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Variations in heterotrophic bacteria and phosphate solubilizing bacteria from Karangadu and Devipattinam coast, Palk Strait, Southeast coast of India

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ABSTRACT

In shallow coastal regions, bacteria are believed to play an important role in the recycling of matter. A study concerning total heterotrophic bacteria and phosphate solubilizing bacterial populations in the water and sediment samples of the Karangadu and Devipattinam, Palk Strait, Southeast coast of India were carried out from July 2008-May 2009. THB and PSB were high in November month. THB was ranged between 5.23-9.75x10^5 cells ml^{-1} and 2.36-11.21x10^5 cells g^{-1} and PSB was fluctuated between 0.98-5.6x10^3 cells ml^{-1} and 1.12-6.84x10^3 cells g^{-1} from water and sediment samples respectively. Among all the PSB isolates, twenty proficient PSB strains were selected for phosphatase activity and P solubilization efficiency under in vitro condition.

Key words: Total heterotrophic bacteria, Phosphate solubilizing bacteria, P solubilization, Phosphatase activity, Palk Strait.

INTRODUCTION

Bacteria are responsible for much of the mineral cycling in marine ecosystems, and act either as source or sink of nutrients. However, bacterial abundance usually varies less than one order of magnitude over the course of a year, a small range when compared with the seasonal fluctuations of phytoplankton [1]. The knowledge of bacterial regulation is essential to understand how the microbial system functions. Both substrate supply and grazing are currently considered to have the greatest potential among the factors that might control bacterial growth and production [2,3]. Although the effects of virus on bacterial mortality could be significant [4,5], bacterial loss due to virus was not estimated in this study. Since the introduction of the microbial loop hypothesis

by Azam *et al.*, [6], the tropho-dynamics between phospho bacteria and heterotrophic have been widely studied.

However, heterotrophic bacterial action promotes organic degradation, decomposition and mineralization processes in sediments and in the overlying water, and releases dissolved organic and inorganic substances [7]. The mineralization of organic matter, which is derived from primary producers, results in its being recycled, so that these substances are again available for primary producers. Heterotrophic microorganisms are the major agents shaping the organic composition of the ocean. These heterotrophic bacteria comprise the bulk of microbial populations inhabiting the water column of oceans and are responsible for much of the biological transformation of organic matter and production of carbon dioxide [8]. Distribution of bacteria depends on changes in water temperature, salinity and other physicochemical parameters [9]. Bacteria also serve as an important source of food for a variety of marine organisms. Thus, bacteria not only maintain the pristine nature of the environment, but also serve as biological mediators through their involvement in the biogeochemical processes.

Phosphorus (P) is one of the major essential macronutrients for plants, which is applied to the soil in the form of phosphaticmanure. However, a large portion of the applied phosphorus is rapidly immobilized, being unavailable to plants [10]. In average, the content of phosphorus of soil is about 0.05% (w/w); however, only 0.1% of them are usable for plants [11]. Saline-alkali soil-based agriculture develops quickly in recent years. Similar to the fertile soil-based agriculture, the intensive culturing of salt-tolerant and even saltresistant plants has dramatically decreased the availability of phosphorus in saline-alkali soil. The free phosphatic ion in soil plays a crucial role; the orthophosphatic ion is the only ion which can be assimilated in an appreciable amount by plants [12]. Soil microorganisms involve in a wide range of biological processes including the transformation of soil phosphorus. They solubilize soil phosphorus for the growth of plants. The growth of phosphate-solubilizing bacteria (PSB) often causes soil acidification, playing a key role in phosphorus solubilization. Therefore, PSB are considered the important solubilizers of insoluble inorganic phosphate. In turn, plants reimburse PSB with carbohydrates. Since the beginning of last century, many PSB have been isolated including, for example, those in *Bacillus*, *Pseudomonas*, *Erwinia*, *Agrobacterium*, *Serratia*, *Flavobacterium*, *Enterobacter*, *Micrococcus*, *Azotobacter*, *Bradyrhizobium*, *Salmonella*, *Alcaligenes*, *Chromobacterium*, *Arthrobacter*, *Streptomyces*, *Thiobacillus*, and *Escherichia* [13]. The objective of this research was to evaluate the distribution pattern of total heterotrophic bacteria and phosphate solubilizing bacteria from Karangadu and Devipattinam marine environment and to develop a better understanding of the mechanism of P solubilization and phosphatase activity by PSB *in vitro* condition.

