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STUDIES ON PHOSPHOBACTERIA IN COCHIN BACKWATER

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ABSTRACT

The maximum total heterotrophic bacteria (THB-2.92 × 10^4 CFU/ml) and phosphobacteria (PB-1.69 × 10^4 CFU/ml) in water and in sediment (THB-3.57 × 10^4 CFU/g; PB-2.42 × 10^4 CFU/g) were recorded respectively during July 1985. Similarly higher concentrations of adsorbed phosphate in sediment (43 μg PO_4-P/g) and soluble phosphate in water (16μg PO_4-P/1) were noticed during the same month. The phosphate content positively influenced the distribution of both THB and PB and inversely related to the salinity and the minimum salinity (20‰) was observed during July 1985. 188 strains of phosphobacteria were identified to the generic level (*Pseudomonas, Vibrio, Moraxella, Bacillus, Micrococcus* and *Corynebacterium*). Of these *Pseudomonas* predominated both in sediment (45%) and in water (34.8%). Since the phosphate is essential for the productivity of phytoplankters, two potent strains such as *Pseudomonas* and *Vibrio* were selected for the study of the influence of incubation period, carbon sources and the temperature for the maximum release of phosphate from the tricalcium-phosphate and for their optimum growth. Both the isolates released maximum phosphate within 72 hrs. and 0.1% of glucose enhanced the maximum release of phosphate. *Pseudomonas* solubilized maximum phosphate and obtained optimum growth at 37°C whereas in *Vibrio* they were at 29°C. It is understood that bacteria could also be utilised for the leaching of the low grade ore samples and of the three types of ore samples used *Pseudomonas* leached out 0.700 mg/g of phosphate from the sedimentary ore. These observations suggest that the microorganisms play an important role in the metabolism of phosphorus and the removable of impurities from the low grade ore samples.

INTRODUCTION

IT IS well known that the majority of the phosphates in sediments are present as insoluble organic and inorganic phosphates. Studies on phosphate concentration are of primary importance in assessing the productivity of natural waters. Pomeroy *et al.* (1965) indicated the nature of phosphate exchange between the sediments and water, suggesting that the sediments act as a buffer on the phosphate concentration in the overlying water. Microbes may be partly responsible for a number of transformations of phosphorus in soils, waters and sediments and the phosphate ions are reported to be strongly adsorbed by soil with a high content of silt and clay. The solubilization of phosphorus compounds may also be brought about by acids and enzymes of microbial origin (Alexander, 1961 ; Skujins, 1967). The occurrence and distribution of the special group of bacteria which are capable of dissolving insoluble phosphorus compounds in the marine sediments and overlying water have been reported (Ayyakkannu and Chandramohan, 1971 ; Dhevendaran *et al.*, 1974 ; Venkateswaran, 1981). Investigations of decomposition of plankton and regeneration of nutrients particularly phosphorus showed that bacterial population played an indirect role in the regeneration of phosphate in aquatic

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ecosystem (Rajendran and Venugopalan, 1974). Recently Dhevendaran and Valsa Joseph (unpublished) also observed the phosphobacteria for the first time in the primary film. It thus seems probable that the phosphobacteria in the marine sediments may be partly responsible for the phosphate content and regulation of it both in sediment and overlying water. The present study aims at the distribution and cultural conditions of phosphobacteria in Cochin Backwater (S. India).

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MATERIALS AND METHODS

Samples were collected every month from February-September 1985 in the Vembanad Lake (Fig. 1). Overlying water samples at 6 m depth were collected with the help of all sterile glass water sampler and the samples were transferred immediately to sterile McCortney bottles. Sediment samples were collected with the aid of a Peterson grab. The central portion of the grab samples were aseptically transferred to sterile polythene bags. All the samples were then carried in an ice box to the laboratory for further analysis.

The atmospheric and the water temperatures were recorded using Celsius thermometer. The salinity and reactive phosphate of the overlying water and adsorbed phosphate of the sediment were determined following the methods of Strickland and Parsons (1968) and Murphy and Riley (1962) respectively. Enumeration of total viable bacterial population and phosphobacteria were carried out following the method adopted by Dhevendran *et al.* (1974). Identification of pure cultures upto generic level was also undertaken (Simidu and Aiso, 1962).

