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Isolation of phosphate solubilizing bacteria from the sediments of Thondi coast, Palk Strait, Southeast coast of India

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ABSTRACT

Sediment samples were collected from different stations of the Thondi coast, Palk Strait, for the isolation of phosphate solubilizing bacteria (PSB) and to estimates the physico-chemical parameters between October 2008 to March 2009. PSB population ranged between 0.80–2.56x10^4 cells g^{-1}. Pseudomonas, Bacillus, Vibrio, Micrococcus, Flavobacterium, Corynebacterium, Alcaligenes and Enterobacter were isolated. Pseudomonas and Bacillus were found to solubilize more phosphates than others. Further phosphate solubilizing activity and solubilization index were also monitored. The phosphate solubilizing potential of Pseudomonas sp was confirmed as a proficient solubilizer than others, where P solubilization was 1670 µg ml^{-1} associated with reduction of pH. These bacteria were found to be highly adaptive and therefore, can significantly contribute to the phosphate economy of the marine environ.

Key words: Phosphate solubilizing bacteria (PSB), phosphate solubilizing activity and solubilization index, *Pseudomonas sp.*

INTRODUCTION

Phosphorous (P) is one of the major essential macro nutrients for plants. However, a greater part of soil phosphorous, approximately 95-99% is present in the form of insoluble phosphates and hence cannot be utilized by the plants [31]. Microorganisms play a direct role by acting as either a sink or a source for phosphates in different niches [7,30]. Moreover phosphate uptake has been found to be dominated by bacteria [19]. Micro organisms are involved in a range of processes that affect the transformation of soil phosphorous and are thus an integral part of the soil P cycle. P-solubilization ability of the microorganisms is considered being one of the most important traits associated with plant P nutrition.

It is generally accepted that the mechanism of mineral phosphate solubilization by PSB strains is associated with the release of low molecular weight organic acids [7,15], which through their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms [9]. However P-solubilization is a complete phenomenon, which depends on many factors such as nutritional, physiological and growth conditions of the culture [27]. There is experimental evidence to support the role of organic acids in mineral phosphate solubilization [10].

Therefore, the present investigation was designed to study the PSB isolated from the sediments of the Thondi Coast, Palk Strait, Southeast coast of India and the potential PSBs solubilization index and P solubilization were studied *in vitro.*

MATERIALS AND METHODS

Sediment sampling

Samples were collected from different stations viz. Station-1 (Thondi open sea-I), Station-2 (Under the Jetty), Station-3 (Thondi open sea-II), Station-4 (Beach). Sediment samples were collected by sediment sampler (Peterson crab), it was sterilized with alcohol before sampling at each station. The central portion of the top 2 cm sediment samples was taken out with the help of a sterile spatula. The samples were then transferred to a sterile polythene bag and transported immediately to the laboratory. Then, 10-fold serial dilutions of the sediment samples were prepared, using filtered and sterilized 50% seawater. Water samples collected from representing marine biotopes for only physico-chemical parameters analysis (pH, temperature, salinity and DO).

Bacteriological Methods

The serially diluted samples were plated on Pikovskaya's agar media to isolate the phosphate solubilizing bacteria. The plates were incubated at 28±2 ºC. After 3 days, the colony forming units (CFUs) were recorded. The cultures which showed clear zone formation around their colonies were considered to be the phosphate solubilizing bacteria and selected for further studies. The well-developed and morphologically different single colonies were picked out randomly, from those plates with less than 40 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 [4,11].

