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Das, Surajit; Lyla, P.S.; Ajmal Khan, S.
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Biogeochemical processes in the continental slope of Bay of Bengal: I. Bacterial solubilization of inorganic phosphate

Surajit Das*, P.S. Lyla & S. Ajmal Khan

Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai- 608 502, Tamil Nadu, India. *surajit@myself.com

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Abstract: Microorganisms play a vital role in the biogeochemical cycles of various marine environments, but studies on occurrence and distribution of such bacteria in the marine environment from India are meager. We studied the phosphate solubilizing property of bacteria from the deep sea sediment of Bay of Bengal, India, to understand their role in phosphorous cycle (and thereby the benthic productivity of the deep sea environment). Sediment samples were obtained from 33 stations between 10°36' N – 20°01' N and 79°59' E – 87°30' E along 11 transects at 3 different depths i.e. ca. 200 m, 500 m, 1000 m in each transect. Total heterotrophic bacterial (THB) counts ranged from 0.42 to 37.38x10⁴ CFU g⁻¹ dry sediment weight. Of the isolates tested, 7.57% showed the phosphate solubilizing property. The phosphate solubilizing bacterial genera were *Pseudomonas, Bacillus, Vibrio, Alcaligenes, Micrococcus, Corynebacterium* and *Flavobacterium*. These strains are good solubilizers of phosphates which ultimately may play a major role in the biogeochemical cycle and the benthic productivity of the Exclusive Economic Zone (EEZ) of Bay of Bengal, because this enzyme is important for the slow, but steady regeneration of phosphate and organic carbon in the deep sea. Rev. Biol. Trop. 55 (1): 1-9. Epub 2007 March 31

Key words: phosphorous, biogeochemical cycle, microbial loop, THB, phosphate solubilizing bacteria, Bay of Bengal.

Microorganisms play a vital role in the biogeochemical cycles of various marine environments. The microorganisms mediate between the dissolved, particulate and gaseous states of chemical compounds, thereby regulating the distribution of organic and inorganic nutrients throughout the ocean. The microorganisms are efficient in this as they penetrate the water and sediment most intimately by their high number of individuals (Hoppe and Gocke 1993). Microorganisms also possess a variety of catabolic enzymes which allow them to decompose various dissolved and particulate substrates by extracellular enzymes (Meyer-Reil 1994). Dissolved nutrients found in the aquatic environment make life possible since aquatic organisms are to directly or indirectly obtain most of their required nutritional elements from their habitats.

Further, the availability of organic carbon and inorganic nutrients in water limits the biological productivity of the aquatic environment.

Of the essential elements involved in the biogeochemical cycle of the marine environment, phosphorous and nitrogen are most often considered as the potential limiting factors. Most of the phosphate and nitrogen incorporated into the aquatic plants, animals and sediments are usually generated by bacterial action. As a result of this, dissolved and dead organic matter unusable by other organisms is subjected to microbial decomposition. Hence, the 'microbial loop' is important for recycling of phosphate and nitrogen in the water and sediment (Azam *et al.* 1983).

In the marine environment various processes are responsible for recycling of phosphorous.

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Large quantities of the element phosphorous are immobilized in living organisms and locked up in the sediments as insoluble inorganic and organic phosphorous compounds. This form of phosphorous being held in the sediments for long time, remains excluded from recycling. The phosphate solubilizing bacteria (PSB) are capable of dissolving this insoluble inorganic phosphorous into soluble inorganic phosphorous through the organic acid they produce which bind calcium, iron and aluminium into organic mineral compounds, thereby liberating the precipitated phosphate like calcium and apatite. Thus, phosphorous cycle interacts with the carbon cycle (Thingstad and Rassoulzadegan 1995), which may be important for C-limited bacteria in the deep sea (Hoppe and Ullrich 1999).

Studies on occurrence and distribution of such bacteria in the marine environment from India are meager. Ayyakkannu and Chandramohan (1970, 1971) and Venkateswaran and Natarajan (1983) from Porto Novo coastal environment; Promod and Dhevendaran (1987) from Cochin backwater; Kannapiran (1997) from Gulf of Mannar; De Souza et al. (2000) around Indian peninsula and Seshadri et al. (2002) from Chennai, southeast coast of India reported the phosphate solubilizing bacteria. But none of the study was made from the deeper waters. In this backdrop, the present study was undertaken on phosphate solubilizing bacteria (PSB) to understand their role in the phosphorous cycle and thereof the benthic productivity in the continental slope environment.

