Culturable heterotrophic bacteria from the euphotic zone of the Indian Ocean during the summer monsoon

Heterotrophic culturable bacteria
Physiological properties
Euphotic zone
Indian Ocean

Bactéries hétérotrophes cultivables
Caractéristiques physiologiques
Couche euphotique
Océan Indien

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ABSTRACT

Cuturable heterotrophic bacterial counts (viable counts: VC) by surface plating on ZoBell medium and total bacterial counts (TC) by epifluorescence microscopy were carried out in sea water off the southwest coast of India during the monsoon. TC were three orders of magnitude higher than VC. *Pseudomonas/Alteromonas* group I was dominant in both nearshore and offshore regions. Pigmented bacteria were more abundant in offshore stations and showed multiple antibiotic resistance. Statistically there was found to be no significant differences in bacterial number and their activities between offshore and nearshore isolates. Nevertheless, the former tend to be more versatile and resistant to metals and drugs.

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RÉSUMÉ

Bactéries hétérotrophes des couches euphotiques de l'Océan Indien pendant la mousson d'été

Les bactéries hétérotrophes et cultivables (viable counts : VC) et les nombres totaux (total counts : TC) ont été déterminés par dénombrement de colonies en milieu gélosé et par microscopie en épifluorescence, dans les échantillons d'eau de mer prélevés pendant la mousson au large de la côte sud-ouest de l'Inde. Les nombres totaux sont mille fois plus élevés que les nombres viables. *Pseudomonas/Alteromonas* groupe I domine dans les échantillons prélevés dans les eaux littorales et océaniques. Les bactéries pigmentées sont plus abondantes dans les stations océaniques, et sont caractérisées par les résistances aux antibiotiques. Il n'y a pas de différence statistiquement significative entre la quantité et la qualité des bactéries littorales et océaniques. Cependant les bactéries océaniques sont plus versatiles et résistantes aux métaux et aux antibiotiques.

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INTRODUCTION

Studies on bacterial ecology in Indian waters, as elsewhere, have in the past been carried out with the culturable fractions of microorganisms. Although these viable fractions form only a small fraction of total microorganisms, they can still be used for a comparison of data representing different times of the year or different areas (Van Es and Meyer-Reil, 1982). Moreover, these culturable fractions are amenable to laboratory experiments designed to elucidate certain aspects of ecological phenomena. Hence the distribution of heterotrophic bacteria in waters off the west coast of India has been investigated during the hitherto unstudied season, *viz.* the southwest monsoon. The primary aim was to estimate total *vis-à-vis* viable counts and to define certain differences in the physiological and biochemical properties of nearshore and offshore isolates during this season. Given the various implications of widespread use of drugs and metal-based compounds, the incidence of resistance/tolerance to these substances among the bacterial isolates has also been examined.

MATERIALS AND METHODS

During the 33rd cruise of RV *Sakar Kanya*, twenty-one stations were sampled for bacteriological studies (Fig. 1), of which fourteen stations, suffixed 1 and 2, have been referred to as "coastal" in the present study on the basis of their distance from the shore. Chemical parameters (salinity, phosphate, nitrate, nitrite, ammonia and chlorophyll) were estimated using standard methods (Strickland and Parsons, 1972). Sea water was collected from three depths (5, 50 and 100 m) using ZoBell's bacteriological samplers. VC were enumerated by spread plate technique (Simidu *et al.*, 1983) using ZoBell's marine agar. The plates were incubated at 20°C for five days. The colony-forming units (CFU) were expressed as no.ml^{-1}. TC were obtained by epifluorescence microscopy using DAPI (Kirchman *et al.*, 1982; Porter and Feig, 1980).