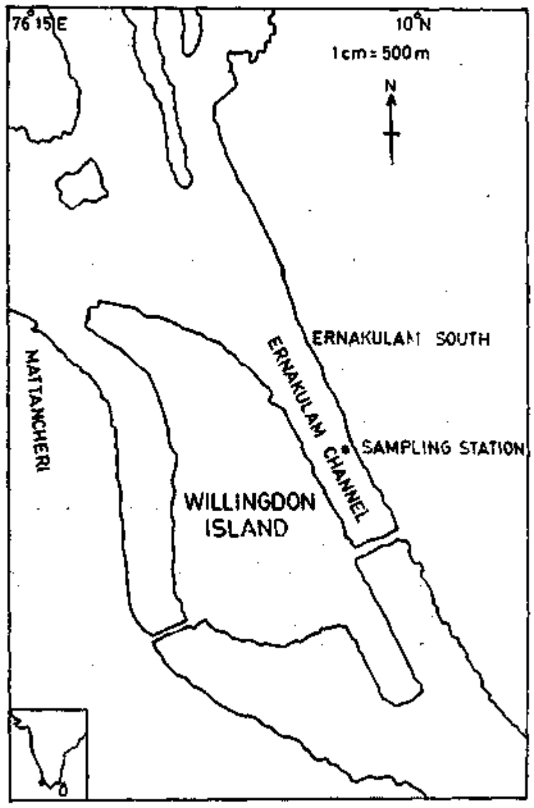

FIG. 1. Sampling station in Cochin Backwater.

RESULTS AND DISCUSSION

Results of the present study on the distribution of total heterotrophic bacterial population (THB), phosphobacteria (PB), salinity, soluble phosphate in the water and the adsorbed phosphate in the sediment during the period from February to September '85 are presented in Figs. 2 & 3. The THB varied from 0.16×10^4 to 2.92×10^4 CFU/ml (Colony Forming Unit), and 0.4×10^4 to 3.5×10^4 CFU/g, in the overlying water and sediment respectively. High counts of THB were seen during February (pre-monsoon) and secondary peak during June (Monsoon) both in water and sediment. Similar pattern of distribution of THB was exhibited on the East Coast (Venkateswaran, 1981). PB varied from 0.02×10^4/to 1.69×10^4 CFU/ml and from 0.10×10^4 to 2.42×10^4 CFU/g, in the water and sediment respectively. PB counts were high during pre-monsoon and a secondary peak during the monsoon season thereby confirming the earlier observation made by Venkateswaran (1981). The soluble phosphate concentration ranged from 9 to 16 μg PO_4-P/l and from 30 to 43 μg PO_4-P/l in water and sediment respectively. The primary peak during the pre-monsoon and secondary peak during the monsoon seasons both in water and sediment were observed. Venkateswaran (1981) recorded the particulate phosphate in the water ranging from 8.54 to 43.68 μg PO_4-P/l and Rajendran and Venugopalan (1973) observed phosphate as much as 20.75 μg PO^9 P/l as adsorbed phosphate in the estuarine sediment. Jitts (1959) has shown that the

