BACTERIOLOGY OF INSHORE SEA WATER AND OF MACKERELS OFF TELLICHERRY (MALABAR) *

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INTRODUCTION

The bacterial flora of marine fish and of sea water has been studied all the world over with a view to explain the spoilage of fish and to control or prevent spoilage by the application of suitable techniques of preservation. The importance of various groups of bacteria can be assessed chiefly on the basis of their biochemical activities such as liquefaction of gelatin, peptonization of milk and production of indole, hydrogen sulphide, etc. (Snow and Beard, 1939; Shewan, 1949; Castell and Mapplebeck, 1952).

Most of the work on fish spoilage has been carried out on marine fish. The existence of specific 'marine' bacteria has been postulated by ZoBell and co-workers (ZoBell, 1946; ZoBell and Conn, 1940; ZoBell and Upham, 1944) and quite recently Canadian workers also support this hypothesis (McLeod *et al.*, 1953). It has been pointed out that gram negative types predominate in marine water in contrast to gram positive soil types. Wood (1950*a*) on the other hand considers the existence of specific 'marine' bacteria to be doubtful but believes in a gradation from soil forms through estuarine to gram negative marine flora. His further work (1950*b*) has shown that the flora (of shark) may be regarded as a modified marine flora in which the gram positive elements have been enriched and the gram negative ones are of little importance. Burke (1934) is of the view that there is an interchange of bacteria between the sea and fresh water. The question of occurrence or not of coliform bacteria in sea water and in guts of fish has been reviewed by Griffiths (1937). Coliforms are not normally inhabitants of the digestive tracts of marine fish from unpolluted areas (Gibbons, 1934 cited by Griffiths, *loc. cit.*).

MATERIAL AND METHODS

The samples were collected off Tellicherry in Malabar. The surface inshore water samples were collected 1½ miles away from the shore, in sterile glass stoppered bottles. Fresh mackerels were taken from the same area, direct from gill nets. Methods of collection and plating were the same as described earlier (Venkataraman and Sreenivasan, 1952). Ability of the organisms to grow in fresh water media was also tested. The procedure for their identification was that as described in the 'Manual of methods for pure culture study of bacteria' (1952), with the modifications needed for specific marine bacteria (ZoBell and Upham, *loc. cit.*). The organisms were classified according to Bergey's Manual (1948). Corynebacteria were identified by the angular arrangement of their cells and 'snapping division' (Jensen, 1952) in addition to the characteristics described in Bergey's Manual.

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Results

Twenty-two bacteria isolated from inshore sea water and one hundred from mackerels were studied. Many of the colonies on the original plates were phosphorescent but on sub-culturing ceased to be so. Contrary to the notion (Burke, 1934) that 'spreader' type of colonies do not occur in sea water media, we observed invariably two-types of spreaders—one, a thin effuse subsurface colony and the other, a rhizoid spreader, especially in low dilution plates. Other marine materials like sea prawns and green mussels also yielded such spreader colonies.

Coliforms: They were absent in the inshore sea water as well as in guts and flesh of mackerels, when tested by enrichment in standard lactose broth and by direct plating on 'V.R.B.' (Violet red bile) agar. In the plate isolates, however, there was a single strain of *Aerobacter aerogenes*, which is not of any significance, since it does not indicate faecal contamination. Three of the isolates were slow lactose fermenting bacteria belonging to the sub-genus *Paracolobactrum*, and one of them was a strictly 'marine' form failing to grow in fresh water media, even after subculturing for months (Venkataraman and Sreenivasan, 1953*a*).

Bacterial flora: Bacteria belonging to the following genera were noted among the 122 cultures studied—*Micrococcus*, *Bacillus*, *Achromobacter*, *Sarcina*, *Flavobacterium*, *Paracolobactrum*, *Pseudomonas*, *Corynebacterium*, *Bacterium*, *Aerobacter*, and *Alcaligenes*. Some of the commonly occurring species are, *Achromobacter superficiale*, *A. thalassius*, *A. aquamarinus*, *A. stenohalis*, *Micrococcus citreus*, *M. flavus*, *M. epidermidis*, *M. varians*, *M. conglomeratus*, *M. caseolyticus*, *Sarcina flava*, *S. lutea*, *Flavobacterium dormitator*, *Fl. halohydrium*, *Fl. diffusum*, *Fl. invisible*, *Fl. maris*, and *Pseudomonas sessilis* (ZoBell and Upham, 1944). Some of the aerobic sporeforming *Bacillus* having rose-red to pink colour resemble *Bacillus subtilis* morphotype *globigii*.

