Indian J. Fish., 46(3) : 227-236, Jul.-Sep., 1999

Major microbial points (MMP) in halobacterial contamination of fish curing environments of Andhra coast, India

M.M. PRASAD[1], K. GOPAKUMAR[2] AND G. SEENAYYA*

Department of Microbiology, Osmania University Hyderabad - 500 007, India

ABSTRACT

The levels of microbial contamination from different environmental sources in the process of fish curing in five coastal districts of Andhra Pradesh are reported. The descending order of halophilic bacterial contamination is (1) solar salt, drying ground (DG), soil, raised cemented platform (RCP), sea sand (SS) and fish skin surface (FSS) for red halophilic bacteria; (2) DG, soil, FSS, RCP, solar salt and SS for *Halomonas* spp. and (3) soil, FSS, solar salt, RCP, DG and SS with respect to slime producing bacteria. A positive correlation was observed with solar salt and SS for red halophiles, soil for *Halomonas* spp., and DG and solar salt for slime producing bacteria. The sources of contamination in relation to cured product showed that (a) solar salt, RCP and soil for red halophilic bacteria, (b) soil, solar salt and RCP for *Halomonas* spp., and (c) DG, soil and solar salt for slime producing bacteria as best sets. An inverse relationship was observed in the occurrence of red halophiles and slime producing bacteria at different fish curing environs. Occurrence of *Halomonas* spp., in fish drying grounds is reported for the first time.

Introduction

Curing of fish is one of the most popular methods of preservation in developed and developing countries. On the global basis 14% of the marine landings are processed by curing (Sanjeev and Surendran, 1996). Cured fish constitutes a major source of animal protein available at cheaper price to economically weaker sections of the society, especially people residing in interior and hill areas. The consumption of this amounts to 32% of the total marine landings in India. This ranks second to fresh fish consumption (Thomas and Balachandran, 1989). Spoilage of cured fish during storage due to rancidity and red discolouration by slime producers and red halophiles amounts to 38 and 50 per cent respectively (Prasad and Rao, 1994).

Fish is harvested from relatively cleaner environments. During subsequent handling bacteria of spoilage type and of public health significance type come in contact with the fish (Chichester

Present address : 1. *Visakhapatnam Research Centre of Central Institute of Fisheries Technology, Visakhapatnam - 530 003, India.*

2. Indian Council of Agricultural Research, Krishi Bhavan, New Delhi - 110 001, India.

* *Corresponding author.*

and Graham, 1973). To prevent or minimise the contamination it is necessary to know the sources of contamination in post harvest handling of fish. However, not much information is available with respect to dry salt cured fish. The quality of commercially cured fish has been reported from east and west coasts of India (Prasad *et al.,* 1994). Forsyth *et al* (1971) reported that a large proportion of bacteria isolated from the intertidal zone could grow in higher salt concentrations than found in sea water and some could grow in saturated or near saturated NaCI. Half of the isolates from the same study could grow in media containing very low (0.06%) NaCI. Bacteria of this kind can play a significant role in the spoilage of salt cured fish. In the light of the above, the present study is undertaken to assess the chances of contamination of 1. red halophilic bacteria; 2. *Halomonas* spp. and 3. slime producing bacteria from the associated environs in the process of salt curing of fish, such as drying ground (DG), soil, sea sand (SS), raised cemented platform (RCP) and fish skin surface (FSS) of the fish used for curing. The flora present in the cured fish and the associated factors are also reported. The correlation of the occurrence of these bacteria to the sources screened has been made.

Materials and methods

Five coastal districts of Andhra Pradesh namely Srikakulam, Visakhapatnam, East Godavari, Krishna and Nellore were chosen for this study. In each district three fishing hamlets of high fish curing activity were selected for sample collection (Fig.l). Sciaenids which are abundant in Andhra Pradesh coast and are most commonly preserved by salt curing were collected in sterile containers and their microbial analysis was carried out in less than an hour's time. The average length of the fish was 203 mm. Drying grounds (DG) considered are those grounds nearer to the landing centre used exclusively for drying of fish.