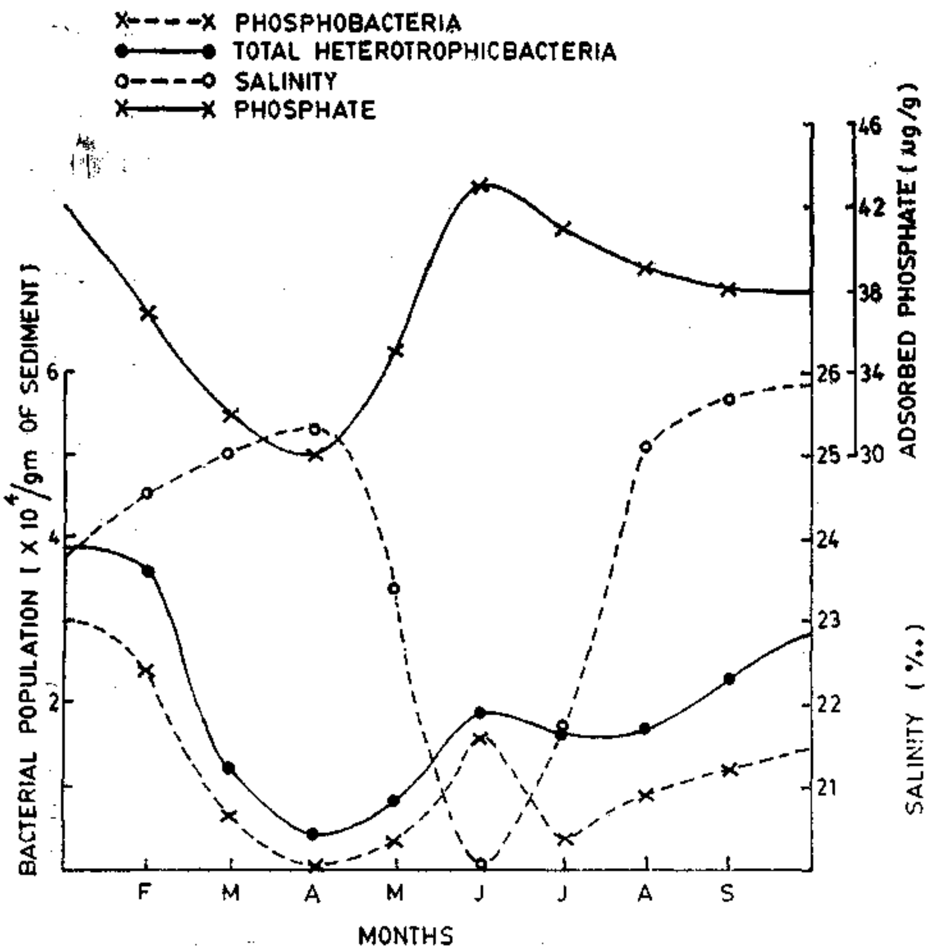

FIG. 2. Seasonal variations in the distribution of total heterotrophic bacteria and phosphobacteria in sediment in relation to environmental factors.

phosphate in solution may be adsorbed to the estuarine silt even upto 90%. Sudden changes in salinity due to tidal flushing seem to influence the concentration of adsorbed and interstitial phosphate in the mud (Rajendran and Venugopalan, 1973). The salinity of the overlying water ranged from 20.0 to 25.70‰ and it showed two peaks. *viz.* one during the pre-monsoon and the another during the post-monsoon seasons, but the minimum salinity was noticed during the monsoon season when there was heavy rainfall. Similar findings have already been reported by Qasim and Gopinathan (1969)

