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SEASONAL VARIATIONS OF AEROBIC HETEROTROPHIC BACTERIA IN COCHIN BACKWATER*

V. CHANDRIKA AND P. V. RAMACHANDRAN NAIR

Central Marine Fisheries Research Institute, Cochin - 682 014

ABSTRACT

The occurrence and seasonal distribution of aerobic heterotrophic bacteria in water and sediments and their ecophysiology and biochemical characters were studied for a period of three years — 1972-73, 1974-75 and 1975-76 in Cochin Backwater. The total counts between one station and the other did not show significant difference while the counts between months did. The distribution was characterised by overdispersion. Among 920 strains selected from this tropical marine environment in different months in the three years period, 23 strains were lost during purification and retransferring procedures and classified into 8 genera. The genus *Alcaligenes* was found to be the dominant genus in all the seasons. Data on temperature related bacterial density indicated that there are very few, true psychrophilic bacteria in Cochin Backwater and sediments. The relationship between bacterial density and nutrients are discussed.

INTRODUCTION

THE IMPORTANCE of heterotrophs in fisheries environmental research has received only very little attention in the marine environment. The present study is on the numerical abundance and seasonal variation of heterotrophic bacteria, their generic composition and biochemical and physiological activity in the estuarine, and inshore marine environment of Cochin in order to assess their ecological importance in aquatic environments. An attempt was also made to correlate their seasonal variations in density with some of the physico-chemical factors such as temperature, salinity, oxygen, pH, rainfall, nutrients (phosphate and nitrate), organic carbon and organic nitrogen and with the seasonal variation and phytoplankton.

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MATERIALS AND METHODS

The study area is situated between 09° 28'-10° 00' N and 76° 13'-76° 31'E (Fig. 1). The length of the area studied is about 65 km and the width varies from 0.5 to about 15 km.

The area of study during 1972-73 is confined to those areas of the backwater system, the first 4 stations being situated between Aroor and the barmouth and the 5th one in neritic waters in the inshore environment of Cochin (Fig. 1). All the sampling stations during 1974-75 were situated in the estuarine environement of Cochin Backwater (Fig. 2). Altogether 6 fixed stations were studied, 4 of them situated in the brackishwater, whereas 2 of them were almost in fresh water condition. During the third year of study, 3 stations were fixed between 09° 28' and 10°00' N in the estuarine area. Although Station II is situated near the barmouth actually it is in estuarine environment dominated by marine conditions which was evident from bacterial and chemical

parameters. The mean salinity is relatively high at this station, as compared to other two stations. Stations II and III do not differ significantly in mean salinity.

During July 1975 to June 1976 data were collected from 3 fixed stations (Fig. 1). The

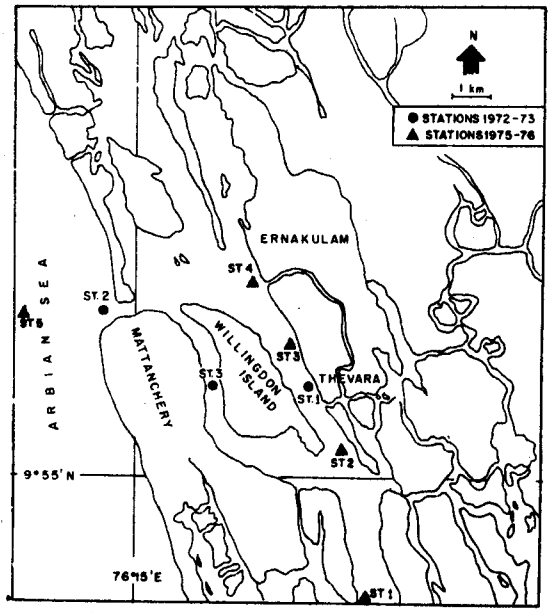

Fig. 1. Sampling stations in Cochin Backwater during 1972-'73, 1975-'76.

first station is very near the sewage outlet of Cochin city and the second one in typical backwater conditions 5 km away from the 1st station in Mattancherry Channel. The Station III was in marine conditions and the mean salinity of Station III near barmouth differed significantly.

Water samples were collected in sterile 300 cc glass bottles and the sediment in fresh polythene bag in aseptic conditions and kept at 4°C until the time of bacteriological investigations. For identification in addition to microscopic examination, numerous physiological and biochemical tests were carried out using standard procedures (Fig. 4 a, b, c). Each bacterial strain tested thus received a profile. With the help of these data, identification was attempted using the scheme of Usio Simidu and Kayuyoshi Aiso (1962) and also the scheme of Shewan *et al.* (1962) and Bergy's manual of determinative bacteriology (1974).