MATERIAL AND METHODS

The sediment samples were collected from the continental slope of Bay of Bengal using Smith McIntyre Grab (0.2 m² mouth area) during Cruise No. 225 of Fishery and Oceanography Research Vessel Sagar Sampada in 2004 from latitude 10°36' N to 20°01' N and longitude 79°59'E to 87°30'E, covering 33 stations from 11 transects (Table 1). The physicochemical parameters of the water viz. salinity,

dissolved oxygen and pressure were determined by the on-board Sea-Bird's *911 plus* CTD unit. Besides, the sediment parameters- water content, pH, temperature, total organic carbon (El Wakeel and Riley 1956), texture (Krumbein and Pettijohn 1938) and porosity (Danovaro *et al.* 1999) were also determined.

The sediment samples were analyzed immediately on board onto Zobell's Marine Agar 2216e medium plate (Peptone: 5.0g; Yeast extract: 1.0g; Ferric citrate: 0.1g; K₂HPO₄: 0.5g; pH: 7.5±0.3; Agar: 15.0g; Sea water: 1000 ml) by spread plate method. The plates were then incubated on board at 27°C and after 3 days colony forming units were counted and expressed as CFUg-1 dry sediment weight. The representative colonies were picked up and further purified by successive restreaking. The strains were stored in Zobell's marine agar slants at 4°C. The bacterial isolates were then screened for phosphate solubilizing activity. Macro colonies of the isolates were developed onto Zobell's Marine Agar 2216e supplemented with CaHPO₄ (5g/l) by inoculating 3µl of overnight nutrient broth culture and incubated for three days at room temperature. The Phosphate solubilization was expressed as negative and positive depending on the halo zone formation around the macro colonies. The cultures which showed halo zone formation around them were considered to be the phosphate solubilizers. The size of the clear zone around the colonies showing phosphate solubilization was measured. The results were expressed as solubilization efficiency as described by Seshadri et al. (2002).

$$E=\frac{Solubilization\ diameter}{Growth\ diameter}\ X\ 100$$

Standard bacteriological procedures were carried out to identify the isolates up to generic level following the scheme given by Baumann *et al.* (1972), Oliver (1982) and Holt *et al.* (1994).

Simple correlation coefficient was calculated between the physico-chemical parameters of the water and sediment with the total

TABLE 1 Location, water depth, organic carbon and texture of sediments for the stations

Transects	St. No	Latitude (N)	Longitude (E)	Depth (m)	Total Organic Carbon (mg/g)	Texture of the sediment samples
	S48	10°34'96"	80°26'70"	248	2.5	Sandy clay loam
Karaikkal	S49	10°36'64"	80°30'67"	521	2.3	Sandy clay loam
	S50	10°36'16"	80°36'23"	950	4.5	Clay
	S51	11°31'82"	79°59'01"	214	2.6	Sandy clay
Cuddalore	S52	11°31'44"	80°02'16"	512	4.5	Clay
	S53	11°32'03"	80°07'55"	912	3.9	Silty clay
	S54	12°18'81"	80°29'35"	216	3.0	Sandy loam
Cheyyur	S55	12°21'68"	80°33'49"	490	3.5	Silty clay
	S56	12°21'34"	80°36'10"	937	4.0	Silty clay
	S57	13°09'78"	80°36'66"	225	4.3	Silty clay
Chennai	S58	13°09'95"	80°41'99"	516	3.5	Silty clay
	S59	13°10'21"	80°43'18"	970	3.8	Sandy clay
	S60	14°10'65"	80°24'97''	228	4.5	Clay
Tammenapatanam	S61	14°10'99"	80°26'37"	477	5.5	Clay
	S62	14°10'23"	80°27'04"	1000	4.9	Silty clay
	S63	15°15'19"	80°31'68"	240	4.6	Clay
Singarayakonda	S64	15°15'32"	80°33'47"	507	4.8	Clay
	S65	15°16'33"	80°35'82"	960	5.5	Clay
	S66	16°00'09"	81°29'57"	223	4.9	Clay
Divipoint	S67	16°04'70"	81°49'67''	502	5.5	Clay
	S68	16°00'67"	82°03'29"	917	4.9	Silty clay
	S69	17°00'16"	83°01'86"	220	5.5	Clay
Kakinada	S70	17°00'07"	83°12'70"	500	4.8	Clay
	S71	17°01'45"	83°21'18"	901	5.3	Clay
	S72	18°01'49"	84°15'62"	222	4.7	Clay
Bhemuli	S73	18°01'93"	84°16'26''	550	5.3	Clay
	S74	18°01'13"	84°20'28''	850	5.0	Clay
	S75	19°03'37"	85°25'81"	260	4.8	Clay
Barua	S76	19°06'37"	85°32'55"	500	4.8	Clay
	S77	19°05'63"	85°39'03"	994	4.0	Clay
	S81	20°05'29"	87°11'14"	273	5.2	Clay
Paradweep	S82	20°04'32"	87°12'19"	503	4.8	Clay
	S83	20°01'04"	87°30'95"	958	4.3	Silty clay