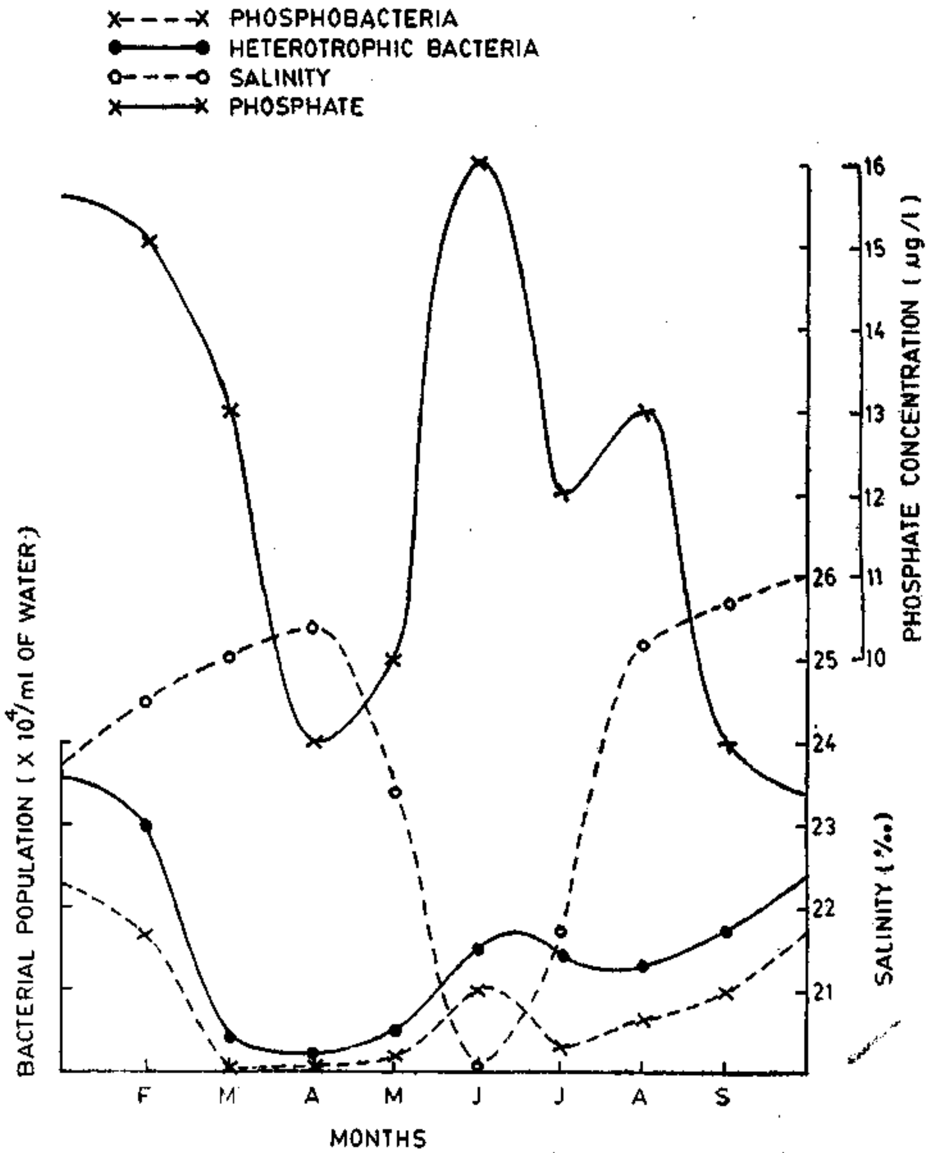

FIG. 3. Seasonal variations in the distribution of toal heterotrophic bacteria and phosphobacteria in water in relation to environmental factors.

from Cochin Backwater and by Dhevendran (1978) and Venkateswaran (1981) in Vellar Estuary.

A positive correlation was established between THB and PB in water (r = 0.95) and also in sediment (r = 0.93). Similarly the same pattern of correlation was observed between phosphorus content and PB (r = 0.45) in water and that of also (r = 0.41) in sediment. But the salinity and phosphorus content are inversely correlated (r = — 0.56) in water. These findings agree with the earlier observations in the marine and estuarine regions (Ayyakkannu and Chandramohan, 1971 ; Dhevendaran, *et al.*, 1974). This indicates that the release of phosphate is mostly governed by microbial activities (Dhevendaran *et al.*, 1974).

Total 188 pure cultures were isolated and were identified to the generic level. Genera like *Pseudomonas* and *Vibrio* among Gram negative and *Moraxella, Micrococcus, Bacillus* and *Corynebacterium* among Gram positive groups were isolated from both water and sediment sources (Table 1). This observation confirms the earlier findings made by Gak (1959), Niewolak (1971) and Venkateswaran (1981). High percentage of occurrence of *Pseudomonas* spp. in the marine environment has already been reported by Kobori, *et al.* (1979) and by Venkateswaran (1981). In the present study also *Pseudomonas* spp. were found to be the dominant bacteria in the marine environment, and *Corynebacterium* spp. were found to be less in concentration in the sediment thereby confirming the earlier report of Dhevendaran *et al.* (1985). This is the first report of the generic composition of bacteria in Cochin Backwater which are capable of solubilizing phosphate.

