Bull. Fish. Res. Stn., Ceylon, Vol. 17, No. 2, pp.–151–157, December, 1964.

A Comparison of the Bacterial Flora of Surface and Sub-Surface Water in the Sea off Ceylon

By

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Introduction

Tropical waters have been characterised by the presence in them of a shallow and intense thermocline which is relatively stable and corresponds to the discontinuity layer (Watts, 1958). In the tropical Indian Ocean a sharp oxygen minimum (less than 0·2 ml/l.) with its upper boundary coinciding with a sudden density change has also been reported. This oxygen minimum layer extended from a depth of 125–200 meters to 1,000–1,250 meters and appeared to influence markedly the distribution of plankton in the Arabian Sea (Vinogradov & Voronina, 1962). Under such stable conditions of oceanic stratification some evidence of the physiological adaptation of the bacterial flora relative to the oxygen content has been reported earlier (de Silva, 1963).

The use of bacteria as indicators of water masses in the oceans has been suggested. Kriss (1960) reported that the bacterial flora of oceans indicated latitudinal variations, the equatorial flora consisting of a large proportion of heterotrophs compared to that of the higher latitudes. The conclusion drawn by Kriss *et al* (1960) regarding the hydrological structure of the Indian Ocean on the basis of its bacteriology was that it was a homogeneous mass of water characterised by a large proportion of heterotrophs penetrated by tongues of polar and sub-polar water containing large numbers of autotrophs. This view was in contradiction to the classical theories of oceanic circulation and no attempt was made to correlate the bacteriological data with those on the distribution of temperature, salinity and other hydrochemical characteristics. Indeed Bogoyavlenskii (1964) showed that results on which Kriss based his conclusions may as well be determined by the quality of the water bottles and the condition of their inner surfaces and that these results did not indicate any evidence of circulation. But the use of marine bacteria as sensitive indicators of the hydrochemical condition of the associated water mass has still to be investigated and may well prove to be a useful technique for the study of the productivity of the sea. The present work is an attempt to investigate the qualitative vertical distribution of bacterial genera with particular reference to their biochemical properties.

Such a study was also thought to be necessary in view of the theory that has been suggested that the bacterial flora of newly caught fish reflects that autochthonous to the environment in which they are caught (Shewan, 1944 ; 1949). Though much experimental study and speculation have centred round this theory (Liston, 1957 ; Georgala, 1958 ; de Silva, 1960 ; Colwell, 1961 ; Colwell and Liston, 1962) much of the data cannot be resolved until a more precise characterisation of the water masses by bacteria is available so that a more precise definition can be given of what constitutes the ' autochthonous flora ' and in turn the related commensal flora of marine fish.

Methods

Samples of water were collected from three stations off the west coast of Ceylon between 200 and 250 miles off-shore. These stations were selected after density and bathythermographic measurements had indicated a marked stratification of the water mass with a clearly defined thermocline. The surface water and the deep water were collected using the same Nansen bottle internally lined with plastic. Six samples were taken at each station, each surface sampling alternating with a deep water sampling. Further at each sampling the Nansen bottle was suspended

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for 5 to 8 minutes for stabilization with the water mass. By this means errors due to the nature of the inner surface of the bottle were minimised. In addition precautions suggested by Kriss (1963) were also taken.

Within 5 minutes of hauling bacterial counts were done on the water samples using membrane filters as well as the pour-plate technique. Zobell's medium 2213 (Zobell, 1945) with aged sea water was used with 1·5% agar for the pour plates and without agar for the membrane filters. The plates were incubated at 20°C. for 5 days and overnight at 30°. The membrane filters contained in aluminium cans were incubated at 25° ±2° for 6 days.

Colonies were picked off at random from the membrane filters. For this purpose a blank filter pad marked with squares (0·5 sq. cms.) similar to the one used for sampling was serially numbered. After selection of a number from a table of random numbers, all the colonies on the square on the sampled filter pad corresponding to the chosen number on the blank grid were picked off.