MATERIALS AND METHODS

Sampling site

Karangadu and Devipattinam non-rhizophere marine ecosystem were selected for this study based on the dominant ecosystems prevalent in these sites. During the on set of Northeast monsoon (October to December) turbulent conditions prevail in Palk Strait. Karangadu (station 1) (Lat 9^0 36'N and Long 78^0 83'E) is one of the important mangrove region in the Palk Strait.

Devipattinam (station 2) (Lat 9^0 28'N and Long 78^0 54'E) is situated 35 km north of station 1 by road. The shallow coast is dominated by a muddy bottom thereby supports luxuriant growth of seagrasses. Predominant seagrasses occurring in this area are *Cymodocea serrulata, Enhalus acroides, Halophila ovalis* and *H. baccarii*. A thick patch of mangrove dominated by *Avicennia marina*. Two sampling points (Karangadu open sea I and II and Devipattinam open sea I and II) were fixed for each station with an interval of 50-100m on the respective ecosystems.

Collection and analysis of samples

Collection of water and sediment were made alternate month for a period of six months from July 2008-May 2009. For bacteriological assessment, water samples were collected in clean polypropylene bottles. Water samples for the estimation of abiotic parameters were also collected for the same sampling points. Surface water temperatures were measured using standard mercury filled centigrade thermometer. Salinity was estimated with the help of a hand refractometer (Model E-2) and pH was measured using a Elico pH meter (Model LC-120). Dissolved oxygen was estimated by the modified Winkler's method as described by Strickland and Parsons [14]. Sediment samples were collected employing an alcohol rinsed and air-dried Petterson grab. The central portion of the collected sample was aseptically transferred into new polyethelene bags using a sterile spatula for bacteriological analysis.

Bacteriological methods

Serial dilutions of the water and sediment samples were prepared, using fittered and sterilized 50% seawater. One ml aliquots of 10^{-3}-10^{-6} dilutions were transferred to petriplates containing Zobell's marine Agar 2216 (HiMedia, India) for enumerating THB and enumeration of phosphate solubilizing bacteria by using Pikovskaya's agar media (HiMedia, India). Plating was done and incubated at room temperature 28±2°C. After 72 hours the colony forming units (CFUs) were recorded. The well-developed and morphologically different single colonies were picked out randomly and restreated on appropriate agar plates for obtaining pure cultures. Bacteria were studied for their morphological and biochemical characteristics following standard techniques and their identification confirmed [15,16]. Phosphate solubilizers were isolated based on the holozones produced around the colonies.

Determination of phosphatase activity in sediments and by PSB

For phosphate solubilization, PSB produce phosphatase enzyme. In an attempt to study the phosphatase activity in response to P enrichment, experiments were done using β-glycerophosphate as a substrate. Culture filtrates were centrifuged and subjected to estimate phosphatase activity following the procedure of Tabatabai and Bremner [17]. The considerable quantities of the sediment samples were air-dried and the phosphatase activity was estimated by a modified method of Kramer and Erdei [18]. The samples were incubated with phenyl disodium orthophosphate in appropriate salinities and phosphatase activity was directly measured by the amount of phenol released.

Estimation of phosphorous contents

The potential of PSB strains was tested invitro by estimating available phosphorous in the Pikovskaya's broth amended with known amount of tricalcium phosphate as sustrate. The Flasks were inoculated with culture broth of cultures at OD 2 (A_{600}). The flasks were incubated at 30 °C for 7 days and centrifuged at 15000 rpm for 15 mins. Phosphorus was determined in supernatant

following the procedure of Natarajan and Buvana [19]. Total phosphates in four stations sediment samples were also estimated adopting the method of Strickland and Parsons [20]. Suitable controls were maintained in all analyses and the results represent the averages of three replicates.

Measurement of p^H and titrable acidity

A change in pH of the medium due to the growth of phosphate solubilizing bacteria was measured with a pH paper after three days of incubation. In order to study the titrable acidity of growth medium, three days old culture filtrates were certrifuged at 1000 rpm for 10 min, 5ml of supernatant was added with 2-3 drops of phenolphthalein indicator and titrated against 0.01N NaOH. The titrable acidity was expressed as mL of 0.01N NaOH consumed per 5 ml of culture filtrate.

RESULTS AND DISCUSSION

The annual mean surface water temperature for station 1 was 30.12 °C with minimum of 27 °C in the month of November 2008. The temperature rose up to 32 °C in May 2009. The annual mean surface water temperatures for stations 2, 3 and 4 were 30 °C, 30 °C and 30.5 °C, the minimum of 26 °C, 25 °C and 27 °C in the month of Nobermber 2008 and the temperature rose upto 31.5 °C, 31.5 °C and 32.5 °C in the month of May 2009 respectively. The mean values of pH fluctuated between 7.6 and 8.2; salinity, 28 and 36.0% and DO, 4.1-7.6 ml L^{-1}. However, there were little variations in these parameters between the months (Table 1).

