Studies on Lipolytic Bacteria in Stored Fish *Etroplus suratensis* (Bloch)

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The distribution of total hectrotrophic bacteria (THB) and lipolytic bacteria in various regions (body surface, gill, intestine and flesh) of fish *Etroplus suratensis* (Bloch) during storage at 28±2°C and 4°C was studied. *Pseudomonas* dominated at reduced temperature whereas at 28±2°C and in fresh condition *Vibrio, Aeromonas,* and *Acinetobacter* dominated. Lipolytic activity was elaborated by the members of various genera and their activity varied in different lipid compounds (tributyrin, tween 80, tween 60, tween 40 and tween 20). Tributyrin was utilized by majority of the isolates. All the selected isolates preferred a temperature of 35°C and pH 6.0 for their maximum growth. *Aeromonas* and *Vibrio* showed maximum growth at 0.5% NaCl concentration while 3% NaCl was found to be optimum for *Pseudomonas.*

Lipase enzyme produced by microorganism plays a vital role in degrading the fat in food and thus pave the way for quick deterioration. In chilled food, the lipolytic activity of psychrotrophic microorganisms can give rise to quality changes (Alford & Pierce, 1961) and moist fat will spoil rapidly than normal muscle tissue (Gill & Newton, 1977). This enzyme is stable and active during storage at low water activity (Anderson, 1980). Hence the study on lipolytic bacteria is important in spoilage.

Lipolytic microorganisms associated with finfish and shellfish are reported (Kelly *et al.,* 1978; Mary, 1977; Teplinskaya, 1981). However, information on the occurrence and distribution of lipolytic bacteria in fish during storage at various non-freezing temperatures is not available. In the present study, an attempt was made to find out the changes of bacterial flora and lipolytic bacteria during storage of *Etroplus suratensis.* The aspects dealt with are (a) total heterotrophic bacteria and generic composition (b) occurrence and distribution of lipolytic bacteria and (c) growth and physiology of lipolytic bacteria.

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Materials and Methods

Etroplus suratensis was collected from Cochin backwater, killed by shock treatment and stored in sterile containers at room temperature (28 ± 2°C) and at 4°C. The samples were drawn at regular intervals. Five fishes were taken for each analysis and the total heterotrophic bacterial population (THB) was estimated for the body surface, gill, alimentary canal and flesh following the standard plate count (SPC) method, using ZoBell's 2216 e agar. Isolation of the bacterial strains was made at random from the plates. The purified isolates were identified upto the generic level (Simidu & Aiso, 1962).

All the isolates were tested for lipolytic activity by employing five different substrates such as tributyrin, tween 80, tween 60, tween 40 and tween 20 added to a basal medium of Harringen & McCance (1972). Inoculation was made by surface spot streak method. Appearance of a waxy material and transparent area around the colony in tween agar and tributyrin agar respectively were considered positive.

The effect of environmental factors on the growth of selected lipolytic bacteria (*Vibrio* sp., *Pseudomonas* sp., *Aeromonas* sp.) was tested in nutrient broth. Studies

Table 1. *Total heterotrophic bacterial population of E. suratensis stored at different temperatures*

Storage temperature	Storage period	Surface THB/cm²	Gill THB/g	Gut content THB/g	Flesh THB/g
28±2°C	0 hour	3.00 x 10^5	1.98 x 10^7	6.09 x 10^8	1.21 x 10^2
	4 „	3.10 x 10^7	2.50 x 10^7	1 19 x 10^8	1.73 x 10^6
	8 „	2.94 x 10^7	1.02 x 10^8	1.37 x 10^8	1.05 x 10^7
	12 „	5.46 x 10^7	7.57 x 10^8	2.17 x 10^8	2.57 x 10^8
	24 „	9.70 x 10^6	2.31 x 10^8	4.54 x 10^7	8.15 x 10^6
4°C	0 days	3.00 x 10^5	1.98 x 10^7	6.09 x 10^8	1.21 x 10^2
	2 „	4.00 x 10^5	2.49 x 10^7	1.25 x 10^7	2.03 x 10^6
	5 „	1.70 x 10^6	4.46 x 10^7	1.41 x 10^7	1.54 x 10^7
	10 „	5.70 x 10^6	3.75 x 10^7	3.82 x 10^7	2.56 x 10^7
	15 „	2.25 x 10^7	9.83 x 10^7	7.56 x 10^7	7.37 x 10^7
	22 „	1.47 x 10^7	8.56 x 10^7	5.52 x 10^7	6.37 x 10^7