The colonies were inoculated into nutrient broth with aged sea water and later into nutrient broth with distilled water. Thus the sea water requirements of the cultures were checked. Those requiring sea water were examined for their biochemical activities in media made up with aged sea water. However they constituted only a very small proportion.

The identification of the bacteria was done using the determinative scheme of Shewan, Hobbs, and Hodgkiss, (1960). Other biochemical tests were done according to the methods enumerated in the Manual of Methods of the American Society of Bacteriologists (1957).

Results

The surface flora consisted mainly of Gram negative rods with a predominance of pseudomonads and achromobacters (81·2%), as compared to the Gram positive micrococci and coryneforms (6·6%). At 200 meters depth the population of Gram negative bacteria was considerably smaller (57·1%) in comparison to the Gram positive types (15·8%). The proportion of *Vibrio* sp. in the sub-surface water ('intermediate water' Wyrtki, 1961) was 21·2% and exceeded that at the surface (7·9%). (See Table 1).

TABLE I

PERCENTAGE DISTRIBUTION OF BACTERIAL GENERA IN SURFACE AND SUB-SURFACE WATER

Genus	*Surface*	*Sub-surface*
Pseudomonas	56·6	43·9
Achromobacter	24·6	13·2
Coryneforms	1·9	5·9
Flavobacteria	2·7	—
Micrococcus	4·7	9·9
Bacillus	—	—
Vibrio	7·9	21·2

The distribution of biochemical properties in the two populations also differed significantly. Bacteria at the surface showed a greater proportion of oxidase positive, motile groups compared to the bottom flora. However in other biochemical activities such as the ability to hydrolyse starch, break down urea and gelatine, the deeper flora showed greater activity. This was particularly marked in the case of the fermentative breakdown of glucose (Hugh and Leifson test (1953)). The greater capacity for deamination of amino acids was also evident in the deeper flora. This sub-surface flora also showed a marked ability to peptonise milk—an ability which appeared to be co-related

with their proteolytic properties towards fish muscle. On the assumption that growth in Koser's citrate medium constitutes an indication of the ability of micro-organisms to synthesise most of their nutritional requirements (Colwell, 1962) there appeared to be a greater preponderance of autotrophs in the sub-surface water than in the surface water.

In general it may be stated that while the surface water showed a greater variety of bacterial types, the sub-surface flora appeared to possess a greater range of biochemical activity. (Table 2).

TABLE 2

COMPARISON OF THE BIOCHEMICAL ACTIVITIES OF SURFACE AND SUB-SURFACE WATER

Test	*Surface Total number of cultures examined = 103*		*Sub-surface Total number of cultures examined = 94*	
	Number + ve	*%*	*Number + ve*	*%*
Oxidase	79	76·6	21	22·3
Motility	81	78·6	47	50·0
Penicillin Sensitivity	21	20·3	36	38·2
Hugh and Leifson test—				
Fermentative	9	8·7	29	30·8
Alkaline	7	6·7	23	24·4
Acid	11	10·7	9	8·7
Urease	16	15·5	24	25·5
Starch hydrolysis	24	23·3	49	52·1
Gelatin liquefaction	23	22·3	23	24·4
Litmus Milk—				
Acid	35	33·9	25	26·5
Alakaline	37	35·9	8	8·5
Peptonised	5	4·8	29	30·8
Nitrate reduction	16	15·5	15	15·9
Utilization of citrate	6	5·8	29	30·8

As regards temperature of growth, nearly 56% of the cultures grew at 20°C. and 37°C. in the case of the surface flora : in the sub-surface flora 48% grew at 20° and nearly 60% of the cultures were also capable of growth at 37°C. All cultures from the surface and sub-surface water grew at room temperature (28° to 30°C.).

