Bacteriology of Indian Oil Sardine *(Sardinella longiceps* Valenciennes) Stored in Chilled Sea Water*

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The changes in the bacteriology of Indian oil sardine (*Sardinella longiceps* valenciennes) and the medium surrounding the fish during storage in chilled sea water ($2 \pm 1^{\circ}C$) were determined by the identification of bacterial isolates upto their generic level. The flora of the freshly caught fish consisted mainly *Micrococcus* spp., *Bacillus* spp., *Flavobacterium* spp., *Pseudomonas* spp., *Acinetobacter* spp. and *Vibrio* spp., While that of the freshly collected sea water consisted mainly *Micrococcus* spp., *Vibrio* spp., *Pseudomonas* spp., *Arthrobacter* spp., *Bacillus* spp. and *Aeromonas* spp. The flora of the fish and the medium surrounding the fish after 10 days storage consisted mainly of *Pseudomonas* spp., *Vibrio* spp., *Flavobacterium* spp., *Acinetobacter* spp. and *Aeromonas* spp.

In the course of the last two decades, the world's annual marine fish catch increased from around 55 to 80 million tonnes. A substantial increase in the catch of small species accounted for the major part of this increase. As the individual catches are often large, the conditions allow no gutting or other elaborate treatments of such catches. To overcome the disadvantage of icing bulk catches of small fishes, chilled sea water (CSW) storage technique has been developed and successfully tried in commercial fisheries, (Hiremath *et al.*, 1979, 1980; Salian *et al.*, 1985). Although work has been done on physical biochemical and organoleptic aspects of CSW preservation, very little information is available on the bacteriological aspects. It has been suggested that besides quantitative differences, qualitative differences also occur in the bacteriology of fish during storage in CSW (DeSilva & Mendis, 1963; Shewan, 1965). To understand these differences, the bacteriology of fish and the surrounding medium during CSW storage was studied and the results are presented in this paper.

Materials and Methods

Chilled sea water (CSW) was prepared by adding sea water to ice in the ratio 1:2 and mixed thoroughly. Freshly caught Indian oil - sardines (*Sardinella longiceps* Valenciennes) were immediately transferred to the precooled medium in the ratio of 4:3 (fish to slush). Samples of fresh fish and sea water were collected in sterilized polythene bags and bottles respectively, kept under crushed ice and brought to laboratory for analysing the initial bacterial load and the flora. Temperature of the fish was maintained at $2 \pm 1^{\circ}C$ in CSW throughout the storage.

Samples of fish and the surrounding medium were drawn at regular intervals for total plate counts. Total plate counts were done using spread plate technique and the plates were incubated at $2 \pm 1^{\circ}C$ for a week. After taking the counts, the plates with isolated colonies were selected and 100 to 120 colonies were picked up, using random table, for isolation and identification of cultures. All the selected isolates were identified upto their generic level (Shewan *et al.*, 1960; Lechevallier *et al.*, 1980). Care was taken not to expose these isolates to higher temperature, during the whole course of the study. All media and broths were prepared in the laboratory according to the composition and procedures given in APHA (1976).

*Formed part of the Ph.D. thesis of the first author, approved by the University of Agricultural Sciences, Bangalore.

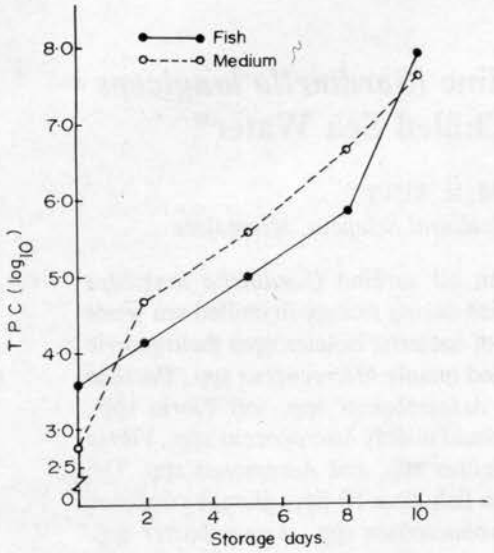

Fig. 1. Changes in total plate count of fish and surrounding medium during storage in CSW.

Results and Discussion

The initial total plate count (TPC) of freshly caught fish was found to be 3.6 x 10^3/g which increased to 8.1 x 10^7/g during storage of 10 days (Fig. 1). The initial total plate counts of freshly collected sea water were found to be 5.9 x 10^2/ml (Fig.1) which was in good agreement with the results of Durairaj *et al.*, (1983) and Krishnakumar *et al.*, (1985). During storage of fish in CSW, the total plate counts of the medium surrounding the fish increased steadily as that of the fish (Fig. 1). Baker & Hulme (1977) and Krishnakumar *et al.*, (1985) also noticed a similar trend. Eventhough there was a difference of one log count between the fish and the surrounding medium initially, there was no much difference in counts towards the end of the storage. The lower bacterial counts of the CSW preserved fish, as compared to ice stored fish, seem very likely to be linked to faster initial cooling and ower storage temperature during the CSW preservation (Showan, 1965).

