

BACTERIA ISOLATED FROM SEA-WATER AND MARINE MUD OFF MANDAPAM (GULF OF MANNAR AND PALK BAY)*

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THE heterotrophic bacteria commonly present in the marine environment have received little attention, though specialised groups such as the agar digesters have been extensively investigated. Existing knowledge of marine bacteria is comprehensively summarised by Zobell and Upham (1944) and Zobell (1946), a significant addition since then, being the report of extensive investigations on bacteria from marine sources in Australia by Wood (1953). Qualitatively, the bacterial flora of marine environments in different parts of the world recorded by different researchers shows some differences. How far this is due to seasonal and environmental differences, and to differences in the techniques employed in the investigations, is not known.

Bacteria of the Indian coastal waters have not been studied so far.† A study of these bacteria and their comparison with the marine bacteria recorded in other regions would be interesting, particularly from a consideration of the temperature differences. Moreover, a knowledge of these bacteria is necessary in the processing and preservation of fish, since fish spoilage flora is derived largely from the bacterial flora associated with the living fish (on the slime, gills and in the gut) and hence has a marine origin (Wood, 1940, 1952, 1953; Castell and Anderson, 1948).

During the course of a quantitative investigation of the bacterial population of sea-water, plankton and mud from the inshore environment at Mandapam the author isolated a number of bacterial strains. These bacteria are described in this paper.

MATERIAL AND METHODS

Well separated colonies distinguished by such characters as the shape, size, opacity and pigmentation, were selected for isolation from the colonies appearing on sea-water agar counting plates used in the quantitative work. Cultural characteristics and the biochemical reactions of the bacteria were studied by standard procedures, employing sea-water in the

* Abstract in *Proc. Indian Sec. Congress*, January, 1954.

† Since this paper was submitted for publication some observations on bacteria in the sea-water off the West Coast have been reported.

preparation of the media except in the case of a few strains which could grow satisfactorily on fresh-water media. Gram-staining was carried out using Huckers modification. For staining the flagella different methods were attempted; a technique which often proved successful was the procedure described by Patel *et al.* (1950).

The bacteria are described in a tabular form (Table I). While some are obviously related to and are possibly identical with types described in Bergeys Manual (1948), complete identification is not possible in most of the cases. Various difficulties arise in the classification of marine bacteria with reference to the previously described types. The necessity of employing sea-water in the preparation of media for the study of marine bacteria renders a strict comparison with types described with the use of standard fresh-water media difficult, since the composition of the medium significantly influences the biochemical reactions of bacteria. Wood (1950), in the light of his very considerable experience of bacteria from marine sources, has discussed in detail the difficulties encountered in classifying these organisms with reference to the existing determinative systems and has tentatively suggested a new scheme. It is felt, therefore, that instead of an attempt at assigning them to genera and species at this stage of the work, a description of the bacteria studied would be more useful and might avoid confusion in comparing the flora with that described from other marine environments by different investigators.

RESULTS

The bacteria are separated arbitrarily into four groups for convenience of discussion.

(i) Gram-negative, non-sporing, achromic rods which produce acid from one or more sugars.

(ii) Gram-negative, non-sporing achromic rods which do not ferment sugars.

(iii) Gram-negative, non-sporing, chromogenic rods.

(iv) Spore-forming rods, micrococci and a few other organisms.

About half of the total cultures are included in Groups I & II, *i.e.*, Gram-negative, asporogenous rods which do not produce any pigment. Among these only two are non-motile. The cultural characters of these organisms are non-distinctive; for their classification, separation into two divisions based on the nature of their flagellation, peritrichous or polar, is a prerequisite. All those which were successfully stained showed polar flagellation and, therefore, belong to the family Pseudomonadaceæ. In

TABLE I (GROUP I)