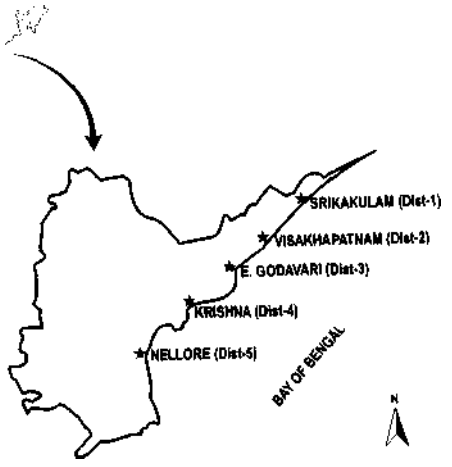

Fig. 1. Areas of study in each district samples were collected in replicates from three hamlets of high fish curing activity.

Raised cemented platforms (RCP) are platforms specially constructed for drying offish. Soil adjoining the drying ground where fish are put in heaps after drying is considered as soil. Sea sand (SS) is the sand in the shore area where fish is placed immediately after landing. Solar salt is the salt used for curing of fish obtained from the same site. Fish after curing and before marketing is given here as cured product (CP).

The surface of DG, RCP and FSS were swabbed over an area of 9 cm^2 using a sterile aluminium template and sterile cotton swabs and transferred into the diluent. 20 grams sample was taken from solar salt, soil, sea sand and cured fish and added to 180 ml of a

specific diluent. Cured fish was cut into small pieces with sterile scissors under sterile conditions. 20 g cut pieces were aseptically triturated in sterile mortar with sterile pestle using part of 180 ml specific diluent which was later mixed with the rest.

Isolation methods : Tryptose yeast extract salt media with a modification of employing pure NaCl (Analytical grade, Qualigens, India) for the isolation of red halophiles (Larsen, 1984). For isolation of *Halomonas* spp. the medium used was that of Vreeland *et al.* (1980) and for slime producing bacteria it was Lindeberg (1958) with glucose replacing sucrose and the levels from 50 to 5 g per litre of the medium. Moisture and salt contents of the cured product were estimated by standard methods (AOAC, 1995). Total volatile nitrogen (TVN) of the cured product was analysed by the method of Conway (1947). The sterile diluents used throughout this study were normal (0.85%), 10 and 20% solutions of NaCl in distilled water for *Halomonas* spp., slime producers and red halophiles respectively.

The incubation period for slime producers and *Halomonas* spp. was one week and for red halophiles it was three weeks. Drying of the plates was checked by keeping the plates in 200 gauge polythene covers and sealed air tight. The suspected *Halomonas* were identified based on characters described by Vreeland, 1992 and Holt, 1994. The statistical analysis of the data was carried out by the methods described by Visweswara Rao (1996). Chemical and media components used in this study were of Qualigens (India) and Hi-Media (India) make.

Results and discussion

The occurrence of red halophiles, their population size and mean values are presented in Table 1. It is clear from the Table that the red halophiles occurred in all the samples screened from DG and solar salt and population size was high. They were present only in some samples of soil, SS, RCP and FSS. Their occurrence is 27, 33,60, 66% for SS, FSS, RCP and soil, respectively. Interestingly, red halophiles were observed only in 40% of the cured fish samples as compared to 46, 55 and 62% in Malabar and Kanara coasts (Joseph *et al.,* 1983), east coast (Prasad and Rao, 1994) and in Maharashtra coast (Joseph *et al.,* 1988) of India, respectively. Such a low occurrence of red halophiles in the present study may be attributed to the freshness of the samples which were free from contamination.