Distribution and composition of heterotrophic bacterial flora — 1972-73

The estuarine microflora generally consist of marine, brackishwater, freshwater and intermediate forms. The important bacterial

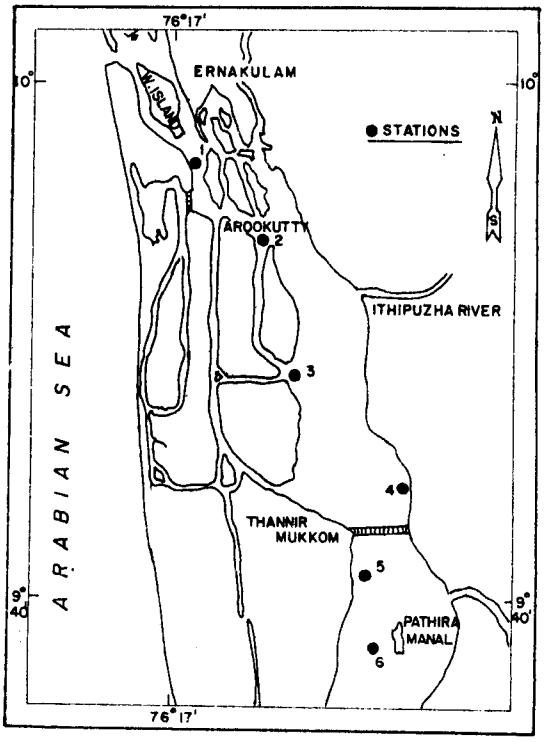

Fig. 2. Sampling stations in Cochin Backwater system during 1974-'75.

genera encountered during the present investigations were *Alcaligenes, Vibrio, Pseudomonas, Aeromonas, Flavobacterium* and *Micrococcus.* In addition *Bacillus* spp. was found in surface sediments. The bacterial

population based on numerical counts showed wide variation in their distribution in different stations both in sea water and in surface sediments. Quantitative distribution of microflora as determined by numerical abundance in surface sea water and sediments in all stations is given in Figs. 1a and b. Seasonal cycle in the total bacterial flora revealed the highest count (656 x 10^3/ml) in December 1972 and the minimum (95 x 10^3/ml) during November 1972. The period of maximum abundance of bacteria was encounted during December to March. In all the stations except the fourth, the minimum total counts were recorded during the monsoon period. In Station IV, minimum counts were observed during the premonsoon period, with an increasing trend from the premonsoon to the postmonsoon seasons. In Stations III and V, the total counts did not show large fluctuations indicating that the bacterial flora were not subjected to large seasonal variations in abundance. The abundance attained the maximum during the postmonsoon season for all the stations except Station V, where the difference between the lowest and highest count was not large.

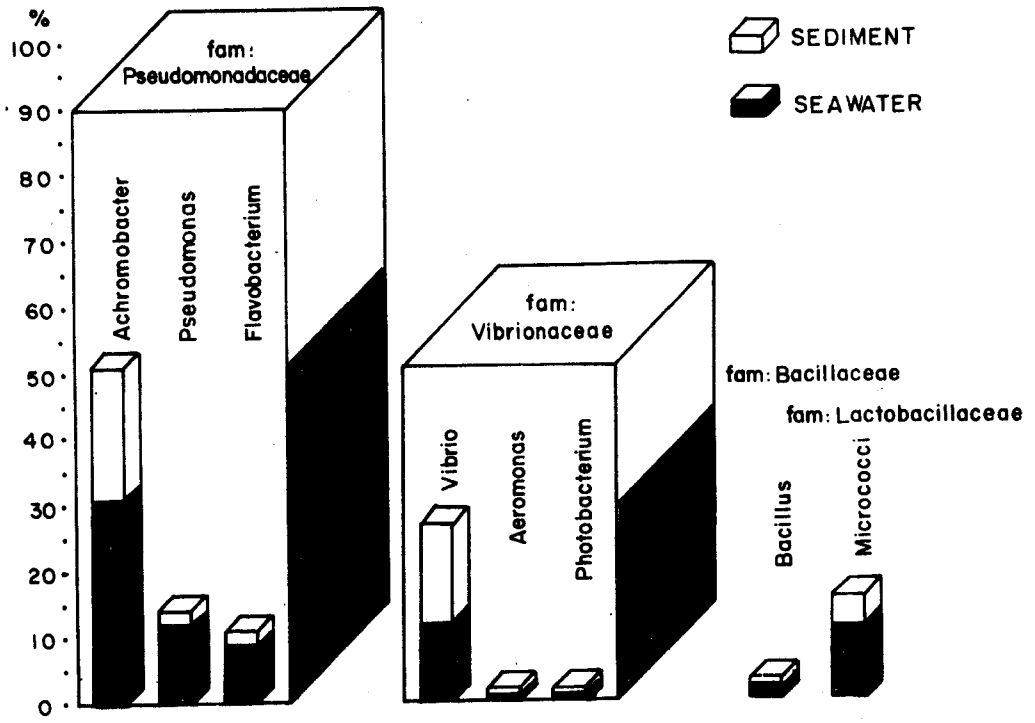

Fig. 3. Generic composition of 296 bacterial strains isolated during 1974-'75.

Qualitative analysis

Altogether 319 strains were isolated, identified and briefly studied physiologically and classified into 6 genera. Almost all of the isolates (99.4%) were asporogenous gram-neative rods somewhat pleomorphic (Fig. 4). Gram-positive bacteria were rare, only 0.7% were isolated. Pigmented bacteria isolated formed 3.2% of the total isolates. All the strains isolated were, actively motile except the *Micrococcus.* Dextrose was fermented oxidatively (40.7%) and fermentatively (30.0%).

The rest of the isolates were unable to utilise dextrose. 60.8% of the isolates were capable of reducing nitrate into nitrite. 78.3% of the total isolates liquified gelatin indicating active proteolytic activity of the flora isolated.

