

## Microbiological Studies on the Sediments of Andaman Sea

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Total bacterial population in the sediments widely fluctuated from  $2.1 \times 10^3$  to  $3.7 \times 10^6$ . There was no significant correlation between organic carbon values and heterotrophic activity. Ratio of gram positive to gram negative bacteria was 1:15.4 and the majority of isolates (51.1%) were coccobacilli followed by pleomorphic forms (26.26%). Most of these isolates utilised sugars, alcohols and carboxylic acids for their growth.

Information about different aspects of marine bacteria in Indian Seas is rather fragmentary<sup>1-7</sup> and some areas such as the Andaman Sea have not been studied previously.

During the oceanographic cruises 51 and 52 of the *R V Gaveshani*, in Feb. 1979, a number of sediment samples have been collected from different parts of the Andaman Sea (Fig. 1) and analysed for microbiological characteristics. The present communication deals with certain aspects of the distribution and physiology of heterotrophic bacteria.

### Materials and Methods

Sediment samples obtained using a van Veen Grab from selected stations and central portions were transferred to sterile glass bottles and stored on board at  $0 \pm 1^\circ\text{C}$  for further analysis. Serial dilutions of the sediment samples were made in sterilised sea water, surface plated on agar medium<sup>8</sup> for total counts of bacteria and incubated at  $29 \pm 2^\circ\text{C}$  for 4 days. Counting was done on a colony counter and the populations were expressed on wet weight basis. Colonies were isolated at random from each plate for further studies and checked for their purity. The cultures were maintained on marine-agar slants.

For morphological characterisation of isolate shape, size, appearance, colour and margin of the colony Gram staining and spore staining of the cells were done. Organisms were tested for motility in motility-agar. After morphological characterisation, isolates were tested for oxidase production, nitrate reduction, gelatin liquification, glucose fermentation and catalase, cellulase, amylase and pectinase activity.

Ability of isolates to grow at temperature  $10-15^\circ\text{C}$ , pH 4-10 (adjusted with HCl and NaOH) and salinity 7 to 20‰ (adjusted by diluting the sea water) was also tested. Effect of low oxygen tension on bacterial isolates was recorded by stab-inoculating into marine-agar butts<sup>9</sup>.