No.	Original colony on Zobell's agar	Source	Morphology	Sea-water peptone broth	Fresh-water peptone broth	Nitrate reduction to nitrite	Gelatin liquefaction
1	Bean-shaped, translucent smooth colony	Sea-water, mud	Non-sporing, Gram-negative, motile rods. Single polar flagellum. Size $0.4 \mu \times 1.6 \mu$	Pellicle; medium cloudy	No growth	+	+
2	Small, opaque, amorphous colony	Sea-water, mud, plankton	Non-sporing, Gram-negative, motile rods. Size $0.6 \mu \times 1.4 \mu$	No pellicle; medium cloudy	No growth	+	+
25	Large (1.5 cm. diam.), dull colony	Sea-water	Non-sporing, Gram-negative, motile rods; both straight and curved cells present, polar flagella. Size $0.5 \mu \times 1.4 \mu$	No pellicle, but thin film at surface; medium cloudy	Poor growth	+	+
51	Circular (2 mm. diam.), colony with bluish iridescence	Sea-water	Non-sporing, Gram-negative, motile rods. Flagella at both poles. Size $0.4 \mu \times 1.2 \mu$	Uniform cloudiness	No growth	+	+
52	Opaque, irregular, whitish colony	Sea-water	Non-sporing, Gram-negative motile rods. Size $0.5 \mu \times 2.0 \mu$	Cloudy; granular turbidity	Poor growth	+	+
56	Dull, opaque, circular (6 mm. diam.) colony	Sea-water	Non-sporing, Gram-negative, motile rods; curved cells present. Size $0.4 \mu \times 1.8 \mu$	Uniformly cloudy	Moderate growth	+	+
58	Circular (5 mm. diam.) colony with filamentous edge	Sea-water	Non-sporing, Gram-negative, slender, motile rods; curved cells also present. Stain unevenly. Size $0.3 \mu \times 1.8 \mu$	Uniform turbidity; no pellicle	No growth	+	+ slow
44	Large oval (2 cm. \times 0.5 cm.) whitish opaque colony	Mud	Non-sporing, Gram-negative, motile rods; short, almost spherical, cells also present. Single polar flagellum. Size $0.4 \mu \times 1.6 \mu$	Heavy uniform turbidity; no pellicle	No growth	+	+ slow

46	Irregular shaped large colony with wrinkled surface	Mud	Non-sporing, Gram-negative, motile rods; coccoid cells also present. Size $0.5 \mu \times 1.6 \mu$	Uniform turbidity; no pellicle	No growth	+	
37	Dull; white, moist, circular (2 mm. diam.) colony	Mud	Non-sporing, Gram-negative, motile rods. Coccoid cells also present. Size $0.4 \mu \times 2.2 \mu$	Moderate turbidity	Very good growth	+	+
59	Arborescent, dull colony	Sea-water	Non-sporing, Gram-negative, motile rods and coccoid cells. Size $0.8 \mu \times 1.8 \mu$	Moderate turbidity; granular pellicle	No growth	+	+
47	Thin, spreading, dull colony	Sea-water and mud	Non-sporing, actively motile, curved rods. Single polar flagellum	Heavy uniform turbidity; granular pellicle	Slight cloudiness	+	+
4	Thin spreading colony with bluish iridescence	Sea-water and mud	Non-sporing, Gram-negative, motile rods. Single polar flagellum. Size $0.4 \mu \times 1.4 \mu$	Heavy uniform turbidity; no pellicle	No growth	+	+
53	Circular (1 cm. dia m.) dull, filamentous colony	Sea-water and mud	Non-sporing, Gram-negative, motile rods. Long chains present. Stain unevenly. Size $0.5 \mu \times 2.0 \mu$	Uniform turbidity; no pellicle	Good growth	+	+
22	Amorphous, opaque, wrinkled surface colony, turning brownish later	Mud	Non-sporing, Gram-negative, thin rods; long chains also present. Non-motile. Size $0.3 \mu \times 2.2 \mu$	Buff coloured cushion-like pellicle; medium clear	Moderate growth	+	-
60	Pinkish, opaque, amorphous colony	Sea-water	Short, medium size rods, non-motile, Gram-negative, bipolar staining. Size $0.5 \mu \times 1.0 \mu$	Faint ring at surface; medium slightly cloudy	No growth	+	+; rose pink pellicle

TABLE I (GROUP I)

