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STUDIES ON PHOSPHATE SOLUBILISING BACTERIA FROM SAKHARTAR CREEK, RATNAGIRI

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ABSTRACT

Many microorganisms by virtue of their phosphate solubilising activity, can increase the fertility of soils. Phosphate solubilising bacteria were isolated from rhizospheric soil and water of mangroves of Sakhartar creek, Ratnagiri. Large number of phosphate solubilising bacteria were isolated on Pikovaskaya's agar medium and most showed very good activity as seen from the zones of clearance on agar medium. The isolates were selected for further studies. Some of the isolates showed ability to fix atmospheric nitrogen and production of indole acetic acid (IAA). The isolates thus have a good potential as plant growth promoting bacteria.

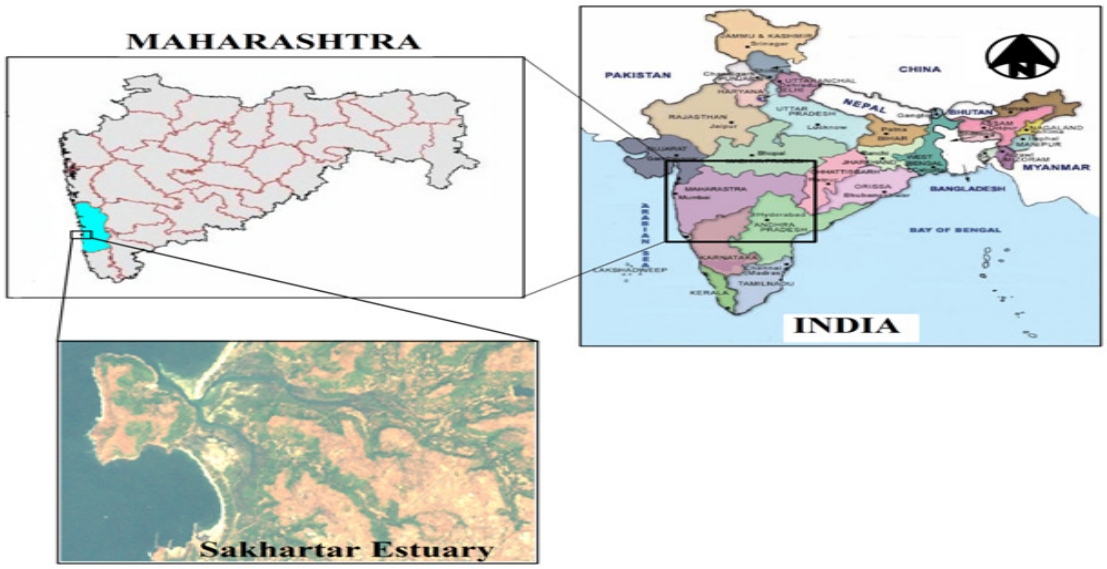
INTRODUCTION

Phosphorus (P) deficiency is a major constraint for crop production. Plants absorb inorganic form of P which acts as an essential element for plant growth and development. The level of inorganic P is very low in the soil and the available P is in insoluble form. The phenomena of fixation and precipitation of P in soil is generally highly dependent on pH and soil type. Thus, in acid soils, phosphorus is fixed by free oxides and hydroxides of aluminum and iron, while in alkaline soils it is fixed by calciumBacteria, fungi and actinomyces present in the soil convert insoluble P into soluble form for plant growth. Among these, bacteria are most predominant. Phosphate is brought about by a number of mechanisms [1], production of organic acids being one of them.

Bacteria belonging to *Mesorhizobium, Rhizobium, Klebsiella, Acinetobacter, Enterobacter, Erwinia, Achrobacter, Micrococcus, Pseudomonas* and *Bacillus* isolated from different soils have been reported as efficient phosphate solubilizers[2]. The beneficial effects of phosphate solubilizing bacteria on crops have been well documented[3].