In all, 132 isolates from ZoBell medium were randomly selected from various stations. The purified isolates were identified on the basis of Simidu's scheme (Simidu, 1985). The isolates were tested for the various physiological and biochemical characteristics listed in the tables. The ability to grow in 0-10 % (w/v) NaCl, at 4, 10, 40 and 50°C, in the presence of 0.1 mg.l^{-1} of heavy metals and antibiotics was recorded after four days of incubation on nutrient medium [composition: 0.0015 % yeast extract, and 0.015 % peptone in 9-salt solution (Hermansson *et al.*, 1987)]. A carbon utilization study was carried out using a modified basal medium with filter-sterilized sugars (2 % w/v). The hydrolysis of polymers such as starch, chitin, casein and gelatin was studied by supplementing the marine agar medium with the respective polymers (2 % w/v). Biochemical tests including the production of sulphide and ammonia from peptone broth and the reduction of nitrate were carried out as described in the Manual of Methods for general Microbiology (Gerhardt *et al.*, 1981).

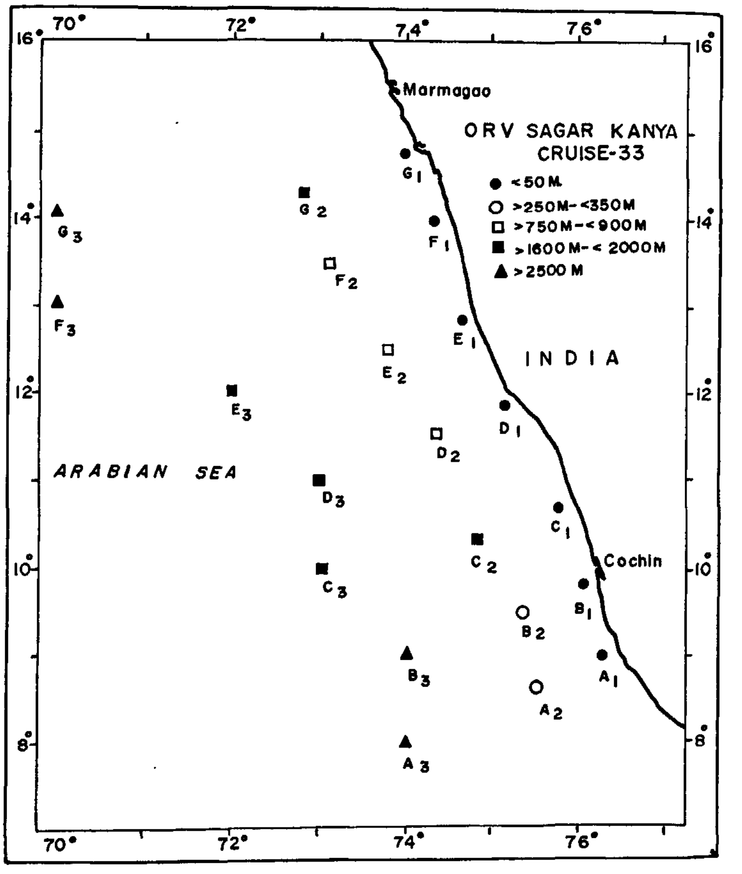


Figure 1

Microbiological stations in the Arabian Sea (A-G transect).

RESULTS

Physico-chemical parameters

Table 1 summarizes the hydrophysical and chemical data of all the stations investigated during the cruise. There were no significant changes in their range and vertical distribution. This suggests that the surveyed area was a mixed layer during the period of investigation.

Bacterial numbers

The distribution of direct and viable bacterial counts is presented in Figure 2. Direct counts were three orders higher than the viable counts. There was no particular trend with distance from the shore. The highest number was not restricted to the surface layer, but varied from station to station. There was no correlation between direct counts and CFU, except at the 50 m depth, where significant correlation was observed ($r = 0.99$, $P < 0.001$). The viable bacterial density of the offshore stations showed correlation with the inshore stations (r =

Table 1

Physico-chemical parameters off the west coast of India.

