Asian Fisheries Science 6(1):91-97.
Asian Fisheries Society, Manila, Philippines

https://doi.org/10.33997/j.afs.1993.6.1.009

Studies on the Psychrotrophic Bacteria from Croaker Fish (*Johnius dussumieri*) Treated with Propyl Paraben

KARTAR SINGH*, T.M.R. SETTY and T.S. SHETTY**

Department of Fish Processing Technology
College of Fisheries, Hoige Bazar
Mangalore 575 001, India

**Present address:*
British High Commission, British Council Division
Anna Salai, Madras 600 002, India

***Address for correspondence:*
Agricultural Station, Ankola-581 314
Karnataka, India

Abstract

Psychrotrophs belonging to the genera *Flavobacterium*, *Pseudomonas*, *Vibrio*, *Achromobacter*, *Pseudobacterium* and *Micrococcus* were isolated from croaker fish (*Johnius dussumieri*) treated with preservative, propyl p-hydroxy 4-benzoate (paraben). The optimum growth temperature for the selected cultures was 27±2°C and visible growth was discernable at 0°C within 8-10 days. A generic succession was observed as the spoilage of fish advanced. *Pseudomonas*, *Achromobacter* and *Vibrio* dominated over other genera as late spoilers. Biochemical activities of these genera were also tested.

Introduction

Fish spoilage is a complex process governed by factors such as species differences, catching methods, fishing grounds, seasons, handling methods, nature and load of bacterial contamination. While a general similarity exists between the bacterial flora of fish and of its marine surrounding, it is also known that bacteria belonging to the genus *Pseudonomas* are the most important among fish spoilers followed by *Vibrio*, *Aeromonas* and *Achromobacter* to a lesser degree (Shetty and Setty 1990). The purpose of this study has been to determine the role of psychrotrophs in the spoilage of fish treated with

propyl paraben and to compare the different groups with particular reference to their biochemical activity and growth characteristics.

Materials and Methods

Marine fish, the croaker (*Johnius dussumieri*), were collected by trawling at depths of 30 to 40 m off Mangalore, along the west coast of India. The fish were immediately iced and brought to the laboratory.

Seawater agar (SWA) was used for isolation of psychrotrophs, and nutrient agar fortified with 3% sodium chloride, for all other studies. The preservative used was propyl p-hydroxy 4-benzoate (propyl paraben) obtained from E. Merck. All the other chemicals and media were obtained either from BDH (India) or Hi-media (India).

Whole fish washed in freshwater were dipped in 0.1% propyl paraben solution for 30 minutes then stored in ice (1:1 ice to fish) at 3±2°C throughout. Re-icing was done wherever necessary. Quality of the fish was evaluated organoleptically by trained panelists. Representative samples of fish were taken on the 1st day and on the 14th day, when the fish was considered as spoiled organoleptically, and were plated on SWA plates after suitable dilution. The plates were incubated at 3±2°C until growth was discernable. About 40-50 colonies were picked up randomly from the plates with isolated colonies and were further purified and maintained on nutrient agar slants at 3±2°C for further study.

The selected isolates were identified up to generic level using the schemes of Shewan (1963) and Kriss (1967). The identity of the isolates was confirmed by repeated morphological and physiological examinations, following the procedures of Conn et al. (1957) and using Bergey's Manual (Breed et al. 1957).

Different biochemical tests were conducted to study the spoilage potential of the cultures of late spoilers, according to the methods described in the manual of microbiological methods (Conn et al. 1957).

The optimum growth temperature of the isolates was determined by incubating the cultures on slants at 0±2°C, 8±2°C, 27±2°C, and 37±1°C and the time of appearance of growth of different isolates was noted at different intervals of time as a qualitative index of growth.

Results and Discussion

The distribution pattern of bacterial genera in the fish during storage is shown in Table 1. *Flavobacterium* spp. dominated the initial flora, followed by *Pseudomonas* spp. The major flora of the spoiled fish were *Pseudomonas* spp. and *Achromobacter* spp. The incidence of *Flavobacterium* spp. dropped during the spoilage period and *Vibrio* spp., which were not encountered initially, constituted 16% at the end.

The results suggest a generic succession as spoilage advanced. *Flavobacterium* spp., which dominated the early spoilers, gave way to *Achromobacter*, *Pseudomonas* and *Vibrio* at later stages of spoilage. Velankar and Kamasastri (1956) found no generic succession but Liston (1980), Barile et al. (1984), Shetty and Setty (1990) amongst others were of the view that a generic succession existed in the case of chill-stored fish. Liston (1980) reported that bacterial groups which can grow rapidly at low temperatures and utilize a variety of substrates from fish muscle, dominate the flora of fish during later storage and hence the generic succession.