MATERIALS AND METHODS

Sampling site: The coastal belt of Ratnagiri has number of invaginations in the main land which are called the creeks. These being abundant in mangroves form a very rich ecosystem. Sakhartar estuary is located in Ratnagiri district of Maharashtra state on the west coast of India. It extend between 17°03’14.95”N latitude and 73°16’18.93”E longitude to 16°59’36.56”N latitude and 73°16’21.90”E longitude. Wetland comprises intertidal mudflats, mangroves, salt marsh, sand beach, dunes, tidal creeks,etc.It experiences semi-diurnal tides, with two high-two low tides daily.



Sample collection soil and water samples were collected from the mangrove cover of Sakhartar creek of Ratnagiri. Soil samples were collected in sterile polythene bags and water samples were collected in sterile bottles. Collected samples were stored in refrigerator till use.

Isolation and screening of phosphate solubilisers:

Water sample (0.1 ml) was directly plated on nutrient agar (NA) and Pikovaskaya's agar[4] (PA) plates. Pikovskaya's medium containing Tricalcium phosphate (0.5 g Yeast extract, 10 g Dextrose, 5 g $Ca_3(po_4)_2$, 0.5 g $(NH_4)_2SO_4$, 0.20 g KCl, 0.1 g $MgSO_4.7H_2O$, 0.0001 g $MnSO_4.H_2O$, 0.0001 g $FeSO_4.7H_20$, 18 g Agar in 1000 mL distilled water). Soil sample (0.1g) was added to 100ml of sterile saline, kept on shaker for 1hr. The suspension was then allowed to settle. The supernatant was diluted serially upto 10^{-3} and 0.1ml of each dilution was spread plated on NA and PA media plates. All plates were incubated for 24-48 hrs at R.T.

Phosphate solubilising efficiency:

Isolates obtained on PA were purified on NA media plates by quadrant streak method. The purified cultutes were then spot inoculated on PA. Plates were incubated at R.T. and zones of clearance were measured after 24 and 48 hrs incubation. The phosphate solubilising activity was calculated using the formula[5]:

$$\text{Phosphate solubilising efficiency} = \frac{\text{Zone of clearance}}{\text{Zone of growth}} \times 100$$

RESULTS

Phosphate solubilising bacteria were isolated on Pikovaskaya's agar medium (Figure 1). The colonies were colourless to white with clear zones around the colonies. The viable count of the phosphate solubilisers in water samples from site 1, 2 and 3 was 2×10^3, 2.6×10^3 and 1.1×10^3 cfu/ml, respectively, while the total bacterial count for the three sites was 3×10^3, 3.6×10^3 and 2.1×10^3 cfu/ml, as seen on NA plates. The count of phosphate solubilisers from soils samples of site 1, 2, 3 was 8×10^2, 2.8×10^3, and 2.1×10^3cfu/ml, respectively, while the heterotrophic count was 2×10^3, 5×10^3, and 8×10^3 cfu/ml, respectively. The isolates showing clear zones were selected and maintained on Pikovaskaya's agar slants.

Fifteen phosphate solubilising bacteria each were isolated from the site 1 water sample (W1) and soil sample (S1). From site 2 water and soil samples, 25 and 12 phosphate solubilisers were isolated while from site 3 water and soil samples, 21 and 19 isolates were obtained. These isolates were maintained on NA slants and used for further work.

The zones of clearance and growth of selected isolates upon spot inoculation on PA were measured and the phosphate solubilising efficiency was calculated (Table 1-3). Isolates W1-15, S1-2, W2-2, 11, 13, 20, 21, W3-7, 8, 9, 11, S3-14, 15 showed good phosphate solubilising efficiency, with the best being of W2-2 (533).

Of the isolates, 90% of all the isolates showed growth on Ashby's Mannitol agar after 24 hrs of incubation.

DISCUSSION

Mangrove ecosystem is known to be a rich and diverse niche harbouring microorganisms with varied potential. Apart from the medicinal use of mangroves, this ecosystem has agricultural and industrially applicable microbial treasure that needs to be bioprospected.

Growth on NA and PA was close, showing that most of the heterotrophic bacteria, almost 90%, can solubilise phosphate. Similar results have been observed by Venkateswaran & Natarajan[6] and De Souza[7]. 10-15% of the total viable counts have been reportrd by workers to be capable of solubilising inorganic phosphate[6]. The variation in numbers depends on the bacterial abundance and the nature of the samples. Around the islands and coastal areas the number of PSB was higher as compared to those in the sandy beaches and offshore areas. It is probable that the offshore organisms are poor solubilizers of inorganic phosphate because these organisms are generally low in the uptake of carbon and therefore, do not change the pH of the medium drastically.

