

SOME BACTERIA ISOLATED FROM MARINE PLANKTON AND MUD

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Fiftyseven bacterial strains isolated from marine plankton and mud from the inshore waters of Cochin are described. The flora of the plankton was dominated by chromogens while mud samples failed to produce chromogens. The different strains are divided into five arbitrary groups for the convenience of description. The flora is similar to that of Mandapam (east coast) while it differed from the flora of Calicut (west coast) in the paucity of *Bacillus* *sp* and other sporeformers.

INTRODUCTION

Heterotrophic bacteria occurring in the marine environment have been receiving considerable attention in recent years. Marine bacteriological investigations have been carried out both on the west and the east coast of India by different workers. Velankar (1950) studied the bacterial population of the surface water, relative abundance of bacteria in the seawater and in association with plankton off Madras city (Bay of Bengal). He also made qualitative and quantitative studies on the bacteria occurring in the inshore environment at Mandapam (Velankar, 1955, 1957). Venkataraman and Sreenivasan (1954a, 1954b, 1956) examined the bacterial flora of the seawater off Malabar coast and off Tuticorin on the east coast. Santhakumary (1966) and Gore (1971) made some observations on the bacterial flora of the Cochin backwaters and Cochin beach respectively. While studying the quantitative abundance of bacteria in the mud and in the plankton tows off Cochin, the au-

thor isolated a number of bacterial strains and this paper includes detailed description of morphological and biochemical features of these isolates.

MATERIAL AND METHODS

Samples of plankton and mud were collected from a fixed station in the inshore region. Plankton was collected in organic die net and mud from a depth of 6 fathoms was collected in a grab. The samples were carried to the laboratory in sterile glass containers, immediately after sampling. Standard procedures (Karthiayani and Mahadeva Iyer, 1967) were employed in studying the morphological and biochemical features. Plankton samples were carefully ground in a mortar and then plated. Generic identification of the isolates was done according to a modified scheme of Simidu and Aiso (1962).

RESULTS AND DISCUSSION

A total of 57 strains were isolated,

25 from plankton and 32 from mud. Five of the isolates did not grow well and hence could not be studied further. The different isolates are classified into 5 arbitrary groups for convenience of description, mainly based on their gram stain reaction and ability to ferment sugars.

- i. Gram negative non-sporing motile rods capable of fermenting two or more sugars.
- ii. Gram negative non-sporing motile rods capable of fermenting only one sugar.
- iii. Gram negative non-sporing motile rods not capable of fermenting any of the sugars.
- iv. Gram negative cocci capable of fermenting at least one sugar.
- v. Gram positive sporeforming rods.

These different groups are dealt with in tabular form. It is obvious from the tables that majority of the strains fell in group I. All the isolates were aerobic and showed satisfactory growth at room temperature ($30^{\circ}\text{C} + 1^{\circ}\text{C}$). In general the flora is fairly reactive. While gelatin liquifaction, nitrate reduction, ammonia production from peptone and fermentation of sugars are exhibited by majority of the isolates, indole is produced only by a few. Gram negative nonsporing motile rods predominated the flora, a few gram negative cocci and two gram positive spore-forming strains (*Bacillus* sp.) were encountered. All the *Flavobacterium* sp. producing yellow, red and orange pigments were isolated only from plankton and none from the mud. They were less saccharolytic and only one of them produced indole. Velankar (1955) also had isolated chromogens only from surface waters and none from mud samples from the east coast. All the 8 denitrifiers, viz; strain nos, 3a, 4a, 14a, 15a, 16a, 27a, 28a, 29a, isolated were of *Pse-*

udomonas sp. and all were from mud samples. Velankar (1955 *loc cit*) also reported denitrifiers from mud samples only.

Nitrate reducers were present both in plankton and mud. However, they were more frequent in mud samples than in plankton. 7 strains showed good growth on freshwater agar medium (strain nos: 1a, 7a, 16a, 17a, 27a, 31a, and 32a) suggesting their probable non-marine origin. In general the flora is similar to that reported from Mandapam (east coast.) There was no abundance of *Bacillus* sp. and other sporeformers unlike at Calicut as observed by Venkataraman and Sreenivasan (1954 *loc cit*).

