BACTERIAL FLORA OF FRESH AND ICED INDIAN MACKEREL *(RASTRELLIGER KANAGURTA)* AND ITS RESPONSE TO CHLORTETRACYCLINE (CTC) TREATMENT

P. K. SURENDRAN AND K. MAHADEVA IYER
Central Institute of Fisheries Technology, Cochin-682003

Quantitative and qualitative studies on the bacterial flora of fresh Indian mackerel *(Rastrelliger kanagurta)* have been made. The total native flora as well as 5 ppm. CTC insensitive flora of the fish showed variations with season. About 90 % of the fresh fish flora was sensitive to 5 ppm. CTC. The natural flora of the fresh fish consisted of *Vibrios*, *Pseudomonas*, *Achromobacter*, *Flavobacterium*, *Corynebacteria*, *Micrococci*, *Bacillus* and yeasts. In the CTC insensitive flora, *Vibrios* predominated followed by yeasts. The selection of bacterial genera during storage of the fish in ice and in 5 ppm. CTC incorporated ice has also been investigated. At the time of spoilage, *Pseudomonas* were found to be the dominant flora of the fish stored in both types of ice.

INTRODUCTION

The literature available on the nature of the bacterial flora of fish from the tropical waters of India is rather limited. Venkataraman and Sreenivasan (1952, 1954), Velankar (1955, 1956) and Velankar and Kamasastri (1956) investigated the bacterial flora on fish caught in the waters off the west and east coasts of India. Qualitative and quantitative studies on the bacterial flora of sardine *(Sardinella longiceps)* caught off the coast of Cochin and on the seasonal variation of such flora had been carried out by Karthiayani and Mahadeva Iyer (1967, 1971).

The effectiveness of bacteriostatic chemicals, especially the antibiotics, in the preservation of fish depends very much on the composition of the native flora of the fish. Flora of different types of fish, though caught from the same waters, may show considerable variations in the generic distribution and in their behaviour towards preservative antibiotics. In an earlier

communication, we had reported on the behaviour of marine bacteria, isolated from sardine, towards the antibiotic. (Surendran and Mahadeva Iyer, 1971 a). In the present work, apart from the studies made on the generic distribution of bacteria on the fresh fish, the changes in the composition of the microflora of the fish during its storage in ordinary ice as well as in 5ppm. chlortetracycline (C T C) incorporated ice have also been followed. The findings reported herein form part of a study aimed at extending the knowledge on the bacterial flora of tropical fishes (from Indian waters) and on the changes which the flora undergoes during icing and/or antibiotic treatment of the fish.

Material And Method

Fresh mackerel (*Rastrelliger kanagurta*) from boats landed at Manassery, Cochin, were transferred to sterile bottles and brought to the laboratory without delay. Skin with muscle was cut from either side of the fish and about 10gm. were used for determination of total viable plate count, using sea water agar (SWA) as the plating medium (Surendran and Mahadeva Iyer, 1971 b). The plates were incubated at room temperature (RT) (28 ± 2°C) for 48 hours and counts taken. In order to determine CTC insensitive strains, CTC at 5 ppm. level was incorporated in the plating medium (Surendran and Mahadeva Iyer, 1976). The colonies which developed after 48 hours on this medium were assumed to be insensitive to 5 ppm. CTC.

The colonies appearing on the plates after 48 hours at RT were picked at random into tubes of sea water peptone (SWP) and cultures which grew in SWP within 72 hours were subsequently transferred to SWA slants. Each isolate was re-streaked three times to ensure purity before their morphological and biochemical characteristics were studied. The pure cultures were maintained on SWA slants for subsequent studies.

Morphology and gram stain were observed on 16-24 hrs. cultures grown on S W A slants. Motility was observed by the hanging drop method (Manual of Microbiological Methods, 1957). The ability of the cultures to reduce nitrate, to produce indol from tryptone, to liquefy gelatin and to ferment various sugars like glucose, lactose, sucrose, maltose and mannitol was studied by the standard methods (Salle, 1954). Sensitivity to Penicillin and CTC was determined by observing the zone of inhibition produced around filter paper discs (impregnated with the respective antibiotic solution), placed on cultures grown on agar medium. The mode of attack of glucose by cultures was determined by using the Hugh and Leifson's oxidative and fermentative medium. (Hugh and Leifson, 1953). The presence of oxidase in the culture was detected by Kovacs' test. Sterile cooked fish meat medium was employed to screen the putrifiers. Pigmentation was observed on SWA slants after one week's incubation at RT. Growth temperature tests at 0°C and 30°C were done using SWP and SWA slants.

