THE OCCURRENCE AND ACTIVITIES OF CERTAIN BACTERIAL GROUPS IN OFF-SHORE MARINE ENVIRONMENTS OF GULF OF MANNAR, OFF TUTICORIN[1]

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INTRODUCTION

Very little work has been done on the marine bacterial groups in off-shore regions in India, though comprehensive accounts are available about these in the U.S.A. (ZoBell, 1946). Earlier, Waksman and collaborators (1933*a*) demonstrated the presence of certain physiological groups, such as cellulose digesters, algin digesters, agar digesters, denitrifiers and 'copper bacteria', in the Gulf of Maine. Some workers have studied single groups, such as chitin digesting bacteria (reviewed by Sreenivasan, 1955), agar digesting bacteria (Lundestad, 1928; Hidake and Samesima, 1953), sulphate reducing bacteria (ZoBell and Rittenburg, 1948), cellulose digesting bacteria (Stanier, 1940; Waksman *et al.*, 1933*a*), algin digesting bacteria (Waksman *et al.*, 1934), and urea splitting bacteria (ZoBell and Feltham, 1935). Wood (1953), in Australia, has also given an account of some of these groups in his paper which contains an excellent account of fish spoilage bacteria also. In our earlier work on the off-shore sea-water of the west coast, bare mention was made of the abundance of denitrifiers and absence of coliforms. Velankar (1955) has studied the presence of a few physiological groups, but only in the shallow inshore bay in the Gulf of Mannar. In connection with the study of the productivity of pearl banks and chank (conch-shell) grounds, investigation was carried out on the bacterial flora of sea-water, pearl oysters, chanks and bottom sand, 10 to 12 miles off Tuticorin. The periods of investigation were in September, 1953 and in January, 1955. The existence of these groups may explain the biological cycles in the sea, the deterioration of submerged materials, such as ship's bottoms, netting, etc., and spoilage of marine products.

EXPERIMENTAL PROCEDURES AND MATERIALS

The pearl banks were visited during the periods of pearl bank inspections in September, 1953, March, 1954 and January, 1955. Water samples were taken from south, north, central and eastern sections of Tolayiram par, and also from Kanava par and Kadayan par. The average of bacterial counts of these and those of the pearl oysters is indicated in Table I. The sand and chanks were collected from the chank beds near Tolayiram par. The temperature of water ranged from 27·3°C. to 29·5°C.

Water samples were collected 12″ beneath the surface and from the bottom (8 to 12 fathoms depth), and plated out on board the vessel itself. A Nansen water-bottle was used and the water at once transferred to a sterile glass-stoppered bottle.

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TABLE I

Bacterial groups in different areas of pearl banks and the bottom materials, in the Gulf of Mannar

Bacterial groups	Sea-water (off-shore)						Sand (off-shore)	Chanks		Pearl oysters
	September, 1953		March, 1954		January, 1955			I	II	
	Surface	Bottom	Surface	Bottom	Surface	Bottom				
Total counts/c.c. or gm. on sea-water agar	392	33	<30	<30	456	23	99	TNC	>3,000,000	649,000
Total counts/c.c. or gm. on fresh-water agar	Spr.	26	..	..	..	..	..	..	45,000	..
Coliforms, 'M.P.N.' per 100 ml.	Nil	Nil	Nil	Nil	..	..	Nil	>10,000	>10,000	17
Denitrifiers	+	..	+	−	+	−	+	1,000	1,000	1,000
Ureafermenters	−	−	−	−	−	−	+	+	+	..
Agar digesters	−	−	−	−	−	+	+	+	+	+
Algin digesters	+	−	+	+	+	+	±	±	±	−
Chitin digesters	−	−	−	−	−	−	+	1,000	1,000	1,000
Cellulose digesters	−	−	−	−	−	−	−	1,000	1,000	1,000
Sulphate reducers	−	−	−	−	−	−	+	1,000	1,000	+
'Copper bacteria'	−	−	..	..	..	..	..	7,300	..	5

+ = Present.
− = Absent.
.. = Not tested.
± = Rare.
TNC = Too numerous to count.
Spr. = Spreaders.