heterotrophic bacterial population and percentage of phosphate solubilizing bacteria.

RESULTS

In the present study, total heterotrophic bacterial (THB) counts ranged from 0.42 to 37.38x10⁴ CFU g⁻¹ dry sediment weight (Fig. 1). A total of 541 isolates obtained from all the stations were screened for the phosphate solubilizing property.

Salinity ranged from 34.73 to 35.02 psu. Higher salinity was observed at the depth of ca. 500 m. Dissolved oxygen was in the range of

0.12 to 2.46 ml/l and was found to be higher at ca. 1000 m depth stations. While the temperature showed a regular trend of decreasing from ca. 200 m to ca. 1000 m stations, the pressure increased. Water content and pH of the sediment were in the range of 44.73-68.54% and 6.6-7.0 respectively. Total organic carbon varied in relation to the texture of the sediment and was in the range of 2.3-5.5 mg/g dry weight of sediment (Table 1). The textural characteristics are presented in Table 1 and the sediments were mostly clayey type.

Among all the bacterial isolates tested, only 41 isolates (7.57%) showed the phosphate solubilizing property (Fig. 2) and the diameter

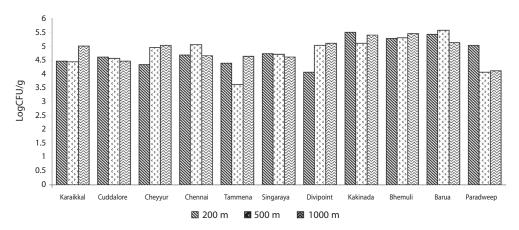


Fig. 1. Bacterial population in different stations located along various transects of Bay of Bengal.

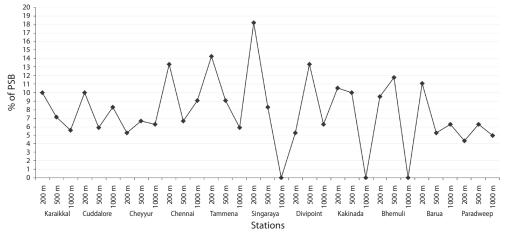


Fig. 2. Percentage contribution of phosphate solubilizing bacteria (PSB) in different stations.

of the halo zone varied from 0.5 cm to 2.4 cm (Table 2). Phosphate solubilization index of bacterial isolates showing halo zone is given in Table 2, which ranged from 113.63 to 184.61. The number of isolates and the solubilization index varied from station to station depending on the depth. The correlation coefficient values of the different physico-chemical parameters with the total heterotrophic bacterial population and the percentage of phosphate solubilizing bacteria are also given in Table 3.

Totally 7 genera, namely *Pseudomonas, Bacillus, Vibrio, Alcaligenes, Micrococcus, Corynebacterium* and *Flavobacterium* were observed as phosphate solubilizing bacteria and the percentage contribution of these genera in PSB are presented in Table 4.

DISCUSSION

PSB and physicochemical parameters

The higher solubilization index was noticed in the bacterial strains isolated from ca. 200 m depth whereas lower solubilization index was found in the strains isolated from ca. 1000 m depth. De Souza et al. (2000) reported the percentage occurrence of phosphate solubilizing bacteria from different niches viz. beaches (8.1%), Islands (16.7%), coasts (15.6%) and offshore (8.7%) and it was found that phosphate solubilizing bacterial population is higher in coastal areas than offshore areas. It is probably that the offshore organisms are poor solubilizers of inorganic phosphate because these organisms are hampered by an insufficient supply of easily degradable organic compounds (Hoppe and Ullrich 1999) and low uptake of carbon (De Souza et al. 2000). Hence, the lower percentage occurrence of phosphate solubilizing bacteria observed in the present study may be due to the location of the stations in the deeper waters. But, in terms of phosphate solubilization efficiency, the isolates showed a good result. The solubilization index in the present study ranged from 113.63 to 184.61 with an average of 143.04, whereas,

from the coastal and offshore waters of India, the index varied from 105.56 to 205.86 with an average of 141.73 (Seshadri *et al.* 2002).

