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SOME ASPECTS ON THE DISTRIBUTION OF 'PHOSPHOBACTERIA' IN MARINE ENVIRONMENT AT PORTO NOVO*

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ABSTRACT

The occurrence and distribution of a special group of bacteria called 'Phosphobacteria' capable of dissolving insoluble phosphorus compounds in marine environment at Porto Novo were studied and reported. Various bottom sediments of marine, estuarine, and fresh water zones, intertidal sands, soils from hydrogen sulphide area and different waters like seawater, estuarine water, interstitial water and sub-terranian water were analysed. Totally 20 samples were taken for analysis. Total and phosphobacterial populations were estimated in each sample. Phosphobacteria were present in all the samples irrespective of the variations in salinity, pH, texture of the soil and depth of sampling. Clayey substrata harboured more phosphobacteria when compared to sandy ones. Sub-terranian water also contained phosphobacteria but the population was very low when compared to other waters. Facultative anaerobes capable of dissolving insoluble phosphate were also isolated from the hydrogen sulphide region. In general bottom deposits contained more phosphobacteria than in the corresponding overlying water. Preliminary staining and biochemical studies revealed that majority of the isolates belong to one genus, *Bacillus*. The horizontal and vertical distribution of phosphobacteria is discussed in relation to other environmental factors.

INTRODUCTION

STUDIES on the phosphate concentration are of primary importance in assessing the productivity of natural waters. It is well known that most of the phosphorus is locked up as insoluble inorganic and organic phosphates and that microorganisms may bring about a number of transformations of the element (Alexander, 1961). These include (i) altering the solubility of inorganic compounds of phosphorus, (ii) mineralizing organic compounds with the release of orthophosphate, (iii) converting the inorganic available anion into cell protoplasm and (iv) bringing about an oxidation or reduction of inorganic phosphorus compounds. Many fungi and bacteria are capable of solubilizing the insoluble phosphorus compounds and the solubilization may be brought about by acids or enzymes of microbial origin (Alexander, 1961 ; Skujins, 1967 ; Ayyakkannu and Chandramohan, 1970 c). It is logical to expect that the activities of such specialised microorganisms may influence considerably the organic production in marine environments too. Very recently Ayyakkannu and Chandramohan (1970 a) reported for the first time about the occurrence of phosphobacteria (phosphate solubilizing bacteria) along with their distribution in marine environment at Porto Novo. Later the same authors (1970 b) reported that phosphobacteria not only occur in seawater and marine sediments,

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but also in freshwater and freshwater river sediments. The present investigation was undertaken to study the phosphobacterial population in relation to environmental factors such as pH, salinity, oxygen, type of sediment, etc. Water samples from open well and bore well were also analysed inorder to understand the phosphobacterial concentration in underground waters.

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Materials and Methods

The present investigation was carried out during the month of July, 1970 in and around the Vellar Estuary (Fig. 1) at Porto Novo, S. India (11° 29′ N-79° 49′ E). Twenty-two samples were analysed from ten stations selected on the basis of the nature of the sediments and salinity. From stations 1 to 4, the water samples at

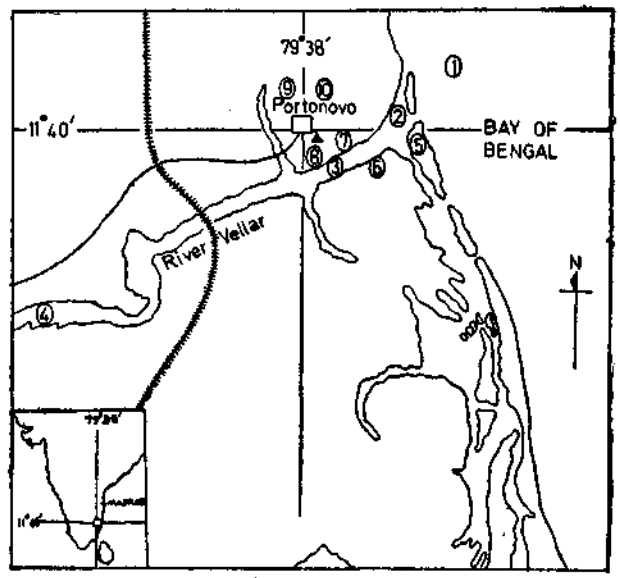

Fig. 1 Station positions in the Vellar Estuary at Porto Novo.