Discussion

According to Wyrtki (1961) the conception of circulation in the sea is based on a four-layered ocean. In the lower and mid-latitudes the upper mode of this circulation was thought to consist of the intermediate waters, the thermocline and the wind driven surface circulation. The lower mode was formed of the " bottom layer " and the deep layer. The circumpolar current and the Antarctic polar front formed the link between these two modes. Though such a picture is particularly characteristic of the Atlantic there is no doubt that in the Indian Ocean the picture is broadly similar. If these water masses have characteristic hydrochemical properties, it should be possible

to detect them by the associated bacterial flora particularly by the differences in their biochemical activities, for such differences are no doubt related to the origin of and to the hydrological conditions prevailing in the particular water mass.

For instance the predominance of Gram negative rods especially *Pseudomonas* sp. and *Achromobacter* sp. in the surface water is no doubt related to the content of dissolved oxygen for example in the surface water. Nielsen and Jensen (1957) found that under tropical conditions in the Pacific the photosynthetic layer was restricted to 78 to 81 meters from the surface. Further Hasle (1959) also showed that in tropical water the bulk of the photosynthetic plankton was in depth ranging from 50 to 100 meters. This upper layer of high oxygen appears to rest on an oxygen minimum layer particularly in the intermediate latitudes. Indeed a sharp oxygen minimum with less than 0·2 ml/l. has been recorded from the Arabian sea. The upper boundary of this layer has been shown to approximate to a layer of sudden density change (Carruthers *et al*, 1959 ; Neiman, 1960). This oxygen minimum layer extended from about 125 to 200 meters to a depth of about 1,000 meters.

With a well established thermocline which shows characteristics of a discontinuity layer (Watts, 1958) under stable hydrological conditions as in tropical seas, there appears a sudden environmental change at a depth ranging from 150 to 200 meters. The sub-surface water is thus characterised by a high content of decaying organic matter due to plankton fall-out from the surface layer thus maintaing reducing conditions. There is no doubt that such conditions are of great consequence to the associated bacterial flora, for whatever the content of free oxygen, since the bacteria tend to grow attached to particulate matter it renders the available environment physiologically micro-aerobic.

These hydrological conditions appeared to be reflected in the nature of the bacterial flora characteristic of the surface and sub-surface water. Such a relationship has been shown to exist in the case of the oxidase reaction of bacteria (de Silva, 1963). This has once again been confirmed. In addition adaptations to these environments are also indicated by the greater preponderance of fermentative groups (e.g. *Vibrio* sp.) which occurs in sub-surface water. The variety of substrates which are attacked by the bacterial flora in this layer is also reflected in the corresponding range of their biochemical activities (Table 2). Their ability to deaminate amino acids, hydrolyse starch as well as their proteolytic activities combined with their ability to synthesise most of their nutritional requirements confirm such a view.

This variety in biochemical activities in the sub-surface water is comparable with the observations made by Kriss (1960) regarding the greater activity of the bacterial flora at higher latitudes. Perhaps in addition to the content of decaying organic matter at higher altitudes and in lower depths, such a versatility in the enzymic constitution of the flora compensates in some measure, the depression of the activity rates under the lowered conditions of temperature.

The similarity between the sub-surface flora and the antarctic flora on the one hand and the surface flora and the tropical flora on the other is indicated in Table 3.

TABLE 3

BIOCHEMICAL ACTIVITIES OF MICRO-ORGANISMS ISOLATED FROM THE INDIAN OCEAN

Location	*Gelatine liquefaction*	H_2S	*Peptonisation of milk*	*Glucose utilization*	*Starch hydrolysis*	*Citrate utilization*	*Denitrification*
Antarctic* ..	19·3	5·9	16·6	28·6	57·7	91·1	40·8
Southern sub-tropical*	21·5	4·2	23·2	23·4	67·5	95·1	22·2
Equatorial* ..	5·3	0·8	6·7	8·7	89·3	99·2	43·9
Northern tropical* ..	6·6	0 0	5·2	12·0	89·5	98·7	26·0
Northern tropical (Ceylon)							
sub-surface ..	24·4	0·0	30·8	30·8	52·1	30·8	15·9
surface ..	22·3	0·0	4·8	8·7	23·3	5·8	15·5

* Data obtained from Kriss (1963).

