Phosphatase Producing Microbes Associated with Fish and Shellfish in Trivandrum Coast

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Total heterotrophic bacteria and fungi and the phosphatase producing bacteria and fungi in the gill, guts and muscle of fish (*Etroplus* spp., *Gerres* sp. and *Glossogobius* sp.) and viscera and mantle of shellfish (*Vellorita* sp. and *Perna* sp.) were studied. The hydrographic parameters of the environment from where the fishes were caught were also studied. Except shellfish *Perna indica*, all the species studied harboured phosphatase producing bacteria. The gut portion of finfish contained high amount of total heterotrophic as well as phosphatase producing bacteria. The present study showed that the phosphatase producing bacteria mainly belonged to *Bacillus, Vibrio, Micrococcus* and *Corynebacterium* spp. *Moraxella* showed maximum growth at 5% salt concentration. *Moraxella* and *Aeromonas* were sensitive towards mercury while 45% of *Vibrio* showed growth at 0.4 ppm mercury.

Phosphorus is present in sediment, water and living organisms including microbes and plays an important role in primary production of the shallow water regions (Dhevendaran *et al.*, 1974). Occurrence and distribution of phosphatase producing bacteria in the marine environment (Ayyakkannu & Chandramohan, 1971; Chandramohan & Natarajan, 1971; Dhevandaran *et al.*, 1986; Dhevendaran & Valsa, 1987), in clayey sediments (Ayyakkannu & Chandramohan, 1974), in mangrove sediments (Dhevendaran *et. al.*, 1974) and on the surface of algae (Aaronson & Patni, 1976) have been reported. A high percentage of *Pseudomonas* and *Vibrio* produced phosphatase enzyme in the marine environment (Venketeswaran & Natarajan, 1983). Promod & Dhevendaran (1986) studied the occurrence and distribution of phospho-bacteria in Cochin backwaters, and utilised the microorganisms for the leaching of low grade ores. Dhevendaran & Valsa (1987) studied the phosphobacteria in the primary film and characterised certain groups of bacteria for the physiological activities as well as for the tolerance towards certain heavy metals. It is thus probable that the phosphatase activity by microorganisms either in sediment or in water or associated with finfish or shellfish may regulate the phosphate content of the respective environment.

The present study is an attempt to know the pattern of distribution of phosphatase producing bacteria in different parts of the finfish and shellfish inhabiting the Veli Lake and adjacent coastal waters.

Materials and Methods

The present investigation was carried out during the month of January 1987, from Veli Lake and Valiathura. Surface water samples were collected fortnightly by immersing polythene bottles and fishes were collected with the help of cast nets and drift gill nets. The salinity, reactive nitrate and nitrite were estimated by the method of Strickland & Parsons (1968). pH of the water was noted with Elico pH meter and dissolved oxygen by Winkler's method. The reactive phosphate was estimated by the method of Murphy & Riley (1962). Total heterotrophic bacteria (THB) and phosphatase producing bacteria (PPB) were isolated from different parts of fish and shellfish, following the procedure adopted by Dhevendaran *et al.* (1974). The selected bacterial strains from both THB and PPB

were identified to the generic level following the scheme adopted by Simidu & Aiso (1962). The effect of sodium chloride on the growth of potent strains were also carried out. For this nutrient broth was prepared in distilled water. Sodium chloride was added at the concentration of 0.5, 1, 2, 3, 5 and 7%. Simultaneously a control was also maintained without sodium chloride. The media were sterilized and allowed to cool. The cultures were inoculated and incubated at room temperature (28±2°C) for 48 h. To find out the toxicity of mercury on the survival of selected isolates nutrient broth was prepared in 50% seawater and sterilized and then mixed with mercuric chloride at 0.2, 0.4, 0.5, 1 and 2 ppm concentration. Simultaneously a control without mercuric chloride was also maintained. Mercuric chloride containing sterile media was pipetted out into sterile cultures tubes. After cooling the medium, the culture were inoculated and incubated at 29 ± 2°C for 48 h. After the incubation period was over, the cultures were diluted to constant volume and the turbidity of the cultures was measured using a Spectronic-20 at a wave length of 500 nm.

Results and Discussion

Results of the present study on the hydrographical parameters and total heterotrophic microbial population from fishes namely, *Etroplus suratensis*, *Etroplus maculatus*, *Gerres abbreviatus* and *Glossogobius giuris* and from shellfishes namely, *Vellorita cyprinoides* and *Perna indica* are presented in Table 1.