No.	Litmus milk	Indol production	Starch hydrolysis	Carbohydrate fermentation	Growth on potato	Casein digestion	H ₂ S production	Remarks
1	Nil	+	-	Acid but no gas from glucose mannitol, sucrose, maltose. No acid from lactose, xylose, glycerol	-	-	++ (24 hrs.)	
2	Nil	+	-	Acid but no gas from glucose mannitol, sucrose, maltose and lactose. No acid from xylose; slight acid from glycerol	-	+	+(3 days)	
5	Nil	-	+	Acid but no gas from glucose mannitol, sucrose, maltose. No acid from lactose, xylose, glycerol	-	-	++ (24 hrs.)	
51	Nil	+	+	Acid but no gas from glucose mannitol, sucrose, maltose, glycerol No acid from lactose, xylose	-	+	Slight (4 days)	
52	Nil	+	-	Acid but no gas from glucose, mannitol, sucrose, maltose No acid from lactose, glycerol, xylose	-	+	+(48 hrs.)	
56	Nil	-	+	Acid but no gas from glucose, mannitol, sucrose, maltose. No acid from lactose, glycerol, xylose	-	+(slight)	+(48 hrs.)	
58	Peptonized, neutral	-	-	Acid but no gas from glucose, sucrose, maltose, mannitol, lactose, xylose, glycerol, not fermented	-	-	-	

-44	Peptonised, slightly reddened	-	+	Acid but no gas from glucose, sucrose, maltose, glycerol, lactose, Mannitol, xylose, not fermented	-	+	+ slow (6 days)
-46	No change	-	-	Acid but no gas from glucose, mannitol, sucrose, maltose, xylose. Lactose, glycerol, not fermented	-	-	+ (48 hrs.)
-37	Nil	-	-	Acid but no gas from glucose, mannitol, glycerol. Lactose, sucrose, xylose, maltose, not fermented	-	-	+ (48 hrs.)
-59	Nil	-	-	Acid but no gas from glucose, mannitol, sucrose Lactose, glycerol, maltose, xylose, not fermented	-	-	+ (4 days)
-47	Nil	-	+	Acid but no gas from glucose, mannitol, sucrose, maltose. Lactose, glycerol xylose not fermented	-	-	+ slow (1 week)
4	Decolorised, peptonised	-	+	Acid but no gas from glucose, mannitol, sucrose, maltose, glycerol Lactose, xylose, not fermented	Dull brownish growth	-	++ (48 hrs.)
-53	Decolorised, peptonised	+	+	Acid but no gas from glucose, Mannitol, sucrose, maltose, glycerol Lactose, xylose not fermented	-	+	++ (24 hrs.)
-22	Decolorised, peptonised	-	-	Acid but no gas from glucose, mannitol sucrose, maltose, xylose, glycerol, lactose, not fermented	-	-	+++ (24 hrs.)
-60	Peptonised, slightly pink	-	-	Acid but no gas from glucose sucrose, maltose ; slight acid from mannitol. Lactose, glycerol, xylose not fermented	-	++	-

TABLE I (GROUP II)

No.	Original colony on Zobell's agar	Source	Morphology	Sea-water peptone broth	Fresh-water broth	Nitrate reduction to nitrite	Gelatin liquefaction
3	Large, irregular, smooth, moist, translucent colony	Sea-water and mud	Non-sporing, Gram-negative, motile rods. Single polar flagellum. Size $0.3 \mu \times 2.0 \mu$	Waxy, firm, pellicle	No growth	+	-
22	Irregular, large, wrinkled colony	Mud	Non-sporing, Gram-negative, motile rods. Single polar flagellum. Size $0.4 \mu \times 1.4 \mu$	Uniform, turbidity	Moderate turbidity	+	-
5	Circular (2 mm. diam.) colony with bluish iridescence	Sea-water, and plankton	Non-sporing, Gram-negative, motile rods. Single polar flagellum. Size $0.5 \mu \times 1.8 \mu$	Pellicle; heavy turbidity	No growth	+	+
55	Circular (0.7 cm. diam.) colony; opaque centre and edge translucent	Sea-water	Non-sporing, Gram-negative, motile rods. Stain unevenly. Size $0.6 \mu \times 1.4 \mu$	Thin film at surface; medium cloudy	No growth	-	+
42	Circular (0.8 cm. diam.) opaque colony	Mud	Non-sporing, Gram-negative, motile rods; coccoid cells also present. Stain unevenly. Size $0.6 \mu \times 2.0 \mu$	Turbidity at surface mainly; medium clear	No growth	-	+
43	Irregular, large, dull colony with greenish-yellow tinge	Mud	Non-sporing, Gram-negative, motile rods. Stain unevenly. Single polar flagellum. Size $0.4 \mu \times 1.8 \mu$	Uniform turbidity; medium slightly yellow	No growth	-	-
31	Round, raised (3 mm. diam.), moist, translucent	Sea-water	Non-sporing, Gram-negative, motile rods. Single polar flagellum. Size $0.5 \mu \times 1.6 \mu$	Waxy pellicle medium turbid	Opalescent growth	Reduced to nitrite and to free N_2	++
32	Circular, glistening, (2 mm. diam.) with yellowish tinge	Sea-water	Non-sporing, Gram-negative, motile rods. Single polar flagellum. Size $0.4 \mu \times 1.6 \mu$	Thin pellicle; medium turbid	Opalescent growth	Reduced to nitrite and to free N_2	-
33	Circular (2 mm. diam.), moist, glistening	Sea-water and plankton	Non-sporing, Gram-negative, motile rods. Size $0.4 \mu \times 1.5 \mu$	Pellicle; medium turbid	Opalescent growth	Reduced to nitrite and to free N_2	-