The *Halomonas* spp. similar to red halophiles, occurred in all the samples screened from the DG and the population size was also high. On the other hand they were observed only in 40% of the samples screened from SS and the population density was low. Although the population size of *Halomonas* spp., was almost similar on RCP, FSS, soil and solar salt, their occurrence was 93, 80, 73 and 60% respectively, whereas the occurrence of *Halomonas* spp., was observed in all the cured product samples (Table 2). Vreeland (1992) reported that no *Halomonas* was isolated from saline soils and lakes. Occurrence of *Halomonas* spp. in the present study from the drying grounds is reported for the first time. Ability of these bacteria to retain rod shape in the changing NaCl concentration is one of

TABLE 1. *The range, mean, SD and CV of red halophilic bacteria from different environs of curing processes and in the cured products*

	Curing environs						
	DG	Soil	Sea sand	RCP	FSS	Solar salt	CP
District 1	4.8 (3.90-5.28)	2.4 (0.00-4.90)	0.82 (0.00-2.47)	0.67 (0.00-2.00)	0.49 (0.00-1.47)	6.84 (6.56-7.07)	1.86 (2.14-3.43)
District 2	4.69 (4.47-5.10)	2.05 (0.00-4.00)	ND -	1.26 (1.77-2.00)	0.56 (0.00-1.69)	5.76 (5.30-6.18)	ND -
District 3	4.52 (4.23-5.04)	1.56 (0.00-2.43)	0.8 (0.00-2.44)	3.32 (2.2-3.96)	0.65 (0.00-1.95)	6.29 (6.02-6.49)	ND -
District 4	4.62 (3.90-5.34)	2.2 (0.00-4.30)	0.43 (0.00-1.30)	1.83 (2.25-3.25)	0.63 (0.00-1.90)	5.62 (5.11-6.60)	0.53 (0.00-1.60)
District 5	4.62 (3.82-5.38)	1.58 (0.00-2.41)	0.87 (0.00-2.60)	1.11 (0.00-3.32)	0.63 (0.00-1.90)	6.4 (5.78-7.41)	3.01 (2.30-3.40)
	All districts						
Range	3.82-5.38	0.00-4.90	0.00-2.60	0.00-3.96	0.00-1.95	5.11-7.41	0.00-3.43
Mean	4.63	1.96	0.59	1.64	0.59	6.18	1.08
Standard deviation	0.56	1.67	1.05	1.53	0.87	0.69	1.45
Coefficient of variation	12.1	85.2	178	93.2	147.5	11.2	134.2

In each district samples were collected from three hamlets of high fish curing activity, in replicates.
Values given in parentheses are range of bacterial count of each district per gram of soil, sea sand (SS), solar salt and cured product (CP) and per cm of drying ground (DG), raised cemented platform (RCP) and fish skin surface (FSS).
All bacterial counts are expressed in log cycles. ND . not detected.

TABLE 2. *The range, mean, SD and CV of* Halomonas *spp. from different environs of curing processes and in the cured products*

	Curing environs						
	DG	Soil	Sea sand	RCP	FSS	Solar salt	CP
District 1	2.84 (1.90-3.38)	4.52 (3.99-4.90)	0.81 (0.00-2.44)	1.69 (0.00-2.60)	3.02 (2.30-3.41)	3.26 (3.00-3.47)	3.22 (2.20-3.86)
District 2	2.71 (1.77-3.47)	4.28 (3.78-4.77)	0.82 (0.00-2.47)	3.52 (3.25-4.00)	1.84 (0.00-3.25)	ND -	3.26 (3.04-3.60)
District 3	3.57 (2.76-4.12)	2.8 (2.27-3.69)	1.62 (0.00-2.46)	2.89 (2.34-3.07)	1.16 (0.00-3.47)	2.77 (0.00-5.00)	2.94 (2.44-3.90)
District 4	2.73 (2.23-3.32)	1.2 (0.00-3.60)	1.49 (0.00-2.47)	2.28 (2.00-2.43)	3.17 (2.44-3.66)	2.99 (0.00-5.50)	3.26 (3.04-3.41)
District 5	3.42 (3.25-3.46)	0.66 (0.00-2.00)	ND -	2.49 (2.00-3.44)	3.7 (3.62-3.75)	2.5 (0.00-4.00)	2.72 (2.11-3.27)
	All districts						
Range	1.77-4.12	0.00-4.90	0.00-2.47	0.00-4.00	0.00-3.75	0.00-5.50	2.11-3.90
Mean	3.06	2.69	0.95	2.57	2.58	2.3	3.1
Standard deviation	0.68	1.89	1.21	0.93	1.42	2.05	0.58
Coefficient of variation	22.22	70.26	127.37	36.19	55.04	89.13	18.7

In each district samples were collected from three hamlets of high fish curing activity, in replicates.
Values given in parentheses are range of bacterial count of each district per gram of soil, sea sand (SS), solar salt and cured product (CP) and per cm of drying ground (DG), raised cemented platform (RCP) and fish skin surface (FSS).
All bacterial counts are expressed in log cycles. ND : not detected.

the characteristic features observed in this study. This is in agreement with the earlier studies (Martin *et al.,* 1983).

