

BACTERIA IN THE INSHORE ENVIRONMENT AT KARWAR AND VENGURLA

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Qualitative and quantitative studies of the bacterial population in two stations, viz. Karwar and Vengurla, have been made for a period of eight months, which revealed the pattern of distribution to be almost similar to that from east coast and rest of west coast.

INTRODUCTION

Marine bacteriological investigations have received considerable attention in the present day ocean research in view of the importance of bacteria in the chemistry and fertility of the marine environment. Considerable work has been done on the heterotrophic bacteria in the past by various Indian workers from east and west coasts of India. The pioneering work of Velankar (1950, 1955, 1957) from the east coast of India is the only one of its kind which covered a few years from a particular area. He studied the heterotrophic bacteria of the Madras waters, surveyed the bacterial population of the inshore waters at Mandapam and reported a number of bacteria which he isolated during the course of his work. Venkataraman and Sreenivasan (1954, 1954b 1956), and Gore (1971, 1972, 1973) studied the bacterial flora from the west coast of India.

Qualitative and quantitative distribution of bacterial population is interesting in the inshore environment compared to the waters of the ocean remote from land. With a view to determining the trend of qualitative and quantitative bacterial distribution in the inshore environments at two stations along the central west coast of India (Vengurla and Karwar) studies were carried out from November 1971 to June 1972 covering the post monsoon and premonsoon period.

The present paper deals with the quantitative distribution of bacteria of surface seawater, seawater from 13-14m. depth and mud from inshore waters at these two stations and, also some important physiological groups.

MATERIAL AND METHODS

Monthly sampling of seawater and mud from the above two stations was done.

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Samples were collected from a fixed station 1 mile away from the shore, surface samples were collected in sterile glass containers and mud was collected using a grab.

Water samples from bottom were collected in Van Daon Sampler aseptically (the walls of this sampler were polythene-lined). Collected samples were plated immediately (*i. e.* within one hour). Standard procedures (Karthiayani and Mahadeva Iyer 1967) were employed in the enumeration and other biochemical aspects concerned with the study of bacteria. The quantitative abundance of different physiological groups was determined by specific media

using different dilutions. Incidence of 'Coliforms' was tested using MPN method.

BACTERIAL FLORA (Quantitative)

The surface seawater counts ranged from 100 to 440 per ml. at Karwar while the same at Vengurla ranged from 212 to 300 per ml. Bottom seawater counts ranged between 80 to 260 per ml. in Karwar while the same at Vengurla ranged from 90 to 300 per ml. Total aerobic counts per g. of mud (wet basis) from Karwar ranged from 98,000 to 2,39,000 while that from Vengurla ranged from 68,000 to just over a lakh. Table 1 shows surface counts,

TABLE I

THE BACTERIAL COUNTS (AVERAGE OF 4 PLATINGS) IN INSHORE ENVIRONMENT AT VENGURLA & KARWAR

Month	Place	Bacteria per ml. of surface seawater	Bacteria per ml. of bottom seawater	Bacteria (aerobic counts) per g. of mud (wet basis)
November '71	Karwar	320	120	Over one lakh
	Vengurla	300	202	Over one lakh
December '71	Karwar	240	180	90,000
	Vengurla	212	168	88,000
January '72	Karwar	340	192	Over one lakh
	Vengurla	No data	No data	No data
February '72	Karwar	310	180	80,000
	Vengurla	220	190	Over one lakh
March '72	Karwar	290	220	Over one lakh
	Vengurla	274	300	Over one lakh
April '72	Karwar	380	260	1,29,000
	Vengurla	290	90	68,000
May '72	Karwar	440	230	2,39,000
	Vengurla	No data	No data	No data
June '72	Karwar	100 ml.	80	98,000

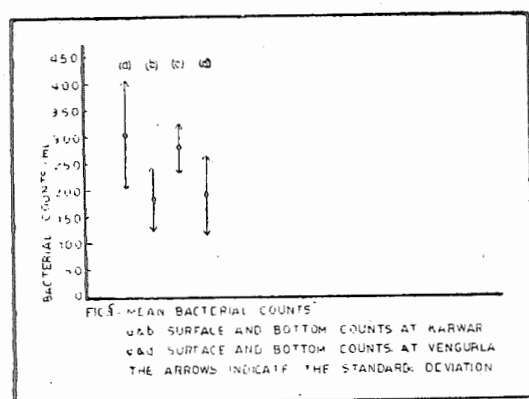


Fig. 1 Showing the two stations under study along the central west coast of India.

bottom counts and mud counts in Karwar and Vengurla. Fig. 1 shows the mean counts of surface and bottom along with Standard Deviation (SD) for Karwar and Vengurla. From the graph it is seen that surface counts are not significantly different for both Karwar and Vengurla at 95%

confidence limit (calculated 't' 0.6 which is less than 't' from table, 2.20). Similarly, bottom counts are not significantly different for Karwar and Vengurla at 95% confidence limit. (calculated 't' 0.16, 't' from table 2.20). However, surface and bottom counts of Karwar are significantly different at 95% confidence limit (calculated 't' = 3, 't' from table 2.1). Similarly surface and bottom counts of Vengurla are significantly different at 95% confidence limit (calculated 't' 2.52, 't' from the table 2.26).