A dominance of Gram-negative (60.0%) over Grampositives (40%) was observed in freshly caught Indian oil sardine. Among the Gram- positives, *Micrococcus* spp. and *Bacillus* spp. Were the dominant genera, while among Gram-negatives, *Flavobacterium* spp., Were dominating, followed by *Pseudomonas* spp., *Acinetobacter* spp., *Vibrio* spp. and *Aeromonas* spp. (Fig. 2). The dominance of Gram- negatives in the freshly caught topical marine fish has been reported by many workers (Mary *et al.*, 1975; Anand & Setty, 1977; Surendran & Gopakumar, 1981, 1983). The general composition of the initial microflora of tropical marine fish as reported by the above authors is in good agreement with the present findings.

In the present study, a generic succession was observed as the spoilage of fish advanced. The Gram-negatives dominated the flora in the following order, *Pseudomonas* spp., *Vibrio* spp., *flavobacterium* spp., *Acinetobacter* spp., *Aeromonas* spp. and *Moraxella* spp. (Fig. 2). De Silva & Mendis (1963) Shewan (1965) and Barile *et al.*, (1984) were also of the view that a generic succession existed, in the case of fish stored in CSW. De Silva & Mendis (1963) reported that the *Pseudomonas* spp., *Achromobacter* spp. and coliforms were the major genera in a Singhalese fish stored in chilled fresh water, while Shewan (1965) recorded the dominance of *Pseudomonas* spp. and *Achromobacter* spp. during the storage of cod in CSW. *Pseudomonas* spp., *Moraxella* spp., *Acinetobacter* spp. and *Flavobacterium* spp., were reported to be the dominant genera after

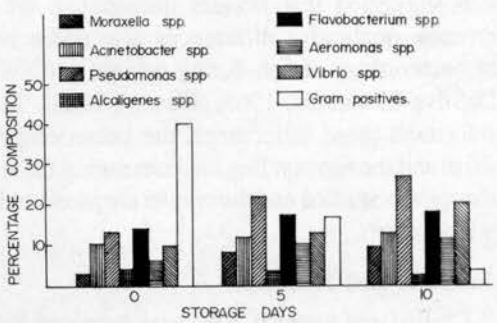

Fig. 2 Pattern of changes in the bacterial flora of fish during storage in CSW

68 h, in shrimp stored in RSW (Lee & Kolte, 1982), while Barile *et al.*, (1984) in a study on mackerel (*Rastrelliger faughni*) stored in CSW, observed *Pseudomonas* spp., *Alteromonas* spp., and *Aeromonas* spp. as major spoilers. In the present study, dominance of one or two bacterial genera as reported for most of the ice stored fishes, was however not observed but the results indicate that the spoilage of fish in CSW was due to the combined action of bacterial genera like *Pseudomonas, Vibrio, Flavobacterium, Aeromonas* and to some extent *Moraxella* and *Acinetobacter*. The *Vibrio* spp. and *Aeromonas* spp. appear to play a significant role in the spoilage of CSW preserved fish, although next in importance to *Pseudomonas* spp. The dominance of vibrios in the west coast waters of India has been reported and their importance in fish spoilage has also been demonstrated (Devaraju & Setty, 1985).

There was a dominance of Gram-negatives in the flora of freshly collected sea water. A higher incidence of *Vibrio* spp. and *Arthrobocter* spp. in the sea water is observed (Fig. 3). Durairaj *et al.*, (1983) & Chandrasekharan *et al.*, (1985) reported *Vibrio* spp as the major genus among the Gram-negative flora of the sea water samples around south-west coast of India. Lee & Kolbe (1982) have reported an abundance of *Arthrobacter* spp. in the tropical sea water among the Gram-positive flora.

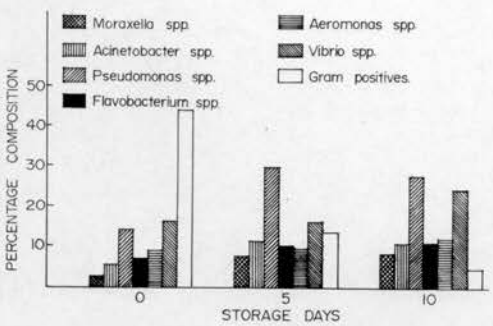

Fig. 3. Pattern of changes in the bacterial flora of medium surrounding the fish during storage in CSW.

The distribution pattern of different bacterial genera (Fig. 3) of the CSW media surrounding the fish, during the storage, is almost resembling that of the fish (Fig. 2). The two differences noted were a lower incidence of *Flavobacterium* spp. and a higher incidance of *Vibrio* spp. and *Aeromonas* spp. towards the end of the storage period. This may be explained by the aerobic nature of the *Flavobacterium* spp. (DeSilva & Mendis, 1963) and the facultative anaerobic nature of the *Aeromonas* spp. and *Vibrio* spp. (Shetty, 1988).

The present study indicates that the spoilage of fish in CSW is brought about by the combined action of *Pseudomonas, Vibrio, Flavobacterium, Aeromonas* and *Moraxella-Acinetobacter*, as compared to *Pseudomonas* only in case of ice stored fishes and these spoilage genera appeared to be facultatively anaerobic in nature.

The authors wish to thank Prof. H.P.C. Shetty, Director of Instruction, College of Fisheries, University of Agricultural Sciences, Mangalore, for the facilities offered. Financial assistance from the Indian Council of Agricultural Research in the form of Senior Research Fellowship to the first author is also gratefully acknowledged.

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