Table 2. *Generic composition of bacteria at different temperatures*

Genus	Temperature 28 ± 2°C	4°C
Bacillus	1	0
Micrococcus	0	1
Coryneform	3	3
Vibrio	7	17
Pseudomonas	2	35
Aeromonas	11	1
Acinetobacter	13	8
Alcaligenes	4	2
Enterobacteriaceae	13	4
Flavobacterium	1	0
Moraxella	2	8
Unidentified	13	0
Total isolates	70	79

were conducted at various temperatures, pH and NaCl concentrations.

Results and Discussion

Maximum counts (6.09 x 10^8/g) of THB was recorded in intestine followed by gill (1.98 x 10^7/g) and the least was in the flesh (1.21 x 10^2/g) (Table 1). It is obvious that the bacterial load in the alimentary canal of a fish in a given time is influenced by the composition of ingested food. This observation is in agreement with the results of Mary *et al.* (1975) and Surendran & Gopakumar (1981) who reported high bacterial population in intestine. The flesh of fresh and healthy fish is normally free of bacteria. However, in some cases flesh seems to harbour few bacteria (Geez, 1930; Bisset, 1948; Bullock & Sniezko, 1969) and in this investigation, the flesh of *E. suratensis* was found to harbour a bacterial population of 1.21 x 10^2/g.

The bacterial population of body surface, gill and flesh of fish stored at 28 ± 2°C and 4°C showed an increase with advancement of time. However, high reduction in the gut flora, was found initially. The fresh fish and the fish stored at room temperature contained a diversified taxonomic group of bacteria, the dominant being *Acinetobacter* and Enterobacteriaceae followed by *Aeromonas* and *Vibrio* (Table 2). *Pseudomonas* was less than 3%. During the storage at 4°C some of the genera was eliminated and the domination of *Pseudomonas* was evident. Similar pattern of changes in the bacterial flora and domination of *Pseudomonas* during storage at lower temperature was reported by Shewan (1971, 1977) and Surendran & Gopakumar (1981).

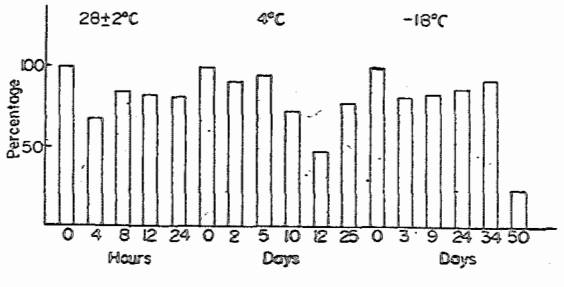

Fig. 1. Percentage of lipolytic bacteria present in fish stored at different temperatures.

Table 3. *Changes in the proportion of lpiolytic bacteria (percentage poistive for lypolysis) isolated from Etroplus suratensis stored at various temperatures*

Storage temperature	Storage period	No. of isolates tested	Tween 80	Tween 60	Tween 40	Tween 20	Tributyrin
	hours						
	0	16	37.50	31.25	37.50	37.50	100.00
	4	3	66.67	—	—	—	66.67
28±2°C	8	12	31.25	50.00	50.00	66.67	83.83
	12	11	9.09	27.27	18.18	45.45	72.72
	24	15	26.67	26.67	26.67	26.67	60.00
	Days						
	2	10	20.00	20.00	20.00	30.00	90.00
	5	14	50.00	36.00	28.57	57.14	85.71
4°C	10	14	21.43	28.57	35.71	42.86	57.14
	15	13	23.08	23.08	23.08	23.08	38.46
	22	28	32.14	39.29	35.71	39.29	75.00