Parameters	Coastal	Offshore
Salinity (x 10^{-3})	33.61-36.92	34.98-36.66
Temperature (°C)	17.81-29.42	20.85-29.48
Phosphate (µg.at.L^{-1})	0.03-0.34	0.17-0.46
Ammonia-N (µg.at.L^{-1})	0.05-0.34	0.05-0.65
Nitrate-N (µg.at.L^{-1})	0.18-26.50	6.82-23.30
Nitrite-N (µg.at.L^{-1})	0.03-0.50	0.06-0.37
Chlorophyll (mg.m^{-3})	0.03-0.23	0.03-0.19

0.46, P < 0.05), whereas no such tendency was observed with respect to direct bacterial number.

Physiological characteristics

The ability to utilize simple sugars as the sole source of carbon varied with the sugars tested (Tab. 2). Some 60 % of the isolates could survive on one or another sugars. The percentage of sugar utilizers varied in offshore and coastal waters but was not significant.

The isolates were more tolerant to higher temperatures with 10.9 % showing growth at 10°C. Most of the isolates could grow over a wide range of NaCl (Tab. 2). The coastal isolates (64.5 %) could grow in a wider range of NaCl concentration compared to those offshore. Isolates that could grow only in a narrow range of NaCl concentration were equally distributed in the coastal and offshore regions (not shown).

Generic composition

Some 10 % of the total isolates were lost during the processes of purification and subculturing. The remainder were all Gram-negative and were grouped into eight genera. *Pseudomonas/Alteromonas* group I was the dominant group in both regions [41.9 and 33.3 % (Tab. 3)]. The generic composition was fairly consistent except that the pigmented *Flavobacterium* and *Chromobacterium* were confined to the offshore stations. The total percentage of pigmented bacteria was 37.5 %, of which 78.9 % were from the offshore area. Of the offshore *Pseudomonas/Alteromonas*, 78.9 % were pigmented.

Biochemical characteristics

There was no significant variation in activities between nearshore and offshore isolates (Fig. 3). The variation was, however, significant for amylase and ammonia production (P < 0.05). *Aeromonas* of the coastal region exhibited chitinase activity whereas those of the offshore regions were devoid of it. Offshore *Vibrio* and *Alcaligenes* were positive for chitin hydrolysis [15.4 and 100 % (Fig. 4)]. Although the percentage of activity in the coastal waters was higher compared to that in offshore waters, the genera of the latter were more diverse and versatile in their ability to degrade polymers.

Most of the isolates (95.1 %) were resistant to antibiotics (Fig. 5). The percentage of resistance was maximum in the case of penicillin (β lactam). Equal proportions of offshore isolates were inhibited by kanamycin and gentamycin. Least