The comparison of the figures obtained during the present study of the generic distribution of bacteria in the sea with those obtained by other workers is shown in Table 4. No marked similarities are evident except perhaps the general observation that tropical waters carry a greater variety of bacterial forms compared to the temperate waters. Any other comparison is made difficult since the hydrology of the water mass from which the sample was drawn has not often been specified by previous workers. For instance, as pointed out by Colwell and Liston (1962) it is likely that the results of Indian workers, Venkataraman and Sreenivasan (1954), Velankar (1955), and Velankar and Kamasastri (1956), reflect certain factors like fresh-water run-off in the inshore areas. The high proportion of *Bacillus* sp. encounted by them confirms such a view.

TABLE 4

BACTERIAL FLORA OF SEA WATER

Group	*Australia* (1) %	*Australia* (2) %	*India* (3) %	*North Sea* (4) %	*North Cape Norway*, (5) %	*Ceylon*	
						Surface %	*Sub-surface* %
Pseudomonas ..	10·0	0·9	18·0	94·0	0·0	56·6	43·9
Achromobacter ..	26·0	0·0	11·6	6·0	14·0	24·6	13·2
Coryneform ..	—	71·0	—	—	25·0	1·9	5·9
Flavobacterium ..	18·0	—	9·7	—	1·0	2·7	9·9
Micrococcus ..	34·0	19·0	18·1	—	55·0	4·7	9·9
Bacillus ..	12·0	7·5	40·3	—	—	—	—
Vibrio ..	—	—	1·4	—	—	7·9	21·2

(1) Wood (1940)
(2) Wood (1953)
(3) Venkataraman and Sreenivasan (1954)
(4) Shewan and Hodgkiss (1954)
(5) Shewan and Hodgkiss (1954)

But on the basis of Shewan's hypothesis regarding the relationship between the bacterial flora of newly caught fish and that of the water mass in which they are caught, results of earlier investigations of the bacterial flora of fish are in conformity with the present findings. For instance the method of catch should reflect to some extent the differences in the bacterial flora of surface and bottom water if a comparison is made between line caught fish and trawled fish. Results of Colwell (1962) confirm this prediction. She found that at Port Orchard there was a greater variety in the case of trawled fish but that the hand-lined fish had a greater proportion of pseudomonads. She also found that fish caught by otter trawl showed a higher incidence of fermentative types than those caught by hand line. Spencer (1959, 1960) had observed a higher proportion of Gram-positive organisms in trawled fish which he suggested were probably derived from net and from bottom muds. Shewan (1949) also observed a high proportion of fermentative *Vibrio* sp. on trawled fish and thought that their incidence was probably related to the contamination by gut contents expressed during the hauling process. While such extraneous contamination cannot be completely ruled out a more likely explanation is that in deeper water the autochthonous flora carry a greater proportion of those groups they encountered as indicated during the present investigation.

Further, the quantitative seasonal variations of the bacterial flora on freshly caught North Sea herring have been reported earlier with summer and autumn peaks linked with plankton variations as well as qualitative differences in these two peaks with a greater proportion of Gram positives in the summer peak as compared to the autumn peak (de Silva, 1960). Though this difference has been co-related with the difference in the constitution of the two plankton peaks it is also likely that the mixing of the deeper water with surface water during winter affects the early summer peak whilst the gradual build up of the thermocline in summer and the resultant stratification of the water is reflected in the autumn peak.

This investigation, necessarily preliminary in character, indicates that in the present state of the study of the microbiology of the sea both in relation to hydrology and fish spoilage, a better characterization of the water masses in terms of generic types and biochemical activities is urgently required.

Summary

Under stable conditions of stratification of the sea, evidence of generic differences of the associated bacterial flora of the water masses has been obtained, between surface and sub-surface water. Gram negative rods, especially pseudomonads and achromobacters were more frequent at the surface. The fermentative and oxidase negative flora was more frequent in sub-surface water. The surface water in general had a greater variety of bacterial types while the sub-surface water had a flora with a greater range of biochemical activity.

These results are discussed in relation to the hydrological condition of the water masses and the bacterial flora of freshly caught fish.

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