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Short Communication

Variations in hetetrophic and phosphate solubilizing bacteria from Chennai, southeast coast of India

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Estuarine and open coastal biotopes along the Chennai coast were sampled bimonthly for total heterotrophic as well as phosphate solubilizing bacteria (PSB) between January and December 1999. THB was high during months January, September and November. THB population ranged from 6.03 – 8.13 × 10^5 cells ml^{-1} and phosphate solubilizing bacterial population from 1.00–1.3 × 10^3 cells ml^{-1}. *Pseudomonas* and *Bacillus* were found to solubilize more phosphates than others. Further the phosphate solubilizing potential of *Pseudomonas* was confirmed in broth cultures where P solubilization was 1700 μg /ml^{-1} associated with reduction of *p*H.

[**Key words :** Total heterotrophic bacteria, phosphate solubilizing bacteria, Chennai coast, Pulicat lake]

Microorganisms distributed in the marine and brackish environments play an important role in the decomposition of organic matter and mineralization. A few studies reported on the occurrence of phosphate solubilizing microbes in the Indian marine environment[1-5]. Recently De Souza *et al.*[6] reported an extensive study on phosphate solubilizing bacteria around the Indian peninsula. In India 12 million hectare of land is salt affected[7]. If bacteria, with salinity tolerance and phosphate solubilization potential, can be isolated from marine environments they could be used efficiently to help the crop plants growing in saline soils through amelioration. As phosphate solubilization is a complex biochemical phenomenon, an understanding of the bacterial populations capable of P-solubilization is a prerequisite in realizing the multiple roles the native bacteria perform. Total Heterotrophic Bacteria (THB) and the constituent phosphate solubilizing bacteria (PSB) from the Chennai coast were enumerated and the phosphate solubilization potential of PSB was studied *in vitro* with a view to obtain an information on the role of native bacteria in P cycle.

Water samples were collected on alternate months (January-December, 1999) from five different stations

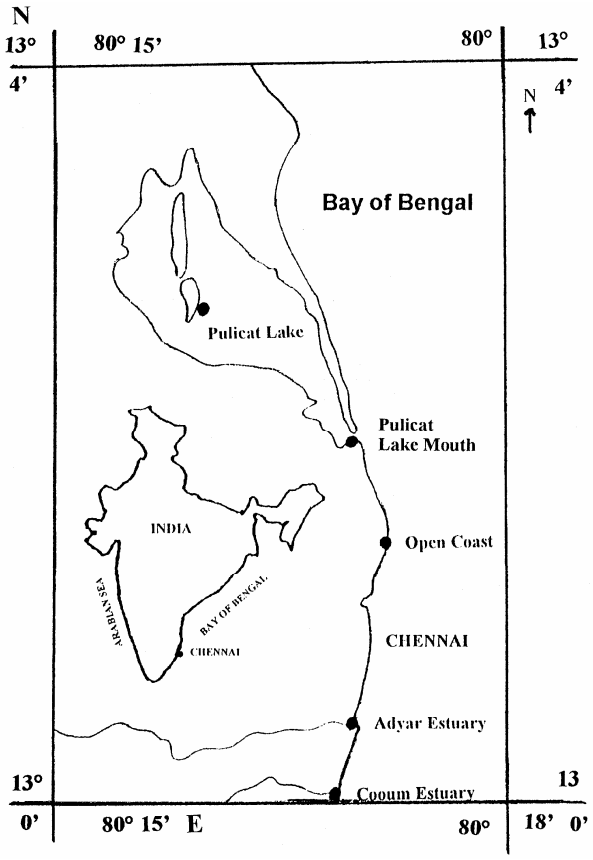

Fig. 1— Sampling stations

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Table 1 — Total heterotrophic bacteria (THB) and phosphate solubilizing bacteria (PSB) at different sampling stations

	Sampling Stations									
	Pulicat lake		Pulicat lake mouth		Cooum Estuary		Adyar Estuary		Open Coast	
Months	THB	PSB	THB	PSB	THB	PSB	THB	PSB	THB	PSB
January	6.46	1.15	6.92	1.07	6.61	1.07	6.92	1.07	6.03	1.02
March	6.61	1.12	6.46	1.05	6.46	1.12	6.46	1.05	6.31	1.05
May	6.92	1.12	6.46	1.00	6.92	1.20	6.92	1.00	6.76	0.96
July	6.76	1.07	6.46	1.05	6.76	1.20	6.92	1.05	6.76	0.98
Sept.	8.13	1.30	7.59	1.23	7.59	1.20	7.59	1.23	7.10	1.02
Nov.	7.94	1.23	7.41	1.12	7.59	1.15	7.41	1.12	6.61	1.07

THB (total heterotrophic bacteria) = No. $\times 10^5$ ml^{-1}
PSB (phosphate solubilizing bacteria) = No. $\times 10^3$ ml^{-1}
Figures are average of six replicates