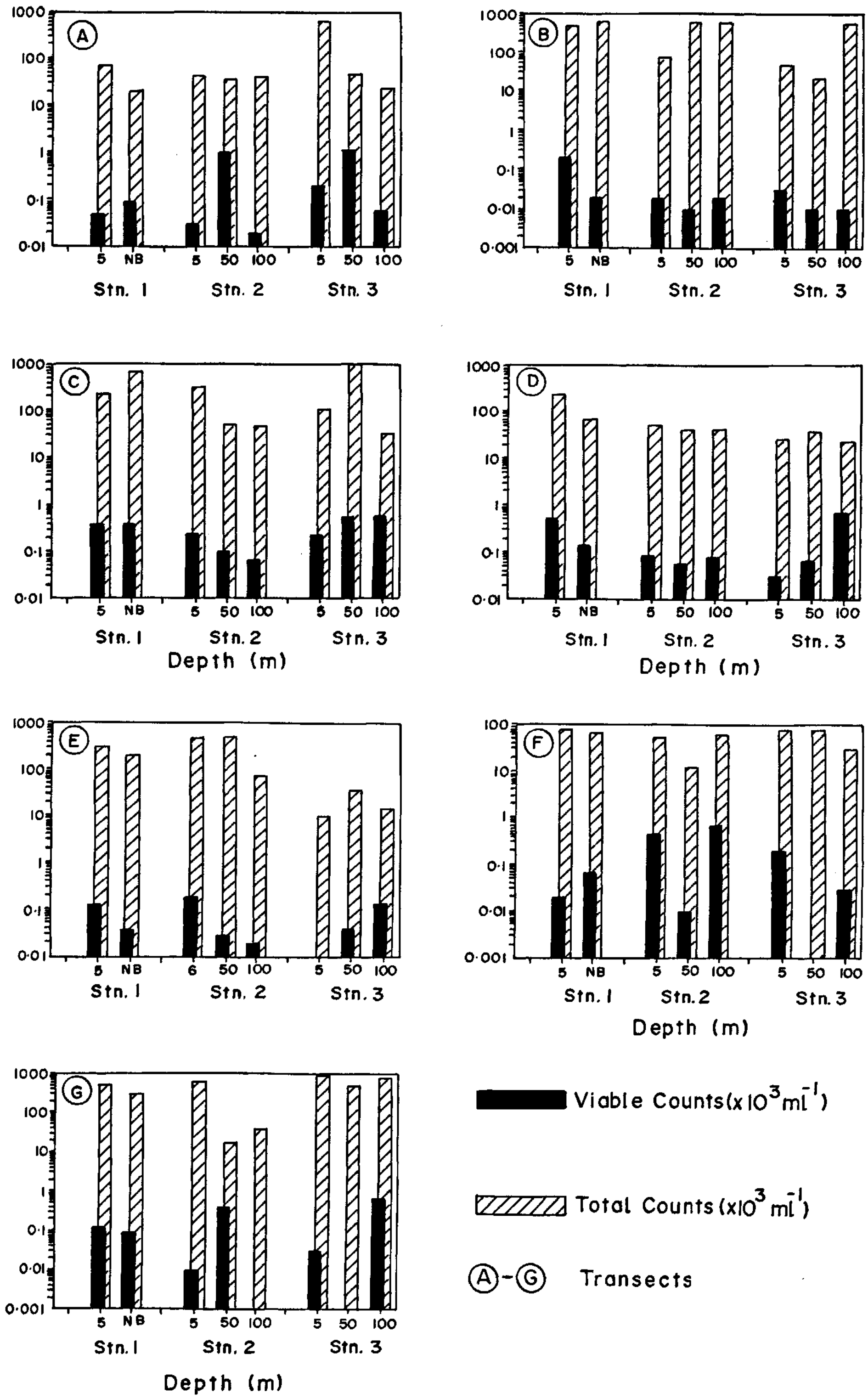
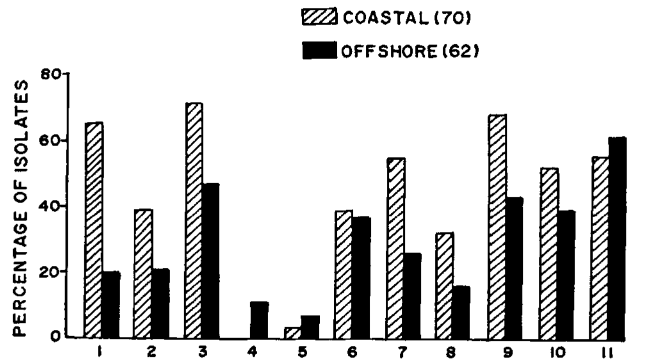


Figure 2

Bacterial number in the Arabian Sea.



1. STARCH HYDROLYSIS. 2. TWEEN 80 HYDROLYSIS. 3. TWEEN 20 HYDROLYSIS. 4. TRIBUTYRIN HYDROLYSIS. 5. CHITIN HYDROLYSIS. 6. CASEIN HYDROLYSIS. 7. GELATIN LIQUEFACTION. 8. HYDROGEN SULFIDE PRODUCTION. 9. NITRATE REDUCTION. 10. UREA HYDROLYSIS. 11. AMMONIA PRODUCTION.

Figure 3

Comparison of biochemical activities of bacteria.