Moreover, the optimum temperature for this enzymatic activity was reported to be 27±2°C (De Souza et al. 2000). That is why the experiment was done at 27°C, eventhough the sediment temperature in the sampling stations varied from 8°C to 17°C and tended to decrease from ca. 200 m to ca. 1000 m depth stations. At the optimum temperature it was believed to found higher solubilization index of PSB which might have lower solubilization property in their native temperature, taking into consideration of the fact that the isolates which had the phosphate solubilization property at lower temperature would show better result in the optimum temperature to ease the enumeration of PSB, and the isolates which showed no activity at the optimum temperature would not be having phosphate solubilization property in their inhabitant temperature also. Hence, it is believed that the influence of the inhabitant sediment parameters render the bacterial isolates to be phosphate solubilizers. Therefore, the lower solubilization index of the bacterial strains isolated from ca. 1000 m depth stations with compare to ca. 200 m and ca. 500 m depth stations may be attributed to the sediment temperature which is again supported by the correlation coefficient values (Table 3).

A significant positive correlation (P<0.01) was observed with the percentage of the phosphate solubilizers and the sediment temperature and a significant negative correlation was observed with the station depth and (P<0.01) the bottom water pressure (P<0.02) (Table 3). Although, significant positive correlation was observed with the total heterotrophic bacteria and the total organic carbon (P<0.05), clay fraction (P<0.01), porosity (P<0.05) and water content (P<0.05) of the sediment, the PSB% showed no significance with these parameters. Benny and Kurup (1991) also reported that the extracellular enzyme (L-asparaginase) producing bacteria decreased with the increase of organic carbon in the estuarine sediment, but in contrast, Avyakkannu and Chandramohan

TABLE 2
Phosphate solubilizing bacterial distribution at different depths in Bay of Bengal