In order to study the selection of bacterial population during storage in ice as well as in antibiotic ice, fresh mackerel was stored in ordinary crushed ice and in 5 ppm. CTC ice in separate ice boxes. At definite intervals, samples of fish were withdrawn from the boxes and plated out for total viable count on SWA and for

TABLE I

Total bacterial counts, of skin with muscle of mackerel, obtained on SWA and 5 ppm. CTC SWA at room temperature.

Month	Count on SWA	Count on 5 ppm. CTC SWA	% of 5 ppm. CTC insensitive flora
September	7.60×10^4	1.50×10^3	2.0%
October	4.10×10^4	$3.66 \times 10_3$	8.9%
November	$4.80 \times 10_5$	3.00×10^4	6.24%
December	2.60×10^5	4.00×10^3	1.6 %
January	4.80×10^5	3.00×10^4	6.2 %
February	1.35×10^6	4.70×10^5	34.7 %
March	2.96×10^5	3.50×10^4	11.3 %
April	1.10×10^5	8.47×10^3	7.7 %
May	2.63×10^4	2.80×10^3	10.7 %

antibiotic-resistant bacterial count on 5ppm. CTC incorporated SWA. Well isolated colonies were picked up from both types of plates. The bacterial cultures collected were classified into genera, according to the scheme of Karthiayani and Mahadeva Iyer (1967).

RESULTS

Fresh fish flora

Table I gives the total bacterial count of skin with muscle on SWA and on 5ppm. CTC SWA for a period of nine months (September to May, corresponding to the fishery season for mackerel along the Kerala coast). The counts on SWA varied between 2.6×10^4 and 1.35×10^6 and on 5 ppm. CTC SWA, between 1.5×10^3 and 4.7×10^5, showing that majority of the fresh fish flora was sensitive to CTC at 5 ppm. level. Maximum suppression by antibiotic was observed during the months of September and December, while in February, maximum growth of CTC insensitive flora was observed.

Table II gives the generic distribution of native flora as well as 5ppm. CTC insensitive flora of mackerel. Majority of the native flora consisted of gram negative asporogenous rods belonging to the genera *Vibrios*, *Pseudomonas*, *Achromobacter* and *Flavobacterium*. *Corynebacterium*, *Micrococci* and *Bacillus* constituted the gram positive types which usually ranged between 10-15% of the total flora. Yeasts were always detected in the flora, usually in the range of 2-7%. In the 5 ppm. CTC insensitive flora, *Vibrios* constituted 50-70%, followed by yeasts, *Pseudomonas* and *Achromobacter*.

TABLE II

Generic distribution of bacteria on skin with muscle of mackerel

Sl. No.	Bacterial genus	% Native flora*	% 5 ppm. CTC insensitive flora**
1.	*Vibrios*	36.5	56.1
2.	*Pseudomonas*	16	9.3
3.	*Achromobacter*	24	5
4.	*Flavobacteria*	3.5	2.5
5.	*Corynebacteria*	2	4
6.	*Micrococci*	6	3
7.	*Bacillus*	5	2
8.	Yeast	7	18

* Picked from colonies on SWA plates.
** Picked from colonies on 5 ppm. CTC SWA plates.

Ice-storage study

The native flora of mackerel underwent significant changes during storage in ice. Results of a typical ice storage study in ordinary ice and in 5 ppm. CTC ice are presented in Table III. The initial flora consisted of 60% *Vibrios*, 21% *Achromobacter*, 12% *Pseudomonas* and the rest *Flavobacteria* and gram positives. After storage for 21 days in ice, 74% of the flora was constituted by *Pseudomonas* and 15% by *Achromobacter*. *Vibrios* had almost completely disappeared. For fish stored in CTC ice, the changes of the flora were quite different; initially a preponderence of *Vibrios* and yeasts was observed, but the flora on the 21st day in ice was similar to the one in ordinary ice stored fish, except that about 90% of the flora was 5 ppm. CTC insensitive.