(Fig. 1) viz. Chennai coast Pulicat Lake (PL), Pulicat Lake mouth (PLM), Cooum estuary (CE), Adyar estuary (AE) and open coast (OC) representing estuarine and marine biotopes. Water sampes collected in sterile McCartney bottles were transported to the laboratory in an icebox immediately for further studies. Serial dilutions were made and one ml aliquots of 10^{-3}-10^{-5} dilutions were transferred to petriplates containing Zobell's Marine Agar 2216(Hi Media, Bombay) for enumerating THB and Zobell's Marine Agar 2216 supplemented with $CaHPO_4$ (5 g/l) for enumeration of phosphate solubilizers. Plating was done in triplicate and incubated at room temperature 28 ± 2°C. After 48 hours, the colony forming units (CFUs) were recorded. The well-developed and morphologically different single colonies were picked out randomly, from those plates with less than 50 colonies, and restreaked on appropriate agar plates for obtaining pure cultures. Bacteria were studied for their morphological and biochemical characteristics following standard techniques and their identification confirmed[8,9]. Phosphate solubilizers were isolated based on the halo zones produced around the colonies[10]. The size of the clear zone around the colonies showing phosphate solubilization was noted. The results were expressed as solubilization efficiency (E)[11]:

$$E = \frac{\text{Solubilization diameter (s)}}{\text{Growth diameter (g)}} \times 100$$

From the isolated strains in this study a *Pseudomonas* strain found to produce a maximum clearing zone in plate assay, was further tested for P-solubilization in broth cultures as described earlier[12]. Single colony was inoculated into 100 ml Pikovskaya's medium[13] (1% glucose, 0.5% $CaHPO_4$, 0.05% NH_4SO_4, 0.05% yeast extract, 0.02% NaCl, 0.02% KCl, 0.01% $MgSO_4$, traces of $MnSO_4$ and $FeSO_4$) and incubated at 28 ± 2° C in a rotary shaker at 200 rpm. All the experiments were conducted in triplicate. For P solubilization, the cultures were harvested on every alternate day, centrifuged at 10000 rpm for 15 minutes and the cell free culture filtrates were subjected for phosphate estimation. From the cell free culture filtrate, 1 ml was used for phosphate estimation by the paramolybdate blue method[14] and the results of three replicate anlayses were presented. *p*H of the culture medium was also recorded simultaneously.

Table 2 — Percentage contribution of different genera of bacteria identified from various locations

	Sampling locations				
Bacteria	PL (70)	PLM (52)	CE (41)	AE (37)	OC (26)
Vibrio	20.2	19.3	31.1	19.5	13.6
Alcaligens	3.5	2.3	ND	4.1	3.8
Bacillus	24	23.7	18.5	20.6	29.8
Pseudomonas	39.2	44.45	41.7	41.1	42.9
Flavobacterium	1.5	2.16	1.2	2.3	ND
Corynebacterium	4.54	3.09	3.3	5.8	4.1
Micrococcus	4.62	3.2	3.2	4.6	4.5
Unidentified	2.44	1.8	1	2	1.3

ND – Not detected
PL - Pulicat Lake, PLM - Pulicat Lake Mouth, CE - Cooum Estuary
AE - Adyar Estuary, OC - Open Coast
Figures in parenthesis are number of strains isolated from each location

Population densities of THB and PSB at different stations during various months are presented in Table 1. THB population in all the stations remained almost between $6.03 - 8.13 \times 10^5$ cells ml^{-1} excepting a very few samples. From 224 isolates selected for identification *Bacillus, Micrococcus, Coryneobacterium, Alcaligens, Pseudomonas, Vibrio,* and *Flavobacterium* were encountered. However, *Alcaligens* from

Table 3—Variations (Range and Annual mean*) of different physicochemical parameters monitored during January-December, 1999

Stations	*p*H	Salinity (‰)	Temp. (°C)
Pulicat lake	7.5-8.2 (7.93)	26-29 (28.2)	25-29 (28.9)
Pulicat lake mouth	7.6-7.9 (7.85)	25-31 (29.6)	25-30 (29.3)
Cooum estuary	7.6-8.1 (7.83)	26-30 (29.3)	26-30 (29.1)
Adyar estuary	7.6-8.2 (8.21)	27-31 (2 8.6)	25-30 (29.2)
Open coast	7.5-7.9 (7.93)	28-32 (29.5)	26-31 (29.2)

*****Figures in parenthesis indicate annual mean

Table 4—Phosphate solubilization index for various bacterial strains on Pikovskaya's agar

Bacteria	Solubilization index
Vibrio sp.	119.44 ± 6.00
Alcaligens sp.	105.56 ± 7.86
Bacillus sp.	184.19 ± 6.43
Pseudomonas *sp.*	205.86 ± 4.11
Corynebacterium *sp.*	117.26 ± 5.52
Micrococcus *sp.*	118.11 ± 10.77