In Veli lake the salinity ranged between 3.5 and 4.85‰ and in Valiathura it was 40‰. Oxygen level was found lower in Veli lake than that in Valiathura. Higher concentrations of nitrate and nitrite was observed at Veli lake while phosphate concentration was lower compared to Valiathura.

Table 1. *Hydrographical parameters of sampling site and microbial population in different fish and shellfish*

pH	Salinity (‰)	NO_3 (µg/l)	NO_2 (µg/l)	Oxygen (ml/l)	PO_4 (µg/l)	Source		Microbial population (x 10^5), g^{-1} Total		Phosphatase producers	
								Bacteria	Fungi	Bacteria	Fungi
7.6	3.5	137.66	127.41	6.33	263.2	*Etroplus maculatus*	Gill	65.76	4.2	17.52	3.5
							Muscle	36.20	-	3.96	-
							Gut	77.72	58.63	66.14	42.4
7.7	3.9	192.5	99.95	4.98	207.79	*Etroplus suratensis*	Gill	13.72	5.5	13.72	1.8
							Muscle	4.32	-	2.16	-
							Gut	128.4	-	42.82	-
7.7	3.9	192.5	99.95	4.98	107.79	*Vellorita cyprinoides*	Mantle	21.52	11.43	11.43	0.71
							Visceral mass	63.15	11.15	11.15	-
7.8	40.1	33.77	13.03	8.30	623.38	*Perna indica*	Mantle	42.19	-	-	-
							Visceral mass	115.94	-	-	-
7.8	4.85	282.88	141.89	4.24	387.88	*Gerres abbreviatus*	Gill	1.39	0.53	0.53	-
							Muscle	0.56	0.30	0.30	-
							Gut	69.65	35.52	35.52	-
7.8	4.85	282.88	141.89	4.24	387.88	*Glossogobius giuris*	Gill	72.32	23.52	23.52	-
							Muscle	21.84	10.59	10.59	-
							Gut	117.76	48.83	48.83	

The microbial population of both total as well as phosphatase producers were recorded from gill, mucsle and gut regions of fish and mantle and visceral mass of shellfish. Maximum bacterial population was noticed in the gut and minimum was observed from the muscle of finfish examined. Except *Perna indica* all the fish and shellfish studied harboured phosphatase producers. Phosphatase producing fungi was detected in the gut and gill samples of *Etroplus maculatus* only.

Table 2 shows the generic composition of bacterial populations in the selected fish and shellfish. Of these *E. maculatus and Gerres abbreviatus* showed wider diversity of microbial population, where as *P. indica* exhibited a specific type of population. The predominance of a particular group of bacterial population in fish and shellfish varied. The results show that *E. suratensis, Gerres abbreviatus* and *Vellorita cyprinoides* harbour a higher percentage of *Micrococcus, Aeromonas* and *Corynebacterium* respectively.

Table 3 shows the physiological characteristics of the selected isolates from both THB and PPB. Of these the higher percentage of population showed activity towards starch when compared to lipids. Gnanam *et al.* (1982) also observed similar trend of amylase and lipase enzyme activity, in bacteria associated with fish and in sediments. Srikumari & Lakshmanaperumalswamy (1986) noticed higher percentage of lipase producers rather than amylase producers in *Vellorita.*

Since the three bacterial cultures were isolated from the estuarine environments, the possible effect of sodium chloride concentration on the growth of the selected bacterial cultures were studied (Fig.1). Important genera selected were *Bacillus, Moraxella, Aeromonas, Corynebacterium* and *Vibrio.* Invariably all the cultures grew in the medium without sodium chloride. But the optimal growth was exhibited by the bacterial cultures from 1-5% NaCl concentrations. Of all the cultures *Moraxella* showed maximum growth at 5% sodium chloride concentration. *Corynebacterium and Bacillus* showed optimum growth at 2 and 3% NaCl concentration respectively. These two cultures are considered to be