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GROUP I a) MORPHOLOGICAL FEATURES OF THE ISOLATES

S. No	Source	Shape of the colony on seawater agar slant.	Growth on seawater peptone	Growth on seawater agar slant	Growth on freshwater agar slant	Morphology
1	2	3	4	5	6	7
1	Plankton	Greenish yellow, round surface colony.	Moderate growth, medium turbid.	Filiform	Very poor growth	Gram negative, non-sporing, motile, stumpy, short rods with polar flagellation.
2	"	Red coloured surface colony.	Medium slightly turbid, a thin reddish pellicle at the top.	Effuse, dry.	No growth	Gram negative, non-sporing motile rods
3	"	Orange, round surface colony.	Medium turbid	Filiform luxuriant growth.	No growth	Gram negative, non-sporing motile rods.
4	"	Mucoid ridged, white, surface colony.	Medium turbid, a pellicle at the top.	Effuse	No growth	Gram negative, non-sporing motile short rods.
5	"	Mucoid ridged, orange surface colony.	Medium turbid, a thick pellicle at the top, which crumbled into pieces on shaking.	Filiform	No growth	Gram negative, non-sporing motile elongated rods.
6	"	Rose coloured large (1.7mm) surface colony	Uniform turbidity	Effuse (very poor growth).	No growth	Gram negative, non-sporing motile short rods.
7	"	Pale white almost transparent, round, surface colony with bluish iridescence	Uniform turbidity	Filiform luxuriant growth	No growth	Gram negative non-sporing motile elongated rods.

1	2	3	4	5	6	7
8	"	Greenish yellow, large (1.5mm) surface round colony.	"	Beaded luxuriant yellowish in colour	No growth	Gram negative non-sporing short, stubby motile rods.
8a	Mud	Dull white large (1.6mm) colony with irregular margins	Medium almost clear. Poor growth	Echinulate	No growth	Gram negative non-sporing motile short rods
9a	"	Dull white round deep colony	Uniform turbidity	do (bluish iridescence)	"	Gram negative non-sporing elongated motile rods
10a	"	Cream coloured colony	Uniform turbidity	do	"	Gram negative non-sporing short rods, passive.
11a	"	Dull white large (1.7mm) colony with irregular margin	Medium turbid a pellicle at the top	do	No growth	Gram negative non-sporing motile short rods
13a	"	Large (1.4mm) white colony	Uniform turbidity	-do- (bluish iridescence)	No growth	Gram negative non-sporing motile short rods
15a	"	Pale whit deep colony	Uniform turbidity	Effuse	Slight growth	Gram negative non-sporing motile rods
16a	"	Dirty white amoeboid colony	Uniform turbidity, a thin pellicle at the top	Echinulate	Good growth	Gram negative motile rods
17a	"	Pure white colony with crenulated margins	Medium highly turbid	-do- (membranous)	Good growth	Gram negative non-sporing motile short rods

1	2	3	4	5	6	7
19a	"	Punctiform grey colony	Uniform turbidity	-do- (luxuriant)	No growth	Gram negative nonsporing motile short rods
24a	"	Large (1.7mm) white deep colony	Heavy turbidity	Filiform, moist	No growth	Gram negative nonsporing motile elongated rods
13	Plankton	White rough colony with concentric rings	Medium turbid, a thin pellicle at the top	Filiform	No growth	Gram negative nonsporing motile elongated rods.
15	"	Rose coloured large (1.4mm) raised colony	Medium turbid	Filiform dry growth	"	Gram negative nonsporing motile rods.
17	"	Pure white transparent raised colony	Uniform turbidity	Beaded	Moderate growth	Gram negative nonsporing short stumpy rods, passive movements.
18	"	Dull white opaque surface colony	Medium slightly turbid	Effuse	No growth	Gram negative nonsporing elongated motile rods.
19	"	White large (1.4mm) opaque surface colony	Uniform turbidity	Filiform	"	Gram negative nonsporing elongated rods, motile, with pointed ends.
20	"	Orange, spreading colony	Medium turbid, a pellicle on the top.	Filiform	"	Gram negative nonsporing motile rods
22	"	Orange, large (1.2mm) round colony	Heavy turbidity	Filiform	"	Gram negative nonsporing short rods
25	"	Dull white amoeboid colony	Uniform turbidity	Effuse, moist	"	Gram negative nonsporing short motile rods