From Table I it is seen that the flora of slime and gills (external parts) are to some extent identical with regard to the genera and even the ratio of these. *Achromobacter*, *Bacillus* and *Paracolobactrum* are present in nearly the same percentage in both. The gut flora differs from these, in that the *Micrococcaceae* account for 60% of the strains, while *Bacillus* is the next important group with 17·5%. Other genera are poorly represented in guts and *Alcaligenes* was present. A red *Bacterium* was present in the gills.

Table I

Showing the numerical distribution of bacteria according to genus in various parts of fish and in inshore water

Genus	Inshore sea water	Gills	Guts	Slime	Flesh	Total
Achromobacter ..	3	6	1	15	3	28
Flavobacterium ..	4	..	1	3	4	12
Bacillus	2	3	3	6	16	30
Micrococcus ..	11	1	6	1	11	30
Sarcina	1	..	4	..	7	12
Miscellaneous ..	1 (Aero-bacter)	3 (Pseudo-monas 2 Paraco-lobact-rum 1)	2 (Bact-erium 1 Alcali-genes 1)	2 (Para-colo-bact-rum)	2 (Coryne-bact-erium)	10
	22	13	17	27	43	122

'Marine' species: As regards their ability to develop on fresh water media, the results are quite interesting. In the inshore water flora only three *Flavobacter* failed to grow in fresh water media, and can therefore be regarded as 'marine' forms. Similarly from the gill flora, five *Achromobacter*, two *Pseudomonas*, and one *Paracolobactrum* were of marine species, while from the slime cultures only eight *Achromobacter* belonged to this category. Among the gut flora, one *Achromobacter* and one *Flavobacterium* and from the flesh, three *Achromobacter* were of marine species.

Active motility, a characteristic of marine bacteria (ZoBell, 1946) was noted in 69 (57%) of the 122 cultures, including one micrococcus. Fermentative powers were very poor, only about 50% of the cultures produced just a faint acidity from at least one sugar. Only four cultures were aerogenic in sugars. The thermal sensitivity (ZoBell and Conn, 1940) was not evident as most of the cultures grew luxuriantly at 37°C. and at slightly higher temperatures. Growth at room temperature was a little less than at 37°C. There was no growth at 4°C. for 12 weeks.

Discussion

Bacteria belonging to *Micrococcaceae* form the largest group, accounting for nearly a third of the isolates. Wood (1940) in Australia, and Dyer (1947) in Canada also obtained large numbers of micrococci in fish. Shewan (1949) recorded that 30 to 35% of fresh slime flora were micrococci. *Bacillus*, the aerobic sporeformer formed between 18 to 24% of the flora of slime, guts and gills of mackerels and to a lesser extent in sea water. Next in numerical importance were the *Achromobacter* and *Flavobacterium* respectively. Fluorescent rods and *Proteus* were conspicuous by their absence, though both formed a large percentage of the flora of green mussels in the sea, examined by us (unpublished results).

The fact that some of the bacteria fail to grow in fresh water media, even after repeated subculturing confirms ZoBell's views regarding the existence of specific 'marine' bacteria. Seventeen *Achromobacter*, four *Flavobacter*, two *Pseudomonas* and one *Paracolobactrum* did not grow in fresh water media and may therefore be regarded as 'marine' forms. All these are gram negative, non-sporing rods and are actively motile. Very few of them are fermentative and only one produced gas from lactose after 9 days. In contrast, all the cocci, *Bacillus* and *Bacterium* grew equally well in fresh water and sea water media. Most of these are gram positive and thus resemble the soil flora. Wood (1950*a*, *b*) who has recorded a large number of gram positive bacteria in sharks, is also of the view that these are soil types. Further, from his latest work (Wood, 1953), it will be seen that cocci, *Bacillus* and *Corynebacterium*, the gram positive types, account for 55% of the total isolates from marine environments. In view of the above findings it is possible to reconcile the views of ZoBell and Wood. There may be a specific 'marine' bacterial flora in the same sense as there are specific milk, soil and sewage forms and this may quite often be modified by environment. It seems reasonable to believe that many fresh water bacteria carried into the sea can maintain themselves and carry on their activities as in fresh water (Burke and Baird, 1931). However, the prospect of survival of the 'marine' bacteria in fresh water seems very remote.