Phosphate solubilization efficiency

Bacterial isolates were then employed for phosphate solubilization by streaking them on the Pikovskaya's agar medium [25] and incubated for 7 days at 28±2 ºC. The phosphate solubilization was expressed as positive and negative depending on the halo zone formation. The size of the clear zone around the colonies showing phosphate solubilization was noted. The results were expressed as solubilization efficiency (E) [23].

$$E = \text{solubilization diameter (s)/Growth diameter (g)} \times 100$$

P-solubilization activity by isolated PSB strains

P-solubilization in broth cultures as described by [1,20,26,28]. Single colony was inoculated into 100 ml Pikovskaya's medium (Pikovskaya's, 1948) (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 ^{0}C in rotary shaker at 200 rpm. All the experiments were conducted in triplicate. 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 estimate by the paramolybdate blue method [24] and the results of three replicate analyses were presented. pH of the culture medium was also recorded simultaneously.

Statistical analysis

All the experiments were performed in triplicate and average values with ± SD was reported in tables. Separate statistical analysis (ANOVA) was done for each organism and different sets of experiments.

RESULTS AND DISCUSSION

The physico-chemical parameters of all the stations are presented in table-1. The pH range varied from 7.3 to 8.2, maximum was present in the station-1 followed by 3, 2 and 4. The salinity was ranged between 28 to 36 ‰, the temperature ranged between 25 to 31.5 ^{0}C and the DO was between 2.05 to 6.8 ml l^{-1}. However, little variations observed in these parameters between the months. Population densities of PSB at different stations during various months are tabulated in table-2. PSB population in all the stations remained almost between 0.80 to 2.56 x 10^4 cells g^{-1}. The bacterial population densities of the open sea soil varied between the station. It is generally observed that there was a significant different on the population density. It is found to be higher in the soil samples collected from station-1. Six months collections were employed at all the 4 stations soil samples from the month of October 2008 to March 2009. The results thus throw light on the existence of microbial solubilizing of phosphorous in soils of different stations. Seshadri et al., [29] carried out an investigation on microbial dynamics in the soil samples of Chennai coast reported that there was a significant difference on the population level of PSB in Chennai coast. De Sousza et al., [5] reported that the occurrence of PSB around Indian peninsula, they were recorded also its phosphatase activity. Similar studies were observed earlier in marine sediments from Porto Novo region by (Ayyakkannu and Chandramohan, 1971).

From 118 isolates selected for identification, selected only efficient phosphate solubilizers (table.3), they were *Pseudomonas, Bacillus, Vibrio, Micrococcus, Alcaligenes, Enterobacter, Corynebacterium* and *Flavobacterium*. However *Pseudomonas, Vibrio* and *Bacillus* were repeated genera *Enterobacter* was recorded from station 1 and 3 only. *Alcaligenes* from station 1, 3 and 4 only. *Flavobacterium* was absent in station 3. *Micrococcus* was recorded from all the stations. These genera are common in the marine environment and undergo seasonal fluctuation [12,22]. *Pseudomonas* sp. was found to be the predominant genus at all the four stations followed by *Bacillus* sp., and *Vibrio* sp. Venkateswaran and Natarajan [32] while studying the Porto Novo waters *Pseudomonas* spp., and *Bacillus* spp., as dominat inorganic phosphorous compounds solubilizing microbes. Dhevendran and Joseph [6] indicated *Vibrio* spp., as a potent strain for maximum solubilization of tricalcium phosphate than *Pseudomonas* and *Alcaligenes.*

The phosphate solubilizing efficiency of isolated strains of PSB indicated that all the strains were solubilized inorganic phosphate contents effectively in the Pikovskaya's medium. (Table-4) results shows that *Pseudomonas* sp. was most efficient phosphate solubilizer on Pikovskaya's agar plates with solubilization index 228±6.12 at 7th day incubation. Measurements of SI ranged from 96.24±4.32 to 228.26±6.12. Generally, halo zone increased with increase in colony diameter. Fluctuations in solubilization index were observed during the seventh day observation period. In most of the cases it gradually increased, while in few cases (*Micrococcus, Alcaligenes, Corynebacterium* and *Flavobacterium*) increased initially and later decreased; it was observed that the solubilization index. Similar results have been reported from various niches [9,20,21,26].