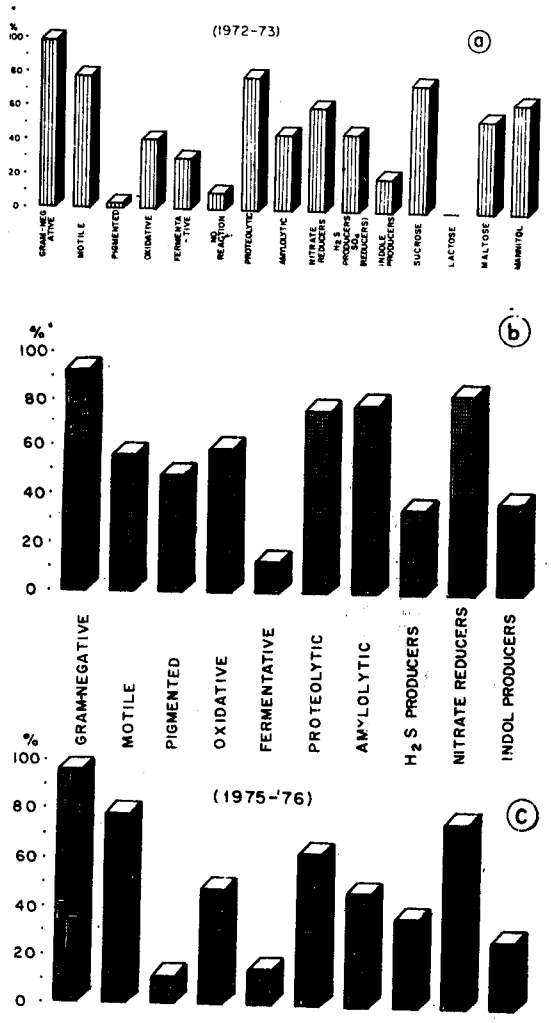

Fig. 4. Biochemical and physiological activity of bacteria isolated during : a. 1972-'73 b. 1974-'75 and c. 1975-'76.

Approximately 44.5% of the isolates hydrolysed starch and 45.7% strains reduced sulphates forming H_2S. None of the isolates fermented

TABLE 1. *Comparison of generic percentage distribution of heterotrophic bacteria isolated from different environments by various investigators with the present study (1972-73)*

Genus	Cochin Backwater		Chesapeake Bay		Long Island Sound Conn.	Narangasett Bay R.I.	Kamogawa Bay Japan
	Seawater	Mud	Bay	Mia	(Conover, 1956)	(Murochelano, 1967)	(Simidu & Aiso, 1962)
	Present Study		(Lovelace *et al.*, 1968)				
Alcaligenes	32.0	28.0	13.0	42.0	28.6	12.2	21.3
Vibrio	22.0	22.0	56.0	17.0	4.9	13.3	37.3
Pseudomonas	18.0	16.0	18.0	—	40.6	28.3	29.8
Bacillus	7.0	14.0	—	—	0.1	0.5	5.5
Flavobacterium	8.0	8.0	6.0[b]	8.0[b]	23.1	40.7[b]	2.1
Aeromonas	7.0	5.0	—	—	—	—	—
Micrococcus	2.4	5.0	—	—	0.3	1.2	0.4

lactose and produced gas from any of the sugars. Most of the isolates fermented sucrose, maltose and mannitol. The above observations indicated that the heterotrophic micro-organisms are actively involved in the degradation and total turn over of organic matter in the Cochin Estuary.

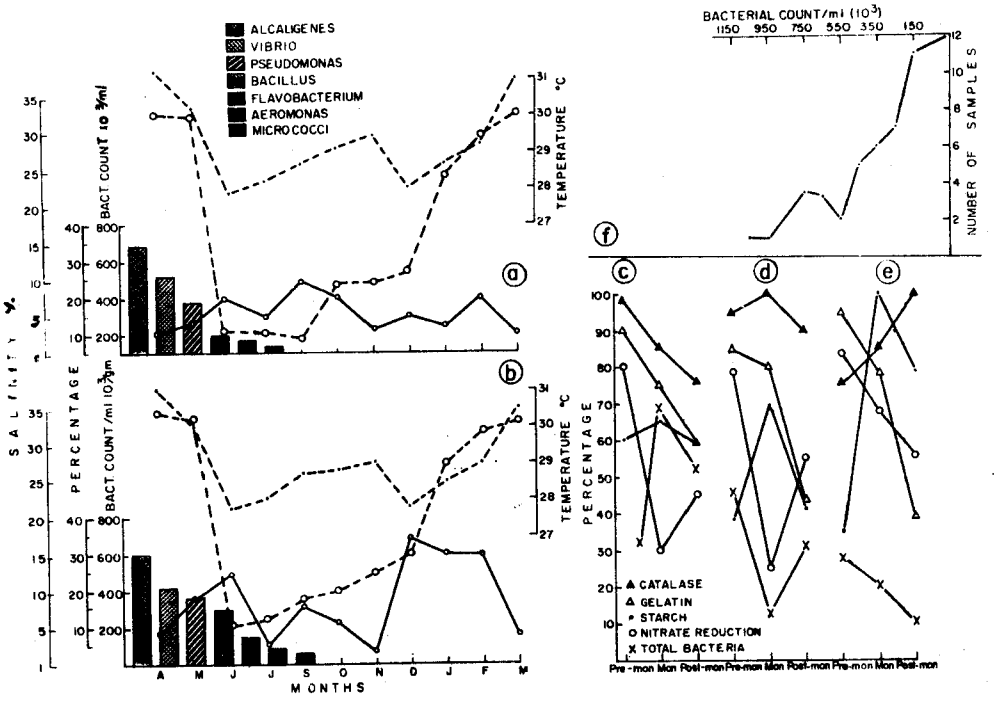

Fig. 5. Total plate count along with percentage occurrence of the 6 genera isolated during 1972-'73 : a. in water, and b. in sediment, seasonal percentage variation during premonsoon and postmonsoon of c. *Alcaligenes*, d. *Vibrio*, e. *Pseudomonas* and f. *bacterial* density in sampling stations during 1972 - 73.