1	2	3	4	5	6	7
1a	Mud	White round surface colony	Medium slightly turbid a pellicle at the top	Effuse	Good growth	Gram negative non-sporing motile rods
3a	"	White surface colony crenulated margins	Uniform turbidity	Filiform, bluish iridescence	Poor growth	Gram negative non-sporing motile rods
7a	"	White spreading surface colony	Uniform turbidity	Echinulate	Good growth	Gram negative nonsporing motile elongated rods with pointed ends
27a	Mud	White, deep colony, irregular shape	Heavy turbidity	Filiform, moist	Good growth	Gram negative nonsporing motile rods with few curved cells
28a	"	Pure white oval colony	Uniform turbidity	- do - - do -	No growth	Gram negative nonsporing motile rods.

b) Biochemical features of the isolates

S.No.	Source	Nitrate reduction to nitrite.	Ammonia production from peptone	Gelatin liquefaction	Indole production	Sensitivity towards penicillin 2.5 IU/ Disc	Fermentation of sugars (glucose, maltose, mannitol & lactose)	Hugh and Leifson's medium (to study mode of attack of glucose)	
1		2	3	4	5	6	7	8	9 10
1.	Plankton	+	+	+	+	-	+	Acid from glucose, maltose & mannitol, did not ferment lactose	NF
2.	"	-	+	+	+	-	+	Acid from glucose & maltose only	NF
									<i>Pseudomonas</i> sp
									<i>Flavobacterium</i> sp

1	2	3	4	5	6	7	8	9	10
4.	"	+	+	-	-	-	Acid from glucose, maltose & mannitol, did not ferment lactose	NF	<i>Pseudomonas</i> sp.
5	"	-	+	-	-	+	Did not ferment lactose; acid from the rest	NF	<i>Flavobacterium</i> sp.
6	"	+	+	+	+	+	Did not ferment lactose; acid from the rest	NF	<i>Flavobacterium</i> sp.
7	"	+	+	+	(slow, 10 days)	-	Did not ferment lactose; acid from the rest	NF	<i>Pseudomonas</i> sp.
8	"	+	+	+	-	-	Acid from glucose, slight acid from maltose and mannitol. Did not ferment lactose.	NF	<i>Pseudomonas</i> sp.
13	"	+	+	+	-	-	Acid from glucose, maltose and mannitol. Did not ferment lactose.	NF	<i>Pseudomonas</i> sp.
15	"	+	+	-	-	+	Acid from glucose, maltose and mannitol. Did not ferment lactose.	NF	<i>Flavobacterium</i> sp.
17	"	+	+	+	-	-	Acid from glucose, maltose and mannitol. Did not ferment lactose.	NF	<i>Flavobacterium</i> sp.
18	"	+	-	+	-	-	Acid from glucose and lactose. Did not ferment mannitol and lactose.	NF	<i>Pseudomonas</i> sp.