Almost 53% of the isolates showed efficiency of more than 200. Isolate W2-2 had the highest phosphate solubilising efficiency of 533. Kukreja[5] reported a highest efficiency of 202 among *Pseudomonas* sp.

The undisturbed, nutritionally rich habitat of the mangroves thus houses bacteria of immense potential as seen in the isolates. Apart from the high phosphate solubilising activity, all the isolates were nitrogen fixers. These bacteria are therefore promising candidates as plant growth promoting bacteria. Although several phosphate solubilizing bacteria occur in soil, usually their numbers are not high enough to compete with other bacteria commonly established in the rhizosphere. Thus, the amount of P liberated by them is generally not sufficient for a substantial increase in situ plant growth. Therefore, inoculation of plants by a target microorganism at a much higher concentration than that normally found in soil is necessary to take advantage of the

property of phosphate solubilization for plant yield enhancement[8]. There have been a number of reports on plant growth promotion by bacteria that have the ability to solubilize inorganic and/or organic P from soil after their inoculation in soil or plant seeds. The production by these strains of other metabolites beneficial to the plant, such as phytohormones, etc. The present work is the first report of phosphate solubilisers from Sakhartar creek.

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Table 1. Phosphate solubilising efficiency of the isolates from site 1

Water sample isolates	Phosphate solubilising efficiency (E)	Soil sample isolates	Phosphate solubilising efficiency (E)
W1-1	-	S1-1	200
W1-2	175	S1-2	**333**
W1-3	233	S1-3	200
W1-4	233	S1-4	250
W1-5	166	S1-5	267
W1-6	200	S1-6	233
W1-7	200	S1-7	175
W1-8	200	S1-8	200
W1-9	250	S1-9	250
W1-10	178	S1-10	200
W1-11	275	S1-11	200
W1-12	250	S1-12	-
W1-13	-	S1-13	267
W1-14	300	S1-14	267
W1-15	**467**	S1-15	167
		S1-16	267
		S1-17	150
		S1-18	167
		S1-19	150
		S1-20	200
		S1-21	200
		S1-22	200
		S1-23	233

Table 2. Phosphate solubilising efficiency of the isolates from site 2

Water sample isolates	Phosphate solubilising efficiency (E)	Soil sample isolates	Phosphate solubilising efficiency (E)
W2-1	250	S2-1	200
W2-2	**533**	S2-2	200
W2-3	-	S2-3	300
W2-4	300	S2-4	300
W2-5	367	S2-5	167
W2-6	333	S2-6	300
W2-7	-	S2-7	233
W2-8	275	S2-8	300
W2-9	275	S2-9	167
W2-10	100	S2-10	233
W2-11	**400**	S2-11	300

W2-12	200	S2-12	200
W2-13	**400**		
W2-14	250		
W2-15	367		
W2-16	350		
W2-17	200		
W2-18	250		
W2-19	333		
W2-20	**467**		
W2-21	**500**		
W2-22	167		
W2-23	233		
W2-24	300		
W2-25	200		

Table 3. Phosphate solubilising efficiency of the isolates from site 3

Water sample isolates	Phosphate solubilising efficiency (E)	Soil sample isolates	Phosphate solubilising efficiency (E)
W3-1	200	S3-1	300
W3-2	267	S3-2	200
W3-3	200	S3-3	175
W3-4	150	S3-4	175
W3-5	240	S3-5	300
W3-6	300	S3-6	100
W3-7	**400**	S3-7	200
W3-8	**400**	S3-8	200
W3-9	**467**	S3-9	233
W3-10	350	S3-10	150
W3-11	**433**	S3-11	-
W3-12	267	S3-12	350
W3-13	325	S3-13	367
W3-14	333	S3-14	**400**
W3-15	175	S3-15	**500**
W3-16	267	S3-16	200
W3-17	375	S3-17	200
W3-18	-	S3-18	133
W3-19	325	S3-19	100
		S3-20	250
		S3-21	200