BACTERIOLOGY OF OFF-SHORE SEA-WATER OF THE WEST COAST *

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Received July 14, 1954
(Communicated by Prof. P. S. Sarma)

In a recent communication Velankar (1954) has given an account of the bacteria in the inshore environment of the East Coast of India and Venkataraman and Sreenivasan (in Press) have described the flora of inshore sea-water of the West Coast. We have presented here the bacterial flora of Off-shore waters of the West Coast—off Tellicherry and off Calicut.

MATERIALS AND METHODS

A sample was collected 25 miles off Tellicherry and the other 16 miles off Calicut. Plates were poured on sea-water agar as well as on fresh-water agar and also on VRB agar for coliforms. 10 c.c. quantities were also inoculated into double strength lactose broth for detection of coliforms (presumptive test). Presence of denitrifiers and urea fermenters was also tested in Calicut water samples.

RESULTS

Coliforms were not present in either of the samples of water. Even enrichment in lactose broth did not indicate any positive presumptives.

Denitrifiers were shown to be present in 1 c.c. quantities of sea-water. Of the 95 strains studied, as many as 13 were denitrifiers, producing gaseous nitrogen from nitrates. Occurrence of such large numbers of denitrifiers is interesting since Waksman et al. (1933 a) have stated that they were not present in the surface-waters and hence do not play any part in the loss of nitrogen from the sea. However if we consider the large numbers that occur, there appears to be a chance of nitrogen being lost and probably this is the reason for the poor nitrate content of surface-waters of the tropics which have high temperatures. Of these 13 strains nine were 'marine' species originally isolated from sea-water agar and failing to grow in fresh-water media. Two were from fresh-water agar plates and grew equally well on sea-water as well as on fresh-water media. There was

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practically no difference in their morphological, cultural and biochemical characteristics but notable differences were found in their amino acid utilisation. The remaining two strains were isolated from sea-water agar plates incubated at 4°C for 4 months and produced greenish fluorescent soluble pigment. These failed to grow at 37°C but grew slowly at room temperature of 28-30°C. They also grew well in fresh-water media and they appear to be identical with *Ps. denitrificans*. A detailed account of the denitrifiers is given elsewhere.

*Psychrophiles.*—When 1 c.c. of sea-water was inoculated into sea-water agar plates and incubated at 4°C, only three colonies developed after 4 months. This, in contrast to the 106 mesophiles per c.c. on the same agar, is a poor number. Two of these were the fluorescent *Pseudomonas* mentioned above and one was a *Micrococcus*.

*Bacterial flora.*—Ninety-five strains were isolated from Calicut sea-water and their morphological and cultural characteristics studied. 72 of them were further studied in detail for their biochemical properties. In general pleomorphism appeared to be a common feature and two of the *Bacillus* species were involution forms. The pattern of bacterial flora is the same as described in our earlier work on inshore sea-water and in mackerels. There were numerous aerobic spore-forming *Bacillus* and of these, as many as 10 were distinctly chromogenic, a fact noted in our earlier work. These rose-red *Bacillus* thus seem to be part of the natural bacterial flora of the sea. *Achromobacter* were also found in large numbers and seven of them produced indole. The next important group was *Pseudomonas* and all the 13 were denitrifiers but only two of them produced greenish fluorescent pigment. *Micrococi* were the next numerically large group, *Flavobacter* following up. *Sarcina, Vibrio* and *Bacterium* were also encountered in very small numbers. The results are tabulated in Table I.

Yellow was the dominant colour, 16 of the organisms belonging to *Flavobacterium, Micrococcus, Sarcina* and *Bacillus* having this colour. Red and rose shade was next evident, with a large number of *Bacillus* developing this colour as well as fewer number of *Micrococcus* and a *Bacterium*. Two greenish *Pseudomonas*, one yellowish-orange *Bacillus* and one brownish-black *Bacillus* were also noted.

It could be seen that none of the *Pseudomonas*, only one *Micrococcus*, two *Flavobacter* and four *Achromobacter* were gelatinolytic. They were much less active in digesting casein of milk. On the contrary 25 of the *Bacillus* liquefied gelatin, and 17 digested casein. These were also saccharolytic to a great extent as against very negligible fermentative activity
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**Table I**

*Bacterial flora of off-shore sea-water (Calicut) and their biochemical properties*

<table>
<thead>
<tr>
<th></th>
<th>Gelatin liquefiers</th>
<th>Milk peptoniizers</th>
<th>Milk acid coagulated</th>
<th>Fermentation of Dextrose</th>
<th>Hydrolysis of starch</th>
<th>Chromogenesis</th>
<th>Nitrate reducers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achromobacter</td>
<td>8</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>..</td>
<td>6</td>
</tr>
<tr>
<td>Flavobacterium</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>..</td>
<td>7 (Denitrifiers)</td>
</tr>
<tr>
<td>Micrococcus</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Sarcina</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Bacillus</td>
<td>29</td>
<td>25</td>
<td>17</td>
<td>3</td>
<td>21</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>13</td>
<td>..</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>13 (Denitrifiers)</td>
</tr>
<tr>
<td>Vibrio</td>
<td>1</td>
<td>1</td>
<td>..</td>
<td>..</td>
<td>1</td>
<td>..</td>
<td>1</td>
</tr>
<tr>
<td>Bacterium</td>
<td>1</td>
<td>1</td>
<td>..</td>
<td>1</td>
<td>1</td>
<td>..</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>72</td>
<td>36</td>
<td>23</td>
<td>5</td>
<td>33</td>
<td>14</td>
<td>31</td>
</tr>
</tbody>
</table>

Of the *Flavobacter*, *Achromobacter* and *Pseudomonas*. *Micrococci* were slightly more active in fermenting sugars.

