J. mar. biol. Ass. India, 1975, 17 (1) : 96 - 100

THE BACTERIAL FLORA OF CERTAIN MARINE FISHES AND PRAWNS IN COCHIN WATERS IN RELATION TO THEIR ENVIRONS*

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ABSTRACT

Commercially important fishes like sardines, prawns, lobster and seer fish caught off Cochin, sea water samples gathered from different depths (Surface, middle and bottom) and bottom mud were subjected to bacteriological analysis. Qualitatively the predominant organisms found associated with these marine animals were the gram negative, asporogenous, rod-like bacilli of the genera *Pseudomonas*, *Achromobacter* and *Vibrio*. Sea water and bottom mud samples also showed a predominance of gram negative rods, though with considerable variation in generic composition with depth.

Quantitatively the 'bacterial density' of the sea water increased with depth, the highest aerobic count being shown by bottom mud (2.7×10^4 organisms/gm) and the least by surface water upto 14 metres depth (0–90 organisms/gm). But compared to bottom mud, a 10 to 100 fold increase in the bacterial count was observed on the body of the fishes in general and a higher increase (10^3 to 10^4 fold) at the gills and the highest (10^4 to 10^5 fold) in the guts of sardines have been constantly recorded. The significance of the observation has been discussed in relation to the keeping quality of the fish. In the case of sardine studies extending over a period of one year revealed seasonal variation in the total and luminoscent bacterial counts and in the biochemical characteristics.

INTRODUCTION

THE free swimming fishes and prawns normally carry a population of commensal bacteria the nature of which has been the subject of investigation by workers in the northern temperate zone (Snow and Beard, 1939; Reay and Shewan, 1949; Georgala, 1958; Colwell and Liston, 1960). A knowledge of the characteristics of these bacteria is of help in understanding the role of these organisms in bringing about spoilage in sea foods. Being marine in origin, the data on the nature and distribution of these microorganisms in the sea are of fundamental significance to workers studying the correlation between the flora on the fish and of the fishing grounds. Colwell and Liston (1960) have established that a distinct commensal bacterial flora is associated with the Pacific Oyster (*Crassostrea gigas*) and that habitat conditions, to a limited extent, affect the nature of the microflora in the species.

Some earlier work on the east coast of India refers to the quantitative and qualitative nature of the bacteria in the sea off Madras and Mandapam Coasts (Velankar, 1950, 1957). A description of the activities of certain physiological groups of bacteria off Tuticorin Coast had been presented by Venkataraman and Sreenivasan (1956). The same authors (1954) had collected some preliminary data on the bacteriology of off-shore waters of Calicut. The paucity of similar data in other areas of the Indian Coast, especially off Cochin, prompted the present authors to take up an investigation designed (1) to study the bacterial distribution in the

*Presented at the 'Symposium on Indian Ocean and Adjacent Seas—Their Origin, Science and Resources' held by the Marine Biological Association of India at Cochin from January 12 to 18, 1971.

off-shore waters of Cochin (2) to obtain a full picture of the microflora in fresh fish and prawns caught therein and (3) to examine correlation, if any, btween the above two.

The authors are thankful to Dr. C. V. Kurian, Professor of Marine Biology, Cochin University, for affording facilities for collection of samples in the research vessel CONCH and to Late Dr. V. K. Pillai, former Director of our Institute for his keen interest in this investigation.

MATERIALS AND METHODS

Sea water and mud samples

Surface, middle and bottom sea water samples were collected 4 miles off the Cochin Coast under aseptic conditions. The water samples (1 ml and 0.5 ml) were immediately plated in duplicate on board the vessel using sea water agar as plating medium. One surface, two middle, two bottom water samples and four mud samples were collected. About 1 gm of mud sample was suspended in 100 ml sterile sea water and agitated. The supernatant was then decimally diluted and the dilutions plated out in duplicates using sea water agar.

FISHES AND CRUSTACEANS

Sardine (*Sardinella longiceps*)

Fresh specimens of sardines, caught off the coast of Cochin and brought to the landing place, were transferred aseptically into sterile bottle. The specimens on arrival at the laboratory were sampled as follows:

10-12 gm of muscles amples along with the skin were cut aseptically from 6 to 8 specimens. The entire gills from 4 to 5 specimens and guts were also removed. These were pour plated separately in sea water agar.

Prawns (*Penaeus indicus, Metapenaeus dobsoni, M. affinis*)

These were usually plated on board the trawler immediately after catch. The shells from 6 to 8 specimens were removed as also the vein. Small pieces were cut off aseptically from each peeled and deveined individual and about 10 gm of the muscle were then pour plated in sea water agar. For sampling the veins, about 0.5 gm of the vein, obtained by pooling from a few individual specimens, was used.

Seer fish (*Cybium guttatus*)

The specimens were caught in the Cochin waters by the experimental vessel of CIFT and brought to the laboratory in ice. The sampling for this was confined only to the skin with a little of the underlying muscle.

Lobster (*Panulirus dassypus*)

This was obtained from a local prawn canning factory. The mode of sampling of this was similar to that of prawn.

RESULTS AND DISCUSSION

Quantitative aspects

Sea water and mud samples

The sampling of sea water, fish and prawns were carried out through December, 1967 to April, 1968. The data (Table 1) show that the maximum bacterial density is observed in the case of mud samples which is of the order of 10^3 to 10^4 of aerobic bacteria. Since sufficient data is lacking, no general conclusion could be drawn as to the relative quantitative distribution of bacteria with depth. One notes that the middle layer (11 to 14 m) of water harbours least number of bacteria as compared to surface and bottom waters. This observation may have some significance in determining the bacterial load on fish habitating at different depths.