Bottom sand was scooped out by a sampler devised by Chidambaram *et al.* (1951). Pearl oysters and chanks were taken out by naked divers and were handled on shore according to the usual procedures for the examination of shell fish (U.S. Public Health Service, 1946). Dilutions of sea-water, sand, chanks, etc., were inoculated into appropriate enrichment media for each of the physiological groups. When the presence of these groups was proved pure, cultures were isolated and representative strains were studied.

Bacterial population of sea-water.—The surface and bottom sea-water examined on a number of occasions and in different locations such as *Tolayiram par*, *Kanava par*, *Kadayan par*, etc., indicated that the bacterial populations were sparse, being a few score. There is no variation, in the numbers between different areas of off-shore sea. Marine sand, which mainly consisted of silica and calcareous shell fragments, did not harbour a rich population of bacteria, though very reactive types were isolated. The counts on ZoBell's medium were greater than on fresh-water agar. It was also noted that some of the bacteria failed to grow on salt-free media indicating a special ecological group, viz. 'marine' bacteria. A few could, by gradual laboratory cultivation, be 'trained' to grow on fresh-water media but a majority was halophilic. These mostly belonged to *Pseudomonas*, *Achromobacter*, *Bacterium*, and *Corynebacterium*. *Bacillus*, *Micrococcus* and *Sarcina* did not fail to grow on fresh-water media.

Bacterial action of sea-water.—The very low population of off-shore sea-water may be due to the inherent bactericidal property, especially towards the autochthonous organisms of soil, sewage, etc. In Tuticorin, the town sewage and the effluents from the Harvey Mills are let into the sea. A study was made of the influence of sea-water on the survival of the coliform organisms. This was necessary because the beds of shell fish, such as pearl oysters and chanks, are situated 8 to 12 miles off this discharge site. Further, edible fish are also caught nearby. Results tabulated in Table II clearly bring out the fact that even within a distance of half a mile the coliforms are drastically reduced in population and, at a distance of five miles, there is practically no coliform organism traceable. Vaccaro *et al.* (1950) also found that the number of bacteria decreases much more rapidly than could be accounted for by dilution alone. The 'Total' counts also were progressively reduced with the increasing distance from the land, indicating the inability of terrestrial flora to establish themselves in the open seas.

TABLE II

Coliform and total counts of the sewage outfall and of sea-water opposite

	Distance						
	Near the outfall opposite Harvey Mills	⅛ mile	½ mile	1½ miles	3 miles (near Hare Island)	5 miles	8 miles
September, 1953:							
Coliforms MPN/100 c.c.	160,000	..	920	..	..	..	Nil
Total counts ..	60,000	..	390	..	..	..	18
March, 1954:							
Coliforms ..	1,600,000	1,600	..	49	49	4	Nil
Total counts ..	300,000	3,000	..	50	87	29	..
On (*a*) Sea-water, (*b*) Fresh-water agar	TNC	470	..	Spreader	27	18	..

PHYSIOLOGICAL GROUPS

Agar digesting bacteria.—2 to 3% of the colonies from sea-water and 7 to 10% from marine sand were agar digesters. They were profusely pigmented, pink, yellow orange to red, brown and also non-chromogenic. They all liquefied agar remarkably rapidly. On the agar plates, they initially showed a depression which widened into a saucer-shaped crater and finally spread out liquefying the entire plate. These agar digesters were slender, long, C- or S-shaped curved rods as well as pleomorphic, and straight sluggishly motile or non-motile, non-sporing and gram negative. Two cultures from sea-water were polar flagellated. The others probably belong to the genus *Bacterium*. 'Reducing sugars' were identified, when agar was liquefied. A description of the cultures is given in Table III. Great difficulty was experienced in preserving these cultures, since they die off rapidly in the liquefied (agar) medium, probably due to acid formation from the sugars. A large percentage of colonies isolated from chanks were also agar digesters. ZoBell (1946) computed that 1 to 2% of bacteria occurring in the sea are capable of agar digestion, while Wood (1953) found between 2 to 5% in Australian waters. Waksman *et al.* (1933*a*) noted them mostly in diatom tows. The cultures described by us in Table III differ from those described by Lundestad (1928).