BACTERIAL FLORA (Qualitative)

Physiological groups of bacteria occurred are Nitrifiers, Nitrate reducers, Denitrifiers, Nitrogen fixing bacteria, Agar digesters and Sulphate reducers.

Table II shows the numerical abundance of the different physiological groups at Karwar and Vengurla.

TABLE II

NUMERICAL ABUNDANCE OF GROUPS OF BACTERIA (AVERAGE)

Place	Physiological group	Surface seawater per ml.	Bottom seawater per ml.	Mud/per g.
Karwar	Nitrifiers	15	10	70
	Nitrate reducers	120	90	40,000
	Denitrifiers	15	5	300
	Sulphate reducers	—	—	2 (Only in one sample of Feb. '72)
	Agar Digesters	—	—	4 (only in one sample of Nov. '71)
Vengurla	Nitrifiers	9	5	50
	Nitrate reducers	75	30	10,000
	Denitrifiers	10	5	100
	Sulphate reducers	—	—	—
	Agar Digesters	—	—	—

NITRIFYING BACTERIA

10 to 15ml. quantities of surface seawater and bottom seawater from Vengurla and Karwar when inoculated in standard ammonium sulphate medium, produced nitrite in about 15 days; while 2 to 3 g. of bottom muds produced nitrite in 10 days; on further incubation for another 10 days, nitrate was formed showing thereby presence of nitrifying bacteria. The numerical abundance of nitrifying bacteria ranged between 10 to 15 per ml. in surface and bottom seawater at Karwar while that at Vengurla ranged from 5 to 7 per ml. In mud samples they occurred in 50 to 100 per g. both at Karwar and Vengurla.

NITRATE REDUCERS

Nitrate reducers were present in all the samples studied, from surface seawater, bottom seawater and also from mud samples both from Karwar and Vengurla. Numerical abundance varied between 100 to 300 per ml. of seawater and 1000 to over a lakh per g. of mud at both the places.

DENITRIFIERS

Denitrifiers were present in seawater and mud samples throughout the investigation at both Karwar and Vengurla. Numerical abundance varied between 1 to 10 per ml. of seawater at both the places and that of mud was between 100 to 1000 per g.

NITROGEN FIXING BACTERIA

Bacteria which grew on nitrogen free mannitol medium were assumed to be having the capacity to fix atmospheric nitrogen. These were common in mud samples throughout the investigation in both Karwar and Vengurla.

AGAR DIGESTERS

4 colonies showing agar digestion capacity were isolated from mud sample of Karwar in the month of November 1971.

SULPHATE REDUCERS

Two strains of sulphate reducers which produced hydrogen sulphide were isolated employing Baars' medium from mud samples of Karwar in February 1972. They were uncommon in surface seawater and bottom seawater.

COLIFORMS (Enterobacteriaceae)

Coliforms were totally absent at both the places throughout the period of investigation.

While studying the numerical abundance of bacteria from Karwar and Vengurla about 25 strains of bacteria were isolated and studied in detail. 3 of them did not grow in the laboratory while the rest 22 grew well at room temperature; morphological and biochemical features could be studied and is reported here in tables III & IV.

Majority of the isolates grew well at room temperature on seawater agar medium (SWA); strains 21 and 22 however recorded good growth on fresh water agar medium (FWA) and hence may be of terrestrial origin. Strain No. 1 lost pigmentation after 2nd sub-culture. Majority of the strains are gram negative actively motile rods. The flora in general is very reactive. Saccharolytic and proteolytic properties are common. 12 strains fermented 3 sugars each, 6 of them fermented only one sugar each, 2 of them fermented 2 sugars each, while 2 of them fermented all the four sugars. It has been mentioned earlier (Velankar, 1955, Gore 1972) that based on the ability to ferment sugars and the gram staining reaction, the

TABLE III

BACTERIAL STRAINS INSOLATED FROM KARWAR AND VENGURLA
Morphological characteristics of the isolates