No.	Litmus milk	Indol production	Carbohydrate fermentation	Starch hydrolysis	Growth on potato	Casein digestion	H ₂ S production	Remarks
3	Peptonised, neutral	-	Glucose, mannitol, sucrose, maltose, lactose, xylose, glycerol, not fermented	+	-	-	-	
22	Decolorised	-	Glucose, mannitol, sucrose, maltose, lactose, xylose, glycerol, not fermented	+	Brownish-yellow, dry growth	-	+ slow (4 days)	
5	Peptonised, neutral	-	Glucose, mannitol, sucrose, maltose, lactose, xylose, glycerol, not fermented	+	-	+	-	
55	Peptonised, neutral	-	Glucose, mannitol, sucrose, maltose, glycerol, not fermented. Slight acid from lactose, xylose	+	-	+	-	
42	Peptonised, neutral	-	Glucose, sucrose, maltose, lactose, xylose, glycerol, not fermented. Slight acid from mannitol	+ (slight)	Creamy white	-	-	
43	Peptonised, alkaline	-	Glucose, mannitol, sucrose, maltose, lactose, xylose, glycerol not fermented	-	Yellowish white	+	-	
31	Decolorised, neutral	-	Glucose, mannitol, sucrose, maltose, lactose, glycerol, xylose, not fermented	+	Nil	-	+(48 hrs.)	
32	Decolorised, neutral	-	Mannitol, sucrose, maltose, lactose, glycerol, xylose, not fermented. Acid but no gas from glucose	-	Nil	+	+(3 days)	
33	Decolorised, neutral	-	Glucose, mannitol, sucrose, maltose, lactose, xylose, glycerol, not fermented	-	Heavy, dirty-brown growth	-	+(3 days)	

TABLE I (GROUP III)

No.	Original colony on Zobell's agar	Source	Morphology	Colonies on sea-water agar	Sea-water agar slope	Sea-water peptone broth	Sea-water broth
14	Orange-yellow, opaque raised. Circular (2 mm. diam.) colony	Sea-water and plankton	Non-sporing, Gram-negative, motile rods. Single polar flagellum. Size $0.4 \mu \times 1.5 \mu$	Circular (1.5 mm. diam.), smooth, entire, convex, golden-yellow	Filiform, glistening, golden-yellow	Heavy orange pellicle; medium golden-yellow	Moderate growth
8	Orange-yellow, circular (2 mm. diam.), smooth colony; darkening of the medium around the colony	Sea-water	Non-sporing, Gram-negative, motile rods; curved cells also present. Single polar flagellum. Size $0.4 \mu \times 1.8 \mu$	Circular (1 mm. diam.), smooth orange-yellow	Beaded, abundant, glistening, orange-yellow; darkening of the medium after 48 hrs.	Orange-yellow, pellicle; medium clear, later turning slightly dark	No growth
11	Circular (1.5 mm. diam.), translucent, smooth, pale yellow colony	Sea-water and plankton	Non-sporing, Gram-negative, motile rods. Polar flagella. Size $0.3 \mu \times 2.0 \mu$	Circular (1.6 mm. diam.), low convex translucent, moist, pale-yellow	Moderate, spreading, dull, yellowish	No pellicle, heavy, uniform turbidity. Medium tinged yellow	No growth
12	Greenish-yellow, thin, shining, smooth, circular (1 mm. diam.) colony	Sea-water	Gram-negative, non-sporing, motile rods; curved cells also present. Single polar flagellum. Size $0.3 \mu \times 1.5 \mu$	Circular, greenish-yellow, translucent (1 mm. diam.)	Scanty, spreading, greenish, glistening	Uniform turbidity, no pellicle; medium greenish	No growth
7	Bright-yellow, smooth, circular (2-6 mm. diam.) colony	Sea-water	Gram-negative, non-sporing, non-motile, rods. Size $0.4 \mu \times 2.0 \mu$	Punctiform, convex yellow, translucent	Moderate, beaded, yellow butyrous	Bright-yellow pellicle	Moderate growth
20	Orange-yellow, circular (1 mm. diam.) colony	Sea-water	Gram-negative, non-sporing, non-motile rods and coccoid-cells. Size $0.4 \mu \times 1.0 \mu$	Round (1 mm. diam.), orange colony edge entire	Moderate, filiform, viscid, moist, yellow-orange	Orange-yellow pellicle	No growth