Alginic acid digesters.—Bacteria digesting alginic acid were invariably present in 1 c.c. of sea-water, both surface and bottom, but to a smaller extent only in pearl oysters, and rarely in chanks and in sand. On algin agar plates, they cleared the opacity, formed transparent zones or 'halos' due to the utilization of alginates and even softened the agar in some cases. In liquid enrichment cultures gas was also produced. All the isolates studied were gram negative non-spore-forming peritrichous or non-motile rods, belonging to the genus *Bacterium*. Some of them could be identified with *Bacterium alginovorum* (Waksman *et al.*, 1934). A description of these is given in Table IV.

Cellulose digesting bacteria.—The sea-water and sand were devoid of bacteria digesting cellulose or utilizing it as a sole source of carbon. But the flesh of chanks (*Xancus pyrum*) and pearl oysters (*Pinctada vulgaris*) showed the presence of this group in 10^{-3} dilution. Growth of the organisms occurred on the filter paper partially dipping in the cellulose enrichment medium (Waksman *et al.*, 1933*c*) but only at the exposed and not at the submerged portion. Growth was evident in 2 days and in 4 days, the paper was cut up at the liquid-paper interface. Gradually the filter paper turned transparent due to digestion till it finally broke down. Purification of the culture was made by serial dilution. Two types of organisms were noted—one, a minute gram negative, non-sporing, actively motile, monotrichous rod, and the other, a very actively motile spindle-shaped organism, resembling *Cell-fascicula*.

Chitin digesters.—They were frequently recovered from chanks and pearl oysters, but less often from sand and sea-water. After enrichment and purification by serial dilution, the ability of these organisms to utilize chitin as the sole source of carbon and nitrogen was tested. Pure colonies were isolated on chitin agar plates and studied in detail. An account of one new species of 'marine' chitin digesting bacterium *Pseudomonas chitinovora* is given by Sreenivasan (1955). It was shown to solubilize chitin very rapidly, liberating ammonia. Subsequently many more cultures have been isolated and studied. All these were monotrichous gram negative rods belonging to *Pseudomonas*. Surprisingly, they were all denitrifiers, rapidly liberating nitrogen from nitrates. This explains the gas production observed in chitin enrichment media during primary isolation.

Copper bacteria.—Waksman *et al.* (1943) isolated some bacteria from sea-water and submerged slides tolerating a maximum of 200 mg./L. of $CuSO_4.5H_2O$. In view of the rôle of film forming bacteria in fouling submerged materials (ZoBell, 1946) and their rôle in affecting the antifouling paints, the copper tolerance of

marine bacteria was studied by plating out sea-water, chank flesh and pearl oyster flesh on nutrient sea-water agar containing 250 p.p.m. $CuSO_4.5H_2O$. The sea-water bacteria did not grow in this concentration of $CuSO_4$, but a large number of colonies appeared from chanks (7,300 per gm.) and a few from pearl oysters (5 per gm.). After purification on $CuSO_4$ agar, pure cultures were studied in detail. These 'copper' bacteria formed brownish copper coloured colonies. Some tolerated even 1,000 p.p.m. of $CuSO_4.5H_2O$, while four cultures from pearl oysters tolerated up to 750 p.p.m. This tolerance of high concentration of $CuSO_4$ is highly interesting, since very few workers have investigated these groups of bacteria. Wood (1953) noted that primary film forming bacteria tolerated a maximum of up to 250 p.p.m. of $CuSO_4$. Of further interest is the fact that these organisms are also denitrifiers. They belonged to the genus *Pseudomonas*, *Flavobacterium* and *Achromobacter* (Sreenivasan, 1956).