Sl. No.	Place	Source	Growth on SWA plates	Growth on SW Broth	Growth on S W A Slant	Growth on FWA Slant	Pigmentation
1.	Vengurla	Surface seawater	Red, surface round colony	Uniform turbidity; a thin granular pellicle at the top.	Filiform reddish luxuriant growth	No growth	Reddish Pigmentation disappeared after 2nd sub-culture.
2.	Vengurla	„	Yellow, surface round colony.	Uniform turbidity; thin granular pellicle at the top.	Filiform yellowish growth	No growth	Yellow
3.	Vengurla	Mud	Dirty, brown coloured, surface round colony.	Moderately turbid medium, pellicle.	Echinulate growth	No growth	—
4.	Vengurla	Mud	White, round, surface colony.	Medium turbid, a thick pellicle	Filiform	No growth	—
5.	Vengurla	Mud	Surface, rhizoid colony, white in colour.	Uniform turbidity	Beaded	No growth	—
6.	Vengurla	Surface seawater	Surface, blue coloured, raised colony.	Turbidity with a thick, bluish pellicle.	Filiform	No growth	—
7.	Vengurla	Mud	Dark brown, surface colony	Granular pellicle at the top, medium turbid	Beaded	No growth	Bluish
8.	Vengurla	Mud	Dark black round surface colony.	Uniform turbidity thin pellicle.	Filiform	Slight growth	—
9.	Karwar	Surface seawater	Red, surface, round, colony.	Uniform turbidity.	Filiform, Reddish	No growth	Red

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10.	Karwar	Surface seawater	Pale white, surface, spreading colony	Moderate turbidity	Filiform	No growth	—
11.	Karwar	Surface seawater	Deep, light blue round colony	Moderate turbidity	Filiform	No growth	—
12.	Karwar	Surface seawater	Cream coloured raised colony	Turbidity with a membranous pellicle	Effuse	No growth	—
13.	Karwar	Surface seawater	Pinkish, oval colony	Turbid medium, a granular pellicle at the top.	Effuse	No growth	Pinkish
14.	Karwar	Mud	Dirty white, surface, oval, colony	Granular pellicle at the top, medium turbid	Effuse	No growth	—
15.	Karwar	Seawater	Punctiform yellow colony	Turbid medium, membranous pellicle	Filiform	No growth	—
16.	Karwar	Seawater	Large light blue (1.4mm) surface colony	Membranous pellicle at the top	Filiform	No growth	—
17.	Karwar	Mud	Pure white, raised colony	Uniform turbidity	Filiform luxuriant	No growth	—
18.	Karwar	Mud	Brown, surface, round colony	Thin pellicle, medium turbid	Filiform	No growth	—
19.	Karwar	Mud	White, crenulated colony	Medium turbid, granular, pellicle	Filiform	No growth	—
20.	Karwar	Seawater	Light blue, surface colony	Poor growth, slight turbidity	Filiform	No growth	—
21.	Karwar	Seawater	Pinkish surface colony	Pink coloured turbid medium	Filiform moist growth	Good growth	—
22.	Karwar	Mud	Dark red colony	Reddish pellicle at the top of the medium	Filiform moist growth	Good growth	—

TABLE IV

BIOCHEMICAL REACTIONS OF THE ISOLATES

Sl. No.	Nitrate reduction to nitrite	Ammonia production from peptone	Hugh and Leifson's reaction	Sensitivity towards penicillin (2.5IU/Disc.)	Fermentation of Sugars (glucose, sucrose, maltose, lactose)	Indole production	Gelatin liquefaction	Acid Test	Digestion of starch	Gram staining	Bacterial genus
1	+	—	NF	+	Acid from G, S, M. Lactose not fermented	—	+	—	+	Gram negative motile rods	<i>Flavobacterium</i> sp.
2	+	—	NF	+	Slight acid from G&S M&L not fermented	—	+	—	+	Gram negative motile rods	<i>Flavobacterium</i> sp.
3	+	+	NF	—	Acid from G&Sof lactose not fermented	—	+	— (Slow 5 days)	+	Gram negative motile rods	<i>Pseudomonas</i> sp.
4	+	+	NF	—	Acid from G,S,M; lactose not fermented	—	+	—	+	Gram negative cocci	<i>Pseudomonas</i> sp.
5	+	+	Acid	—	Slight acid from glucose only	—	+	—	+	Gram negative coccoid cells	<i>Vibrio</i> sp.
6	+	+	Acid	—	Slight acid from G, S and M	—	+	—	+	Gram negative motile short rods	<i>Vibrio</i> sp.
7	+	+	NF	—	Acid from G,S,M; lactose not fermented	—	+	—	—	Gram negative motile short rods	<i>Pseudomonas</i> sp.
8	+	+	NF	—	Acid from G,M,S; lactose not fermented	—	+	—	+	Gram negative motile elongated rods	<i>Pseudomonas</i> sp.
9	+	+	NF	+	Slight acid from G. only	—	+	—	+	Gram negative short rods	<i>Flavobacterium</i> sp.
10	+	+	—	—	Acid from G,S,M; lactose not fermented	—	+	—	—	Gram negative motile rods	<i>Pseudomonas</i> sp.
11	—	—	—	—	Acid from G,S,M; lactose not fermented	—	+	—	+	Gram negative motile short rods	<i>Vibrio</i> sp.
12	—	—	NF	—	Acid from G. only	Trace of indole	+	—	+	Gram negative short rods	<i>Pseudomonas</i> sp.

