striata* solubilized about 66 % and found to be best among bacteria under normal growth conditions. *Candida aqualica* also showed high solubulization ability solublizing about 68 % of the total insoluble tri-calcium phosphate added.

Table 2 : Zone of phosphate solubilizatin and % phosphate solubilization shown by selected mocroorganisms.

Efficient phosphate solubilizing Microorganisms	Zone of clearance (Dim. in cm)
Aspergillus niger	4.5/ whole plate
Pseudomonas striata	3.5
Pseudomonas aeruginosa	3.2
Pseudomonas fluorescence	2.5
Alkaligens species	2.4
Arthrobacter species	2.6
Beijerinkia mobilis	3.2
Enterobacter species	2.2
Erwinia species	2.3
Corynebacterium species	2.6

Arthrobacter and *Corynebacterium* species were found to be weaker strains showing only 49 % and 48 % solubilzation respectively of the insoluble phosphate added as compared to other isolates. The amount of phosphate released by the different microorganisms increased significantly with period of incubation upto 15 days. These results are confirmatory with those recorded by Chhonkar and Subba Rao (1967) and Mehta and Bhide (1970).

The pH of the culture filtrate as shown in Table 3 became acidic with all the cultures indicating production of organic acids (Sperber, 1957 ; Gaur and Sachar. 1980), which facilitate the solubilization of tri-calcium phosphate. The release of soluble phosphate was accompanied by a lowering of medium pH, indicating the development of acidity in the culture medium of during bacterial growth. *Aspergillus niger* lowered the pH of the medium upto 1 or even below range of detection by microprocessor pH analyser. After thorough investigation and confirmation of solubilization capacity of tri-calcium phosphate from solid as well as liquid Pikovaskaya's meduim, the above eleven (11) potent strains (Table 2) were selected for further investigation of their efficacy in fields when they are amended with synthetic fertilizers.

Table 3 : Estimation of P_2O_5 released from Pikovaskaya's liquid midium containing 0.5% tricalcium phosphate by phosphate solublizing microorganisms.

Organisum tested	3rd day		6th day		9th day		12th day		15th day	
	% P_2O_5 released	Change in pH	% P_2O_5 released	Change in pH	% P_2O_5 released	Change in pH	% P_2O_5 released	Change in pH	% P_2O_5 released	Change in pH
Aspergillus niger	2.0	5.5	15	3.1	41	2.5	78	1.5	98	2.1
Pseudomonas striata	6.4	3.5	22.9	3.2	44	2.0	53	3.8	66	3.8
Ps. aeruginosa	5.1	5.0	10.4	3.5	28	3.0	42	3.5	58	3.0
Ps. fluorescence	5.0	5.5	9.5	4.0	18	4.0	36	3.5	51	4.0
Candida aquatica	6.6	3.5	10.6	3.0	24	3.5	51	4.0	68	4.8
Cornebacterium sp.	5.0	5.5	10.1	5.0	29	4.2	36	4.0	48	4.5
Alkaligens sp.	5.2	5.0	11.4	4.3	31.6	4.0	42	40	56	4.5
Arthrobacter sp.	4.0	4.0	8.0	4.2	25	4.0	34	5.5	49	5.0
Beijerinkia mobilis	4.0	6.0	9.5	5.5	25.8	4.0	34	4.5	49	5.5
Enterobacter sp.	5.2	6.0	10	5.0	31.0	4.0	42	5.5	57	5.5
Erwinia sp.	6.0	5.5	11.5	5.0	29.0	5.5	38.2	4.0	52	4.8

The acidic soils of Manipur contain eficient indigenous phosphate solubilizing microorganisms which could be used as biofertilizer for sustainable crop production.

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