**Marine species.**—Nine *Pseudomonas* (*Ps. marinodenitrificans* n.sp.), four *Achromobacter* and one *Flavobacterium* failed to grow in fresh-water media and may be considered to be 'marine' according to ZoBell (1946). All of them were gram negative, non-spore-forming rods which were actively motile. They exhibited very poor saccharolytic and proteolytic tendencies as noted in the case of 'marine' bacteria from mackerels (Venkataraman and Sreenivasan, in Press). None of the *Bacillus*, *Micrococcus*, *Sarcina* or *Bacterium* were 'marine' forms in the above sense. Marine flora in general are characterised by the presence of only a few types, without much diversity.

A study of 32 cultures isolated from off-shore sea-water off Tellicherry showed the presence of *Bacillus*, *Micrococcus*, *Sarcina*, *Flavobacterium*, *Achromobacter*, *Bacterium*, *Corynebacterium* and *Alcaligenes*. Here also yellow was the dominant colour but there were also as many as seven rose-red *Bacillus* species similar to the ones found in Calicut sea-water. These rose-red spore-forming *Bacillus*, thus have been repeatedly obtained from marine environment over a wide area examined. In this sample also
the *Flavobacter* were inert, never acting on sugars or on proteins and thus confirm their unimportant role in fish spoilage (Castell and Mapplebeck, 1952). The *Bacillus* on the other hand showed rapid proteolytic action on gelatin and casein, and equally active saccharolytic powers. The species commonly noted were *Achromobacter superficiale*, *Flavobacterium marinum*, *Fl. solare*, *Fl. flavus*, *Bacterium zopfi*, *Sarcina flava*, *Micrococcus flavus* and the rose-red *Bacillus* belonging to the *subtilis* group.

Nitrate reduction by sea-water bacteria seems to be very common. About 50% of the organisms from Calicut and Tellicherry sea-water reduced nitrates. Nitrate reduction was marked in species of *Micrococcus*, *Pseudomonas* and *Corynebacterium*. In the case of *Bacillus* in many cases there was an inverse correlation between gelatin liquefaction and nitrate reduction. In *Micrococi* and *Pseudomonas* also there was a similar trend, more of them reduced nitrates but did not liquefy gelatin. Of the *Pseudomonas* all the 13 produced free nitrogen from nitrates.

Starch hydrolysis was not uncommon and quite a few of the species possessed amylase. Very few species were acido-proteolytic in milk.

**DISCUSSION**

The bacterial flora of the off-shore sea-water of the West Coast is not a varied one but consists of a limited number of types, which are characteristic. As in the case of inshore sea-water and of marine fish (Venkataraman and Sreenivasan, *loc. cit.*) the dominant groups were *Achromobacter*, *Micrococcus*, *Pseudomonas* (non-chromogenic), *Bacillus* and *Flavobacter* with *Sarcina*, *Bacterium* and *Corynebacterium* occurring in smaller numbers. The absence of coliforms only confirms the inability of this group to establish itself in marine environment (ZoBell, 1946; Ketchum *et al.*, 1949; Vaccaro *et al.*, 1950). Though Waksman *et al.* (1933) consider the occurrence of bacteria reducing nitrates to free nitrogen in open sea to be limited, we found that 13 of the 95 cultures isolated from off-shore water, 16 miles off Calicut were denitrifiers and 9 of them requiring sea-water for growth. Though the organic matter in surface-water may be poor, it is possible for these organisms to obtain their requirements of nutrients as commensals on plankton.

Though fluorescent *Pseudomonas* were not encountered by us in earlier studies, two were isolated from Calicut sea-water, and these were from plates incubated at 4° C. for 4 months. Only three colonies appeared on this plate which clearly shows the poverty of psychrophilic types in tropical waters. These two fluorescent rods in fact did not grow at
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37°C. but grew slowly at 28–30°C. Though the non-fluorescent Pseudomonas were 'marine' forms, the two fluorescent ones grew in fresh-water media with equal facility. Since we isolated greenish fluorescent Pseudomonas more frequently from green mussels near shore (unpublished results), it should be considered that the fluorescent rods are not 'marine' types.

Repeated isolation of the chromogenic (rose-red) Bacillus, from both the waters, viz., from Tellicherry and Calicut, and also from other samples reported earlier, emphasizes the fact that these are not adventitious forms but are the types which have established themselves well in the marine environment. Wood (1953) also reported the occurrence of red Bacillus in Australian sea-water.

When we consider that all the Bacillus, Micrococci and Bacterium so far studied grow well in fresh-water as well as in sea-water media but that some of the Achromobacter, Flavobacterium and Pseudomonas fail to grow in the absence of sea-water in media, it appears probable that there is a specific 'marine' flora as suggested by ZoBell (1946). These were all gram negative, actively motile, non-spore-forming rods with very poor fermentative properties as emphasized by ZoBell (loc. cit.). However as suggested by Wood (1950, 1953) there might be a gradation from the soil types through estuarine to typically marine flora. This is in keeping with the views of Burke (1934) and Burke and Baird (1931), who suggested that there is an exchange of marine and fresh-water forms near the sea and that some of the fresh-water forms maintain themselves in sea-water without their physiological activities being affected.

SUMMARY AND CONCLUSIONS

The bacterial flora of the off-shore sea-water of Tellicherry and Calicut was comprised of a limited number of types—Achromobacter, Bacillus, Micrococcus, Flavobacterium, Pseudomonas, Bacterium, Corynebacterium and Sarcina. All the Pseudomonas were denitrifiers, including the two fluorescent ones. There were only very few psychrophiles but a large number of thermoduric Bacillus species. For preservation use of cold is indicated because of the poverty of psychrophiles and abundance of mesophilic forms.

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