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Biochemical Characteristics of Tropical Fish Spoilage Bacteria Isolated from Indian Oil Sardine (*Sardinella longiceps*)

T.S. SHETTY, T.M.R. SETTY and C.N. RAVISHANKAR

Department of Fish Processing Technology
College of Fisheries
University of Agricultural Sciences
Mangalore, 575002 Karnataka
India

Abstract -Biochemical characteristics of fish spoilage bacteria isolated from Indian oil sardine (*Sardinella longiceps* V alenciennes) stored in chilled sea water were studied to understand their role in spoilage of fish.

Cultures of *Vibrio* spp. and *Aeromonas* spp. were biochemically very active, followed by *Pseudomonas* spp., *Acinetobacter* spp., *Moraxella* spp. and *Flavobacterium* spp. There was no significant change in the biochemical activities of cultures compared to the activity of the bacteria under aerobic conditions as in iced fish. However, under semianaerobic or microaerophillic conditions, these spoilage bacteria use trimethylamine oxide (TMAO) as an electron acceptor or as an oxidizing agent.

Bacterial activity is generally accepted as the primary cause of fish spoilage. Although considerable work has been done on this aspect, most of it refers to the spoilage potential of *Pseudomonas* spp. (Chai et al. 1968; Herbert et al. 1971, 1975; Miller et al. 1973; Shewan 1974; Herbert and Shewan 1976) since they dominate the spoilage flora of temperate marine fish and, to some extent, of tropical marine fish. However, little attention has been given to understand the role of other bacterial genera like *Vibrio* and *Aeromonas* in the spoilage of tropical fish in particular. Recent studies on tropical fish (Chandrasekharan et al. 1985; Surendran and Gopakumar 1985; Shetty 1989) have shown the presence of appreciable quantities of *Vibrio* and *Aeromonas* spp. in spoiling fish.

According to Lerke et al. (1965), Singh (1978) and Devaraju and Setty (1985), results of standard biochemical tests on cultures obtained from spoiling fish serve as a basis for drawing inferences regarding the role of particular genera in fish spoilage. This study

was made to understand the biochemical nature and spoilage potential of bacterial isolates belonging to different genera, important in the spoilage of tropical fish stored in chilled seawater (CSW).

Freshly caught Indian oil sardines (*Sardinella longiceps* Valenciennes) in prime condition, were procured from the brails of purse-seine boats operating off Mangalore, and brought to the laboratory in CSW storage. The temperature of the CSW storage was maintained at 2±1°C throughout.

After 10 days storage in CSW, when the fish were found to be organoleptically unacceptable by panelists, a sample homogenate (in physiological saline) after suitable dilution was spread on plate count agar (0.5% W/V NaCl) and incubated at 28±2°C for 48 hours. The plates with isolated colonies were selected and 350-400 colonies were picked up using a random table for isolation and purification. All the selected cultures were identified up to their generic level making use of the schemes suggested by Shewan et al. (1960) and Lechevallier et al. (1980) (Table 1). To assess the spoilage potential of these cultures, their biochemical activities (listed in Table 2) were studied according to methods described in APHA (1976) and by Conn et al. (1957). Motility and Gram reaction were also determined.

Table 1. Bacterial genera investigated.

Bacterial genera	Number of cultures
Vibrio spp.	32
Aeromonas spp.	29
Pseudomonas spp. Group I	20
Pseudomonas spp. Group II	30
Pseudomonas spp. Group III	32
Pseudomonas spp. Group IV	18
Moraxella spp.	27
Acinetobacter spp.	24
Flavobacterium spp.	21
Alcaligenes spp.	12

The reduction of trimethylamine oxide (TMAO) was tested according to Wood and Baird (1943). Production of acetyl methyl carbinol (AMC) was tested according to Mossel and Taminga (1973, cited in Van Spreekens 1977). Nuclease activity was tested on DNase agar (APHA 1976).

Table 2. Percentage of bacterial cultures showing positive reactions to various tests.

Biochemical tests	Bacterial genera									
	Vibrio spp.	*Aeromonas* spp.	*Pseudomonas* spp. Group I	*Pseudomonas* spp. Group II	*Pseudomonas* spp. Group III	*Pseudomonas* spp. Group IV	*Moraxella* spp.	*Acinetobacter* spp.	*Flavobacterium* spp.	*Alcaligenes* spp.
Gelatin hydrolysis	100	79.31	0	100	100	100	59.26	100	85.71	0
Ammonia production	100	79.31	100	100	100	100	77.77	100	52.38	0
Indole production	0	0	0	0	0	100	0	0	100	0
Production of AMC*	0	0	0	0	0	0	0	0	28.57	0
Production of DNase	100	0	20	0	87.50	100	69.96	66.66	0	0
Production of H_2S	100	62.07	100	0	100	100	0	0	57.14	100
Oxidase activity	100	100	100	100	100	100	100	0	0	0
Phosphatase activity	100	100	0	0	100	100	100	100	0	0
Action on glucose	100	100	100	100	100	0	0	100	0	0
Action on maltose	100	0	100	0	0	0	0	0	0	0
Action on sucrose	100	0	0	0	0	0	0	0	0	0
Action on maltose + sucrose	100	0	0	0	0	0	0	0	0	0
Ornithine decarboxylation	0	55.17	0	0	0	0	0	0	0	0
Arginine decarboxylation	0	72.41	80	100	0	0	0	0	0	0
Lysine decarboxylation	0	62.07	0	0	0	0	0	0	0	0
TMAO reduction	87.50	75.86	40	66.66	81.25	100	66.66	75	57.14	0
Catalase activity	100	100	100	100	100	100	100	66.66	100	100
Motility	100	100	100	100	100	100	0	0	0	100
Morphology	G-ve rod	G-ve rod	G-ve rod	G-ve rod	G-ve rod	G-ve rod	G-ve rod	G-ve rod	G-ve rod	G-ve rod

*Acetyl methyl carbinol

Sodium chloride at a level of 0.5% W/V was added to all the media, as recommended by Van Spreekens (1977), since the bacterial cultures were of marine origin. For inoculating the media, cells of logarithmic growth phase were used.

The results of the biochemical tests are presented in Table 2. Cultures of *Vibrio* spp. and *Aeromonas* spp. were found to be the most active spoilers. The organisms belonging to *Pseudomonas* spp., considered to be very active spoilers by many authors (Herbert et al. 1971, 1975; Miller et al. 1973; Van Spreekens 1977), were confirmed as such in the present study, particularly those belonging to Groups III and IV. They follow *Vibrio* spp. and *Aeromonas* spp. in biochemical activities under these conditions. Cultures of *Acinetobacter* spp., *Moraxella* spp. and *Flavobacterium* spp. were considered to be less important than the other genera. The role of these bacterial genera appears to be during the initial stages of fish spoilage as reported by Singh (1978) and Shetty (1989).

Shewan (1965) was the first to suggest that either the types of bacteria surviving, growing and operating under semianaerobic or anaerobic conditions in CSW are different from those in well aerated conditions with melting ice, or, if the types are not different, then the spoilage characteristics are markedly altered. The findings of the present investigation indicate no such marked changes on the biochemical activities of these bacteria.

In the present study, all the cultures, except that of *Alcaligenes* spp., were able to reduce TMAO, supporting the assumption that the spoilage organisms growing in a semianaerobic or microaerophilic environment use TMAO as an electron acceptor or as an oxidizing agent (Sakaguchi and Kawai 1976; Strom and Larsen 1979; Strom et al. 1979; Hobbs and Hodgkiss 1982).

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