Table 2. *Generic composition of bacteria in percentage*

	Etroplus suratensis		*Etroplus maculatus*		*Vellorita cypronoids*		*Gerres abbreviatus*		*Glossogobius giuris*		*Perna indica*	
	THB	PPB	THB	PPB	THB	PPB	THB	PPB	THB	PPB	THB	PPB
Total No.of isolates	140	150	250	130	30	40	60	60	60	60	80	-
Pseudomonas	-	-	4	-	-	-	-	-	-	-	-	-
Bacillus	14.2	6.66	8	23.07	-	-	16.66	16.66	16.66	-	12.5	
Vibrio	-	13.33	16	23.07	-	-	-	-	-	16.66	-	-
Micrococcus	50	66.66	48	30.76	33.33	25	16.66	33.33	16.66	50	37.50	-
Corynebacterium	35.7	6.66	12	15.38	33.33	25	16.66	-	16.66	33.33	50	-
Aeromonas	-	-	-	-	-	25	33.33	33.33	33.33	-	-	-
Moraxella	-	6.66	-	7.60	-	25	16.66	16.66	16.66	-	-	-
Enterobacteriaceae		-	-	-	-	-	-	-	-	-	-	-
Flavobacterium	-	-	4	-	33.33	-	-		-	-	-	-
Cyptophaga	-	-	4	-	-	-	-	-	-	-	-	-
Alcaligenes	-	-	4	-	-	-	-	-	-	-	-	-

THB = Total heterotrophic bacteria; PPB = Phosphatase producing bacteria

Table 3. *Physiological characteristics of selected total heterotrophs and phosphatase producing bacteria*

Name of fish/ shellfish	Total isolates	Physiological characters of total heterotrophs (%)		No. of isolates	Physiological characters of phosphatase producers (%)	
		Amylase +ev	Lipase +ev		Amylase +ve	Lipase +ve
Etroplus maculatus	75	32.00	24.00	39	69.23	15.38
E. suratensis	42	42.85	7.14	45	80.00	6.67
Vellorita cyprinoides	9	33.33	-	12	75.00	-
Perna indica	24	25.00	37.50	-	-	-
Gerres abbreviatus	18	50.00	25.00	18	100.00	25.00
Glossogobius giuris	18	33.33	25.00	18	50.00	25.00

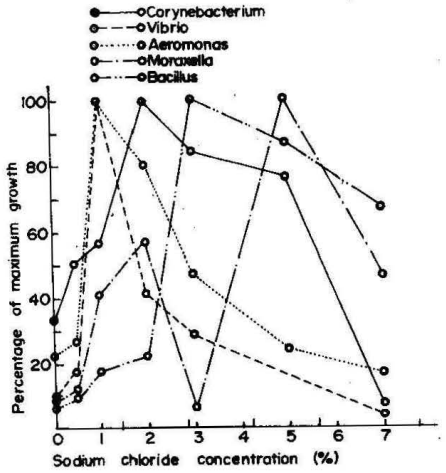

Fig. 1. Effect of sodium chloride on the growth of selected isolates.

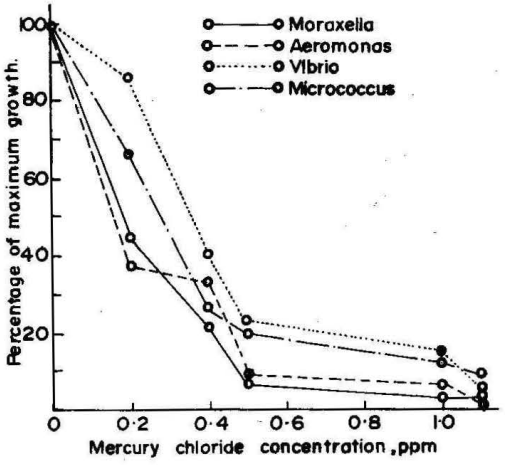

Fig. 2. Effect of mercury chloride on the survival of selected isolates.

typical marine bacteria. The bacterial cultures isolated from the sediments of Veli lake exhibited wider range of tolerance between 2 and 5% NaCl (Dhevendaran & Valsa, 1987).

Fig.2 shows effect of mercury toxicity on the survival of the selected isolates, such as *Vibrio, Micrococcus, Moraxella* and *Aeromonas.* Of all these four cultures *Moraxella* and *Aeromonas* showed more sensitivity towards mercury. 90% *Vibrio* showed growth at 0.2 ppm concentration and 45% at 0.4 ppm concentration. In most *mercury* resistant bacteria studied to date, resistance is associated with flavoprotein mercuric reductase which reduces Hg^{++} to HgO (Booth & Williams, 1984).

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