Among the *Bacillus* a large number were chromogenic, being rose-red to pink. A few were pale yellow, cream coloured or brown. Since these rose-red *Bacillus* were frequently isolated from marine environments, it is quite possible that they are autochthonous to the sea. They tolerate high concentrations of salt (Venkataraman and Sreenivasan, 1954) although growing profusely in salt free media. The presence of chromogenic *Bacilli* in marine environment have not been so far reported except by Wood (1953). The probable explanation for the frequent occurrence of *Bacillus* is the high temperatures of surface waters. Wood (1953) has correlated

the high surface temperatures with thermotolerance of bacteria of Australian waters. He further emphasised that the differences observed in bacterial flora by various workers 'largely reflect the differences in different parts of the world, and in different seasons'. Thjotta and Somme (1943) and Gee (1930) also suggested the possibility of geographical and seasonal variations in the bacterial flora.

The absence of coliform group, especially *E. coli*, in sea water and guts of mackerels confirms ZoBell's (1946) findings. The occurrence of *Paracolobactrum*, however, is interesting, since, apart from a report of their occurrence in fresh water fish (Venkataraman and Sreenivasan, 1953*b*), there is no reference in the literature about their presence in marine fish (Venkataraman and Sreenivasan, 1953*a*).

A comparison of the present work with that of Bhat and Albuquerque (1953) on Bombay-duck will be useful. These authors noted only the presence of *Micrococci*, *Sarcina* and *Bacillus* in the stomach, which is similar to what we found in the guts. Similarly, their flora of 'skin, gills and flesh' were also recorded by us in slime, gills and flesh, with very slight variations. While they have mentioned the presence of *Escherichia*, we did not find any. Since they state that their cultures did not ferment lactose but fermented only glucose and other sugars with 'gas' it is to be considered that they were dealing with the paracolon bacteria. If this is true, it agrees with our results on the occurrence of *Paracolobactrum* in fish. However, the fact that they isolated *Sarcina littoralis* from the alimentary tract in large numbers is surprising inasmuch as these are obligate halophiles, requiring a minimum of 16% NaCl for growth. On the whole, the flora of Bombay-duck and those of our mackerels are not much different.

The significance of the bacterial flora associated with fish lies in the fact, that it must explain the pattern and probable extent of its spoilage. The occurrence of a high percentage of proteolytic types points to the high spoilage potential. As shown in Table II about 75% of the isolates were gelatinolytic, while 37% peptonized casein. The presence of indole producers and hydrogen sulphide producers indicates the possible putrefactive types of spoilage. The occurrence of a large number of *Achromobacter*, which include all the indole producers listed in Table II, and a few which produced hydrogen sulphide, points to the same conclusion. The *Flavobacter*, however, exhibited poor proteolytic and saccharolytic tendencies, and as pointed out by Castell and Mapplebeck (1952), their rôle in spoilage is not of much importance. *Bacillus*, also exhibited proteolytic properties on gelatin and casein. This and its euryhaline nature point to its capacity in playing an adverse rôle in fish spoilage, in fresh as well as in salted condition.

TABLE II

Showing the Biochemical types in various parts of fish and inshore water

Type	Inshore water	Gills	Guts	Slime	Flesh	Total 122
Gelatin liquefiers ..	14	10	14	15	36	89
Milk peptonizers ..	13	3	6	6	17	45
Indole producers ..	..	5	..	1	..	6
Nitrate reducers ..	11	11	8	15	28	73
H_2S producers ..	..	..	..	5	..	5
Saccharolytic ..	13 (1 aerogenic)	10 (1 aerogenic)	7	12 (2 aerogenic)	22	64
Chromogenic ..	11	2	11	9	23	56

Summary

Bacteria belonging to the genera, *Micrococcus, Bacillus, Achromobacter, Flavobacterium, Sarcina, Paracolobactrum, Pseudomonas, Corynebacterium, Bacterium* and *Alcaligenes* are associated with mackerels and sea water off Tellicherry. *E. coli* was not detected in sea water or in guts of mackerels but a few slow lactose fermenting paracolons were isolated. The existence of 'marine' bacteria incapable of growing under hypotonic conditions is confirmed, and these were all gram negative, actively motile rods with faint fermentative properties. Many of the isolates were proteolytic on gelatin and casein and a few produced indole and hydrogen sulphide. Nitrate reduction was very common. Apart from chromogenic cocci and flavobacter, spore-forming *Bacillus* also were seen to possess chromogens quite often. The rôle of bacterial flora in fish spoilage is discussed.

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