required depth were collected with sterile glass bottles while the surface layer of sediments with the aid of a Peterson's grab. In the estuary samples were collected from the middle of the river as far as possible. The samples from stations 5 to 8 were taken with a sterile metal corer (35 cm long). The central portions of the core sample were taken for analysis. The water samples from stations 9 and 10 were collected directly in sterile glass bottles.

The locations of the various stations are given in Table 1. The pH of the various water samples was estimated with a Philips pH meter and the salinity with a Salinity temperature bridge type M.C. 5 (Electronic switchgear, London). The

oxygen content was estimated by adopting the method as outlined by Strickland and Parsons (1968). In stations 5 to 8, where the intertidal samples were collected, the pH, salinity and oxygen content were measured in the interstitial waters of the respective stations.

TABLE 1. *Sampling stations*

Station No.	Description
1	10 fathom line in the sea, clayey sediment.
2	Mouth of the Vellar Estuary, sandy sediment (3.5)*
3	Middle of the estuary - opposite to Biological Station, clayey sediment (5.5).
4	Freshwater region of the estuary at Adanavur, sandy sediment (14.5).
5	Intertidal sands - High water mark of the sea (3.0).
6	Intertidal sands - High water mark of the estuary (3.1).
7	Intertidal sands in hydrogen sulfide region at mid water mark of the estuary (5.2).
8	Intertidal clay - mid water mark of the estuary, opposite to Biological Station (5.5.).
9	Open well water (6.2).
10	Bore well water (6.3).

* Distance in Kilometres from the first station is given in parenthesis.

The total and phosphobacterial population in different samples were estimated by a modified method of Sperber (1958) as employed earlier (Ayyakkannu and Chandramohan, 1970 a). Serial dilutions of the samples were made with sterile water blanks with appropriate salinities and plated in sterile petridishes containing a special hydroxy apatite medium, of the following composition :

Yeast extract	..	0.2 g
Ammonium sulphate	..	0.5 g
Magnesium sulphate	..	0.1 g
Potassium chloride	..	0.2 g
Glucose	..	10.0 g
Soil or sediment extract	..	200.0 ml
Water (at required salinity)	..	1000.0 ml
Agar	..	15.0 g

After sterilizing the above medium at 15 lb pressure for 20 minutes 60 ml of sterile calcium chloride (10%) and 40 ml of potassium dihydrogen phosphate (10%) were added. The pH of the medium was slowly raised to the required level with sterile IN NaOH when white insoluble calcium phosphate precipitated. After thorough shaking, the medium was poured into dishes containing the sample. The dishes were incubated for 3 days at 26 ± 2°C and the phosphobacterial colonies were identified by the clear solubilization zones around them.

The data represent the averages of triplicates.

Results and Discussion

The total and phosphobacterial population in different samples were estimated (Table 2) and phosphobacteria were present in all the samples. Of all the samples analysed, the marine and estuarine sediments (stations 1-3) revealed the occurrence of a high percentage of phosphobacterial population than the corresponding overlying water. It appears that the ratio between phosphobacteria and total bacteria is not influenced by depth and though there are differences in population densities, the ratios between phosphobacteria and total bacteria seem to be the same (stations 1 and 3 inTable 2). Minimum total bacterial population was obtained in bore well water samples. Perhaps, this may be due to the fact that the underground water is not directly exposed to atmosphere and so free from the terrestrial contamination. While the pH had no effect on the phosphobacterial concentration, the oxygen levels seem to exert a marked effect on the phosphobacterial population (Table 2).