Percentage occurrence of each genus is given in (Fig. 5 a-b) *Alcaligenes, Vibrio, Pseudomonas, Bacillus, Flavobacterium, Aeromonas* and *Micrococcus* constituted the genera occurring both in sea water and sediment. The percentage occurrence of Gram-positive bacteria like *Micrococcus* and *Bacillus* was more in sediment than in sea water.

In the absence of any detailed work on the percentage occurrence of bacteria at generic level in Cochin area, the present data have been compared with the data available elsewhere (Table 1). A perusal of the Table 1 shows that the genus *Alcaligenes* predominates the inshore environment of Cochin, while *Vibrio* was found to be the dominant genus in Chesapeake Bay and Kamogawa Bay. *Pseudomonas* was generally encountered in moderate numbers except in the Long Island Sound. The *Flavobacterium* occurred as a predominant genus in Narragansett Bay (Rhode Island), while it was seen in lesser quantities in other environments. The *Micrococcus* was found to be the only genus moderate to rare in all the environments included in Table 1 which indicated that it may not be an indigenous genera, but may be an exotic one which survive in the marine environment.

It is difficult to integrate the data of Table 1 and present meaningful quantitative synopsis of the bacterial genera indigenous to sea water from coastal environments. It may not be desirable to do so because varied procedural methodologies may somewhat affect the outcome of bacterial taxonomic studies. But the observed generic diversity is more likely caused by environmental and seasonal diversity rather than by any other factor. The six genera represented a very small percentage of order Eubacteriales and Pseudomonadales although their relative abundance varied considerably, due to various environmental and biological factors, but they are consistently found in coastal waters. Table 1 illustrates comparison of generic percentage data with the results of other workers in different environments.

Micrococcus was found to increase associated with phytoplankton blooms and during red tide, as red tide was found to contain coloured aggregations of algae, bacteria, ciliates, diatoms, flagellates and other pigmented organims. Total heterotrophic counts in Station V corresponding to the period of red water was found to decrease (64×10^3) when compared to the counts recorded in June (732×10^3) in monsoon months. Steeman Nielsen (1955) has suggested that algal antibiotics keep down bacteria, but phagotrophs such as dinoflagellates may well require bacteria to reach bloom proportions. Oppenheimer (1963) found that bacterial numbers were high in the Prorocentrum red tides of California, but the meaning of this is equivocal. The bacteria can live on the exocrines from the dinoflagellates and also can provide growth factors such as Vitamin B_{12}. In the present case, the decline of bacterial counts during red tide may be due to production of toxin by dinoflagellates.

The distribution of the bacterial counts showed some clustering. In order 'to judge' whether the clustering is in space or in time the method of 'analysis of variance' was applied to the logarithm of the counts. From the results given in Table 2 the variation between months (significant at 1% level) is found to be more than that between the stations. Thus the clustering appears to be relatively more in time than in space.

TABLE. 2. *Analysis of variance of logarithm of bacterial counts* (1972-73)*

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares
Between months	3.7017	9	0.4113
Between stations	0.5432	4	0.1133
Error	7.8346	36	0.2176
Total	11.9895	49	

* Counts for all the stations were available only for 10 months.

Assuming that 1 ml of surface water used for counting the bacteria from 5 stations are random samples, the sampling distribution of bacterial density is given in Fig. 5 f. This distribution has a mean (m) of 414 counts/ml with a variance (S^2) of 495 - 594. As $S^2 > m$ the distribution is characterised by over-dispersion. When overdispersion is present, a clustering of the samples in some ranges of the counts can be expected. It can be seen from Fig. 5 f that more samples occurred with counts at 50 and between the counts 650 and 850 on an average rate. Cassio (1971) had described an approximate relationship between the mean and variance of distribution with overdispersion. This gives S^2 in terms of m

as approximately $S^2 \sim m + cm^2$ where 'c' is a constant which gives the characteristics of the population. The value of 'c' works out to 2.9 in the present case. This coefficient can be used to compare different bacterial populations, as larger values of 'c' indicate a greater overdispersion.