Cooum estuary and *Flavobacterium* from marine environment were not represented. These genera are common in the marine environment and undergo seasonal fluctuation[15-17]. *Pseudomonas* was found to be the predominant genus at all the stations (Table 2) followed by *Bacillus* sp. and *Vibrio* sp. The physicochemical parameters are presented in Table 3. Between different locations, annually, the mean values of *p*H fluctuated between 7.83 and 8.21; Salinity, 28.2 and 29.6 ‰ and temperature, 28.6 and 29.2°C. However, there were little variations in these parameters between the months (Table 3).

The phosphate solubilizers recorded were less in number and were found to fluctuate between 1-1.3 $\times 10^3$ cells ml^{-1}. *Pseudomonas* and *Bacillus* spp. were found to have more phosphate solubilizing capacity than the other genera (*Vibrio*, *Alcaligens* and *Corynebacterium*). On plate assays, *Pseudomonas* showed larger zones of solubilization indicating an efficient solubilization of insoluble and fixed phosphates than the other strains we studied (Table 4) and hence it was selected for further assays. Venkateswaran & Natarajan[1] while studying the Porto Novo waters reported *Pseudomonas* spp. and *Bacillus* spp. as dominant inorganic phosphorus compounds solubilizing microbes. However, the findings of Dhevendaran & Joseph[18] indicated *Vibrio* spp. as a potent strain for maximum solubilisation of tricalcium phosphate than *Pseudomonas* and *Alcaligens.*

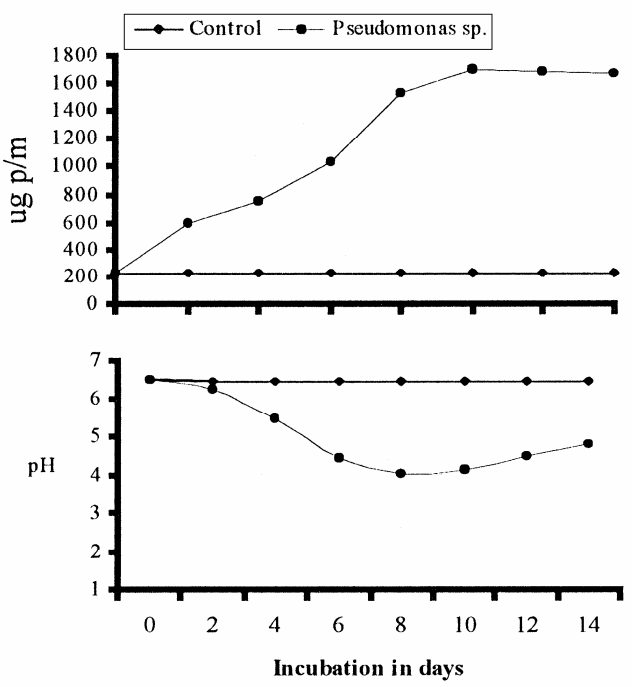

Fig. 2—Solubilization of inorganic phosphate (a) and changes in *p*H (b) in the Pikovskaya medium by a strain of *Pseudomonas* sp. as against non-inoculated control. Values are averages of three replicates.

Broth culture studies were promising in establishing *Pseudomonas* as an important P-solubilizing strain. Although the *p*H of the medium decreased from 6.5 to 4.8 through the growth of bacteria, phosphate solubilization generally increased with prolonged incubation (Fig. 2). Estimates of phosphate solubilization in medium revealed that *Pseudomonas* sp. solubilized phosphates from tricalcium phosphate. It solubilized a maximum of 1700 μg ml^{-1} by day 10 beyond which no further solubilization was seen. This may be due to strong acidic conditions resulting from the metabolic processes. The phosphate concentration in solution increased rapidly after day 6 with a gradual increase initially. The fluctuation in PO_4 concentration and *p*H could be due to initial formation of metabolites and subsequent modification of the same by the bacteria for nutrient use[12,19,20]. *Pseudomonas* has been reported to be a potential PO_4-solubilizing bacterium by various workers[21,22]. This forms the first report to study the *in vitro* PO_4 solubilization by a marine strain of *Pseudomonas.* Production of halo zones on solid agar and efficient release of PO_4 in solution is attributed to the release of organic acids viz. citric, glyoxalic, malic, α ketobutyric, succinic, fumaric, tartaric by various microbes[10]. The *p*H of the media also decreased reaching a minimum after 8 days (*p*H 4.0) and

later recovered slowly. Solubilization of phosphates by *Pseudomonas* spp and various genera examined in this study indicates their potential to participate in the phosphorus cycle in marine waters. Further studies would add new dimensions to their role in any particular area.

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