13	Orange, circular (1 mm. diam.) colony	Sea-water	Short rod and coccoid cells. Gram-negative, non-motile, non-sporing. Stain unevenly. Size $0.4 \mu \times 0.8 \mu$	Punctiform, irregular, orange, translucent	Moderate, beaded, orange growth	Orange-yellow pellicle	No growth
27	Brick-red, punctiform colony	Sea-water	Non-sporing, Gram-negative, non-motile rods. Size $0.3 \mu \times 1.5 \mu$	Punctiform, glistening, brick-red	Moderate, beaded, dry, brick-red growth	Reddish pellicle	No growth
18	Dull red, round (1.6 mm. diam.) colony	Sea-water	Non-sporing, Gram-negative, non-motile rods. Uneven staining. Size $0.6 \mu \times 2.0 \mu$	Round (2 mm.), dull-red	Moist, filiform dull-red growth	Red pellicle slowly formed (4 days)	Poor growth
16	Deep-pink, circular (1 mm. diam.), concentrically ridged colony	Sea-water	Non-sporing, Gram-negative, motile rods; curved cells also present. Size $0.6 \mu \times 1.4 \mu$	Circular (1 mm. diam.) red centre with whitish margin, convex, smooth	Abundant, echinulate, glistening, pink-red	Deep-pink pellicle	No growth
17	Deep-violet, circular (2 mm. diam.) colony	Sea-water	Non-sporing, Gram-negative, motile rods Size $0.4 \mu \times 1.6 \mu$	Circular (1 mm. diam.), violet centre with whitish margin	Abundant, glistening, violet	Deep-violet pellicle	No growth
10	Orange, viscid, raised colony	Sea-water	Non-sporing, Gram-negative, non-motile rods; curved cells also present. Size $0.4 \mu \times 5.2 \mu$	Circular (2 mm. diam.) raised, orange, viscid colony	Orange-flesh coloured, gummy growth	Light orange pellicle slowly (1 week) formed	No growth

TABLE I (GROUP III)

No.	Nitrate reduction to nitrate	Gelatin liquefaction	Litmus milk	Indol production	Starch hydrolysis	Carbohydrate fermentation	Growth on potato	Casein digestion	H ₂ S production
14	+	+	Decolorised,	-	+	Glucose, mannitol, sucrose, maltose, lactose, xylose, glycerol, not fermented	Heavy, moist orange	+	-
8	-	+	Peptonised, neutral	-	+	Glucose, mannitol, sucrose, maltose, lactose, xylose, glycerol, not fermented	No growth	+	-
11	-	+	Peptonised, neutral	-	+	Glucose, maltose, sucrose, mannitol, lactose, xylose, glycerol, not fermented	Heavy, brownish, moist	+	+
12	-	+	Peptonised, neutral	-	+ moderate	Glucose, mannitol, sucrose, maltose, lactose, xylose, glycerol, not fermented	No growth	+	-
7	+	-	Decolorised, neutral	-	+ slight	Glucose, mannitol, sucrose, maltose, lactose, xylose, glycerol, not fermented	No growth	-	-
20	-	-	No change	-	-	Glucose, sucrose, maltose, mannitol, lactose, xylose, glycerol, not fermented	No growth	-	-