1	2	3	4	5	6	7	8	9	10
19	"	+	..	+	-	-	Acid from glucose, maltose and mannitol. Did not ferment lactose.	FNG	<i>Vibrio</i> sp.
20	"	+	+	+	-	-	Acid from glucose and mannitol. Did not ferment maltose and lactose.	FNG	<i>Flavobacterium</i> sp.
22	"	-	..	+	-	+	Acid from glucose maltose and mannitol. Did not ferment lactose.	NF	<i>Flavobacterium</i> sp.
25	"	+	+	+	-	-	Acid from glucose, maltose and mannitol. Did not ferment lactose.	NF	<i>Pseudomonas</i> sp.
1a	Mud	+	-	-	-	-	Acid from glucose, maltose and mannitol. Did not ferment lactose.	FNG	<i>Vibrio</i> sp.
3a	"	+	(reduced + nitrite to free N)	+	-	-	Acid from glucose and maltose. Did not ferment mannitol and lactose.	FNG	<i>Pseudomonas</i> sp.
7a	"	+	-	+	+	-	Acid from glucose, maltose and mannitol. Did not ferment lactose	FNG	<i>Vibrio</i> sp.
8a	"	+	-	+	+	-	Acid from glucose and maltose. Did not ferment mannitol and lactose.	FNG	<i>Vibrio</i> sp.

I	2	3	4	5	6	7	8	9	10
9a	"	+	+	+	+	+	Acid from glucose, maltose and mannitol. Did not ferment lactose	FNG	<i>Achromobacter</i> sp.
10a	"	+	+	+	+	+	Acid from glucose and maltose. Did not ferment mannitol and lactose.	NF	<i>Achromobacter</i> sp.
11a	"	+	+	+	+	+	Acid from glucose and maltose. Did not ferment mannitol and lactose.	FNG	<i>Achromobacter</i> sp.
13a	"	+	-	+	+	+	Acid from glucose, maltose and mannitol. Did not ferment lactose.	FNG	<i>Achromobacter</i> sp.
15a	"	+	+	+	+	-	Acid from glucose and mannitol. Did not ferment maltose and lactose.	NF	<i>Pseudomonas</i> sp.
16a	"	+	+	+	-	+	Acid from glucose and maltose. Did not ferment mannitol and lactose.	NF	<i>Pseudomonas</i> sp.
17a	"	+	-	+	+	+	Acid from glucose, maltose and mannitol. Did not ferment lactose.	NF	<i>Achromobacter</i> sp.
19a	"	+	-	+	+	+	Acid from glucose maltose & mannitol. Did not ferment lactose	NF	<i>Achromobacter</i> sp.

1	2	3	4	5	6	7	8	9	10
24a	"	+	-	-	+	+	Acid from glucose and maltose. Did not ferment mannitol and lactose.	NF	<i>Achromobacter</i> sp.
27a	"	+	(reduced + nitrite to free N)	+	-	-	Acid from glucose and maltose. Did not ferment mannitol and lactose	NF	<i>Pseudomonas</i> sp.
28a	"	+	(reduced + (slow) nitrite to free N)	-	-	-	Slight acid from glucose maltose and mannitol. Lactose not fermented	NF	<i>Pseudomonas</i> sp.
NF = non-fermentative FNG = Fermentative no gas									

GROUP II a) MORPHOLOGICAL FEATURES							FNG: Fermentative, no gas.	
S. No:	Source	Shape of the colony on seawater agar slant	Growth on seawater peptone	Growth on seawater agar slant	Growth on freshwater agar slant	Morphology		
1	2	3	4	5	6	7		
10	Plankton	Orange coloured, large round colony.	Medium moderately turbid, a thin pellicle at the top	Filiform dry growth	No growth	Gram negative non-sporing short motile rods.		
11	"	Greenish yellow, punctiform, surface colony	Medium almost turbid	Beaded, moist growth, yellowish in colour	No growth	Gram neative non-sporing motile short rods.		
14	"	Pure white, myceloid large colony (1.4 mm)	Uniform turbidity	Echinulate, bluish iridescence	No growth	Gram negative non-sporing motile elongated rods arranged in twos.		