TABLE 1

Month	Sample	Bacterial count	Genera (percentage)			
			Pseudomonas	*Vibrio*	*Achromobacter*	Gram positive organisms
January, '68	Water from 14 m	Nil	—	—	—	—
,,	Water from 25 m	40/ml	34	9	39	18
,,	Mud sample No. 1	3.6×10^4/gm	16	Nil	56	28
,,	Mud sample No. 2	1.8×10^4/gm	38	,,	47	15
April, '68	Surface Water	90/ml	68	,,	28	4
,,	Water from 11 m	20/ml	11	,,	22	67
,,	Water from 21 m	110/ml	10	70	Nil	20
,,	Mud sample No. 1	3.5×10^3/gm	Not determined			
,,	Mud sample No. 2	2.5×10^3/gm	25	Nil	15	60
December, '67	Prawn muscle	2.3×10^5/gm	35	5	35	25
January, '68	Sardine skin and muscle	1.6×10^5/gm	20	45	30	Nil
	Sardine gills	2.2×10^7/gm	50	15	30	5
	,, guts	3.1×10^8/gm	63	21	7	Nil
February, '68	Prawns muscle	1.5×10^5/gm	Not determined			12
,,	,, vein	1.0×10^7/gm	Not determined			8
March, '68	Sardines skin and muscle	1.3×10^6/gm	20	50	30	Nil
	Sardines gills	7.4×10^7/gm	41	53	6	,,
	,, guts	5.4×10^8/gm	55	10	35	,,
April, '68	Prawn muscle	9.3×10^3/gm	25	30	20	25
	,, vein	3.8×10^7/gm	20	70	10	Nil
	Sardine skin and muscle	1.8×10^5/gm	52	16	32	,,
	Sardine gills	3.2×10^7/gm	30	Nil	40	15 Unidentified 15%
	Sardine guts	1.1×10^9/gm	80	5	15	Nil
	Lobster	2.1×10^5/gm	11	36	36	Nil Unidentified, 17%
	Seer fish	1.7×10^5/gm	22	3	9	56

Prawns

The bacterial load per gram of ocean fresh prawns (*P. indicus, M. dobsoni, M. affinis*), was found to range from 9.3×10^3 to 2.3×10^5 organisms. The bacterial counts in the vein of prawn shows a higher value *viz.*, 1.0×10^7 which is similar in range to that found in guts of sardines. More gram positive organisms are found associated with bottom mud and prawns whereas fresh sardines harbour very low counts or none at all of these organisms.

Sardines

In sardines three sites on the fish were chosen for bacterial samplings *viz.*, the skin with underlying muscle, gills and guts — the places where bacteria are known to concentrate. The counts obtained with monthly samplings of sardines are shown in Table 2.

TABLE 2

Month	Skin with muscle	Gills	Guts
December 1967	5.4×10^4	—	7.5×10^7
January 1968	3.5×10^5	3.2×10^6	4.8×10^8
February	2.2×10^5	2.9×10^6	4.8×10^8
March	1.3×10^6	7.4×10^7	5.4×10^8
April	1.8×10^5	3.2×10^7	1.1×10^9

It is found that maximum count is observed in the guts, the ranges being 10^4-10^6 for skin with muscle, 10^6-10^7 for gills and 10^7-10^9 for guts. As compared to the count of the bottom mud, this is a 10 to 100 fold increase for the skin, 10^2-10^3 fold increase for the gills and 10^3 to 10^5 fold for the guts. Further a three year study (under publication) has established a seasonal variation in the counts; a regular rise in the bacterial counts on skin is noted during July to October with a similar rise in the guts count in October. The higher bacterial counts during July to October may be attributed to the effect of monsoon. The incidence of a large number of luminous strains of bacteria in guts has already been reported (Karthiayani and Mahadeva Iyer, 1967). The strains are present in the guts almost throughout the year and their highest counts are recorded during July to October (under publication).

Lobster and Seer fish

Both carry a bacterial load in the same range as prawn and sardine.

Qualitative aspects

The generic distribution of the microflora in sea water, bottom mud, fishes and crustaceans is given in Table 1. The results show a wide variation in the percentage composition of the various bacterial genera in the three layers of water. The floral composition of bottom mud seems to show a more or less regular pattern with the absence of *Vibrio* and the constant presence of gram positive organisms.

The generic composition of the microflora of sardines and prawns as seen from Table 1 show a preponderance of gram negative rods of the *Pseudomonas, Vibrio*

and *Achromobacter* groups. These gram negative rods act as casuative agents in the spoilage of these fishes after catch. This in turn depends on the biochemical capabilities of these organisms. In this connection, it is pertinent to mention that activities of these organisms follow a seasonal pattern; biochemically less active groups are present during the months of December and January (under publication).

A striking observation as seen in Table 1 is the prevalence of gram positive organisms in fresh prawns, a bottom feeder, and their absence in sardine, a pelagic fish. This may be explained by the occurrence of gram positive organisms in bottom mud and bottom water and their paucity in surface waters (Table 1). This observation lays emphasis on the necessity for a look-out on *Clostridium* species, a gram positive sporeformer (known to cause fatal food poisoning) in bottom muds and in bottom feeders. The occurrence of such organisms in the bottom mud has been reported elsewhere (Ward *et al.*, 1967).

The distribution of *Vibrio* in the water and on fish calls for further investigation. It is seen that though there is a paucity of *Vibrio* in surface water and in bottom mud, the percentage of this genera on fresh sardines and prawns seems to be fairly high.

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