In the present study the concentration of phosphorous released into the Pikovskaya's broth medium. The broth culture studies were promising in establishing all the strains as an important P-solubilizing strain; it has been varied from strain to strain. Although the pH of the medium decreased from 6.6 to 4.6 through the growth of bacteria, phosphate solubilization generally increased with prolonged incubation. (Fig.1) Phosphate mineralization in the liquid medium revealed that *Pseudomonas sp.* solubilized phosphates from the medium containing tricalcium phosphate. It solubilized a maximum of 1670 µg ml^{-1} by 10th day of incubation (beyond which no further solubilization was seen); it was the maximum solubilization values in all the strains. This may be due to strong acidic conditions resulting from the metabolic processes. The phosphate concentration in solution increased rapidly after 6-7th day with a gradual increase initially. The fluctuation in phosphate concentration and pH could be due to initial formation of metabolites and subsequent modification of the same by the bacteria for nutrient use [3,13,28,33]. *Pseudomonas* sp. and *Bacillus* sp. has been reported to be a potential phosphate solubilizing bacterium by various workers [14,17].

Table.1. Variations (Range) of different physiochemical parameters monitored during Oct 2008 - Mar 2009

S. No.	Station	pH	Salinity (‰)	Temperature (^{0}C)	DO ml l^{-1}
1.	Station-1	7.3-8.2 (7.73)	29-36 (32.5)	26-31.5 (29.0)	3.25-6.68 (4.92)
2.	Station-2	7.2-7.9 (7.62)	29-34.5 (32.08)	25-30 (27.5)	3.0-4.85 (3.85)
3.	Station-3	7.3-8.1 (7.72)	29-36 (32.33)	26-31.5 (29.0)	3.15-6.59 (4.78)
4.	Station-4	7.1-7.8 (7.33)	28-33 (29.83)	25-31 (28.0)	2.05-2.64 (2.31)

Values in parenthesis indicate mean value

Table.2. Phosphate solubilizing bacteria (PSB) at different sampling stations

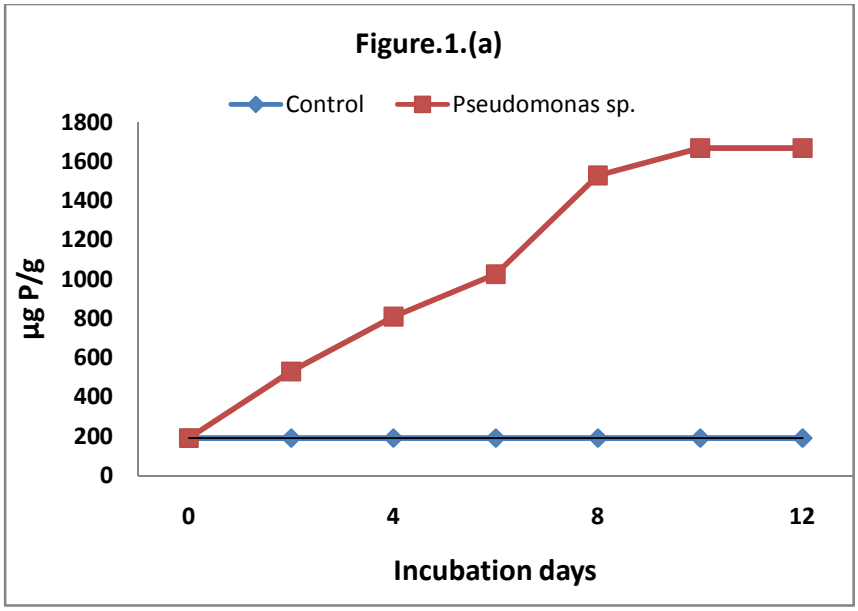
Months	Station-1	Station-2	Station-3	Station-4
October	2.32	2.12	2.25	1.80
November	2.56	2.28	2.40	1.65
December	2.30	2.02	2.23	1.30
January	2.01	1.56	1.98	0.96
February	1.30	1.02	1.15	0.98
March	1.08	0.94	1.00	0.80