Potent strains, *Pseudomonas* and *Vibrio* were selected for the study of the influence of the incubation period, carbon sources and the temperature for the optimum release of phosphate and for the maximum growth of the isolates. Fig. 4 shows the effect of incubation period over the release of phosphate and the growth of bacteria. Growth and phosphate concentration were estimated at every 24 hr. of interval. Both *Pseudomonas* and *Vibrio* released maximum phosphate from C_3 $(PO_4)_2$ after 72 hrs. as well as optimum growth was also observed on the same period of time. Of these two isolates *Pseudomonas* released more phosphate (550 μg. PO4/1). Craven and Hayasaka (1982) found that the bacteria associated with rhizosphere of *Zostera marina* released maximum phosphate after 72 hrs. For the first time we reported the release of phosphate along with the growth of the culture. It is clear that the glucose at 0.1% concentration as well as the 100 mg of Ca_3 $(PO_4)_2$ in 100 ml of the medium supported the growth of the bacteria which enhances the production of organic acid. This in turn solubilizes the tricalcium phosphate to the maximum extent. Work is on progress to identify the organic acid produced by bacteria while growing on the carbon sources.

TABLE 1. *Generic composition (%) of phosphobacteria*

Source	Total number of isolates	*Pseudomonas*	*Moraxella*	*Vibrio*	*Micrococcus*	*Bacillus*	*Corynebacterium*
Water	66	34.8	16.6	10.8	16.6	13.6	7.6
Sediment	122	45.0	13.1	4.9	16.6	13.9	6.5

Acids commonly detected by Craven and Hayasaka (1982) included acetic acid, gluconic acid, glucuronic acid, glycolic acid, lactic acid and succinic acid. Their evidence indicated that bacteria in, *Z. marina* rhizosphere released acetic acid as they solubilized mineral phosphate. Acetic acid deteced was, therefore, the product of direct glucose metabolism.

optimum growth at 37°C whereas *Vibrio* released maximum concentration of phosphate and attained optimum growth at 29°C. This indicates that *Vibrio* is mainly from the marine environment thereby confirming earlier obser-vation made by Dhevendaran (1978). Harrison *et al.* (1972) conducted the experi-ment at 15°C and Craven and Hayasaka