Isolate Nos.	Genera	Station Depth	Area	Diameter of zone (mm)	Solubilization index
DSB 225.005	Vibrio	214 m (11°31 N-79°59 E)	Off Cuddalore	1.8	128.57
DSB 225.016	Pseudomonas	214 m (11°31 N-79°59 E)	Off Cuddalore	2.5	125.00
DSB 225.027	Bacillus	248 m (10°34 N-80°26 E)	Off Karaikkal	1.7	170.00
DSB 225.036	Micrococcus	248 m (10°34 N-80°26 E)	Off Karaikkal	2.1	123.52
DSB 225.053	Vibrio	521 m (10°36 N-80°30 E)	Off Karaikkal	1.5	115.38
DSB 225.070	Alcaligenes	512 m (11°31 N-80°02 E)	Off Cuddalore	2.3	119.52
DSB 225.080	Pseudomonas	912 m (11°32 N-80°07 E)	Off Cuddalore	1.5	115.63
DSB 225.085	Corynebacterium	216 m (12°18 N-80°29 E)	Off Cheyyur	1.1	137.50
DSB 225.108	Pseudomonas	490 m (12°21 N-80°33 E)	Off Cheyyur	0.5	119.04
DSB 225.125	Alcaligenes	937 m (12°21 N-80°36 E)	Off Cheyyur	1.1	114.58
DSB 225.143	Flavobacterium	1000 m (14°10 N-80°27 E)	Off Tammenapatanam	1.5	115.38
DSB 225.156	Vibrio	225 m (13°09 N-80°36 E)	Off Chennai	0.8	160.00
DSB 225.160	Bacillus	225 m (13°09 N-80°36 E)	Off Chennai	1.8	150.00
DSB 225.172	Pseudomonas	516 m (13°09 N-80°41 E)	Off Chennai	2.0	125.00
DSB 225.190	Alcaligenes	228 m (14°10 N-80°24 E)	Off Tammenapatanam	1.3	173.33
DSB 225.193	Pseudomonas	228 m (14°10 N-80°24 E)	Off Tammenapatanam	2.1	140.00
DSB 225.198	Pseudomonas	937 m (12°21 N-80°36 E)	Off Chennai	0.5	113.63
DSB 225.211	Vibrio	477 m (14°10 N-80°26 E)	Off Tammenapatanam	0.8	133.33
DSB 225.220	Pseudomonas	273 m (20°05 N-87°11 E)	Off Paradweep	0.9	180.00
DSB 225.246	Micrococcus	503 m (20°04 N-87°12 E)	Off Paradweep	1.6	118.51
DSB 225.267	Vibrio	958 m (20°01 N-87°30 E)	Off Paradweep	1.9	135.71
DSB 225.279	Micrococcus	500 m (19°06 N-85°32 E)	Off Barua	2.1	123.52
DSB 225.297	Vibrio	260 m (19°03 N-85°25 E)	Off Barua	2.5	156.26
DSB 225.304	Bacillus	260 m (19°03 N-85°25 E)	Off Barua	0.9	180.00
DSB 225.315	Pseudomonas	994 m (19°05 N-85°39 E)	Off Barua	1.5	115.38
DSB 225.334	Micrococcus	550 m (18°01 N-84°16 E)	Off Bhemuli	2.2	169.23
DSB 225.339	Bacillus	550 m (18°01 N-84°16 E)	Off Bhemuli	1.3	162.50
DSB 225.348	Pseudomonas	222 m (18°01 N-84°15 E)	Off Bhemuli	1.8	180.00
DSB 225.362	Bacillus	222 m (18°01 N-84°15 E)	Off Bhemuli	2.3	153.33
DSB 225.384	Alcaligenes	500 m (17°00 N-83°12 E)	Off Kakinada	1.9	146.15
DSB 225.394	Corynebacterium	500 m (17°00 N-83°12 E)	Off Kakinada	2.1	127.27
DSB 225.398	Micrococcus	220 m (17°00 N-83°01 E)	Off Kakinada	1.3	144.44
DSB 225.403	Corynebacterium	220 m (17°00 N-83°01 E)	Off Kakinada	0.7	175.00
DSB 225.434	Bacillus	223 m (16°00 N-81°29 E)	Off Divipoint	2.4	184.61
DSB 225.458	Corynebacterium	502 m (16°04 N-81°49 E)	Off Divipoint	1.12	140.00
DSB 225.465	Bacillus	520 m (16°04 N-81°49 E)	Off Divipoint	2.0	153.84
DSB 225.468	Bacillus	917 m (16°01 N-82°03 E)	Off Divipoint	1.2	150.00
DSB 225.485	Flavobacterium	240 m (15°15 N-80°31 E)	Off Singarayakonda	2.1	161.53
DSB 225.487	Bacillus	240 m (15°15 N-80°31 E)	Off Singarayakonda	1.2	171.42
DSB 225.494	Pseudomonas	507 m (15°15 N-80°33 E)	Off Singarayakonda	0.7	140.00
DSB 225.531	Alcaligenes	950 m (10°36 N-80°36 E)	Off Karaikkal	1.4	116.66

Correlation co-efficient matrix of the different physico-chemical parameters with the THB and the percentage of phosphate solubilizing bacteria TABLE 3

BWP														1
BWDO													-	0.71
BWS												1	-0.65	-0.23
Clay%											-	0.01	-0.24	0.03
Silt%										1	-0.31	-0.28	0.48	0.52
Sand%									-	-0.47	69:0-	0.20	-0.15	-0.42
Porosity								П	-0.58	0.01	0.62	-0.01	0.08	0.23
TOC							1	0.40	-0.71	90.0	0.72	-0.08	-0.08	0.23
Temp. of sediment						1	-0.24	-0.43	0.45	-0.43	-0.13	0.03	-0.48	-0.80
pH of sediment					1	0.21	-0.30	60.0	0.22	-0.02	-0.22	-0.04	0.18	-0.03
Water				1	0.25	-0.27	0.26	0.78	-0.30	-0.07	0.38	0.02	90.0	0.13
PSB%			н	-0.25	-0.05	0.52^{c}	-0.08	-0.14	0.04	0.04	-0.08	0.001	-0.23	-0.48b
THB		_	-0.21	0.41^{a}	0.00	-0.13	0.43^{a}	0.43^{a}	-0.30	-0.25	0.53°	0.01	-0.20	0.01
Depth (m)	1	-0.01	-0.54°	0.14	-0.09	-0.85	0.14	0.26	-0.36	0.50	-0.02	-0.18	89.0	0.95
Parameters	Depth (m)	THB	PSB%	Water content	pH of sediment	Temp. of sediment	TOC	Porosity	Sand%	Silt%	Clay%	BWS	BWDO	BWP