	1	2	3	4	5	6	7
		Mud	Dull white, punctiform colony	Medium almost clear, poor growth	Effuse	Slight growth	Gram negative, non-spo- ring motile elongated rods, arranged in twos.
2a							
4a		"	Dull white, deep colony	Uniform turbidity	Filiform luxuriant	Poor growth	Gram negative non-spo- ring short rods
5a		"	Pale yellow, round colony with smooth surface	Uniform turbidity slightly yellowish	Arborescent growth	No growth	Gram negative, non-spo- ring motile short rods.
6a		"	Large, dull white, surface colony	Uniform turbidity	Filiform	No growth	Gram negative non-spo- ring motile short rods.
12a		"	Dirty white, round surface colony	Uniform turbidity a pellicle at the top	Echinulate	"	Gram negative, nonspo- ring motile short rods.
14a		"	Dull white round colony	Uniform turbidity	do	"	Gram negative nonspo- ring motile short rods.
21a		"	White large (1.3 mm) colony, having convex surface	Uniform turbidity	do	"	Gram negative nonspo- ring motile short rods.
22a		"	Large semitransparent dull white, colony	Medium turbid, a pellicle at the top	Filiform	Poor growth	Gram negative nonspo- ring motile short rods.
26a		"	Dull white, deep colony	Slight turbid medium	Effuse, moist	No growth	Gram negative nonspo- ring, motile elongated rods.
29a		"	White, large (1.2mm) colony with enlarged horns.	Medium almost clear, poor growth.	Filiform, membranous	No growth	Gram negative nonspo- ring elongated motile rods.

b) BIOCHEMICAL REACTIONS OF ISOLATES

Source	Nitrate reduction to nitrite	Ammonia production from peptone	Gelatin liquifaction	Indole production	Sensitivity towards penicillin 2.5 IU/Disc.	Fermentation of sugars (glucose, maltose, mannitol and lactose)	Hugh and Leifsons' medium (to study mode of attack of glucose)	Bacterial genus		
S.No:	1	2	3	4	5	6	7	8	9	10
10 Plankton	+	+	+	+	+	—	+	Acid from glucose only. Did not ferment the rest.	FNG	<i>Flavobacterium</i> sp.
11 "	+	+	+	+	+	—	—	Acid from maltose alone. Did not ferment glucose, mannitol and lactose.	FNG	<i>Vibrio</i> sp.
14 "	+	+	+	+	+	—	—	Acid from glucose only. Did not ferment the rest.	NF	<i>Pseudomonas</i> sp.
24 "	+	+	+	+	+	—	—	Did not ferment glucose mannitol and lactose: Acid from maltose only.	NF	<i>Pseudomonas</i> sp.
2a Mud	+	+	+	—	—	—	—	Slight acid from glucose only. Did not ferment the rest.	FNG	<i>Vibrio</i> sp.
4a "	+(reduced nitrite to free N)	+	+	+	+	—	—	Acid from glucose only. Did not ferment the rest	NF	<i>Pseudomonas</i> sp.
5a "	+	+	+	—	+	—	+	Acid from glucose only. Did not ferment the rest	NF	<i>Achromobacter</i> sp.
6a "	+	+	+	+	+	—	+	Acid from glucose only. Did not ferment the rest	FNG	<i>Achromobacter</i> sp.

1	2	3	4	5	6	7	8	9	10
12a	"	+	+	+	-	+	Acid from glucose only, Did not ferment the rest	FNG	<i>Achromobacter</i> sp.
14a	"	+	+(reduced nitrite to free N)	+	-	-	Acid from glucose only. Did not ferment	NF	<i>Pseudomonas</i> sp.
21a	"	+	+	+	+	+	Acid from glucose only. Did not ferment the rest	FNG	<i>Achromobacter</i> sp.
22a	"	+	+	-	-	+	Slight acid from glucose only. Did not ferment the rest	NF	<i>Achromobacter</i> sp.
26a	"	+	-	-	-	+	Slight acid from glucose only. Did not ferment the rest.	NF	<i>Achromobacter</i> sp.
29a	"	+	+(reduced nitrite to free N)	+	-	-	Acid from glucose only not ferment the rest.	NF	<i>Pseudomonas</i> sp.