Denitrifying bacteria.—Bacteria liberating gaseous nitrogen from nitrates appear to be quite abundant in tropical marine environments. They were quite often detected in 1 c.c. quantities of surface sea-water and sometimes in bottom water also. Chank flesh and pearl oysters invariably contained denitrifying bacteria even in 10^{-3} dilutions. They were capable of producing gas from the medium of Waksman *et al.* (1933*a*), as also from nitrate-peptone broth. These 'marine' denitrifiers were all monotrichous, non-fluorescent, gram negative rods belonging to the genus *Pseudomonas*. The denitrifying activities of similar bacteria have been studied in detail (Sreenivasan and Venkataraman, 1956). Utilization of amino acids by some of the denitrifiers has also been reported upon (Venkataraman and Sreenivasan, 1955). An account of chitinophilic denitrifiers is already given on page 360. It was noted that with only mineral salts, plus chitin and potassium nitrate, some of the chitinophilic bacteria liberated gas. So also with only ammonium sulphate, KNO_3 and glucose, gas was liberated by these, confirming our earlier observation that denitrification is possible in marine environments also. Besides these, some members of the genus *Bacterium* which digest alginic acid were also seen to be denitrifiers. The copper tolerant denitrifiers (page 360) may play some part in fouling by virtue of precipitation of $CaCO_3$ due to the denitrifying activities and thereby increasing the *p*H (ZoBell, 1946). The abundance and ubiquity of these denitrifiers and the poverty of nitrates in tropical waters explain the rôle of these in the depletion of nitrates.

Luminescent bacteria.—Quite a number of bacteria from sea-water were phosphorescent, but this property was lost on subculture. Bacteria isolated from pearl oyster showed a large number of highly luminous colonies. Wood (1953) also recorded the occurrence of such bacteria in Australian water.

Lactose fermenting bacteria.—Sea-water 10 miles off-shore (examined in three 'paars' at different periods) did not reveal the presence of coliforms. The chank flesh and pearl oysters contained some coliforms, the latter 17 per 100 ml. It is thought that they are adventitious and further work is necessary.

Sulphate reducing bacteria.—In the denitrification medium of Waksman *et al.* (1943), inoculated with bottom sand, at first nitrate reduction occurred with the liberation of nitrogen. Later, it was seen that there was blackening of the sediment accompanied by the smell of hydrogen sulphide, indicating the presence of sulphate reducing bacteria. Sea-water and other marine materials were inoculated into Van Delden's medium. Surface and bottom sea-water did not show the presence of sulphate reducers but the off-shore sand did. Chanks and pearl oysters showed positive sulphate reduction even in 1: 1,000 dilution indicating the large numbers of sulphate reducing bacteria. These were found to be also denitrifiers. They were long, flexuous, motile rods. It was found difficult to obtain pure cultures of these by cultivation on the laboratory media. Better growth was, however, obtained in media with thioglycollate indicating the low-redox potential under which they grow.

TABLE

A description of agar

Strain No.	Cultural characteristics	Morphology	Broth	Potato
Sd.13	Orange yellow, circular, moist, undulate, smooth, butyrous, deep saucer-shaped depression and liquefaction. Fishy smell.	Rods, curved, C- & S-shaped, motile, long, thin, variable length, gram negative. No spores.	Turbidity, pellicle and thick ring.	Brown, warty growth.
Sd.14	Grey translucent, glistening, smooth, agar depressed.	Rods, short, ellipsoidal, non-motile, gram negative. No spores.	Turbidity and pellicle.	..
Sd.16	Saffron coloured, bright glistening, smooth, liquefying agar.	Rods, gram negative, long, curved, slender, non-motile, single and pairs. No spores.	Uniform turbidity. Orange red ring.	Scanty streaks.
Sd.17	Orange yellow, glistening, circular, smooth viscous. 'Crater' like depression and liquefaction of agar. Amine odour.	Gram negative, very long rods, curved motile, peritrichic. No spores.	Turbidity and pellicle. Broth turns brown.	..
Sd.18	Orange yellow, smooth, glistening, depression, formed and liquefied. Fishy odour.	Rods, straight, curved, etc., gram negative, motile, plemorphic, peritrichous flagella.	Uniform turbidity.	Rose streaks.
SW.8	White, circular, entire depressed colony. Agar liquefied slowly.	Gram negative, single, pairs and chains, motile, monotrichous.	Turbidity and fragile white pellicle.	..
SW.9	White, circular, entire depressed, agar slowly liquefied. Foul amine smell.	Rods, motile, gram negative, coccoid, slightly curved, monotrichous.	Uniform turbidity. Fragile pellicle ray ring.	..

