

## Some Observations on The Bacterial Flora of the Beach Sand and Beach Seawater

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Fifty four strains of bacteria, 28 from the beach sand and 26 from the beach seawater at Cochin, were isolated and their morphological and biochemical features were studied. Enterobacteriaceae (Coliforms) were the most dominant forms in both sand and seawater. These were followed by the genera *Vibrio*, *Pseudomonas*, *Achromobacter*, *Flavobacterium*, *Bacillus* and *Photobacterium*. Most of the strains were gram-negative and motile rods. Only five strains from the flora of the Cochin beach could be recognized as marine and the rest appeared to be of terrestrial origin.

### Introduction

The heterotrophic bacteria occurring in marine environments of India have received little attention. The earlier works of Velankar (1, 2 & 3), Venkataraman and Sreenivasan (4) and Santha Kumary (5) are confined to marine and estuarine flora. Except for a recent paper by Ayyakkannu and Chandramohan (6), no work has so far appeared on the bacteria of marine sandy beaches of India. The present paper deals with the bacterial flora isolated from the beach sand and beach seawater at Cochin.

### Material and Methods

Seawater and beach sand from the Cochin beach were collected in ster-

ile glass containers and transported to the laboratory where these were plated immediately in seawater-agar medium. After 72 hours of incubation at room temperature, colonies of bacteria which had developed and which could be distinguished from their size, shape and pigmentation were selected for further study. Standard procedures were employed for the study of morphology and biochemical properties of the isolated strains; see Zobell (7). The cultures were inoculated both in seawater and freshwater broths and freshwater-agar slants were also prepared. Motility was studied using "hanging drop method" and gram staining was carried out using Hucker's modification; see Walter

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and Mc Bee (8). In most cases, the isolates were classified upto genera according to the modified scheme of Simidu and Aiso (9).

The aim of this investigation was to isolate as many different strains as possible including the most dominant ones. Hence the flora described here represents its natural composition as is found in the beach sand and in the surf-beaten seawater.

## Results

**Morphological features:**— A total of 54 strains, 28 from the beach sand and 26 from the seawater were isolated and their morphological properties were studied.

The form of the colonies on the seawater-agar plates varied in size, shape, opacity and pigmentation. Punctiform, circular, large and raised colonies with complete margins were common. Some forms, however, were of irregular shape, while a few were of spreading type. Most of them were either white or dull white and a few of these included opaque colonies. Three bright red colonies were also seen and isolated.

In general, the growth of bacteria on the seawater-agar and freshwater-agar slants was good. The commonest form of growth was echinulate, and filiform; while beaded, arborescent and diffused growths were also seen on some instances. The culture medium in most cases became uniformly turbid and on some occasions a pellicle developed over it.

All the strains were found to be actively motile cells. Gram-nega-

tive rods were predominant and only three gram-positive spore forming rods were seen.

**Biochemical features:**— Table I shows some of the biochemical properties of the isolated strains. It can be seen from the table that the flora in general responded to different tests extremely well. All the organisms isolated were aerobic and grew well at room temperature. Nitrate reduction, ammonia production, indol production, gelatin liquefaction and chromo-genesis were the common properties of the flora. Carbohydrate fermentation was shown by all the isolated strains and 17 of them produced gas in addition to acid. Cellulose was decomposed by some of the representative genera; *Pseudomonas* sp. were dominant among the cellulose decomposers. Only one strain was found to show luminescence. Five strains (one strain of *Pseudomonas*, two of *Flavobacterium* and two of *Achromobacter*) failed to grow in freshwater media. All these five exhibited poor saccharolytic reaction. While the two strains of *Flavobacterium* and one of *Pseudomonas* did not produce indol, the two strains of *Achromobacter* produced slight indol only after 8 days. All of them produced acid from maltose and mannitol.

## Discussion

From the 54 strains of bacteria isolated from the beach sand and the beach seawater, the family Enterobacteriaceae seems to be most dominant. This was followed by the genera *Vibrio* and *Pseudomonas*. Other organisms, namely *Bacillus*, *Achro-*

TABLE—I BIOCHEMICAL PROPERTIES OF THE BACTERIAL FLORA ISOLATED FROM THE SAND AND SEAWATER OF THE COCHIN BEACH.