No. x 10^4 g^{-1}

Values are average of three replicates

Production of halo zones on solid media and proficient release of phosphate in solution is attributed to the release of organic acids viz. citric, glyoxalic, malic, ketobutyric, succinic,

fumaric, tartaric by various microbes [18]. The pH of the media also decreased reaching a minimum at 8th day and later recovered slowly. It is concluded by the present study that the phosphate solubilizing *Pseudomonas sp.* was the maximum solubilization values and solubilization efficiency in all the strains. Hence these isolates could serve continuously to fertilize a niche by solubilizing insoluble P compounds and this study indicates their potential to participate in the phosphorous cycle in marine environment.



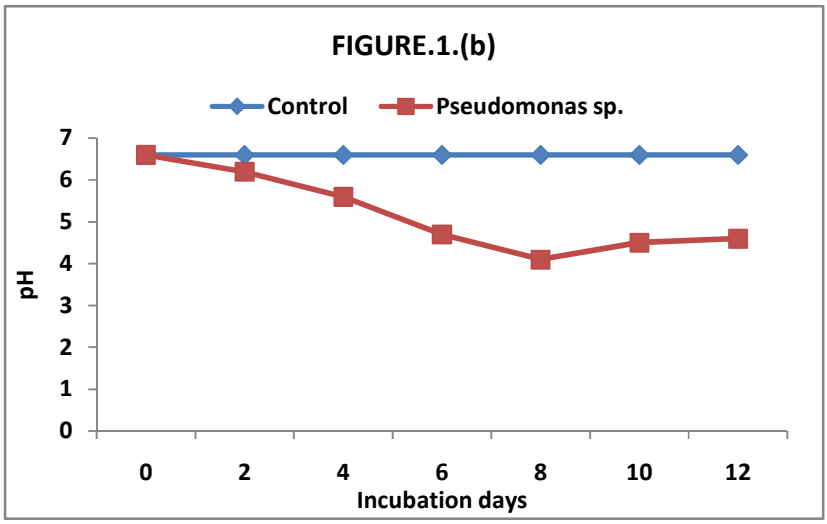


Figure: 1- (a) solubilization of inorganic phosphate and (b) changes in pH in the Pikovskaya's medium by a strain of *Pseudomonas sp.* as against non-inoculated control. Values are average of three replicates.

Table.3. Percentage contribution of different genera of bacteria identified from four stations

S. No.	Bacteria	Station-1 (52)	Station-2 (27)	Station-3 (38)	Station-4 (41)
1.	*Pseudomonas sp.*	38.46	37.04	39.47	36.59
2.	*Bacillus sp.*	21.15	22.22	23.68	21.95
3.	*Vibrio sp.*	15.38	18.52	18.42	17.03
4.	*Flavobacterium sp.*	7.69	7.41	ND	4.88
5.	*Micrococcus sp.*	5.77	3.70	7.89	7.32
6.	*Enterobacter sp.*	1.92	ND	2.63	ND
7.	*Alcaligenes sp.*	3.85	ND	2.63	2.44
8.	*Corynebacterium sp.*	3.85	11.11	5.26	9.76

ND-Not detected

Values in parenthesis are number of strains isolated from each station

Table 4. Phosphate solubilization index (SI) for various bacteria Pikovskaya's agar

S.No.	Bacteria	Solubilization Index (SI)
1.	*Pseudomonas sp.*	228.26±6.12
2.	*Bacillus sp.*	180.35±9.4
3.	*Vibrio sp.*	121.80±3.62
4.	*Enterobacter sp.*	125.10±6.68
5.	*Micrococcus sp.*	102.85±5.02
6.	*Alcaligenes sp.*	105.56±7.86
7.	*Corynebacterium sp.*	103.17±4.50
8.	*Flavobacterium sp.*	96.24±4.32

Values shows the average of triplicates mean±SD

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