The correlation coefficients (Table 3 a) have been worked out separately for each station, because it is possible that the linear relationship between the variables might change from station to station. The coefficients between the bacterial counts and the nitrite showed high positive values at three stations (one coefficient is significant and another highly significant). As can be seen from Table 3 a, phosphates showed positive correlation with the bacterial abundance in station 5 (one significant coefficient and three high values). While at Stations I and II, the nitrates showed some positive relationship, it did not show similar relationship for the rest. Though temperature showed a negative correlation (significant at 5% level with the bacterial abundance at Station I), it is not significant at this level for the other stations. This can largely happen when the temperature tolerance of different species of bacteria differ and when different types of bacteria become abundant at different stations. For arriving at definite conclusions further study of the various parameters affecting the individual species should be made, as the abundance of phytoplankton itself may depend on other factors noted above. It is possible that elevated phytoplankton pigments (Phaeopigments) are indicative of high zooplankton grazing on phytoplankton (Shuman and Lorenzen, 1975) which may make more organic matter available for bacterial growth. Further, it is likely that the different species of bacteria may prefer different species of phytoplankton. But according to Smith *et al.* (1977) increased photosynthesis will cause increased bacterial growth and Fuhrman *et al.* (1980) is of the view that the routes between phytosynthetic carbon fixation and bacterioplankton production are quite complex. Murchelano and Brown (1970) found the annual bacterial cycle positively correlated with the cycle of phytoplankton. In the present case it is not possible to conclude statistically whether there is a negative or positive relationship between total phytoplankton and bacterial counts. Oxygen does not show appreciable linear relationship with the bacterial counts as judged from the correlation coefficients.

Only at Station V, salinity showed (though not significantly) some correlation with the bacterial counts. This may perhaps be due to the mixing of sea water.

TABLE 3 a. *Correlation coefficient between bacterial counts and physico-chemical factors in 4 stations of the Cochin Backwater* (1972-73)

Station No.	Correlation coefficient between bacterial counts and other parameters							
	Phytoplankton	Salinity	Temperature	Oxygen	Phosphates	Nitrites	Silicates	Nitrates
I	-0.3179	-0.1136	-0.6242*	-0.0322	0.5006	0.8377*	-0.4562	-0.3947
II	-0.2111	-0.1983	-0.07995	0.2266	0.5192	0.6045*	-0.3984	0.5657
IV	-0.1734	-0.1620	-0.3106	-0.1734	0.3471	0.5119	0.0712	-0.3298
V	-0.3390	-0.2500	-0.2460	0.0664	0.6803*	-0.0763	-0.2066	0.0499

* Significant at 5% level (In Station No. III the data are not available).

TABLE 3 b. *Correlation coefficient between bacterial counts and physico-chemical factors of the Cochin Backwater (1972-73) (Calculated by pooling the 4 stations)*

Factors	Correlation coefficient
Phytoplankton	- 0.2007
Salinity	- 0.0531
Temperature	- 0.2778
Oxygen	- 0.0028
Silicates	- 0.0326
Nitrates	- 0.0399
Phosphates	0.2690
Nitrites	0.5528*

* Significant at 1% level.

Applying the test for 'homogeneity of correlation coefficients' (Rao, 1952), the correlation coefficients were found to be homogenous. Therefore, by pooling the stations a single coefficient was worked out for each parameter (Table 3 b). Here, only the nitrates showed a highly significant correlation with bacterial counts. However, with phosphate and temperature, the correlation coefficient was also rather high (significant at 1% level).

In the present study the viable bacterial counts showed seasonal fluctuations in numbers at different seasons giving a maximum in postmonsoon and premonsoon months (December 1972 to March 1973). The bacterial counts of the present study were of similar magnitude as given by Zobell (1948) from the Southern California Coast, Velankar (1955) from the Palk Bay and the Gulf of Mannar, Civic (1955) in the Adriatic Sea and Kriss (1961) in the Black Sea. Also no definite seasonal trend was noted as also reported by Velankar (1955) and Lloyd (1930). Wood (1959) found irregular seasonal distribution in the waters of Lake Macquire, but in the waters off Sydney, Brown (1964) recorded higher bacterial counts during summer and spring than at other seasons.

The results of the generic distribution of the micro-flora showed variation in the percentage composition of various bacterial genera in the two layers of water. As reported by Murchelano and Brown (1970) *Alcaligenes, Vibrio* and *Pseudomonas* dominated the bacterial flora. Wood (1953) remarked the abundance of *Micrococcus* in Australian waters which was found contradictory in the present study as *Micrococcus* contributed very little to the total bacterial flora.The enzymatic potential of the isolates of the three dominant genera assayed roughly by using the two substrates such as gelatin and starch indicated that although there was seasonal percentage variation of the three abundant heterotrophic bacterial genera, the enzymatic potential remained essentially constant. Lewis *et al.* (1971) reported the ability of the organisms to liquify gelatin, produce indole and H_2S and decompose urea to form ammonia can be used for qualifying the spoilage potential of that organism. The three dominant genera were found actively degrading gelatin and reducing sulphate indicating enzymatic spoilage potential of the three genera.

Velankar (1955) reported absence of any relationship between temperature and bacterial population. Temperature showed a negative correlation (significant at 5% level with bacterial abundance at Station I) in the present investigation. Brown (1964) found no correlation between bacterial counts and soluble organic phosphorus and counts of phytoplankton in water off Sydney, but Gundersen *et al.* (1972) reported a close correlation between the concentration of proteins and heterotrophic bacteria. In the present study, in addition to the significant correlation between the bacterial counts and nitrite and phosphate, somewhat high correlation between bacterial counts and temperature supported the view (Gundersen *et al.*, 1972) that the distribution of nutrients and temperature play an important role in the distribution of aerobic heterotrophic bacteria.