13	— (Free N)	—	NF	—	Acid from G, S, M; lactose	—	(Slow liquefa- ction 10 days	—	—	Gram negative short rods (a few curved cells)	-do-
14	+	+	Acid	—	Acid from G. only	—	Slow liq- uefaction 4 days	—	—	Gram negative motile rods	<i>Vibrio</i> sp.
15	+	+	Acid	—	Acid from G. only	—	+	—	+	Gram negative motile rods	-do-
16	+	+	NF	—	Acid from G,S,M; lacto- se not fermented	—	+	—	—	Gram negative motile rods	<i>Pseudomo- nas</i> sp.
17	+	+	NF	—	Acid from G,S,M; lacto- se not fermented	—	Slow	—	—	Gram negative short rods	-do-
18	+	+	A;gas	—	Acid from G,S,M; and lactose	—	+	+	—	Gram negative motile rods	<i>Aeromo- nas</i> sp.
19	+	+	A	—	Acid from S only	—	+	—	+	Gram negative motile rods	<i>Vibrio</i> sp.
20	+	+	A	—	Acid from G,S,M; lacto- se not fermented	—	+	—	+	Gram negative motile rods	<i>Vibrio</i> sp.
21	+	+	A	—	Acid from G, S, M; and lactose	+	+	+	+	Gram positive sporing rods	<i>Bacillus</i> sp.
22	+	+	A	—	Acid from G, S, M and L	+	+	+	+	Gram positive sporing forming rods.	<i>Bacillus</i> sp.

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Abbreviations: G = glucose, S = sucrose, M = maltose, L = lactose, NF = non-fermentative, A = acid
F = fermentative, FWA = fresh water agar, SW = seawater agar

isolates could be classified arbitrarily into different groups for the convenience of description. In the present study majority of the strains fall under group I (as suggested by Velankar, 1955, Gore 1972.) Strains nos. 12, 21 and 22 produced indole while majority of them reduced nitrate to nitrite. Strains nos. 3, 8, 13 and 16 reduced nitrite to free nitrogen. Strain no. 18 produced acid and gas in Hugh and Leifson's medium and hence is classified as *Aeromonas* sp. while 8 strains (nos. 5, 6, 14, 15, 19, 20, 21, 22) produced acid in Hugh and Leifson's medium. Gelatin liquefaction is exhibited by most of the strains and only 3 strains (18, 21 and 22) are positive in Acetoin test. *Pseudomonas* sp, *Vibrio* sp, *Flavobacterium* sp, *Bacillus* sp, and *Aeromonas* sp. were the common genera encountered.

DISCUSSION

Qualitatively and quantitatively, the flora of Karwar and Vengurla did not differ much from that of Mandapam and Madras (Bay of Bengal, east coast), and Cochin and Malabar coasts (west coast). The flora presented great similarities quantitatively with the flora of east coast and that of Cochin (west coast). The surface seawater counts were always higher than the bottom seawater counts in these stations so also the numerical abundance. This is in agreement with the counts reported from Mandapam (Velankar, 1955). Qualitatively the present flora showed similarities to that of Mandapam and Cochin in physiological groups, paucity of gram positive sporeforming rods, total absence of coliforms, strains lacking strong saccharolytic and proteolytic properties, stray occurrence of sulphate reducers and agar digesters, good growth on seawater agar medium at room temperature, predominance of gram negative motile rods, occurrence of large numbers of nitri-

strains in the mud than in seawater occurrence of pigmented colonies in surface seawater only, and their total absence in bottom mud.

The present flora differed from the flora of Mandapam in the absence of luminescent bacteria, while it differed to some extent from the flora of Malabar coast especially in the paucity of spore-formers.

It is thus seen that the distribution of marine bacteria both qualitatively and quantitatively in Karwar and Vengurla shows similar trend as on the east and west coasts of India.

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