Pseudomonas spp. which formed a small proportion of early spoilers emerged as a dominant group among late spoilers. A similar trend was also reported by Shewan et al. (1960), Surendran and Gopakumar (1981) and Shetty and Setty (1990). *Achromobacter* spp., which were not encountered among the early spoilers, comprised 20% of late spoilers. Shewan et al. (1960) and Miller et al. (1973) also considered this group to be active spoilers. Other data regarding the importance of *Flavobacterium* spp., which decreased from 76% to 20% during spoilage of fish in the present study, are scarce. Similar observations on sole (*Eopsetta jordani*) by Pelroy et al. (1967) and on oil sardine (*Sardinella longiceps*), Indian mackerel (*Rastrelliger kanagurta*) and shrimp (*Metapenaeus*

Table 1. Generic distribution of initial and final bacterial flora of fish treated with propyl paraben during storage in ice.

Bacterial genera	% composition	
	Initial (day 1)	Final (day 14)
Achromobacter spp.	0	20
Pseudomonas spp.	12	36
Flavobacterium spp.	76	20
Vibrio spp.	0	16
Miscellaneous*	12	8
Total number of isolates identified	46	52

**Pseudobacterium* spp., *Micrococcus* spp.

dobsoni) during storage in ice were made by Surendran and Gopakumar (1981).

In the present study, *Pseudomonas* and *Achromobacter* together accounted for 56% of the flora of the spoiled fish. It was not determined whether all the isolated cultures belonging to these two genera were involved in the process of spoilage, but Adams et al. (1964) were of the view that only a small proportion of the bacterial population were spoilers and that some groups existed as "free riders" or perhaps were involved in some synergism with weak spoilers. The role played by groups such as *Vibrio*, *Micrococcus* and *Pseudobacterium* in the spoilage, if any, is uncertain.

Biochemical activity of the isolates of different genera is given in Table 2. Isolates of *Vibrio* spp. appeared to be biochemically more active than other groups, while the miscellaneous groups (*Pseudobacterium* spp. and *Micrococcus* spp.) showed least activity.

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

Biochemical tests		Bacterial genera									
		Flavobacterium spp.		*Achromobacter* spp.		*Pseudomonas* spp.		*Vibrio* spp.		Miscellaneous+	
Acid and alkaline reaction in	Lactose	11,	5*	40		18,	9*	25		0	
	Glucose	50,	5*	40		45		100		0	
	Sucrose	11,	5*	20,	20*	36		50		0	
Litmus milk		72		80		82		75		50	
Nitrate reduction	Nitrate positive	28		40		36		25		0	
	Ammonia	39		0		0		50		0	
	Gas	5		0		0		0		0	
Gelatin liquefaction		78		80		36		100		0	
Starch hydrolysis		0		0		18		50		0	
Ammonia from urea		22		40		9		50		25	
H_2S production**		39		40		18		50		0	
Triple sugar iron agar utilization		67		60		36		75		25	
Citrate utilization		28		20		18		50		25	
Arginine hydrolysis		33		0		0		25		50	
Methyl red test		5		0		18		0		50	
Indole production		0		0		0		0		0	
Voges-Proskauer test		0		0		0		0		0	
Catalase		100		100		100		100		100	
Number of isolates tested		18		5		11		4		4	

+*Pseudobacterium* spp., *Micrococcus* spp.; *alkaline reaction, **tested by lead acetate strips.

The established spoilage genera like *Pseudomonas*, *Achromobacter* and *Flavobacterium* showed moderate biochemical activity which is presumably due to bacteriostatic action of propyl paraben used in the present study (Singh 1978). Shetty et al. (1992) also reported *Vibrio* spp. as the most active spoiler followed by other groups as seen in this study.

Growth response of bacterial isolates at different incubation temperatures is shown in Table 3. After 96 hours of incubation, most isolates showed growth at 27°C, with lower proportions in both the lower and higher temperatures at 37°C, 42% at 8°C and 7%, at 0°C. All the cultures took 8-10 days for growth at 0°C, 6 days at 8°C and 2 days at 27°C and 37°C. With the exception of a few cultures of *Flavobacterium* spp., the bacterial isolates showed maximum growth at 27±2°C among the different temperatures tried (Table 4). Various investigators (Zobell and Conn 1940; Hucker 1954) have shown that psychrophiles grow better at higher temperatures, generally above 20°C, and hence recommended that they be considered as cold-tolerant (psychrotrophic) rather than cold-loving (psychrophilic). That 88% of the isolates in the present study showing good growth at 27±2°C (Table 3) substantiates this hypothesis. There is a general paucity of data on the optimum growth temperatures of psychrotrophs isolated from tropical fish. However, based on the observations of Anand and Setty (1977), Devaraju and Setty (1985) and that of the present study, it may be stated that these psychrotrophs show an optimum growth temperature in a range similar to that of those isolated from temperate waters and thus

Table 3. Percentage of total number of isolates (42) that exhibited growth at different temperatures.

Incubation temperature (°C)	Period of incubation (hours)	Percentage
0	96	7
	192-240	100
8	96	43
	144	100
27	96	88
37	96	51

Table 4. Growth response of various genera at different incubation temperatures.