III

digesting bacteria

Carbohydrates, sugars	H_2S	Indole	Gelatin	Purple milk	Nitrate reduction	Growth in fresh-water media
Glucose, arabinose, sucrose, mannitol—acid; lactose—no change; starch hydrolysed.	..	..	Rapid liquefaction.	NC	Reduced to nitrate.	..
Starch hydrolysed; glucose, sucrose—acid; lactose—no change.	..	..	Rapid liquefaction.	NC	Reduced to nitrate.	..
Glucose, sucrose, mannitol—acid, lactose—no change; starch hydrolysed.	..	..	Liquefied slowly.	NC	Reduced.	..
Glucose, sucrose—acid; lactose, mannitol—no change; starch hydrolysed.	..	..	Rapid liquefaction.	Alkaline peptonized.	Reduced.	..
Glucose, sucrose, mannitol—acid; lactose—no change; starch hydrolysed.	..	..	Rapid liquefaction.	Coagulated acid (slow).	Reduced.	..
Sugars—no change; starch hydrolysed.	..	..	Liquefied.	No.	Reduced.	..
Sugars—no change; starch hydrolysed.	..	..	Rapid liquefaction.	No.	Reduced.	..

TABLE IV

Alginic acid digesting bacteria

Culture No.	Cultural characteristics	Morphology	Broth	Sugars	Gelatin liquefaction	HS 2	Indole	Milk	Nitrate reduction	Freshwater agar	Remarks
SW.10 SW.20	Grey translucent, flat, moist, circular; agar slant: grey white, moist, glistening, smooth; algin agar; circular convex, drop-like, entire, glistening; aligin digested; agar softened.	Rods, gram negative, actively motile, peritrichous, single and pairs, 0·5–0·8 × 1·0–1·5 rounded ends. No spores.	Turbidity, pinkish fragile pellicle and ring.	Glucose, lactose, mannite, arabinose—NC; starch hydrolysed.	+ Rapid	..	..	NC	..	..	NH from 3 peptone.
SW.43	Grey translucent, flat adherent; agar slant: grey, smooth, glistening; algin plate: cleared the opacity.	Rods, actively motile, single and pairs, and short chains, gram negative. No spores.	Uniform turbidity	Glucose, sucrose, maltose — alkaline; lactose—NC; starch hydrolysed.	+	+	..	NC	+	..	..
SW.44 SW.45	Circular, grey white, entire, smooth, glistening; agar slant: grey white, filiform algin plate: opacity cleared.	Rods, actively motile, gram negative, single and pairs. Medium sized. No spores.	Turbidity, membranous and tenacious pellicle.	Glucose, sucrose, maltose, lactose—no change; starch hydrolysed.	+ Rapid	..	..	NC	Denitrified.	..	..
SW.46	Circular, cream white, raised, entire, smooth; slant: cream yellow, butyrous, abundant, glistening, smooth; algin plate: raised circular, moist, digests algin, softened agar.	Rods, gram negative, non-motile, medium sized. Single and pairs. No spores.	Uniform turbidity	Glucose, sucrose, maltose — no change; starch hydrolysed.	+	..	..	Slowly alkaline.	+	..	..

NC = No change.
— = Negative.

Urea-splitting bacteria.—Bacteria splitting urea and liberating ammonia were present in sea-water in 0·1 c.c. quantities in sand and in chanks. They were also recorded from the surface flora of sharks. The urea-splitting bacteria from chanks were studied in detail. Some of them produced ammonia from urea, but none appeared to be *proteus*. They were all gram negative, non-spore-forming rods, either motile or non-motile with polar or peritrichous flagella. The polar flagellated organism was identical with the denitrifying copper tolerant *Pseudomonas* described earlier.