*Significant level 'a'-P<0.05; 'b'-P<0.02; 'c'-P<0.01 THB- Total Heterotrophic Bacteria (CFU/g) PSB- Phosphate Solubilizing Bacteria (%) TOC- Total Organic Carbon (mg/g) BWS- Bottom Water salinity (ppt) BWDO- Bottom Water Dissolved Oxygen (ml/l) BWP- Bottom Water Pressure (Bar)

TABLE 4
Percentage contribution by different genera
of phosphate solubilizing bacteria

Genera	Total number	% contribution
Pseudomonas	10	24.39
Bacillus	9	21.95
Vibrio	6	14.63
Alcaligenes	5	12.2
Micrococcus	5	12.2
Corynebacterium	4	9.76
Flavobacterium	2	4.87

(1971) observed a positive correlation of the above with the organic carbon and the clay fraction of the sediment. Phosphatase activities in deeper waters have been measured even less than other hydrolytic activities which are due to the fact that phosphate is generally not the growth limiting factor in the euphotic zone of the sea (Rivkin and Anderson 1997).

Generic composition of PSB

Totally 7 genera, namely Alcaligenes, Pseudomonas, Bacillus, Corvnebacterium, Micrococcus, Flavobacterium and Vibrio were observed as phosphate solubilizing bacteria from different stations, among which Pseudomonas contributed more (24.39%) followed by Bacillus (21.95%) and Vibrio (14.63%). Alcaligenes and Micrococcus contributed equally (12.2%), while Corynebacterium and Flavobacterium contributed 9.76% and 4.87% respectively (Table 4). Pseudomonas was found to be the predominant group which was also reported from the Indian west coast by Promod and Dhevendaran (1987) and De Souza et al. (2000) and from east coast of India by Venkateswaran and Natarajan (1983) and Seshadri et al. (2002). Promod and Dhevendaran (1987) and De Souza et al. (2000) reported *Bacillus* as the dominant group in the inshore areas and encountered Moraxella and Klebsiella respectively, which were absent in the present study. Whereas, Venkateswaran and Natarajan (1983) and Seshadri *et al.* (2002) found *Pseudomonas* and *Bacillus* as the most efficient phosphate solubilizing genera which are chiefly responsible for the mineralization of inorganic phosphorous compounds.

Extracellular phosphatase from phosphate solubilizing bacteria is a key enzyme in the marine environment which plays a prominent role in the recycling of organic P and in the avoidance of P limitation in the sea (Hoppe 2003). Thus, phosphate availability in sediment is greatly enhanced through microbial production of metabolites leading to lowering of pH and release of phosphate from organic and inorganic complexes. The distributions of the phosphate solubilizing bacteria in the continental slope sediment of Bay of Bengal emphasize the benthic productivity of that area in terms of nutrients and biogeochemical cycle.

The phosphate solubilizing bacteria have been reported earlier from the different niches but this study gives additional information on the phosphate solubilizing properties of bacteria isolated from the continental slope sediment of Bay of Bengal. It is evident from the present investigation that the bacterial strains isolated from the deep sea are good solubilizers of phosphates which ultimately may play a major role in the biogeochemical cycle and the benthic productivity of the Exclusive Economic Zone (EEZ) of Bay of Bengal, because this enzyme is important for the slow, but steady regeneration of phosphate and organic carbon in the deep sea.

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RESUMEN

Estudiamos la capacidad que tienen las bacterias de sedimentos profundos en la Bahía de Bengala, India, de disolver los fosfatos que juegan un papel clave en los ciclos biogeoquímicos del mar. Recolectamos muestras en 33 estaciones ubicadas entre 10°36'N – 20°01'N y 79°59'E – 87°30'E en once transectos y tres profundidades, i.e. ca. 200 m, 500 m, 1000 m. Los conteos totales de bacterias heterotróficas fueron de 0.42 a 37.38x10⁴ *CFU* g¹ (peso seco) de sedimento. De las cepas evaluadas, un 7.57% disuelven fosfato. Los géneros con esta características fueron *Pseudomonas, Bacillus, Vibrio, Alcaligenes, Micrococcus, Corynebacterium y Flavobacterium.*

Palabras clave: fósforo, ciclo biogeoquímico, ciclo microbiano, bacterias que disuelven fosfato, Bahía de Bengala.

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