Table 4. *Distribution of lipolytic bacteria based on their activity in various substrates*

Substrate	Temperature 28±2°C	4°C	Total
No. of isolates	57	79	136
T 20*	1	1	2
Tri	19	24	43
T 80+T 60	—	—	0
T 80*+Tri	3	2	5
T 60+T 20	—	—	0
T 60+Tri	—	1	1
T 40+T 20	—	—	0
T 40+Tri	1	—	1
T 20*+Tri	3	3	6
T 80+T 40+Tri	—	1	1
T 80+T 20+Tri	1	1	2
T 60+T 20+Tri	1	—	1
T 60+T 40+T 20	—	1	1
T 40+T 20+Tri	—	2	2
T 80+T 60+T 40+T 20	—	2	2
T 80+T 60+T 20+Tri	—	2	2
T 80+T 40+T 20+Tri	1	—	1
T 60+T 40+T 20+Tri	3	3	6
T 80+T 60+T 40+ T 20+Tri	13	16	29
Negative to all	11	20	31

T=Tween; Tri=Tributyrin
* Positive to substrate combination only

All strains isolated from fresh fish were lipase producers and variations in percentage during storage at different temperatures was noticed (Fig. 1). There was a reduction in lipolytic bacteria initially and later it increased at all the temperatures. However at 4°C, after a slight increase, it decreased to the minimum in 12 days and then increased. The experiments carried out with different substrates showed that tributryin was the ideal substrate in detecting lipolytic activity of bacterial isolates (Table 3). The maximum proportion of bacteria capable of producing lipase was recorded in tributryin based media (Table 4). About 32% of the isolates were specific to tributyrin while none was specific to tween 80, tween 60 and tween 40. Only 1.5% of the isolates elaborated lipase enzyme in tween 20. Similar findings of utilization of various tween compounds as sole source of carbon was reported in a fungus *Oospora lactis* and highest production was in tween 80. Also, low number of positive in tween 80, was reported among hydrocarbon degrading bacteria and the results in tween 80 under estimated the lipolytic bacterial population (Nandakumar, 1983). Tween 80 may be favourable for fungus but the present study suggests the affinity of bacteria towards tributyrin. The results show that the lipase enzyme elaborated may be specific to a particular lipid compound and this might be the reason for the variation of activity in different compounds.

Table 5. *Generic composition of lipolytic bacteria isolated from Etroplus suratensis stored at various temperature using various substrates (%)*

Name of the genus	Total no. of isolates tested	Tween 80	Tween 60	Tween 40	Tween 20	Tributyriu
Bacillus	1	0.00	0.00	0.00	100.00	0.00
Micrococcus	1	0.00	0.00	0.00	0.00	100.00
Coryneform	3	16.67	0.00	16.67	16.67	16.67
Vibrio	24	42.42	27.27	30.30	81.81	72.72
Pseudomonas	37	25.80	33.55	38.70	41.28	77.40
Aeromonas	12	46.67	53.33	40.00	66.67	86.67
Acinetobacter	21	24.24	24.24	33.33	54.54	87.88
Alcaligenes	6	50.00	66.67	66.67	66.67	83.33
Enterobacteriaceae	17	10.56	12.67	12.67	27.45	44.36
Flavobacterium	1	100.00	0.00	100.00	100.00	100.00
Moraxella	10	20.00	30.00	10.00	50.00	60.00

The percent distribution of various genera positive to tween compounds and tributyrin is presented in Table 5. All the genera except *Vibrio* which recorded maximum in tween 20, showed positive at high level in tributyrin than tween compounds. There was a variation in percent distribution within the genera for each substrate and no general pattern could be observed. Among the genera, *Alcaligenes,* recorded maximum percent in all the substrates followed by *Aeromonas, Pseudomonas, Vibrio, Acinetobacter* and Enterobacteriaceae.