DISCUSSION

The total bacterial count of skin with muscle of mackerel showed variations with season (Fig. 1). Such seasonal variation was also exhibited by 5 ppm. CTC insensitive flora. Almost in all months of the year, about 90% of the native flora was found to be sensitive to 5 ppm CTC, the maximum susceptible flora being obtained during the months of September and December, during which periods, the qualitative analysis of the flora showed that *Vibrios* constituted a lesser proportion of the total flora. Similarly, in February, when nearly 35% of the native flora was insensitive to 5 ppm. CTC, the proportion of the *Vibrios* in the total flora was maximum. This would mean that majority of the marine *Vibrios* spp. were resistant to CTC at 5 ppm. level. This finding is in

TABLE III

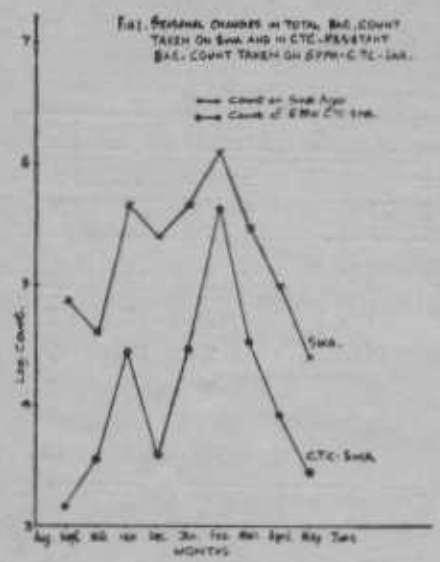
Pattern of change in the bacterial flora of mackerel during storage in ordinary ice and 5 ppm. CTC ice.

Genus or Biochemical Group	% of Micro-organisms at different intervals in ice						
	Ordinary ice				5 ppm. CTC ice		
Days:	0	7	14	21	7	14	21
1. *Pseudomonas*	12	24	39	74	12	29	80
2. *Vibrios*	60	5	5	1	42	17	2
3. *Achromobacter*	21	49	16	15	14	18	9
4. *Flavobacter*	1	4	4	2	2	3	1
5. Gram positives and others, including yeasts	6	18	38	7	30	33	8
Biochemical group							
1. Gelatin liquefiers	96	13	18	92	31	21	81
2. Putrifiers	18	24	22	55	9	16	62
3. Capable of growth at 0°C.	30	95	92	97	64	73	93

consonance with our previous results, where we found that 69% of the *Vibrios* tested were insensitive to 5 ppm. CTC. (Surendran and Mahadeva Iyer, 1971 a).

The native flora of skin with muscle of mackerel was mainly constituted by three genera *viz. Vibrios Pseudomonas* and *Achromobacter* which together comprised about 75% of the flora of the fresh fish. This finding is at variance with the earlier observations of Venkataraman and Sreenivasan (1952), who had reported that 55% of the total native flora of mackerel was constituted by *Bacillus* spp. and that *Vibrios* were absent. Though we isolated *Bacillus* spp. from the native flora of mackerel, it constituted only 5% of the total flora. In the case of sardine caught off Cochin, *Achromobacter*, *Vibrios* and *Pseudomonas* together accounted for 73% of the native flora and *Bacillus* constituted only 1% of the total flora (Karthiayani and Mahadeva Iyer, *loc. cit.*). The CTC resistant strains, as discussed earlier, were mainly *Vibrios*. Majority of the *Achromobacter* spp. and nearly 50% of the *Pseudomonas* spp were sensitive to 5 ppm. CTC. Since it is well-known that *Pseudomonas* and *Achromobacter* spp. include majority of the fish spoiling organisms, their sensitivity to CTC at the levels employed is quite significant from the point of view of fish preservation by CTC.

Succession of bacterial genera during ice storage of fish is of significance from the stand point of fish spoilage. The native



flora on fish undergoes considerable changes during the storage of fish at low temperatures. When mackerel is stored in ice, *Vibrios* which constituted 60% of the original flora, underwent a drastic reduction in proportion and by the 7th day it comprised only 5% of the total flora and by the end of 21 days *Vibrios* practically disappeared (Table III). Whereas *Pseudomonas* and *Achromobacter* which respectively accounted for 12% and 21% of the initial flora, increased to 24% and 49% respectively by the 7th day. Thereafter, *Pseudomonas* rapidly increased and by the 21st day, it established itself as the dominant flora, comprising 74% of the total. *Achromobacter* on the other hand, showed only a slower rate of increase and by the 21st day, only 15% of the flora was constituted by *Achromobacter*. However, at the time of spoilage of the fish in ice *Pseudomonas* and *Achromobacter* accounted for nearly 90% of the total flora.