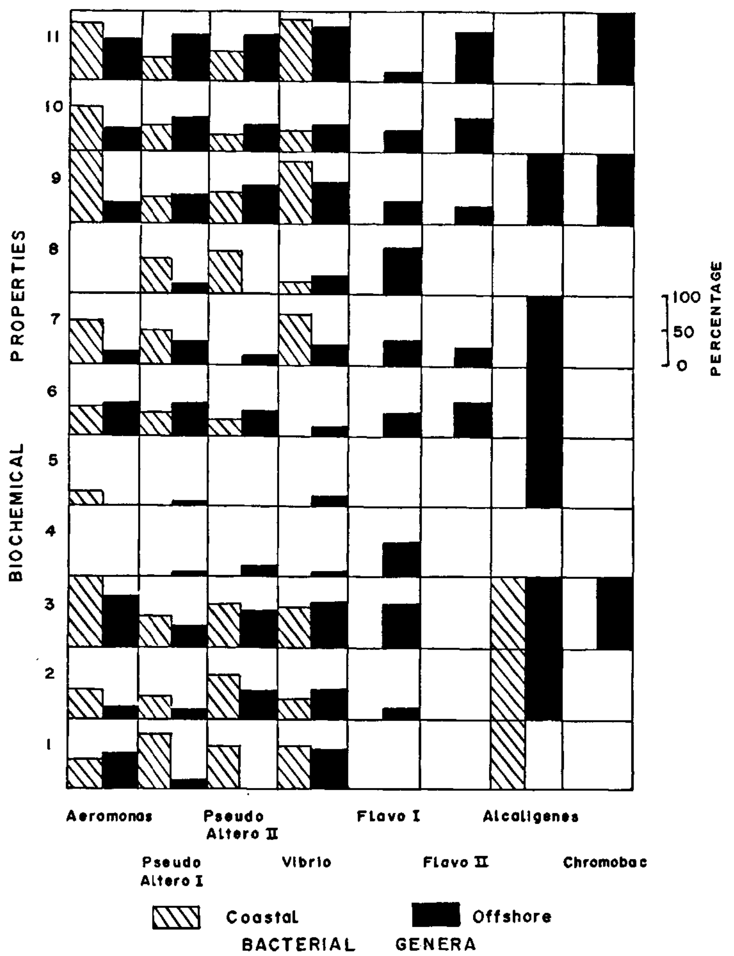


Figure 4

Comparison by genera of biochemical activities of bacteria (activities as in Fig. 3).

resistance was observed with chloramphenicol (3.5 %). Multiple resistance (five antibiotics) was exhibited by 6.8 % of the isolates (Fig. 6). Resistance to three or more antibiotics was greater in the offshore isolates and relatively higher in the case of pigmented bacteria. Metal tolerance, compared to antibiotic resistance, was seen in a higher frequency (Fig. 5). There was no definite demarcation in the resistance to different metals.

Table 2

Physiological characteristics of isolates from the west coast of India.

Characteristics	Percentage of isolates Coastal (70)	Offshore (62)
Sugar utilization		
Ribose	77.0	70.2
Arabinose	74.2	59.7
Maltose	64.5	68.4
Galactose	61.3	61.4
Glucose	83.9	86.0
Fructose	61.3	71.9
Lactose	58.1	64.9
Sucrose	87.1	68.4
Manitol	74.2	63.2
Lactate	61.3	61.4
Malate	74.0	64.9
Growth in % NaCl (w/v)		
0	77.4	87.7
0.1	83.9	96.5
0.5	83.9	100
1.0	96.8	100
2.0	96.8	100
3.0	87.1	86.0
5.0	77.4	63.2
10.0	61.3	40.4
Temperature (°C)		
4	-	-
10	12.9	12.3
40	96.8	93.0
50	45.8	47.4

Table 3

Percentage of generic composition of bacterial isolates from the sea water off the west coast of India.