13	+	-	Decolorised, neutral	-	+ slight	Glucose, mannitol, sucrose, maltose, lactose, xylose, gly- cerol, not fermented	Yellow moderate	-	-
27	-	-	Decolorised, neutral	-	+ slight	Glucose, mannitol, sucrose, maltose, lactose, xylose, gly- cerol, not fermented	No growth	-	-
18	+	+	Peptonised, neutral	-	++	Glucose, mannitol, sucrose, maltose, lactose, glycerol, not fermented. Xylose slightly acid	No growth	-	-
16	+	+	Peptonised, neutral	-	+	Glucose, mannitol, sucrose, maltose, xylose, lactose, gly- cerol, not fermented	No growth	+	-
17	+	+	Peptonised, neutral	-	+	Glucose, mannitol, sucrose, maltose, lactose, xylose, gly- cerol, not fermented	No growth	+	-
10	+	+	Peptonised neutral	-	+	Acid but no gas from glucose, lactose, xy- lose. Sucrose, man- nitol, maltose, gly- cerol, not fermented	No growth	+	-

TABLE I (GROUP IV)

No.	Original colony on Zobell's agar	Source	Morphology	Growth on fresh-water agar slant	Growth at pH 6.0	Peptone broth	Nitrate reduction to nitrite
6	Whitish, opaque, edge filamentous	Sea-water and mud	Spore-forming, Gram positive, motile rods. Central cylindrical spores. Sporangia not bulged. Size $0.6 \mu \times 1.8 \mu$	Non-distinctive, non-adherent dull growth.	Nil	Pellicle; medium opalescent	+
34	Amorphous, moist colony	Mud	Sporeforming Gram-positive, motile rods. Oval, central spores. Sporangia not bulged. Size $0.8 \mu \times 1.8 \mu$	Viscid, moist, moderate growth	++	Granular pellicle; medium opalescent	+
35	Whitish, opaque, rhizoid colony	Mud	Spore-forming, Gram-positive, motile rods. Oval, central spores. Sporangia not bulged. Size $0.8 \mu \times 1.2 \mu$	Moist, abundant, pinkish growth	Nil	Opalescent growth; no pellicle	+
36	Yellowish, moist, raised, amorphous colony	Mud	Gram-positive, short, rather stout rods, motile. Central cylindrical spores. Sporangia not bulged. Size $1.2 \mu \times 2.0 \mu$	Yellowish, butyrous, abundant	Nil	Thin film at surface; medium slightly turbid	-

38	Circular (2 mm. diam.), white, dry, wrinkled surface, colony	Mud	Gram-positive, actively motile rods, in pairs. Central spores. Stain uniformly. Sporangia not bulged. Size $0.6 \mu \times 2.0 \mu$	Dry, white, wrinkled, folded growth	++	Granular pellicle; medium moderately turbid	+
39	Pinkish, raised, moist, amorphous colony	Mud	Gram-positive, large rods, non-motile. Single arrangement. Stain uniformly. Central oval spores; sporangia slightly bulged. Size $1.4 \mu \times 3.4 \mu$	Pink, butyrous, moist, abundant growth	Nil	Medium opalescent	+
45	Large circular (4 mm. diam.), colony with lacerate edges	Mud	Spore-forming, Gram-positive rods. Sluggish motility. Central, oval spores. Size $0.8 \mu \times 2.0 \mu$	Thin, dull, non-distinctive	Nil	Medium opalescent	+
15	Crater-shaped, fleshy orange, coloured depression; large zone of agar digestion seen on flooding the plate with iodine	Sea-water, mud and plankton	Elongated, many curved, Gram-negative, non-motile rods, ends tapering. Aver. size $0.5 \mu \times 12.8 \mu$	Moist, fleshy growth, agar digested. Addition of CaCO_3 necessary for neutralising acid produced from agar hydrolysis	..	Silky growth	+
26	Sulphur yellow, raised, moist bulky colony	Mud	Spherical cells forming cubical packets in all media. Non motile. Gram-positive	Raised, sulphur-yellow, abundant	..	Slow growth	+