Bacterial flora of sea-water.—Representative cultures of surface and bottom sea-water were subjected to detailed morphological, cultural and biochemical tests. As in our earlier studies (Venkataraman and Sreenivasan, 1954*a*, *b*) *Bacillus* was the single dominant group. Six of the *Bacillus* cultures were chromogenic, three being rose red. It is particularly interesting to note that these red *Bacillus* occurred quite often in sea-water and in fish in the west coast also. *Bacterium* and non-chromogenic *Pseudomonas* also occurred in some quantities. *Corynebacterium* and *Achromobacter* were found in lesser numbers. Other genera represented, but in insignificant numbers, are *Flavobacterium, Vibrio, Chromobacterium* and *Microbacterium*. Of the 35 cultures, nine had yellow chromogens and five red. Motility and gelatin liquefaction seem to be a normal property of marine bacteria: 26 cultures out of 35 were motile and 28 liquefied gelatin. As in our earlier studies, we noticed very few 'marine' bacteria fermenting sugars. However, all the cultures hydrolysed starch. Starch hydrolysis is fairly common (ZoBell, 1946; Wood, 1953) despite the fact that this form of carbohydrate does not occur in the sea. Two of the *Pseudomonas* produced indole and three H_2S, while one *Bacterium* produced H_2S. 'Fishy' and foul odours were produced on laboratory media by two *Pseudomonas* indicating the probable rôle in fish spoilage. Nitrate reduction was common among sea-water bacteria, 21 out of 35 reducing nitrates to nitrites and some of them even liberating nitrogen. Twenty per cent of cultures peptonized milk. Sixteen cultures are strictly 'marine' forms failing to grow in fresh-water media.

Discussion

The productivity of the seas depends ultimately on the transformations of nitrogen and carbon compounds, mainly due to bacterial activities in the mud, water and the mud-water interface. The existence of a biochemically versatile flora assists in the mineralization of organic matter, decomposing even inert materials, such as lignin, chitin, cellulose, etc. As a result, mainly of the work of ZoBell and his associates, bacteria are also known to play a prominent rôle in petroleum genesis. A study of marine bacteria is also necessitated by their rôle in spoilage of fish with which we are more concerned.

The existence of a special bacterial flora as an ecological group is evident. The inhospitable nature of sea-water to terrestrial forms is clearly seen from the decrease of 'total' counts as well as coliform counts with progressive distance from the land. The presence in sea-water of agar digesting bacteria has been recorded by Waksman *et al.* (1933*c*), by ZoBell (1946) and by Wood (1953). Their presence in large numbers in the bottom sand and in the chanks will aid in the decomposition of this polysaccharide. The agar and algin digesting bacteria may also attack the algae themselves and decrease their content of these phycocolloids. The association of biochemically active groups of bacteria with chanks and pearl oysters is of benefit to these molluscs. It is indeed an example of 'mutualism'. The presence of cellulose digesting, chitin digesting, agar digesting bacteria, etc., perhaps assists in the digestion of these materials by those molluscs. Such symbiotic chitin digestion is also reported by Jenniaux (cited by Hackmann, 1954). Hungate's (1950) review that the cellulose digesting capacity of termites is due really to the presence of bacteria supports the above reports.

The occurrence of large numbers of denitrifiers on these animals need some explanation. The coral reef formation in tropics has been attributed to the activities of denitrifying bacteria, which precipitate $CaCO_3$ (Bavendamm, 1931; Drew, 1911, 1913). Probably the shell formation in these molluscs is aided by this process. The rôle of denitrifiers and sulphate reducers in calcium carbonate precipitation has been extensively and excellently reviewed by ZoBell (1946). Since sulphate reducers were also present in these molluscs, conditions for calcium carbonate precipitation are favourable, as the removal of acidic radicles like sulphates and nitrates leaves an alkaline medium necessary for calcium carbonate precipitation. At present, pearl formation in pearl oysters is attributed to the irritation caused by certain parasites. The possibility of bacteria initiating chemical 'irritation' and/or subsequently depositing $CaCO_3$ by a process of denitrification and sulphate reduction appears to be an attractive theory. In this connection Drew's experiments (1911) are worth citing—'. . . The addition to this culture of very fine particles of calcium sulphate, or of larger particles of sand, resulted in the aggregation around them of particles of calcium carbonate, forming a concentrically laminated concretion around a central nucleus. These were hard and of almost crystalline appearance . . . . Once this process of concretion has been initiated it appears to progress independently of the presence of particles which act as nuclei . . . . .' More of fundamental research is needed to elucidate the mechanism of pearl formation based on Drew's hypothesis cited above.