Genus	Coastal	Offshore
Aeromonas	16.1	12.3
Pseudomonas/Alteromonas group I	41.9	33.3
Pseudomonas/Alteromonas group II	16.1	10.5
Vibrio	22.5	22.8
Flavobacterium group I	0.0	10.5
Flavobacterium group II	0.0	7.0
Alcaligenes	3.2	1.8
Chromobacterium	0.0	1.8

DISCUSSION

The limitations and advantages of different methods for enumerating bacterial numbers have been critically reviewed by Van Es and Meyer Reil (1982). In ecological studies of heterotrophic bacteria, direct and viable counts have been used for enumeration. The viable count is the number of culturable bacteria in a population, and in our study this was three orders of magnitude less than the total count (Fig. 2). Total counts were 10^4-10^5 in range. A

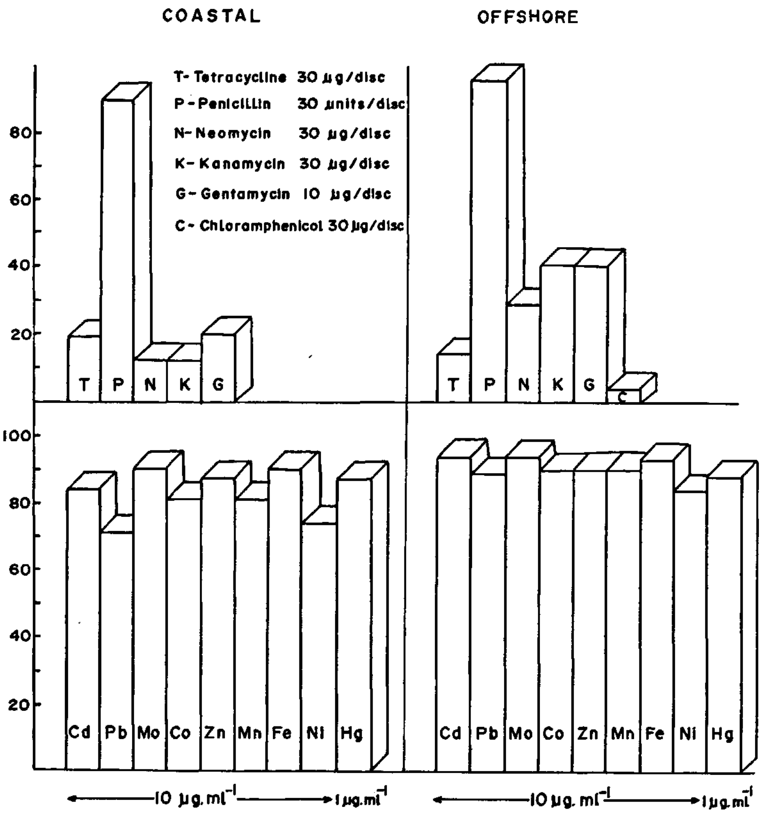


Figure 5

Comparison of metal- and drug-resistant bacteria from coastal and offshore waters.

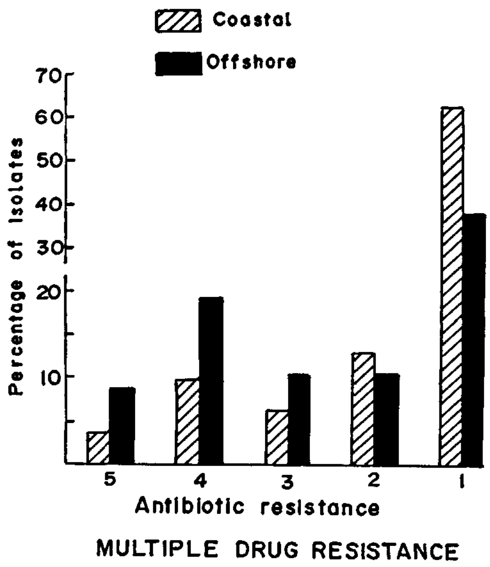


Figure 6

Multiple drug resistance in bacteria (1-5 shows the number of antibiotics to which the coastal and offshore isolates were resistant).