TABLE I (GROUP IV)

No.	Gelatin liquefaction	Acetyl-methyl-carbinol production	Litmus milk	Carbohydrate-fermentation	Starch hydrolysis	Growth on potato	Casein digestion	H ₂ S production	Remarks
6	-	-	Decolorised, peptonised, neutral	Acid but no gas from glucose, mannitol, sucrose, maltose. Lactose, xylose, glycerol, not fermented	-	Nil	++	+ (slight)	
34	-	++	Decolorised, peptonised, neutral	Acid but no gas from glucose, mannitol, sucrose, maltose, lactose. Xylose, glycerol, not fermented	+	Moist, brownish growth	+ (weak)	-	
35	-	-	Decolorised, peptonised, neutral	Acid but no gas from glucose, mannitol, sucrose, maltose. Slight acid from lactose. Glycerol, xylose, not fermented	+ (weak)	Pink, abundant growth	+ (weak)	-	
36	Slow liquefaction	-	Decolorised, peptonised, neutral	No acid or gas from glucose, sucrose, mannitol, glycerol	Nil	Nil	+ (weak)	-	

8	Rapid liquefaction	++	Peptonised, neutral	Acid but no gas from glucose, mannitol, glycerol, sucrose	+	Luxurious, dry, brownish-gray	-	-	<i>B. subtilis</i> (?)
39	-	-	Peptonised, neutral	No acid or gas from glucose, mannitol, glycerol, sucrose	Nil	Pink to red moderate, dry	-	-	
45	-	..	Decolorised, peptonised, slight pink	Acid, but no gas from glucose, sucrose, maltose, mannitol, lactose, xylose, glycerol, not fermented	Nil	Nil	+ (weak)	Nil	
15	+	..	Rapid peptonisation, neutral	Acid but no gas from glucose, sucrose, maltose, lactose, xylose, glycerol. Mannitol, not fermented	Nil	Nil	+	Nil	<i>Cytophaga</i> (?)
26	-	..	Peptonised, neutral	Glucose, sucrose, maltose, lactose, glycerol, mannitol, xylose, not fermented	Nil	Moderate, sulphur-yellow	+	Nil	(?)

TABLE I (GROUP IV)

No.	Original colony on Zebell's agar	Source	Morphology	Sea-water peptone broth	Fresh-water broth	Growth on nutrient agar slant	Utilization of ammonium phosphate as source of N ₂
9	Circular, salmon-pink (2 mm. diam.), colony	Sea-water	Gram-positive, spherical cells in pairs, and non-motile. Size 0.8 μ diameter	Light pink pellicle	Light pink pellicle	Luxurious rose, moist	+; growth poor, pigment poor
28	Circular (1 mm. diam.), translucent	Sea-water	Gram-positive, spherical cells in pairs and small clusters, non-motile. Size 0.6 μ diameter	Scanty growth	Scanty growth	Adherent, apricot, dry	-
72	Circular (1.5 mm. diam.), light-orange colony	Mud	Gram-positive, spherical cells in pairs tetrads and small clusters, non-motile. Size 0.6 μ to 0.8 μ diameter	Light-yellowish pellicle; good growth	Light-yellowish pellicle; good growth	Abundant, light-orange glistening	+; growth poor

No.	Reduction of nitrate to nitrite	Gelatin liquefaction	Litmus milk	Indole production	Carbohydrate fermentation	Starch hydrolysis	Casein digestion	Growth on potato	H ₂ S production	Remarks
9	+	-	Decolorised	-	Glucose, mannitol, sucrose, maltose, lactose, glycerol, xylose, not fermented	-	-	-	-	<i>M. roseus</i> (?)
28	+	-	No change	-	Glucose, mannitol, sucrose, maltose, lactose, glycerol, xylose, not fermented	-	-	-	-	
72	+	-	Coagulated (3 days); peptonised (2 weeks)	-	Acid but no gas from glucose, maltose, sucrose. Lactose, xylose, glycerol, mannitol, not fermented	-	+(moderate)	-	-	