Table 2. *Total and phosphobacterial population in relation to various environmental factors*

Station No.	Sample	Depth of sampling (metre)	pH	Salinity (‰)	Oxygen (ml/l)	Bacterial population (10^6/L or g on oven dry basis)		Percent phosphobacteria
						Phosphobacteria	Total	
1	SW	..	8.2	33.4	4.46	4.42	5.62	79
	BW	16.5	8.3	33.6	3.62	5.06	6.82	74
	S	16.7	..	..	..	6.13	7.23	85
2	SW	..	8.2	33.0	4.72	1.23	2.40	51
	BW	1.8	8.2	33.0	4.24	0.97	1.72	56
	S	2.1	..	..	..	0.99	1.25	79
3	SW	..	7.9	33.0	5.84	2.12	3.08	72
	BW	3.3	8.0	32.8	3.22	4.81	5.89	81
	S	3.5	..	..	..	5 28	6.42	82
4	SW	..	7.4	5.5	4.20	1.20	2.83	42
	BW	1.6	7.4	5.5	3.96	1.42	2.92	49
	S	1.8	..	..	..	1.46	2.62	56
5	SS	..	8.0	32.2	3.26	0.68	0.99	69
	BS	0.3	..	..	..	0.77	1.02	76
6	SS	..	7.9	30.8	3.40	0.87	1.12	78
	BS	0.3	..	..	..	0.92	1.46	63
7	SS	..	7.8	28.4	0.33	1.12	3.25	35
	BS	0 3	..	..	..	0.11	0.29	38
8	SS	..	7.5	29.2	1.45	4.20	5.68	74
	BS	0.3	..	..	..	2.61	3.48	75
9	Water	11.2	7.2	1.0	4.13	0.14	0.18	77
10	Water	7.2	7.1	1.0	3.22	0.02	0.04	50

SW = Surface water. BW = Bottom water. S = Sediment. SS = Surface soil. BS = Bottom soil.

Station 7 was black mud region and rich in hydrogen sulfide (H_2S) and the oxygen content of the interstitial water was extremely low (0.33 ml O_2/L). However, samples from that station were seem to harbour phosphobacteria, the percentage being very low (35% and 38%) compared to other samples and these bacteria may be grouped under facultative anaerobes. The possible role of H_2S producing bacteria in phosphate liberation has been well explained by Alexander (1961) as follows : the H_2S released by the bacteria reacts with ferric phosphate to yield ferrous sulfide liberating phosphate. Likewise phosphate may also be released from iron and aluminium phosphates when the environment becomes oxygen deficient. The same phenomenon may therefore be operating in all H_2S regions of the marine environment. In addition to the facultative anaerobes, obligate anaerobes may also play a major role in phosphate liberation by H_2S production. However no direct correlation was seen between the oxygen content and phosphobacterial population in the present study.

The nature of sediment or soil seems to have an influence on the total and phosphobacterial population. In the present investigation, the clayey samples were seen to contain maximum total bacteria than the sandy ones which is in confirmation with the earlier reports by ZoBell (1938) and Ayyakkannu and Chandramohan (1970 b). Interestingly enough the phosphobacteria also showed a higher concentration in clayey substrata only. The organic matter content in clay is higher than in sand and consequently total phosphorus is also higher in clay (Pomeroy *et al.*, 1965; Ayyakkannu and Chandramohan, 1970 b). The present study has revealed clearly that the phosphobacteria concentrate in places where the total phosphorus is abundant. Such a correlation has already been recorded (Ayyakkannu and Chandramohan, 1970 b). Preliminary staining and biochemical tests showed that out of seven different pure phosphobacterial isolates from marine, estuarine and freshwater regions, five belong to the genus *Bacillus*.

Evidently as recorded in the present study, the phosphobacteria occur under various environmental conditions in marine and freshwater regions and may be involved in a major way in maintaining the phosphate level in different waters and sediments.

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