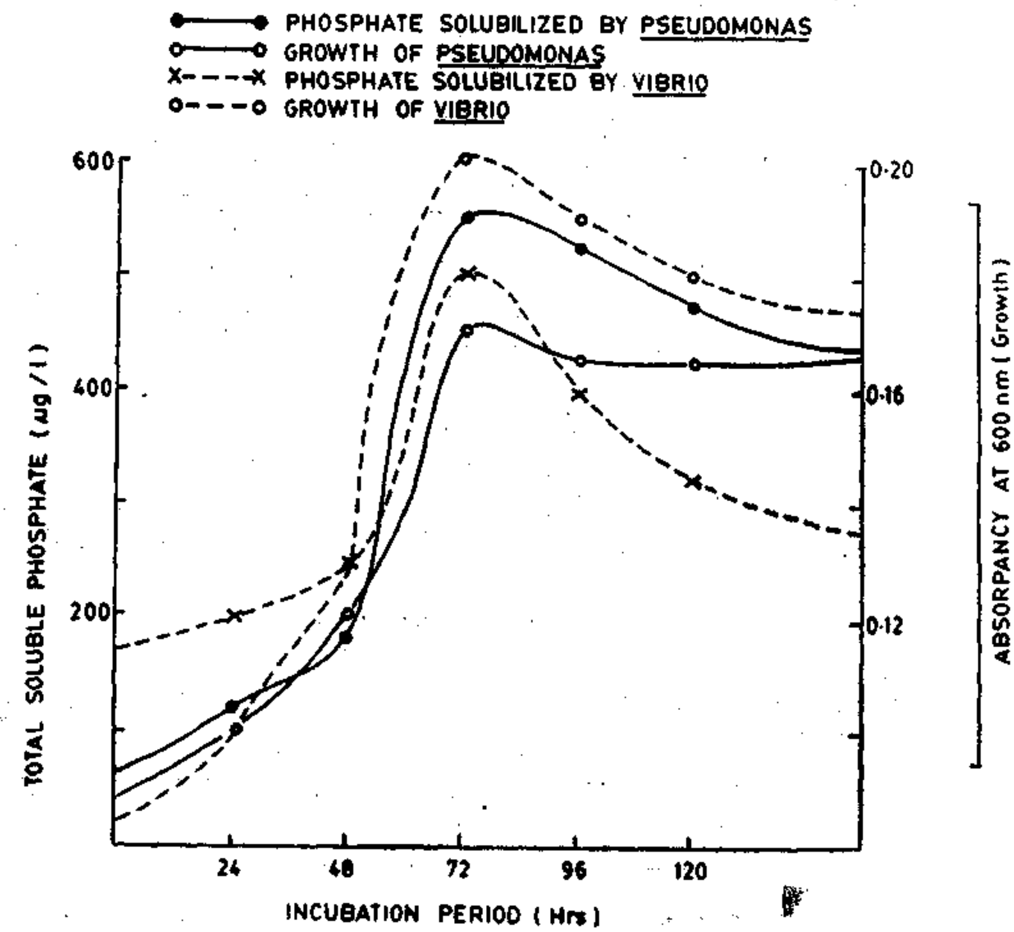

FIG. 4. Effect of incubation period on the solubilization of phosphate and growth of bacteria.

Temperature has got profound influence over the growth and release of phosphate. The cultures were incubated at varying tem-peratures from 4 to 50°C (Fig. 5). *Pseudomonas* solubilized maximum phosphate and attained (1982) incubated the cultures at 25°C. Harrison *et al.* (1972) got maximum phosphate solubilized only after 8 days whereas Craven and Hayasaka (1982) reported the maximum solubilization of phosphate for certain bacteria after 6 days. It is quite interesting that our isolates released maximum phosphate within 3 days when incubated *Vibrio* at 29°C and *Pseudomonas* at 37°C. But at 50°C there as a drastic reduction of phosphate released and the growth. It is almost equal to that at 4°C for both the cultures. It is presumed that fructose, xylose and mannitol were used at 0.1% concentration in the medium and studied the growth of the isolates and the potentiality of releasing phosphate. Of these glucose produced the greatest increase in total soluble phosphate and profused growth of *pseudomonas* and *Vibrio*. Similarly Harrison,

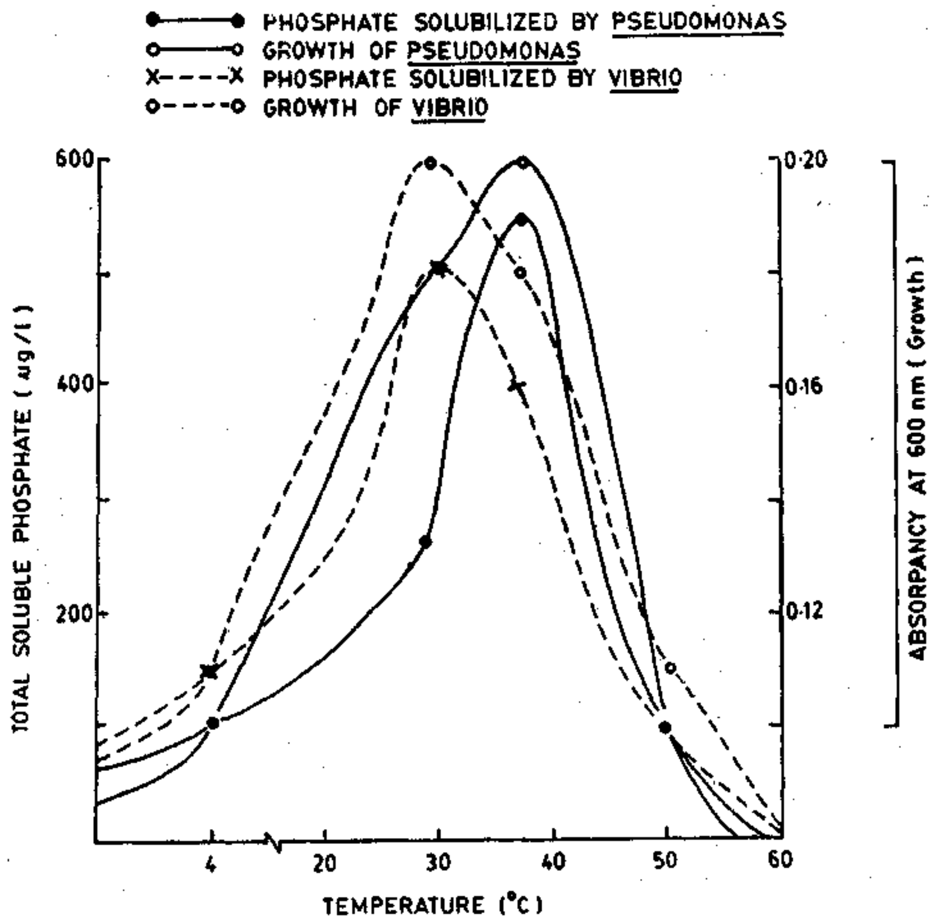

FIG. 5. Effect of temperature on the solubilization of phosphate and growth of bacteria.