No.	Original colony on Zobell's agar	Source	Morphology	Growth on sea-water agar slant	Fresh-water agar	Sea-water peptone broth	Colonies on agar	Nitrate reduction to nitrite
61	Dull-greenish black, opaque, amorphous	Sea-water	Large, yeast-like ellipsoidal cells. Gram-positive, non-motile. Size $1.8 \mu \times 4.8 \mu$	Moist dirty greenish white, slowly turning black	Moist dirty greenish white slowly turning black	Black clumps of organisms at surface; medium clear	Black, circularised, like droplets	-
62	Circular colony with mycelial white surface, yellowish from reverse	Sea-water	Branching mycelium, long hyphae, 0.8μ wide. Gram-positive. Non-motile. Not acid fast	Mealy, yellowish, adherent	Mealy, yellowish, adherent	Granular clumps at surface; medium clear	White filamentous, circular, yellow from reverse	-
63	Circular, white, mealy colony; surrounding medium darkened	Sea-water	Branching mycelium with long thin hyphae, 0.4μ wide. Gram-positive. Non-motile. Not acid-fast	Coarse white, mealy; medium below the growth turns dark	Coarse white, mealy; medium below the growth turns dark	Granular clumps at surface; medium clear, slightly darkened	White, filamentous, circular	-

No.	Gelatin liquefaction	Litmus milk	Carbohydrate fermentation	Growth on potato	Ammonium phosphate agar slant	Casein digestion	Starch hydrolysis	H ₂ S production	Remarks
61	-	Slow peptonisation, neutral	Glucose, mannitol, sucrose, maltose, lactose, glycerol, xylose, not fermented	Heavy, grey-black dry	Good growth	Slight	-	-	Yeast?
62	-	Slow peptonisation, neutral	Glucose, mannitol, sucrose, maltose, lactose, glycerol, xylose, not fermented	Heavy, dull-yellow, dry	Good growth	Slight	-	-	<i>Nocardia?</i>
63	-	Slow peptonisation, neutral	Glucose, mannitol, sucrose, maltose, lactose, glycerol, xylose, not fermented	Heavy, dark, dry growth turning to white powder	Good growth	Slight	-	-	<i>Nocardia?</i>

this family those which show peculiar morphological characters such as pleomorphism, stain irregularly, etc., resemble *Mycoplana* as defined by Wood (1950). Some strains in Group II reduce nitrate to free nitrogen.

Pigmentation of the chromogens (Group III) is a stable character since it was constant through a number of sub-cultures made over a period of two to three years. This group is non-fermentative.

The agar digester, No. 15 (Group IV), resembles *Cytophaga*, but the characteristic creeping motility was not observed; also, it does not digest cellulose under experimental conditions using filter-paper strips suspended in sea-water containing 0.5% peptone.

The flora is fairly reactive. Gelatin liquefaction, milk peptonisation, nitrate reduction and starch and casein digestion are fairly common in all the four groups. Saccharolytic activity is confined mainly to the first group. This group is more reactive than the others; in addition to the saccharolytic activity hydrogen sulphide production is common and indol is produced by six out of the sixteen strains. This is noteworthy, as indol production by strains of marine origin is uncommon. Pigment production, and the ability to ferment sugars or produce hydrogen sulphide from peptone, appear to be properties which are mutually exclusive.

All the organisms are aerobic. Most grow better at room temperature (26° C. to 30° C.) than at 37° C. while some do not grow at the latter temperature. Some grow slowly at 2° C. to 3° C. Ammonium phosphate is utilised as the sole source of nitrogen by a large number, but the growth, except in the yeast and *Nocardia*, is very poor compared with that obtained with peptone as the source.

Most of the strains grow satisfactorily on media prepared with sea-water, or with sea-water diluted with distilled water up to the extent of 50%. One strain (No. 17) failed to grow when the media contained less than 75% sea-water. The *Bacillus* and *Micrococcus* species grow satisfactorily on standard fresh-water media. Calcium and/or magnesium, was essential for pigment production by some.

Since the aim of this investigation was to isolate as many different types as possible and not the dominants alone, the composition of the flora broadly reflects the normal distribution of the bacterial types in the environment. Comparing the flora with that from other marine environments the dominance of Gram-negative, asporogenous, motile rods, the paucity of *Micrococcus* and *Bacillus*, and the absence of organisms producing gas from sugars appear to be common features (Zobell, 1946; Wood, 1953). The

physiological characteristics of the bacteria also agree with those of the marine flora described by these workers.

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