The picture of selection of bacterial genera in the CTC ice stored fish was quite different, at least for the first two weeks in ice. *Pseudomonas* and *Achromobacter* decreased in proportion during the first week, when majority of the flora was comprised by *Vibrios*, gram positive bacteria and yeasts. Subsequently, *Pseudomonas* began to re-establish itself and by the end of 21 days in CTC ice, *Pseudomonas* emerged as the dominant flora, constituting 80% of the total. It shows that a small proportion of CTC resistant *Pseudomonas* strains that remained in the beginning proliferated rapidly and established itself as the dominant flora later, while the cold sensitive *Vibros* died out.

Table III presents another interesting finding. 96% of the native flora of mackerel were gelatin liquefiers, but only 18% of them could produce putrid odour from fish media. During ice storage, there was a drastic reduction in the gelatin liquefiers, parellel with the death of *Vibrios*, while there was no corresponding reduction in the proportion of putrifiers. By the time the *Pseudomonas* and *Achromobacter* had dominated the flora, 92% of the flora liquefied gelatin and 55% of the flora were putrifiers. These findings might indicate that *Vibrios* which were usually gelatin liquefiers, were not generally putrifiers; in other words, they do not presumably have an active role in spoilage of fish. Table III also shows the gradual development of psychrophilic bacteria during storage in ice, as indicated by an increase in the percentage of strains capable of growth at 0°C from the 7th day of ice storage.

Organisms which can grow at low temperature and exhibit proteolytic activity can be assumed to be responsible for spoilage. (Shewan, Hobbs and Hodgkiss, 1960).

A preponderence of a particular genus at a given instant in the bacterial population of spoiling fish muscle is usually indicative of its strong involvement in the spoilage phenomenon. But then, putrifiers need not confine themselves to one major group alone, but they are to be found in all groups. According to Adams, Farber and Lerke (1964) "apparently only a small portion of bacteria of a particular group can cause spoilage; the remainders probably exist as free riders or probably are involved in some synergism with weak spoilers". Hence, eventhough 80% of the flora of mackerel at the time of spoilage in ice was constituted by *Pseudomonas*, only a fraction of them need be actual spoilers.

ACKNOWLEDGEMENT

The authors' grateful thanks are due to Shri. G. K. Kuriyan, Director and Shri. M R. Nair, Project Co-ordinator for their keen interest in the work.

REFERENCES

Adams, R., L. Farber and P. Lerke. 1964. *Appl. Microbiol;* **12**, 3:277.

Hugh, R. and E. Leifson. 1953. *J. Bacteriol*; **66**: 24.

Karthiayani, T. C. and K. Mahadeva Iyer. 1967. *Fish. Technol*; **4**, 2:89.

Karthiayani, T. C. and K. Mahadeva Iyer, 1971. *Fish. Technol*; **8**, 1: 69.

Manual of Microbiological Methods. 1957. Published by Society of American Bacteriologists. McGraw Hill Book Co. Inc. New York.

Salle, A. J. 1954. *Laboratory Manual on Fundamental Principles of Bacteriology*. 4th Ed. McGraw Hill Book Co. Inc., New York.

Shewan, J. M., Hobbs and W. Hodgkiss. 1960. *J. Appl. Bact*; **23** : 3.

Shewan, J. M. and J. Liston. 1956. *Bull. Inst. Intern. Food Suppl*; **1** : 137.

Surendran, P. K. and K. Mahadeva Iyer. 1971a. *Fish. Technol*; **8**, 2: 128.

Surendran, P. K. and K. Mahadeva Iyer. 1971b. *Fish. Technol*; **8**, 1: 55.

Surendran, P. K. and K. Mahadeva Iyer. 1973. *Fish. Technol*; **10**, 2: 110.

Surendran, P. K. and K. Mahadeva Iyer. 1976. *Bull. Dept. Marine Sci., Univ. Cochin*; **7**, *(in Press)*

Velankar, N. K. 1955. *Indian J. Fish*;. **2**: 96.

Velankar, N. K. 1956. *Indian J. Fish*. **3**: 261.

Velankar, N. K. and P. V. Kamasastri. *Indian J. Fish*; **3**: 269.

Venkataraman, R. and A. Sreenivasan. 1952. *Indian. J. Med. Res;* **40**: 529.

Venkataraman, R. and A. Sreenivasan, 1954. *Proc. Nat. Inst. Sci. India*; **20**: 651.