The slime producers were present in all the samples screened from DG, RCP, FSS and in cured product while they were recorded only in 87% of SS, soil and solar salt samples. The number of slime producers were high in all the environs except in solar salt and DG (Table 3). In general when the population of slime producers is dominant at a specific source the population density of red halophiles is low and vice versa. The inverse relationship in the occurrence of red halophiles and slime producers was also observed in solar salt storage (data not given). Slime producers play an important role in the rancidity of salt cured fish. Rancidity spoilage ranks next to red discoloration. According to Prasad and Rao (1994) rancidity amounts to 38% of spoilage of cured fish. Studies of Lindeberg (1958) have shown that enzyme of these bacteria responsible for the spoilage is constitutive in nature. Lindeberg (1958) further reported that among the three cations tested namely sodium, potassium and lithium, only sodium ions are able to activate the slime producing enzyme. The maximum activity was found to occur in the range of 1-3 M NaCl. Considering the levels of sodium chloride in the fish samples (3-4 M. NaCl) of the present study, it is clear that the salt cured fish are vulnerable to spoilage due to these bacteria.

The order of occurrence of these bacteria at different stages of handling is as follows :

1. Red halophilic bacteria : Solar salt>DG>soil>RCP>SS>FSS.
2. *Halomonas* spp.: DG>soil>FSS> RCP>solar salt>SS.
3. Slime producing bacteria : Soil>FSS>solar salt>RCP>DG>SS.

It is clear that the solar salt is the main source of contamination for red halophilic bacteria and occupies third position after soil and FSS for slime producing bacteria. Statistical analysis also shows a positive correlation. Therefore, use of salt, free from spoilage bacteria is a must in the treatment of fishery products.

The quality parameters such as moisture, salt content and total volatile nitrogen observed in cured product in the present study are shown in Table 4. The Indian standard specification for this variety of fish are 45% moisture and 25% salt (ISI 3850, 1973). The moisture content of the cured product ranged from 38 to 47% and one third of the samples had moisture content above 45%. However, the increase is marginal (0.06 to 2.36%) and the mean values are within the permissible limits.

Though 78% of the samples are found to contain above 20% of salt, none of the fish samples is having higher levels than the prescribed ISI specifications. In the previous reports on commercial cured fish 85% of the fish was found to contain below 20% salt (Joseph *et al.,* 1986; Basu *et al.,* 1989; Prasad *et al,* 1994). Higher levels of salt in the present study indicate marked improvement in curing practice offish. The TVN levels in cured product in the present study ranged from 96 to 105 with a mean value of 100 mg N%. High levels of TVN are reported to correlate with high bacterial activity (Vanderzant *et al.,* 1973). And hence, a maximum permissible limit of 200 mg

TABLE 3. *The range, mean, SD and CV of slime producing bacteria from different environs of curing processes and in the cured products*