similar range has been reported from South China Sea and Tokyo waters (Simidu *et al.*, 1983; Fukami *et al.*, 1983). A higher range of 10^6-10^7 from the waters around Tokyo have been reported by Kogure *et al.* (1980). The viable count was comparable to that observed in other regions. Total counts were about the same as those reported from Tokyo bay and elsewhere (Kogure *et al.*, 1980; Watson *et al.*, 1977) but less than those found in other waters (Van Es and Meyer Reil, 1982; Zimmermann *et al.*, 1980). The low numbers found in the present study could be due to grazing by the large number of ciliates observed during sampling (Madhupratap *et al.*, 1992). Protozoan bacterivory involves complex predator-prey interactions, including differential feeding preferences for freely suspended or particle-attached bacteria (Caron, 1987; Albright *et al.*, 1987), and the importance of phagotrophic protozoa as grazers of suspended bacteria is now undisputed (Sherr and Sherr, 1989).

Ramaiah (1989) showed that viable counts declined with distance from shore and depth during more stable pre- and post-monsoon conditions. No such trend was observed in the present distribution of the number of bacteria enumerated by both the techniques. This is partly indicated by correlation in the densities of populations between offshore and nearshore stations. Despite progress in measuring the rate of production and the role of these bacteria in the heterotrophic processes, we are still uncertain of the generic composition of part of the culturable fraction of the bacterial community. However it is to be emphasized that their ecological significance cannot be overlooked (Olsen and Bakken, 1987). *Pseudomonas/Alteromonas* group I was the dominant genus in both nearshore and offshore areas in our study (Tab. 3), and was found to be highly versatile in the hydrolysis of the various substrates. The slight difference in the activity of the offshore *Pseudomonas/Aletromonas* subgroup as compared to the nearshore forms could be attributed to the autochthonous nature of the flora in the offshore regions (Fig. 4).

Vibrios contribute 10-50 % of the total heterotrophic bacteria (Simidu and Tsukamoto, 1985), and their ability to grow in conditions of low nutrient concentration and environmental stress make them a major group in the marine ecosystem. In our study, *Vibrios* were detected (22 %) in both coastal and offshore stations, those of the latter being more versatile in the degradation of various polymers. The chitinoclastic ability was associated with the offshore Vibrios (Fig. 4). The *Aeromonas* which occurred in both coastal and offshore regions, survive over a temperature range of 13-20°C and can grow at limnetic condition (Williams and LaRock, 1985). As mentioned above, the isolates failed to grow at lower temperatures but were active. A similar observation was reported by Simidu and Tsukamoto (1985).

In the present sudy, pigmentation was associated with offshore strains. The genera *Flavobacterium* and *Chromobacterium* were restricted to the offshore waters. Many of the antibiotic-resistant strains were also metal-resistant in our study. The number of metal-resistant strains was higher than that of strains resistant to antibiotics tested. Nakahara *et al.* (1977) reported that the frequency of metal resistance was higher than that of antibiotic resistance and that most metal-resistant strains showed multiple metal and drug resistance. The association of antibiotic resistance and metal tolerance to pigmentation has been discussed by Hermansson *et al.* (1987). Antibiotic-resistant marine bacteria are not limited to coastal waters (Allen *et al.*, 1977; Silva and Herique, 1980; Timoney *et al.*, 1978) but have also been reported from Antarctica (Kabori *et al.*, 1984). The pigmented strains showed higher multiple resistance but the latter was not restricted to any genus (Allen *et al.*, 1977). Nair *et al.* (1992) have demonstrated how the pigmented and non-pigmented isolates from these waters responded differently to both metals and antibiotics, with pigmented bacteria showing resistance to antibiotics particularly at higher concentrations. Thus it appears that many factors are involved in addition to environmental stress. In our study we found no specific difference between the metal- and drug-resistant strains from the two areas. The hydrography and circulation patterns off the west coast of India during this cruise indicate that the mixed layer was observed to extend down to 70 and 20 m in the offshore and nearshore regions respectively (Shetye *et al.*, 1990). It can be inferred that coastal strains have drifted into the offshore location and survived successfully during the monsoon.

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