Distribution and composition of heterotrophic bacterial flora during 1974-75

Quantitative analysis : Seasonal cycle of total heterotrophic bacterial flora revealed the highest count (512 x 10^6/gm) at Station II in sediment

and in sea water (496 x 10^6/ml) in the month of August 1974 and a minimum during February 1975 in Station IV in sea water (46 x 10^6/ml) and in sediment (84 x 10^6/gm). The counts of heterotrophs were generally high during monsoon months in all the stations when fresh water influx is maximum and that any further discharge in subsequent months does not add to bacterial contribution. Altogether 296 strains were isolated, identified and classified into 8 genera (Fig. 3).

Qualitative analysis : Morphological and physiological characteristics of the isolates are studied using various biochemical reactions (Fig. 5 b). The figure representing frequency of occurrence, are self-explanatory and need no further discussion.

Frequency of occurrence of the 7 bacterial genera in 4 different families are illustrated in Fig. 3. *Alcaligenes* was the dominant bacterial genus isolated during the sampling period (1974-75). Together, *Pseudomonas, Vibrio, Flavobacterium, Photobacterium, Aeromonas* comprised 92.3%. *Bacillus* and *Micrococcus* accounted for only 7.3%.

The numbers of *Pseudomonas, Vibrio* and *Flavobacterium* in sea water were low in pre-monsoon period. In mud only, the numbers of *Vibrio* and *Flavobacterium* were low and did show seasonal changes. All the genera are comparatively more in number in sediments than in sea water.

Analysis of variance test : The study of the distribution of bacteria along with environmental parameters was subjected to 'Analysis of variance' to test whether the correlation is with space or with time. The results of analysis of variance are given in Table 4. There was no significant difference in the total plate count between stations, months and regions.

Product-moment correlation coefficient : The purpose of this study was to see which parameters were correlated to the bacterial parameters and which were not, in an attempt to identify some of the mechanisms which influence or are influenced by bacteria. To test whether there were significant independency of the hydrological factors and the different types of bacteria, the product-moment correlation coefficient between each factor were calculated.

Significant positive correlation implies that as one factor increases the other also increases and significant negative correlation implies that as one factor increases, the other factor decreases.

TABLE 4. *Analysis of variance of logarithm of bacterial counts (1974-75)*

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	Variance ratio (F)
Total heterotrophic bacteria	7.9685	127	—	—
Between stations	0.5990	5	0.1198	2.11
Between months	0.8627	10	0.0863	1.52
Between regions	0.2011	1	0.2011	3.54
Error	6.3058	111	0.0568	—

* Significant at 5% level ($P < 0.05$)
** Significant at 1% level ($P < 0.01$)

In sea water

From Table 5 it is clear that in Station I, total heterotrophic bacteria was significantly positively correlated with salinity at 5% level. The positive correlation may be due to dominance of freshwater at Station I, which is situated in the southern side of Thanneermukkom Bund.

Negative correlation of total heterotrophic bacteria with pH was significant at 5% level at Station II (Table 5).

In Stations IV and V heterotrophs showed significant negative correlation with oxygen in sea water (Table 5). But Marty (1981) provided the result opposing this view that heterotrophic aerobic bacteria were detected by him in the Arabian Sea sediments in the Gulf of Aden and Oman Sea (in November, 1978) even beyond 100 cm in core samples. Because of their facultative or microaerophilic nature, generally, it is believed that the aerobic heterotrophs are highly tolerant to anoxic conditions. The negative correlation between bacterial counts and oxygen recorded at Stations IV and V may be due to cumulative effect of some unknown factors.

In Station VI, significant positive correlation was seen at 1% level between heterotrophic bacteria and temperature (Table 5). Nedwell and Floodgate (1971) also found a positive correlation between temperature and seasonal selection of heterotrophic bacteria in an intertidal environment. However, Chan and Kueh (1976) worked out the distribution of heterotrophic bacteria related to some environmental factors in Tolo Harbour, Hong Kong and found temperature was not a limiting factor on the bacterial population even during summer months.

In sediment

In Stations I and IV total heterotrophic counts were significantly ($P < 0.05$) positively correlated with hydrogen ion concentration at 5% level (Table 6).

No significant correlations were seen in Stations III and V between total heterotrophic counts and any of the physico-chemical or bacteriological parameters.

The total counts were significantly ($P < 0.05$) positively correlated with phosphate and nitrate in Stations II and VI respectively. The complexity and variability of estuarine waters and their biota has a control over the establishment of nitrate-phosphate concentration in those waters (Johnson and Sparrow, 1970).

Ishida and Kadota (1975 a, b) analysed bacterial flora by using chemostat and the growth kinetics of bacterial population showed response only to dissolved organic substances as in the present study, but Brown (1964) found no correlation between bacterial counts and organic phosphorus in waters off Sydney.

Distribution and composition of aerobic heterotrophs during 1975-76

In water the highest count was encountered in October 1975 in Station II (256×10^6/ml) and the lowest in March 1976 (36×10^6/ml) in the same station near the Cochin Harbour.

In sediment, highest value of heterotrophic aerobes were obtained in June, 1976 in Station I, near the sewage outlet (274×10^6/ml) and the lowest was encountered in August 1975 in Station I, near the sewage outlet (274×10^6/ml) and the lowest was encountered in August, 1975 in Station II (84×10^6/ml) situated in marine environment.