GROUP III a) Morphological features

S. No.	Source	Shape of the colony on seawater agar slant	Growth on seawater peptone	Growth on seawater agar slant	Growth on freshwater agar slant.	Morphology
3	Plankton	Orange, round surface colony.	Medium turbid	Filiform luxuriant growth	No growth	Gram negative, nonsporng motile rods.
9	"	Dull white, oval, colony with crenulated margins.	Growth mainly at the top, medium almost clear.	Filiform	No growth	Gram negative non-sporng elongated motile rods with pointed ends.
16	"	Golden yellow, surface round colony.	Medium golden yellow with a thick pellicle.	Filiform, dry, yellow in colour.	No growth	Gram negative, non-sporng elongated motile rods.

b) BIOCHEMICAL REACTIONS OF ISOLATES

Source S. No.	Nitrate reduction to nitrite	Ammonia production from peptone	Gelatin liquefac- tion	Indole produc- tion	Sensitivity towards pen- cillin 2.5 IU/ Disc.	Fermentation of sugars (glucose, maltose, mannitol and lactose)	Hugh and Leifsons medium (to study mode of attack of glucose)	Bacterial genus
3 Plankton	+	+	+	-	+	Did not ferment any of the sugars.	NF	<i>Flavobacterium</i> sp.
9 "	+	+	+	-	-	Did not ferment any of the sugars.	FNG	<i>Vibrio</i> sp.
16 "	-	+	-	-	+	Did not ferment any of the sugars.	NF	<i>Flavobacterium</i> sp.

GROUP IV a) MORPHOLOGICAL FEATURES

Source S. No.	Shape of the colony on seawater agar plate	Growth on seawater peptone	Growth on seawater agar slant.	Growth on fresh- water agar slant.	Morphology
18a Mud	White, punctiform colony.	Medium almost clear, poor growth	Effuse	No growth	Gram negative cocci, mesh like arrangement.
20a "	Transparent colony with irregular margins	Uniform turbidity	Echinulate, moist	-do-	Gram negative cocci.
23a "	Grey, large (1.3mm) spreading colony	Medium turbid, a pellicle at the top	Filiform, bluish iridescence.	-do-	Gram negative cocci.

b) BIOCHEMICAL REACTION OF ISOLATES

S. No.	Source	Nitrate reduction to nitrite	Ammonia production from peptone	Gelatin liquifaction	Indole production	Sensitivity towards penicillin 2.5 IU/Disc.	Fermentation of sugars (glucose, maltose, mannitol and lactose)	Hugh and Leifson's medium	Bacterial genus
18a	Mud	+	-	-	-	+	Acid from glucose only. Did not ferment the rest.	NF	<i>Achromobacter</i> sp.
20a	"	+	+	+	+	-	Acid from glucose, maltose and mannitol. Did not ferment lactose.	NF	<i>Achromobacter</i> sp.
23a	"	+	+	+	+	+	Acid from glucose only. Did not ferment the rest.	NF	<i>Achromobacter</i> sp.

GROUP V a) MORPHOLOGICAL FEATURES

S. No.	Source	Shape of the colony on seawater agar plate.	Growth on seawater peptone	Growth on seawater agar slant	Growth on freshwater agar slant	Morphology
31a	Mud	Blackish surface colony	Medium almost clear, growth mainly at the top	Filiform	Good growth turning black after 48 hours	Gram positive spore-forming rods, rather passive.
32a	"	Reddish surface colony	Uniform turbidity medium reddish	Filiform growth reddish in colour	Moderate growth reddish in colour	Gram positive spore-forming motile rods.

b) BIOCHEMICAL FEATURES

Source S. No.	Nitrate reduction to nitrite	Ammonia production from peptone	Gelatin liquefac- tion	Indole produc- tion	Sensitivity towards peni- cillin 2.5 IU/Disc.	Fermentation of sugars (glucose maltose, manni- tol and lactose)	Hugh and Leifson's medium	Bacterial genus
31a Mud	+	+	+	+	—	Acid from glucose and maltose only. Did not fer- ment mannitol and lactose.	NF	<i>Bacillus</i> sp.
32a "	+	+	+	+	—	Acid from glucose, malt- ose, mannitol. Lactose not fermented.	FNG	<i>Bacillus</i> sp.