Organism	Total strains	Nitrate re- duction to nitrite	Ammonia produc- tion from peptone	Cellulose decompo- sition	Indol produc- tion	Gelatin liqui- faction	Carbo- hydrate fermen- tation (in one or more sugars)	Chromo- genesis
<i>Bacillus</i> sp.	3	1	1	Nil	3	3	3	1 (red colour)
<i>Flavobacterium</i> sp.	4	1	1	Nil	2	3	3	4 (yellow colour)
<i>Achromobacter</i> sp.	5	5	2	1	5 (two strains produced slight indol)	4	5	Nil
<i>Photobacterium</i> sp.	1	1	Nil	1	1	1	1	Nil
<i>Vibrio</i> sp.	14	14	6	1	10	12	14	Nil
<i>Pseudomonas</i> sp.	10	9	3	3	7	8	10	1 (green colour)
Fam: Enterobacteriaceae	17	16	6	Nil	14	17	17	3 *(2 red & 1 green) (all produced acid and gas)

\* Of the three strains, two produced red pigmentation and these were identified as *Serratia* sp., belonging to the Tribe Serratiae.

*mobacter*, *Flavobacterium* and *Photobacterium* were also present but in smaller numbers. The abundance of the members of the family Enterobacteriaceae in beach sand and seawater is an indication that the Cochin beach is intensely polluted and there is a contamination of sand with human feces. Ketchum *et al* (10) have given the process of dispersal of coliform bacteria in the sea and have mentioned the viability of these organisms, which according to them is relatively less in seawater than in freshwater. However, in the environments under study, the coliforms represent a major flora. This may possibly be due to the continuous contamination of the beach sand and the beach seawater with human excretion.

It is not clear to the author how many from the 54 isolates are of marine or terrestrial origin. The members of the family Enterobacteriaceae can be regarded as contaminants and are of terrestrial origin. These, to some extent, become adapted to marine conditions. Five strains failed to grow in freshwater media and had poor saccharolytic properties and showed slight or no indol production. These, according to the criteria suggested by Zobell (11), may be considered as marine. The remaining 49 strains do not appear to be of marine origin, although they exhibited some such properties which are similar to those of marine forms. However, the existence of these strains in the beach sand and in the beach seawater may be explained in terms of the periodic exchange which occurs between marine and freshwater forms to main-

tain them well in seawater; see Burke (12) and Burke & Baird (13). This may explain the occurrence of the members of Enterobacteriaceae in sand and seawater of the Cochin beach.

Most of the taxonomic groups of bacteria found in the sea are of terrestrial origin and only about 12% of the total have been recognized as marine; see Breed *et al* (14). Many of the strains found in the sea can be isolated from the inland waters and also from the soil. Therefore, when the organisms are isolated from the shore waters, it becomes difficult to decide whether their presence in the seawater is as a result of terrestrial contamination or they are truly marine forms; see Scholes & Shewan (15). Kriss (16) has given some criteria to distinguish between marine and terrestrial species. In the present flora, none of the forms can be regarded as marine in the light of this criteria. Thus, it is safe to assume that the marine types may simply be the variants, ecotypes or races adapted to seawater conditions; see Scholes & Shewan (15).

The morphological and biochemical features of the flora of the Cochin beach agree in some respects with those described by Velankar (2), Venkataraman and Sreenivasan (4), Simidu and Aiso (9) and Kriss (17). The points of similarities are their appearance as asporogenous rods, motile nature and gram-negative behaviour. In the present study, five strains which were taken as marine, were also recorded by Venkataraman and Sreenivasan (4). A strain of green *Pseudomonas*, a

strain of red *Bacillus* sp. which were repeatedly isolated by Venkataraman and Sreenivasan (4) were found to occur in the beach sand and in the beach seawater. However, despite these common features there seems to be one striking difference exhibited by the beach flora and that is a greater abundance of such strains which are capable of producing acid and gas from sugars.

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