TABLE 5. *Product moment correlation coefficients of all measured parameters in sea water (1974-75) in Stations I, II, III, IV, V & VI.*

Parameters	Temp.	Salinity	O_2	PO_4-P	NO_3-N	pH	TPC
STATION — I							
Temperature	1.00						
Salinity	0.13	1.00					
O_2	-0.27	-0.18	1.00				
PO_4-P	0.41	0.28	0.60[a]				
NO_3-N	-0.06	-0.71[a]	-0.14	-0.25	1.00		
pH	-0.03	-0.12	-0.29	-0.09	0.61[a]	1.00	
TPC	-0.04	0.71[a]	-0.15	0.06	-0.33	0.21	1.00
STATION — II							
Temperature	1.00						
Salinity	0.12	1.00					
O_2	-0.01	0.20	1.00				
PO_4-P	0.35	-0.08	-0.29	1.00			
NO_3-N	0.67[a]	0.42	0.18	0.51	1.00		
pH	-0.33	-0.04	-0.11	-0.00	-0.40	1.00	
TPC	0.30	-0.08	0.15	0.32	0.48	-0.65[a]	1.00
STATION — III							
Temperature	1.00						
Salinity	0.31	1.00					
O_2	-0.48	-0.44	1.00				
PO_4-P	0.90[c]	0.15	-0.60	1.00			
NO_3-N	0.45	0.42	-0.28	0.42	1.00		
pH	0.31	-0.54	0.07	0.39	0.25	1.00	
TPC	0.07	-0.54	-0.35	0.45	-0.23	0.24	1.00
STATION — IV							
Temperature	1.00						
Salinity	-0.41	1.00					
O_2	0.300	0.29	1.00				
PO_4-P	-0.27	0.44	-0.11	1.00			
NO_3-N	-0.65[a]	0.38	-0.13	0.42	1.00		
pH	0.06	-0.54	-0.44	-0.35	-0.61[a]	1.00	
TPC	0.22	-0.21	-0.63[a]	0.31	-0.01	0.04	1.00
STATION — V							
Temperature	1.00						
Salinity	0.38	1.00					
O_2	-0.24	-0.13	1.00				
PO_4-P	0.69[a]	0.27	-0.29	1.00			
NO_3-N	0.10	0.02	0.43	0.03	1.00		
pH	0.21	0.10	0.17	0.47	-0.61[a]	1.00	
TPC	0.77[b]	-0.08	-0.67[a]	-0.21	-0.54	-0.01	1.00
STATION — VI							
Temperature	1.00						
Salinity	0.46	1.00					
O_2	0.30	-0.13	1.00				
PO_4-P	0.31	0.07	-0.59	1.00			
NO_3-N	0.28	-0.14	-0.05	0.35	1.00		
pH	-0.29	0.03	-0.53	0.28	-0.09	1.00	
TPC	-0.11	-0.08	-0.05	-0.55	-0.59	-0.54	1.00

TABLE 6. *Product moment correlation coefficients of all measured parameters in sediments (1974-75) in Stations I, II, III, IV, V & VI*

Parameters	Temp.	Sal.	O_2	PO_4-P	NO_3-N	pH	TPC
STATION — I							
Temperature	1.00						
Salinity	0.29	1.00					
O_2	-0.38	0.31	1.00				
PO_4-P	0.58	-0.04	-0.51	1.00			
NO_3-N	0.53	-0.20	-0.47	0.70[a]	1.00		
pH	0.07	-0.05	-0.37	0.80[b]	0.52	1.00	
TPC	-0.12	0.21	0.07	-0.32	0.10	0.68[a]	1.00
STATION — II							
Temperature	1.00						
Salinity	0.47	1.00					
O_2	-0.25	0.01	1.00				
PO_4-P	0.41	0.46	0.10	1.00			
NO_3-N	0.20	0.01	0.18	0.38	1.00		
pH	-0.03	- 0.27	-0.12	0.32	-0.41	1.00	
TPC	0.05	-0.04	0.29	0.61[a]	0.26	-0.09	1.00
STATION — III							
Temperature	1.00						
Salinity	0.42	1.00					
O_2	-0.76[b]	0.34	1.00				
PO_4-P	0.73[a]	0.48	-0.69[a]	1.00			
NO_3-N	-0.14	0.09	-0.04	0.05	1.00		
pH	0.67[a]	-0.09	-0.62[a]	0.67[a]	-0.34	1.00	
TPC	-0.07	0.35	0.11	0.39	-0.13	0.37	1.00
STATION — IV							
Temperature	1.00						
Salinity	0.44	1.00					
O_2	-0.23	0.08	1.00				
PO_4-P	-0.05	-0.01	-0.00	1.00			
NO_3-N	0.25	-0.00	0.29	0.80[b]	1.00		
pH	0.11	-0.68[a]	-0.39	0.05	0.04	1.00	
TPC	-0.13	-0.48	0.03	0.01	0.19	0.61[a]	1.00
STATION — V							
Temperature	1.00						
Salinity	0.47	1.00					
O_2	0.12	0.11	1.00				
PO_4-P	-0.11	-0.08	-0.35	1.00			
NO_3-N	0.11	-0.12	0.25	0.39	1.00		
pH	0.37	-0.18	0.12	-0.16	0.28	1.00	
TPC	0.52	-0.22	0.37	0.55	0.29	0.55	1.00
STATION — VI							
Temperature	1.00						
Salinity	0.58	1.00					
O_2	0.41	0.11	1.00				
PO_4-P	0.08	0.21	-0.33	1.00			
NO_3-N	0.19	0.25	-0.54	0.57	1.00		
pH	0.15	-0.44	0.35	0.14	-0.11	1.00	
TPC	0.40	-0.26	0.22	0.16	0.62[a]	0.44	1.00