Bacteria tolerating such high concentrations as 750 to 1,000 p.p.m. of $CuSO_4.5H_2O$ have not been reported so far. These bacteria isolated from chanks and pearl oysters are slimy and could probably form films on submerged materials. Their denitrifying faculty is further interesting in that this also assists in film formation (ZoBell, 1946). Thus, these 'copper' bacteria might assist fouling organisms by attacking the copper paint composition, by forming films, by forming the food of fouling organisms and in various other ways mentioned by ZoBell. The ubiquity and abundance of denitrifying bacteria are significant. They might certainly affect the nitrate content of sea-water and cause loss of nitrogen. Existence of bacteria producing gas from nitrates in the presence of chitin, algin, agar, etc., as enumerated in this paper, clearly indicates that denitrification can take place in the sea, despite the poverty of soluble form of organic matter. The part played by the mesophilic saprophytic bacteria in fish spoilage will be evident from the fact that the cultures were proteolytic and produced indole, H_2S and other offensive odours. The presence of large numbers of spore-forming bacteria is again a problem in heat processing of fish.

Summary

The bacterial population of off-shore sea-water is sparse, but there were versatile physiological groups present to a small extent in sea-water and to a greater extent in the bottom sand and in benthic animals. The presence of these groups such as agar digesters, algin digesters, cellulose digesters, urea splitters, sulphate reducers, etc., brings about transformation of organic matter in the sea. By their symbiotic association with the chanks and other molluscs, it is thought that they aid in the digestion of chitin, cellulose and other stable polysaccharides. The presence of denitrifiers in sea-water and in association with the molluscs may bring about the precipitation of calcium carbonates in sea by removing the acid radicles, such as sulphates and nitrates, increasing alkalinity, etc. Whether a similar mechanism may bring about pearl formation needs investigation. The rôle of 'copper' bacteria in the fouling of submerged materials is discussed. Coliforms did not survive in sea-water beyond five miles from shore and the inflow of town sewage had no effect beyond to three miles from the shore. The bacterial flora of sea-water consists of *Bacillus*, *Pseudomonas*, *Bacterium*, *Corynebacterium*, *Achromobacter* and other genera of lesser importance.

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REFERENCES

Bavendamm, W. (1931). Die Frage der bakteriologische Kalkfällung in der tropischen See. *Ber. Deut. Botan. Ges.*, **49**, 282–287.

———— (1931). The possible rôle of micro-organisms in the precipitation of calcium carbonate in tropical seas. *Science*, **73**, 597–599.

Chidambaram, K., Rajendran, A. D. I., and Valsan, A. P. (1951). Certain observations on the hydrography and biology of the pearl bank, *Tolayiram paar*, off Tuticorin, in the Gulf of Mannar. *J. Madras Univ.*, **21**, 48–74.

Drew, G. H. (1911). The action of some denitrifying bacteria in tropical and temperate seas and the bacterial precipitation of calcium carbonate in the sea. *J. Mar. Biol. Assoc.*, **9**, 142–155.

———— (1913). On the precipitation of calcium carbonate in the sea by marine bacteria, and on the action of denitrifying bacteria in tropical and temperate seas. *Ibid.*, **9**, 479–524.

Hackman, R. H. (1954). Studies on chitin. I. Enzymatic degradation of chitin and chitin esters. *Austr. J. Biol. Sci.*, **7** (2), 168.

Hidake, T., and Samesima (1953). Studies on the agar decomposing bacteria isolated from sea-water. *Mem. Faculty, Fisheries, Kagoshima University*, **3**, 158–164.