Genus	Incubation temperature (°C)	Period of incubation (hours)	Percentage showing growth
Flavobacterium (18 isolates)	0	96	11
		192-240	100
	8	96	56
		144	100
	27	96	72
	37	96	50
Pseudomonas (11 isolates)	0	96	0
		192-240	100
	8	96	27
		144	100
	27	96	100
	37	96	64
Achromobacter (5 isolates)	0	96	0
		192-240	100
	8	96	20
		144	100
	27	96	100
	37	96	100
Vibrio (4 isolates)	0	96	25
		192-240	100
	8	96	25
		144	100
	27	96	100
	37	96	50
Miscellaneous (4 isolates)	0	96	0
		192-240	100
	8	96	50
		144	100
	27	96	100
	37	96	100

could be considered as facultative psychrophiles. One would have expected psychrophiles of temperate waters to have lower optimum growth temperatures than tropical psychrophiles in view of the hydrological differences. Such a difference, however, was not apparent in this study.

Acknowledgement

The authors thank the Director of Instruction, College of Fisheries, Mangalore, for providing the required facilities and encouragement throughout this study.

References

Adams, R., L. Farber and P. Lerke. 1964. Bacteriology of spoilage of fish muscle II. Incidence of spoilers during spoilage. Appl. Microbiol. 12:277-279.

Anand, C.P. and T.M.R. Setty. 1977. Chemical control of psychrophilic bacterial spoilage of fish-I. Isolation and identification of psychrophilic bacteria from marine fish. Fish. Technol. 14:98-102.

Barile, L.E., A.D. Milla, A. Reilly and A. Villadi. 1984. Spoilage pattern of mackerel. 2. Mesophilic and psychrophilic spoilage. FAO Fish. Rep. 317 (Suppl.):146-149.

Breed, R.S., E.G.D. Murry and N.R. Smith. 1957. Bergey's manual of determinative bacteriology. Williams and Wilkins Co., Baltimore.

Conn, H.J., H.W. Jennison and O.B. Weeks. 1957. Manual of microbiological methods, p. 140-168. *In* H.J. Conn and M.J. Pelczar (eds.) Society of American Bacteriologists, McGraw Hill, New York.

Devaraju, A.N. and T.M.R. Setty. 1985. Comparative study of fish bacteria from tropical and cold/temperate marine waters. FAO Fish. Rep. 317 (Suppl.):97-107.

Hucker, G.J. 1954. Low temperature organism in frozen vegetables. Food Technol. 8:79-82.

Kriss, A.E. 1967. Microbial population of oceans and seas. Edward Arnold Ltd., London.

Liston, J. 1980. Microbiology of fishery science, p. 138-157. *In* J.J. Connel (ed.) Advances in fish science and technology, Fishing News Books Ltd., Surrey.

Miller, A., R.A. Scanlan, J.S. Lee and L.M. Libbey. 1973. Volatile compounds produced in sterile fish muscle by *Pseudomonas putrifaciens*, *P. fluorescence* and *Achromobacter* species. Appl. Microbiol. 26:18-21.

Pelroy, G.A., J.P. Semen and M.W. Eklund. 1967. Changes in the microflora of irradiated petrale sole (*Eopsetta jordani*) fillets stored aerobically at 0.5°C. Appl. Microbiol. 15:92-96.

Shetty, T.S. and T.M.R. Setty. 1990. Bacteriology of Indian oil sardine (*Sardinella longiceps*) stored in chilled sea water. Fish. Technol. 27:141-144.

Shetty, T.S., T.M.R. Setty and C.N. Ravishankakr. 1992. Biochemical characteristics of tropical fish spoilage bacteria isolated from Indian oil sardine (*Sardinella longiceps*). Asian Fish. Sci. 5:117-122.

Shewan, J.M. 1963. The differentiation of certain gram negative bacteria frequently encountered in marine environments, p. 499-521. *In* C.H. Oppenheimer (ed.) Marine microbiology. Thomas Springfield, Illinois.

Shewan, J.M., G. Hobbs and W. Hodgkiss. 1960. The *Pseudomonas* and *Achromobacter* group of bacteria in the spoilage of marine white fish. J. Appl. Bacteriol. 23:463-468.

Singh, K. 1978. Studies on the use of propyl paraben for the preservation of marine fish at low temperature. University of Agricultural Sciences, Bangalore. (M.F.Sc. thesis).

Surendran, P.K. and K. Gopakumar. 1981. Selection of bacterial flora in the CTC treated oil sardine (*Sardinella longiceps*), Indian mackerel (*Rastrelliger kanagurta*) and prawn (*Metapenaeus dobsoni*) during ice storage. Fish. Technol. 18:133-141.

Valankar, N.K. and P.V. Kamasastri. 1956. The bacterial flora, trimethylamine and volatile base nitrogen of fish muscle at 0°C (in ice). Indian J. Fish. 3:269-289.

Zobell, C.E. and J.E. Conn. 1940. Studies on the thermal sensitivity of marine bacteria. J. Bacteriol. 40:223-238.

Manuscript received 15 November 1991; revised ms received 6 July 1992; accepted 8 March 1993.