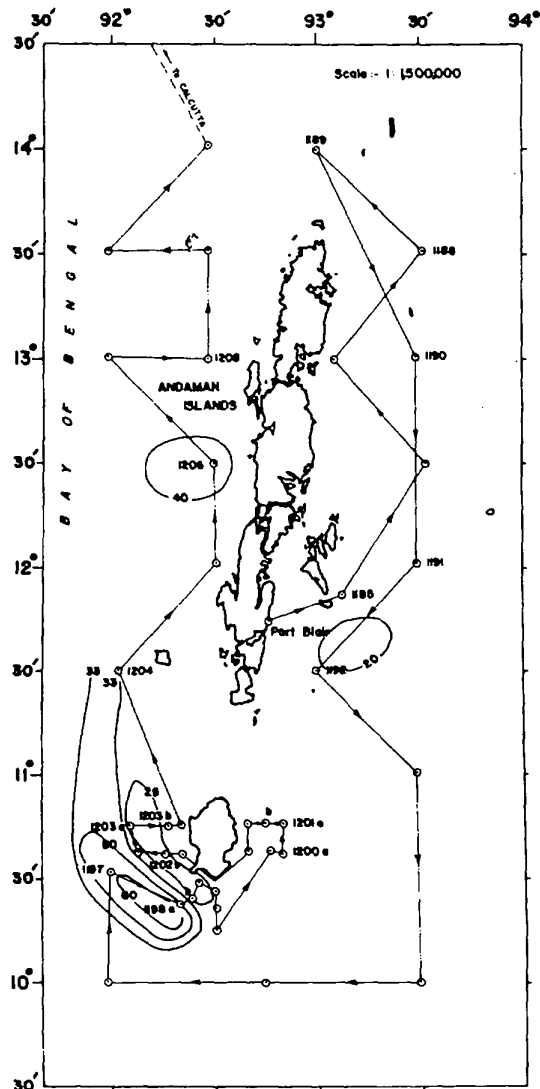


Fig. 1—Locations of sampling stations and zonation of phenol degrading bacteria

Sterilized organic compounds at 1% in basal medium were used as a source of nutrition for bacterial isolates. Compounds tested were glucose, fructose, ethanol, butanol, glycerol, acetic acid, citric acid and phenol. Results were recorded after 7 days incubation at room temperature ( $29 \pm 2$  C). Standard methods were used for all types of characterisations<sup>9</sup>. Organic carbon, total nitrogen and carbohydrate in oven-dried (80 C) and finely powdered sediment samples were determined. Routine methods were employed for other analyses<sup>10, 12</sup>.

**Results**

Maximum heterotrophic bacterial counts were

recorded at st 1200 a where organic carbon was also maximum and minimum at st 1179 where carbohydrate-C concentrations were high in the sediment (Table 1). Generally, where the sediments consisted of yellowish sand, organic carbon values were high (Table 1). Low values of organic carbon were recorded at sts 1185 and 1202 b. Total nitrogen content was maximum at st 1191 and minimum at sts 1206 and 1208. Carbohydrate was lowest at st 1203 b.

Two hundred and thirteen bacterial colonies were isolated initially, of which 180 remained viable during purification and these were used for characterisation studies (Tables 2 and 3).

Table 1 - Biotic and Abiotic Environment in the Andaman Sea (Sediments)

St. No.	Bacterial counts: g wet wt	Sediment C mg: g	Sediment N mg: g	Sediment carbo-hydrate mg: g	Temp. C	Sal. ‰	DO ml: litre	Depth m	Nature of bottom deposits	Meio-faunal density (Ref. 14)
1179	$2.1 \times 10^3$	6.6	0.45	12.62	-	-	-	300	sand, coralline, greenish	204
1185	$3.2 \times 10^5$	4.5	0.38	2.7	27.9	31.88	4.82	32	" " white	390
1188	$1.2 \times 10^5$	10.25	0.95	4.53	5.73	34.89	1.6	1300	clay, yellowish	322
1189	$3.5 \times 10^5$	7.2	0.62	10.51	10.95	34.98	0.45	380	sand, silty	-
1190	$2.1 \times 10^4$	8.8	0.59	5.296	5.02	34.84	1.75	2150	clay, yellowish	68
1191	$1.1 \times 10^4$	13.5	1.25	3.94	5.8	34.85	1.75	1650	clay, greenish	238
1192	$5.2 \times 10^5$	7.2	0.45	9.728	9.8	35.00	1.30	600	clay and coralline sand	436
1197	$2.3 \times 10^5$	7.8	0.45	3.02	6.78	34.90	0.86	1300	clay, yellowish	238
1198a	$1.1 \times 10^5$	5.3	0.45	2.62	8.6	34.92	-	710	sand, coralline	68
1198b	$2.5 \times 10^3$	6.9	0.56	2.12	-	-	-	470	-	68
1200a	$3.7 \times 10^6$	14.8	0.79	8.58	9.35	34.93	-	1500	clay, yellowish	438
1201a	$2.3 \times 10^4$	13.5	0.98	9.4	-	-	-	1440	-	254
1201b	$1.1 \times 10^5$	10.25	0.76	6.89	-	-	-	1225	clay, yellowish	136
1202b	$1.6 \times 10^5$	4.5	0.45	3.56	-	-	-	73	sand, coralline	204
1203a	$5.6 \times 10^3$	9.5	0.38	1.4	-	-	-	825	clay, sand admixture	340
1203b	$3.2 \times 10^6$	5.2	0.45	0.45	-	-	-	140	do	340
1204	$5.1 \times 10^4$	12.4	0.84	1.8	6.83	34.54	1.34	1200	clay	170
1206	$1.4 \times 10^4$	4.5	0.35	3.8	25.38	34.29	2.63	80	sand with clay balls	272
1208	$1.3 \times 10^6$	6.4	0.35	4.53	22.95	34.44	2.6	85	sandy mud	390