	Curing environs						
	DG	Soil	Sea sand	RCP	FSS	Solar salt	CP
District 1	3.17 (2.80-3.38)	4.41 (4.11-4.66)	3.13 (0.00-5.84)	4.01 (3.04-5.53)	3.82 (3.50-4.14)	4.08 (3.60-4.46)	4.01 (3.74-4.53)
District 2	3.37 (3.00-3.73)	4.75 (4.47-4.90)	2.32 (0.00-3.49)	4.65 (3.90-5.14)	3.66 (3.47-3.77)	2.96 (0.00-4.90)	4.03 (3.74-4.60)
District 3	3.75 (3.24-3.73)	4.55 (3.55-5.84)	3.07 (2.41-3.50)	3.51 (3.17-3.90)	4.22 (3.79-4.64)	4.74 (3.90-4.64)	4.79 (4.04-5.30)
District 4	2.88 (2.36-3.27)	4.82 (4.41-5.44)	3.86 (2.84-5.30)	2.54 (2.20-3.07)	4.44 (4.01-4.80)	4.56 (4.00-5.39)	2.58 (3.11-4.25)
District 5	2.96 (2.30-3.38)	1.91 (0.00-5.74)	3.02 (2.69-3.38)	2.13 (2.07-2.17)	4.19 (3.60-4.66)	3.47 (0.00-5.73)	4.14 (3.50-4.74)
	All districts						
Range	2.30-4.36	0.00-5.84	0.00-5.84	2.07-5.53	3.47-4.80	0.00-5.84	3.11-5.30
Mean	3.23	4.09	3.08	3.37	4.07	3.96	4.11
Standard deviation	0.51	1.77	1.54	1.13	0.44	1.74	0.63
Coefficient of variation	15.79	43.28	50	33.53	10.82	43.94	15.33

In each district samples were collected from three hamlets of high fish curing activity, in replicates.
Values given in parentheses are range of bacterial count of each district per gram of soil, sea sand (SS), solar salt and cured product (CP) and per cm of drying ground (DG), raised cemented platform (RCP) and fish skin surface (FSS).
All bacterial counts are expressed in log cycles.

TABLE 4. *Chemical and physical quality of cured product*

	Moisture %	Salt %	T.V.N, mg N%
District 1	44.78 (41.94-46.80)	22.26 (21.92-22.82)	101.82 (97.51-107.26)
District 2	42.71 (39.68-46.21)	22.31 (20.94-23.48)	119.28 (106.48-129.41)
District 3	43.52 (40.76-47.36)	19.82 (18.29-21.26)	101.27 (96.71-104.63)
District 4	42.04 (38.27-44.56)	19.78 (18.26-21.84)	101.75 (99.28-104.51)
District 5	42.02 (40.18-45.2)	21.14 (20.73-21.95)	100.45 (98.3-104.26)
	All districts		
Range	38.27-47.36	18.26-23.48	96.71-129.41
Mean	43.03	21.06	104.92
Standard deviation	2.85	1.57	9.14
Coefficient of variation	6.23	7.45	8.71

In each district cured product (CP) samples were collected from three hamlets of high fish curing activity and from the same sites of fish curing environs, in replicates.
Data given in parentheses are ranges. TVN : Total volatile nitrogen.

N% has been suggested (Sreenivasan and Joseph, 1966; Prasad and Rao, 1994). However, in this study all samples have shown less than the above suggested levels of TVN.

Statistical analysis of the data revealed a positive correlation with solar salt and SS for red halophiles, soil for *Halomonas* spp. and DG and solar salt for slime producing bacteria. The order of sources of contamination in relation to cured product, showed (a) solar salt, RCP and soil for red halophiles; (b) soil, solar salt and RCP for *Halomonas* spp. and (c) DG, soil and solar salt for slime producing bacteria as best sets.

The present study shows that the microbial contamination of fish can come from different sources and this includes raw fish itself. Contamination from these major microbial points (MMP) can result in loss of nutritional quality, spoilage of the fish and finally rendering unacceptability of the product. Though the quality of final product is good in respect to some physical and chemical parameters in all the areas it is found to contain halophilic and halotolerant bacteria.

Acknowledgment

The first author thanks the Director, CIFT, Kochi for granting study leave and the authors are thankful to Dr. K. Visweswara Rao, Deputy Director, National Institute of Nutrition, Hyderabad for useful discussions in statistical analysis of the data.

References

A.O.A.C. 1995. *Official Methods of Analysis.* 16th edition. Association of Official Analytical Chemists, Washington DC.

Basu, S., D. Imam Khasim, S.S. Gupta and C.C.P. Rao 1989. Quality of dry fish from markets in Andhra Pradesh. *Fish Technol.,* 26: 114-118.

Chichester, CO. and H.D. Graham 1973. *Microbial Safety of Fishery Products.* Academic Press. New York AC, USA.