there is a possibility for the solubilization of phosphate in the deeper oceanic waters also.

The addition of various carbohydrates to the cultures as the carbon and energy source (Table 2), caused the changes in the solubilization of phosphate and growth. Glucose, *et al.* (1972) and Craven and Hayasaka (1982) reported glucose had a profound effect over the maximum release of phosphate. Harrison, *et al.* (1972) added the glucose in the growing cultural medium at various interval of time and it resulted in a progressive increase in the concentration of organisms and of soluble phosphate. This invariably retard the precipitation of phosphate, with Ca^{2+} and Fe^{3+} by the production of acid. Mannitol could not influence the solubilization of phosphate. trial cultures can be accounted for such action. This prompted the possibility of utilizing the marine phosphobacteria for leaching out the phosphate from the Igneus, sedimentary and residual phosphate ores. *Pseudomonas* and *Vibrio* were found to grow well in the presence of phosphate ores (500 mg/100 ml) in the semi-synthetic media and solubilized phosphates (Table 3). Of these three samples, maximum amount (0.700 mg/g) of phosphate was released from the sedimentary ores by *Pseudomonas* and 0.480 mg/g of phosphate by *Vibrio* from the same sample and the minimum concentration (0.24 mg/g and 0.120 mg/g) was

TABLE 2. *Solubilization of phosphate* by bacteria using various carbohydrates*

Bacteria	Glucose		Fructose		Xylose		Mannitol	
	Growth	PO_4^{3-}- released	Growth	PO_4^{3-}- released	Growth	PO_4^{3-}- released	Growth	PO_4^{3-}- released
Pseudomonas ..	P	+++	P	++	M	+	F	—
Vibrio ..	P	+++	M	++	M	+	M	—

* Tricalcium phosphate [$Ca_3(PO_4)_2$]
P Profused growth
M Moderate growth
F Feeble growth
+++ Maximum release of phosphate
++ Average release of phosphate
+ Minimum release of phosphate
— No release of phosphate

It is now well established that bacteria can be successfully employed either to leach out contaminating metals or required metals from any ore body. Much work has been done especially on copper, uranium, sulphur and zinc (Tuovinen, *et al.*, 1971 ; Brierley and Brierley, 1978 ; Murr and Brierley, 1978). There is practically no evidence to show that contaminating phosphorus can be removed from the ore body, utilizing microorganisms like bacteria. Dhevendaran *et al.* (1974), Venkateswaran (1981) and Dhevendaran and Valsa Joseph (unpublished) have isolated many bacterial cultures from the marine, estuarine sediments and also in the primary film capable of solubilizing hydroxy-apatite. Even though similar bacteria were already isolated from the cultivated soils (Chandrasekharan, 1966), the interesting aspects with the marine isolates are that they are very active solubilizers than the others. Besides, 80-90% of the marine isolates are capable of dissolving insoluble phosphate whereas only 50-60% of the terres-

TABLE 3. *Solubilization of phosphate from phosphate ore samples by bacteria*

Microorganisms	Phosphate released (mg/g) Phosphate ore sample		
	Igneous ore	Sedimentary ore	Residual ore
Pseudomonas	0.240	0.700	0.300
Vibrio	0.120	0.480	0.200

released from the igneus ore samples both by *Pseudomonas* and *Vibrio* strains respectively, wihtin 3 days of incubation period at room temperature (28 ± °C). Chandramohan (unpublished) observed that some of his cultures were capable of leaching out phosphate from the manganese ores. It is confirmed that the marine phosphobacteria could be employed successfully to leach out phosphorus from the ore samples.

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