Hock, C. W. (1941). Marine chitin decomposing bacteria. *J. Mar. Res.*, **4**, 99–106.

Hungate, R. E. (1950). Mutualisms in Protozoa. *Ann. Rev. Microbiol.*, **4**, 53–66.

Lundestad, J. (1928). Über einige an der Norwegischen Küste isolierte Agar-spaltende Arten von Meer Bakterien. *Zbl. Bakt., II Abt.*, **75**, 321–344.

Sreenivasan, A. (1955). New species of marine chitin digesting bacterium. *Curr. Sci.*, **24**, 270-271.

———— (1956). New species of marine bacteria tolerating high concentration of copper. *Ibid.*, **25**, 92-93.

Sreenivasan, A., and Venkataraman, R. (1956). Marine denitrifying bacteria from South India. *J. Gen. Microbiol.*, **15** (2), 241–247.

Stanier, R. Y. (1941). Studies on marine agar digesting bacteria. *J. Bact.*, **42**, 527–559.

———— (1940). Studies on cytophagas. *Ibid.*, **40**, 619.

U.S. Public Health Service (1946). Manual of recommended practice for sanitary control of the shell fish industry. *Publ. No.* **33**, 1–44.

Vaccaro, R. F., Biggs, M. P., Carey, C. L., and Ketchum, B. H. (1950). Viability of *Escherichia coli* in sea-water. *Amer. J. Publ. Health*, **40** (10), 1257–66.

Velankar, N. K. (1955). Bacteria in the inshore environment at Mandapam. *Ind. Jour. Fish.*, **2**, 96–112.

Venkataraman, R., and Sreenivasan, A. (1954*a*). Bacteriology of inshore sea-water and of mackerels off Tellicherry (Malabar). *Proc. Nat. Inst. Sci.*, **20**, 651–656.

———— (1954*b*). Bacteriology of offshore sea-water of the west coast. *Proc. Ind. Acad. Sci.*, **40**, 161–166.

———— (1955). Utilization of various nitrogenous compounds by certain *Pseudomonas* cultures from marine environments. *Ibid.*, **42**, 31–38.

Waksman, S. A., Reuszer, H. W., Carey, C. L., Hotchkiss, M., and Renn, C. E. (1933*a*). Studies on the biology and chemistry of the Gulf of Maine. III. Bacteriological investigations of the sea-water and marine bottoms. *Biol. Bull.*, **64**, 183–285.

Waksman, S. A., Hotchkiss, M., and Carey, C. L. (1933*b*). Marine bacteria and their rôle in the cycle of life in the sea. II. Bacteria concerned in the cycle of nitrogen in the sea. *Ibid.*, **65**, 1137–67.

Waksman, S. A., Carey, C. L., and Reuszer, H. W. (1933*c*). Marine bacteria and their rôle in the cycle of life in the sea. I. Decomposition of marine plant and animal residues by bacteria. *Ibid.*, **65**, 57–79.

Waksman, S. A., Johnstone, D. B., and Carey, C. L. (1943). The effect of copper upon the development of bacteria in sea-water and the isolation of specific bacteria. *J. Mar. Res.*, **5**, 136–152.

Waksman, S. A., Carey, C. L., and Allen, M. C. (1934). Bacteria decomposing alginic acid. *J. Bact.*, **28**, 213–220.

Wood, E. J. F. (1953). Heterotrophic bacteria in marine environments of E. Australia. *Austr. J. Mar. Freshw. Res.*, **4**, 160–200.

ZoBell, C. E. (1946). 'Marine microbiology'—Chronica Botanica Co., Waltham, Mass., U.S.A.

ZoBell, C. E., and Upham (1944). A list of marine bacteria including descriptions of sixty new species. *Bull. Scripps. Inst. Oceanogr.*, **5**, 239-292.

ZoBell, C. E., and Feltham, C. B. (1935). The occurrence and activity of urea-splitting bacteria in the sea. *Science*, **81**, 234–236.

ZoBell, C. E., and Rittenburg, S. C. (1948). Sulphate reducing bacteria in marine sediments. *Jour. Marine Res.*, **7**, 602–617.

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