Conway, E.J. 1947. *Microdiffuswn Analysis and Volumetric Error.* Crossby, Lock Wood and Sons, London, U.K.

Forsyth, M.P., D.B. Shindler, M.B. Gochnauer and D.J. Kushner 1971. Salt tolerance of intertidal marine bacteria. *Can. J. Microbiol.,* 17: 825-828.

Holt, G.J. 1994. *Bergeys Manual of Determinative Bacteriology.* 9th edition. Williams and Wilkins, Baltimore, USA.

ISI No. 3850. 1973. Indian Standard Specification for dry salted Threadfin *(Dara)* and dry salted Jew fish *(Ghol).* Indian Standard Institution, New Delhi.

Joseph, KG., V Muraleedharan and T.S.U. Nair 1983. Quality of cured fishery products from Malabar and Kanara coasts. *Fish Technol,* 20: 118-122.

Joseph, K.G., V. Muraleedharan, N. Kalaimani and T.S.U. Nair 1986. Quality of cured fish from Tamil Nadu coast. *Fish Technol.,* 23: 63-65.

Joseph, K.G., V. Muraleedharan and T.S.U. Nair 1988. Quality of cured fish from Maharashtra coast. *Fish. Technol.* 25: 120-123.

Lindeberg, G. 1958. Levan - producing halophilic bacteria in sugar-containing herring brines. In: *The Microbiology of Fish and Meat Curing Brines.* B.P. Eddy (Ed.). *Proceedings 2nd International Symposium on Food Microbiology.* London, H.M. Stationary Office, p. 157-173.

Larsen, H. 1984. Halobacteriaceae. In : *Bergy's Manual of Systematic Bacteriology.* N.R. Krieg (Ed.). Williams and Wilkins Co., Baltimore, p. 261-267.

Martin, E., T. Duryea-Rice, R.H. Vreeland, L. Hilsabeck and C. Davis 1983. Effects of NaCl on the uptake of alpha [14C] aminobutyric acid by the halotolerant bacterium *Halomonas elongata. Can J. Microbiol.* 29: 1424-1429.

Prasad, M.M. and C.C.P. Rao 1994. Storage studies on commercial salt cured dry fish with special reference to red-discoloration. *Fish. Technol.,* 31: 163-166.

Prasad, M.M., C.C.P. Rao and S.S. Gupta 1994. Chemical microbiological quality of dry fish from Kakinada. *Fish. Technol.,* 31: 75-78.

Sanjeev, S. and P.K. Surendran 1996. Fate of enteropathogenic staphylococci in fish subjected to curing. *Fish. Technol.,* 33: 66-68.

Sreenivasan, R. and KG. Joseph 1966. A survey on the quality of salt cured fish produced in the Kanyakumari district, Madras state. *Fish. Technol.,* 3 : 03-112.

Thomas, F. and K.K. Balachandran 1989. Fish curing in India. In: *Recent Trends in Processing of Low Cost Fish.* Society of Fisheries Technologists of India, Matsyapuri P.O., Cochin-29, p. 1-10.

Vanderzant, G, B.F. Cobb and C.A. Thompson Jr. 1973. Microflora, chemical characteristic and shelf life of four species of pond reared shrimps. *J. Milk Food Technol.,* 35: 443-446.

Visweswara Rao, K. (Ed.) 1996. Biostatistics. *A Manual of Statistical Methods for use in Health Nutrition and Anthropology.* Jaypee Brothers Medical Publishers (P) Ltd., New Delhi-2.

Vreeland, R.H. 1992. The family, Halomonadaceae. In : *The Prokaryotes: a Handbook on the Biology of Bacteria;*

Ecophysiology, Isolation, Identification and Applications. A. Balows, H.G. Truper, M. Dworkin, W. Harder and K.H. Schleifer (Ed.). Springer-Verlag, New York, p. 3181-3188.

Vreeland, R.H., CD. Litchfield, E.L. Martin and E. Elliot 1980. *Halomonas elongata,* a new genus and species of extremely salt tolerant bacteria. *Int. Natl. J. Syst. Bacterial.,* 30: 485-495.