Population density

The total heterotrophic bacterial loads in all sampling sites were tabulated in Table 2 and 3. The higher density was occurred in sediment than in water samples at all the stations. THB population in all the stations water samples between 5.23-9.75x10^5 CFU ml^{-1}, and in sediment samples remained almost between 2.36-11.21x10^5 CFU g^{-1} expecting a very few samples. The phosphate solubilizing bacteria in water samples were found to be 0.98-5.60x10^3 CFU ml^{-1} and sediment samples were found to fluctuate between 1.12-6.84x10^3 CFU g^{-1}. The higher density of THB and PSB were recorded at station 4 followed by station 2 which are characteriaed by muddy bottom. The distribution and abundance of bacteria in aquatic environments have been reported to be closely related to the physical properties and organic matters in the ecosystem [21,22]. It is generally observed that minimum bacterial counts of the THB and PSB groups were found in summer and maximum counts in monsoon. Similar trends have been recorded in marine environments [23,24,25].

Phosphatase activity

Determination of phosphatase activity by the isolated potential phosphate solubilizers showed that the strain PSB7 was isolated from Sediment samples of station 2 had higher activity (38.84 µmoles/g/h) followed by the strain PSB13 (34.32 µmoles/g/h) isolated from sediment samples of station 4 (Table 3). The phosphatase activity was least in PSB1 isolated from water samples of station 1, followed by PSB10 (isolated from water sample of station 3), PSB3 (isolated from water sample of station 2) and PSB14 (isolated from sediment samples of station 4). However, there was a positive correlation between PSB and phosphatase activity. This might be due to availability of higher amount of phosphorous in the medium and the ability of the PSB strains

[26]. Phosphatase activity of the sediments was recorded from all the sampling stations (Table 4). In general, clayey substrata reveal higher phosphatase activity than sandy mixed substrata. Kobori and Taga [27] have shown that there is an increase in activity from the coastal to the offshore region, but a decrease in activity with depth, which may be due to biotope difference [28]. In general, phosphatase activity at offshore regions and coastal regions sediments showed little variations of values as compared to other locations. It was seen that station 4 showed highter activity followed by station 2, 1 and 3, which may be due to nature of location. Simlar observations have been recorded in marine environment [29,30,31].

Estimation of phosphorous

Phosphorous solubilizing efficiency of isolated strains of PSB indicated that all the strains were solublized phosphate contents effectively in the liquid medium (Table 5). Among the 14 strains, PSB7 was found as the potential in solubilizing phosphate (48.23 ppm mL^{-1}) and PSB13 (44.24 ppm mL^{-1}) was the second second effective solubilizer while PSB14 (20.99 ppm mL^{-1}) was the least solubilizer. Similar results have been reported by many investigators [32,33]. The total phosphate content was higher in station 4 (0.46 mg/g) followed by station 2 (0.38 mg/g). The content of total phosphates was also higher in clayey sediments than sandy sediments. This is substantiated by the fact that where ever the total phosphate content was found to be high, the phosphatase activity was also high. Such correlation has been reported by Alexander [34] and Ayyakkannu and Chandramohan [35]. It should be noted here that the sediments containing a large fraction of soil and clay are rich in phosphate. The present investigations confirm this.

The present results observed that there was reduction in pH of the medium but an increase in titrable acidity. This might be due to secretion of organic acids by PSB [36] . The initial pH of the medium was 7.0, the results on the pH of the different strains in culture medium varied between 4.9 and 6.3. The titrable acidity of the PSB strains in medium was fluctuated between 2.3 and 3.9 ml/0.01N NaOH (Table 5). The control was maintained at 2N. Similar findings were recorded by PSB isolated from rhizophere soils of different field crops [37]. However, the soil conditions and surface layer of waters vary in the amount and type of nutrients, temperature, degree of aeration, available moisture and pH towards the water and soil microorganiars.