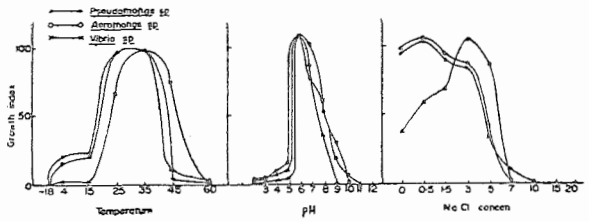

Fig. 2. Effect of temperature, pH and NaCl concentration on growth of *Pseudomonas* sp., *Aeromonas* sp. and *Vibrio* sp.

While testing the effect of temperature, pH and NaCl of the three selected isolates belonging to the genera *Aeromonas* (SE 189), *Pseudomonas* (SE 194) and *Vibrio* (SE 206), they preferred a temperature of 35°C and a pH of 6.0 for their maximum growth (Fig. 2). Both species of *Aeromonas* and *Vibrio* showed maximum growth at 0.5% NaCl concentration and *Pseudomonas* showed maximum growth at 3% NaCl. The data on the utilization of various substrates by these isolates show that species of *Aeromonas* and *Vibrio* exhibited maximum growth in tween 60 while *Pseudomonas* in tween 40 at 1% substrate concentration. From the results it could be inferred that bacterial isolates show activity on specific chemical components available in the substrate. Similar findings of the substrate specificity of the lipase by *Pseudomonas flourescens* was reported by Shabanova *et al.* (1978).

This investigation suggests that lipolytic bacteria may change the quality of the stored marine food and the extent of degradation depends on the nature of lipid and the storage conditions.

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References

Alford, J. A. & Pierce, D. A. (1961) *J. Fd Sci.* 26, 518

Anderson, R. E. (1980) *J. Fd Sci.* 45, 1694

Bisset, K. A. (1948) *J. Hyg. Camb.* 46, 94

Bullock, G. L. & Snieszko, S. F. (1969) *Trans. Am. Fish. Soc.* 98, 268

Geez, A. H. (1930) *Cont. Can. Biol. Fish.* 5, 433

Gill, C. O. & Newton, K. G. (1977) *J. Appl. Bacteriol.* 43, 189

Harringan, W. F. & McCance, M. E. (1972) In *Laboratory Methods of Microbiology,* Academic Press, London & New York, p. 362

Kelly, M. D., Lukaschewsky, S. & Andersson C. G. (1978) *J. Fd Sci.* 43, 1196

Mary, P. P. (1977) *Studies on the Gastro-intestinal Microflora of the Mullet Liza dussumieri (Valenciennes) (Mugiliformes: Teleostei)* Ph. D. Thesis, Annamalai University

Mary, P. P., Chandramohan, D. & Natarajan, R. (1975) *Bull. Dept. Mar. Sci., Univ. Cochin,* 7, 185

Nandakumar, K. (1983) *Studies on Hydrocarbon Degrading Bacteria of Cochin Backwater,* M.Sc. Thesis, University of Cochin

Shabanova, G. F., Dnitrieva, L. N., Witvinko N. M., Firsaeva, T. M. & Buyabova, N.U. (1978) *Prikl. Biokhim. Mikrobiol.* 14, 399

Shewan, J. M. (1971) *J. Appl. Bacteriol.* 34, 299

Shewan, J. M. (1977) in *Proc. Trop. Prod. Inst. Conf. Handl. Process. Mark. Fish.* London

Simidu, U. & Aiso, K. (1962) *Bull. Jap. Soc. Sci. Fish.* 28, 1133

Surendran, P. K. & Gopakumar, K. (1981) *Fish. Technol.* 19, 89

Teplinskaya, N. G. (1981) *Biol. Morya,* 6, 3