TABLE 7. *Analysis of variance of logarithm of total heterotrophic bacterial counts (1975-76)*

Source of variation	Sum of squares	Degree of freedom	Mean sum of squares	Variance ratio (F)
Total heterotrophic bacteria	2.1888	71	—	—
Between stations	0.3063	2	0.1532	6.96*
Between regions	0.2279	1	0.2279	10.36**
Between months	0.5316	11	0.0483	2.20
Between stations x regions	0.1026	2	0.0513	2.33
Between stations x months	0.3318	22	0.0151	0.69
Between regions x months	0.2051	11	0.0186	0.85
Error	0.4835	22	0.0220	—

* Significant at 5% level ($P < 0.05$)
** Significant at 1% level ($P < 0.01$)

The seasonal variations of heterotrophs in sea water, was meagre, whereas in sediments it was prominent during monsoon (Station I) and postmonsoon months (Stations II and III).

Altogether, 282 pure strains were maintained after isolation for further identification and for a brief study physiologically. The morphological and physiological characteristics of the isolates are summarised in the Fig. 4 c. Almost all the isolates (270, 96.1%) were asporogenous gram-negative rods usually pleomorphic. Gram-positive bacteria isolated were only 4.9%. Motile bacteria were more abundant (78.2%) than non-motile bacteria. Gelatinolytic activity was found to be more than starch hydrolysis and the genus *Alcaligenes* was found to be very active in the proteolytic process.

The observations from the analysis of variance test (Table 7) showed that the total plate count has shown significant difference between stations ($P < 0.01$) and between regions ($P < 0.01$). Station III was having significantly higher counts in bottom water and in surface sea water. The variation between months were not significant at 5% level. Also, none of first order interactions was significant at 5% level.

The matrix of correlation framed showed in Station I, no correlation was seen between any of the chemical and microbiological parameters with total heterotrophic counts (Table 8). In Station II, significant ($P < 0.01$) negative correlation was seen with temperature, which showed the true phychrophilic nature of the marine bacteria isolated from Cochin Harbour area. Moreover, this type of inverse association analysis may indicate groups of bacteria particularly useful for comparative physiological ecology. Although, both normal and inverse forms of association analysis may

TABLE 8. *Product moment correlation coefficients of all measured parameters in sea water (1975-76) in Stations I, II, III*

Parameters	Temperature	Salinity	O_2	PO_4-P	NO_3-N	SO_3-Si	Org. Carbon	Org. Nitrogen	TPC
STATION I									
Temperature	1.00								
Salinity	0.38	1.00							
O_2	-0.36	-0.68[a]	1.00						
PO_4-P	0.37	-0.27	0.03	1.00					
NO_3-N	0.50	0.69[a]	-0.51	-0.15	1.00				
SO_3-Si	-0.15	-0.76[b]	0.22	0.28	-0.52	1.00			
Org. Carbon	0.57[a]	0.13	-0.30	0.69[a]	0.05	0.04	1.00		
Org. Nitrogen	-0.48	-0.32	0.21	0.26	-0.62[a]	0.30	-0.02	1.00	
TPC	-0.38	0.27	-0.05	-0.48	-0.11	-0.33	-0.28	0.06	1.00
STATION II									
Temperature	1.00								
Salinity	0.57	1.00							
O_2	-0.53	-0.51	1.00						
PO_4-P	0.71[b]	0.50	-0.65[a]	1.00					
NO_3-N	0.66[a]	0.79[b]	-0.35	0.59[a]	1.00				
SO_3-Si	-0.01	-0.55	0.19	0.01	-0.35	1.00			
Org. Carbon	-0.08	-0.62[a]	0.18	-0.08	-0.25	0.73[b]	1.00		
Org. Nitrogen	-0.01	-0.26	0.50	-0.30	-0.04	-0.12	-0.02	1.00	
TPC	-0.31	-0.16	0.01	0.05	-0.31	0.08	-0.23	-0.32	1.00
STATION III									
Temperature	1.00								
Salinity	0.50	1.00							
O_2	0.47	0.39	1.00						
PO_4-P	0.12	0.12	-0.42	1.00					
NO_3-N	-0.30	-0.19	-0.70[a]	0.27	1.00				
SO_3-Si	-0.12	0.23	-0.29	0.63[a]	0.47	1.00			
Org. Carbon	0.06	0.33	0.16	0.31	-0.04	0.66[a]	1.00		
Org. Nitrogen	0.31	0.41	0.58[a]	-0.42	-0.40	-0.25	0.02	1.00	
TPC	-0.11	-0.29	-0.08	-0.04	-0.10	0.17	-0.17	-0.09	1.00

be ecologically meaningful as separate analysis, it may be of interest to examine the extent to which these saprophytic heterotrophs are tied up with their habitat.

A thorough analysis of the data concerning morphology, physiology and biochemical activity of micro-organisms has revealed that they are structurally and biochemically complex organisms. They may rapidly adapt themselves to different environmental conditions, because of their ability to form adaptive (induced) enzymes produced by the influence of new substrates of the surrounding environment consequently, the production of adaptive enzymes may cause a change in the character of metabolism and biological functions of the new variants which needs a further study.

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