Table 2 - Colony and Cell Morphology of Bacterial Isolates

Shape		% of isolates	
Shape	Circular	66.66	Gram +
	Irregular	19.44	
	Spreading type	8.33	Gram -
	Branching type	5.55	
Size	< 4.5 mm	25.0	Cocci
	4.5-5.5 mm	69.44	Coccobacilli
	> 5.5 mm	5.55	
Appearance	Opaque	58.33	Rods
	Transparent	8.33	
	Translucent	17.77	Sporulating
	Granular	15.55	Pleomorphic
Colour	Colourless	22.22	Motility
	White	61.11	
	Pink	8.33	
	Creamish (yellow)	8.33	
Margin	Smooth	62.62	
	Entire	25.0	
	Rough	12.77	

Table 3-- Biochemical Characteristics, Growth Requirements and Utilization of Carbon Sources in Bacterial Isolates (values represent percent of isolates)

Biochemical characteristics		Growth requirement		Utilization of C-sources	
Oxidase positive	66.22	Temp. 10-15 C	45.5	Glucose	94.44
H <sub>2</sub> S formers	34.44	pH 4	19.44	Fructose	93.33
Nitrate reducers	95.55	6	23.33	Ethanol	66.66
Gelatin liquifiers	73.88	8	100	Butanol	61.11
Glucose fermentators	86.11	10	61.11	Glycerol	42.22
Catalase producers	100	Sal. 7-10‰	82.22	Acetic acid	51.11
Cellulase producers	80.55	17-20‰	100	Citric acid	54.44
Amylase producers	84.44	Low oxygen tension	100	Phenol	13.88
Pectinase producers	40				

### Discussion

Majority of organism were Gram-negative and non-motile (Table 2). Bacteria cultured from these sediment samples exhibited wide range of physiological activities. More than 50% were nitrate reducers, gelatin liquifiers, catalase, amylase and cellulase producers (Table 3). Sieburth<sup>13</sup> and Rheinheimer<sup>7</sup> reported similar bacterial types from different marine environments. The nature of the sediment in this region varied between coralline sand and clay. At sts 1204, 1206 and 1208 the bottom was sandy clay and this appears to favour the distribution of heterotrophic bacteria (Table 1) as far as total counts are concerned.

Although the isolates have not been identified to the species level, the cultures show a great deal of overlap in physiological characteristics. For example at sts 1202 a and 1201 b nitrate reducing activity is shown by 100% and gelatin liquification activity by 100% of the total organisms tested. Morphological examination shows that similar types of colonies were involved in both cases. This suggests that the organisms may be able to synthesize necessary enzymes depending on the nature of their substrates. Such behaviour is typical of facultative chemo-organotrophs.

Sts 1200 a, 1203 b and 1208, showed the highest value for total bacterial counts and meiofaunal density<sup>14</sup> (Table 1). The constituents of organic matter such as carbohydrate carbon and sediment nitrogen do not show any correlation with bacterial total counts at these stations. Total organic carbon does not show any correlation with total counts either. Since the organisms isolated appear to be facultative chemo-organotrophs, the absence of such correlation is perhaps to be expected.

One interesting point of observation is that phenolic degraders are present at only 8 stations out of the 19 samples tested from the Andaman Sea. These 8 stations are situated mostly in the southern part of the Andaman Sea and 35% of the organisms isolated from the stations are phenol degraders. An attempt has been

made in Fig. 1 to show the zonation of phenol degraders in this area.

It is interesting that higher concentrations of such bacteria occur to the northwest of the Ten Degree Channel and south of Little Andaman Island. The reasons for such a distribution are not clear. With the available data, it is difficult to assess whether precursors of phenolic compounds are produced autochthonously by anaerobic degradation of organic matter or whether such compounds, allochthonously produced are transported to this area by physical processes of current combined with bottom topography of the area. It may be significant that, oxidisable organic carbon at these stations is generally low (Table 1) as is the dissolved oxygen content in the overlying water column. The sediment is composed of sand, sand and clay or clay alone and is not significantly different from that of other stations. Physical and chemical data from this region indicate oxygen depletion with depth in the water column and it is possible that the sediment at least in some areas, is oxygen deficient at this time of the year.

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