Table-1 Variations (Range and Annual mean*) of different physico-chemical parameters monitored during July 2008-May 2009

Station	Temperatute (°C)	pH	Salinity (‰)	DO (ml L^{-1})
Station 1	27 – 32 (30.12)	7.6 – 8.0 (7.8)	29 – 33.5 (30.5)	4.1 – 5.98 (4.95)
Station 2	26.5–31.5 (30)	7.6 – 8.2 (7.9)	28 – 35 (33)	4.4 – 7.1 (5.8)
Station 3	26 – 31.5 (30)	7.6 – 8.2 (7.93)	28 – 34 (31.5)	3.52 – 5.65 (4.82)
Station 4	27– 32.5 (30.5)	7.8 – 8.2 (8.1)	28.5 – 36 (34.25)	4.8 – 7.6 (6.2)

**Figures in parenthesis indicates annual mean*

In conclusion, the THB and phosphate solubilizing bacteria are widely distributed in different niches with the coastal area having a high density. Phosphatase production and solubilization of phosphate are linked. It is evident from the present investigation in that phophatase and phosphate solubilizing bacteria may play a major role in increasing the phosphate concentration and consequently the buffering capacities of sediment microorganisms as well as water microbes. Further studies are now in progress relating to the nature of production of plant growth

due to biosynthesis of plant growth substances rather than their action in releasing available phosphorous. Hence these isolates could serve continuously to fertilize a niche by solubilizing insoluble phosphorous compounds especially in environments where a low concentration of phosphorous causes various limitations.

Table-2 THB and PSB in water samples at different samplig stations

Months	Station 1		Station 2		Station 3		Station 4	
	THB	PSB	THB	PSB	THB	PSB	THB	PSB
July 2008	6.76	3.3	6.91	3.0	7.53	3.07	6.83	3.14
September	8.25	3.12	7.8	3.05	7.85	2.98	8.44	3.03
November	9.56	4.12	9.42	4.83	9.32	5.21	9.75	5.6
January'09	7.8	3.07	7.56	3.12	7.16	3.12	7.9	3.35
March	6.62	3.08	6.46	3.17	6.31	3.2	6.03	3.1
May	6.08	1.3	6.12	1.38	6.0	1.96	5.23	0.98

THB = No.x10^5 CFU ml^{-1}, PSB = No.x10^3 CFU ml^{-1}
Figures are average of three replicates

Table-3 THB and PSB in sediment samples at different samplig stations

Months	Station 1		Station 2		Station 3		Station 4	
	THB	PSB	THB	PSB	THB	PSB	THB	PSB
July 2008	9.65	5.1	9.84	5.21	9.72	4.82	9.73	5.3
September	7.86	5.32	8.2	5.13	8.86	5.3	8.25	5.89
November	10.76	5.18	11.21	6.02	10.31	6.76	10.83	6.84
January'09	6.03	4.84	6.46	4.63	7.1	5.1	7.76	5.3
March	5.13	3.9	4.81	3.81	5.26	4.1	5.2	3.63
May	4.25	1.12	4.68	2.08	2.36	2.5	2.49	2.18

THB = No.x10^5 CFU g^{-1}, PSB = No.x10^3 CFU g^{-1}
Figures are average of three replicates

Table-4 Phosphatase activity in four marine stations in relation to total phosphate

Station	Phosphatase activity (µmoles/g/h)	Total phosphate (mg/g)
Station 1	10.61±0.89	0.19±0.008
Station 2	11.92±0.91	0.38±0.013
Station 3	9.28±0.84	0.24±0.012
Station 4	12.8±1.02	0.46±0.016

Values are average of six replicates

Table-5 *In vitro* phosphorous solubilizing capacity and phosphatase activity of PSB strains

Strains	Phosphatase activity (µmoles/g/h)	Available P (ppm mL^{-1} of cultures filtrate)	pH of the culture medium[(a)]	Titrable acidity of the medium[(b)]
PSB1	14.52	24.43	6.0	3.1
PSB2	20.8	34.41	5.9	2.6
PSB3	16.5	26.71	6.2	2.6
PSB4	34.42	44.24	6.0	2.5
PSB5	25.22	36.56	5.9	2.9
PSB6	20.61	31.26	6.1	3.0
PSB7	38.84	48.23	6.3	3.8
PSB8	24.52	43.2	6.1	2.9

PSB9	30.16	40.86	5.9	3.3
PSB10	15.84	24.85	4.9	3.9
PSB11	26.72	30.88	5.7	3.6
PSB12	22.5	40.63	5.8	2.8
PSB13	20.98	41.4	5.6	2.7
PSB14	16.82	20.99	5.3	2.3
PSB15	14.28	23.24	5.5	2.5
PSB16	26.31	30.26	4.9	2.5
PSB17	24.12	34.22	5.6	2.7
PSB18	19.31	22.25	5.8	3.1
PSB19	17.7	26.5	5.5	3.3
PSB20	15.5	21.05	5.4	2.7

[(a)]Initial pH of the medium was 7.0, [(b)]Titrable acidity expressed as mL of 0.01N NaOH consumed per 5 ml of culture filtrate, control was 2N.

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