THE BACTERIAL FLORA, TRIMETHYLAMINE AND TOTAL VOLATILE NITROGEN OF FISH MUSCLE AT 3° C.

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Storage in ice is widely employed as a means of preservation for short durations prior to marketing of fish in the fresh condition. The use of special ices treated with antibiotics for enhancing the period of preservation is under experimentation in different countries. In this connection it is necessary to have some knowledge of the bacteria which are associated with fish at the temperatures obtaining during the storage. Further, bacteriological observations might be useful in ascertaining the freshness of fish in storage. Various tests, including the determination of trimethylamine, which are in use at present, are not thoroughly satisfactory since opinions differ as to how far they are reliable as criteria of freshness (Tarr *et al.*, 1939; Shewan, 1949).

While considerable information is available concerning the bacteria associated with spoilage of marine fishes, most of it is based on studies carried out in temperate regions. Differences might be expected between the bacterial flora of fishes from tropical waters and those from relatively colder waters. The observations made in India on the bacterial flora of sea-water and marine fishes (Velankar, 1955; Venkataraman and Sreenivasan, 1952, 1954; Bhat and Albuquerque, 1953) lend support to this view.

A comprehensive bacteriological and chemical investigation on the storage of fish at low temperatures is in progress at this Station and the observations on fish kept at 3° C. reported in this paper were made as a preliminary to this investigation. During transportation in ice under commercial conditions the actual temperature to which the fish is subjected is likely often to exceed 0° C., and a knowledge of the bacteriological and chemical changes occurring at temperatures slightly above 0° C. is desirable. A few observations made on the bacterial flora of fresh fish and fish allowed to spoil at ordinary temperatures are also reported here for comparison.

Material and Methods

Fish were obtained from shore-seine catches made at Dhanushkodi. The slime, gills and the gut contents were examined by plating on both sea-water and fresh-water nutrient agars, in order that marine bacteria having specific salt requirements may not be excluded. For studies on storage at 3° C., the fish were kept

in a refrigerator and muscle samples were removed aseptically at appropriate intervals for bacterial plate counts and for determination of trimethylamine and total volatile basic nitrogen. The procedure followed for the estimations, etc., was the same as that described previously for the examination of salt-cured fish (Velankar, 1952). For spoilage studies at ordinary room temperature (27° C.–30° C.) the fish were kept in a closed glass jar for 24 hours. Bacterial isolations were made from colonies appearing on the counting plates inoculated with suitably high dilutions of the muscle so that mainly the dominant types were obtained.

Results

The results are shown in Tables I, II and III. Gram-negative asporogenous rods predominated in the fresh fish flora; *Bacillus* and *Micrococcus* were also present (Table I).

TABLE I

Flora of fresh fish

Sample No.	*Achromobacter*	*Bacillus*	*Micrococcus*
	(Number of isolates)		
Perch (*Lethrinus* sp.)			
Slime and Gills ..	3	1	..
Gut	2	1	..
Horsemackerel (*Caranx* sp.)			
Slime and Gills ..	5	..	..
Gut	2	..	..
Seer (*Cybium* sp.)			
Slime and Gills ..	2	..	..
Gut	..	..	..
Dorab (*Chirocentrus* sp.)			
Slime and Gills ..	5	..	..
Gut	2	1	..
Perch (*Psammoperca* sp.)			
Gut	..	2	..
Red Mullet (*Upeneus* sp.)			
Gut	..	1	3

TABLE II

Bacteria, trimethylamine and total volatile nitrogen at 3° C.

Sample	Duration of storage	Tri-methyl-amine	Total volatile N	Bacterial count	Micrococci	*Bacillus*	Achromobacteria
	(mg. N per 100 g. of fish muscle)			(per g. of muscle)		(Number of isolates)	
Seer (*Cybium* sp.) ..	1 week	..	6·0	3,660	3	..	..
	2 weeks	4·7	29·5	400,000	3	1	..
Pomfret (*Stromateus* sp.)	1 week	..	11·2	9,000	4	2	..
	2 weeks	5·0	49·9	5,100,000	4	1	3
	3 weeks	13·6	53·8	Plates crowded	..	..	2
Mullet (*Mugil* sp.) ..	10 days	..	5·4	32,000	..	7	..
	20 days	..	7·6	120,000	..	5	..
	6 weeks	2·6	21·4	270,000	..	3	..
Perch (*Lethrinus* sp.) ..	10 days	..	2·0	3,200	1	3	..
	20 days	1·4	7·2	15,000	1	3	..
	6 weeks	1·8	19·4	144,000	1	5	..
Seer (*Cybium* sp.) ..	10 days	..	10·5	Nil	..	..	..
	20 days	2·0	14·5	35,000	..	2	..
	4 weeks	13·3	49·0	3,400,000	..	4	4

TABLE II (*Contd.*)

Sample	Duration of storage	Tri-methyl-amine	Total volatile N	Bacterial count	Micrococci	*Bacillus*	Achromobacteria
	(mg. N per 100 g. of fish muscle)			(per g. of muscle)	(Number of isolates)		
Dorab (*Chirocentrus* sp.)	10 days	..	7·4	Nil	..	..	..
	20 days	3·0	14·4	64,000	2	3	..
	4 weeks	5·6	22·2	560,000	2	3	1
Perch (*Psammoperca* sp.)	1 week	..	7·9	11,700	..	3	..
	2 weeks	0·12	11·8	5,660	..	2	..
	3 weeks	4·60	29·50	Plates crowded	3	3	4
Red Mullet (*Upeneus* sp.)	1 week	2·8	23·6	5,200	..	2	1
	2 weeks	6·8	40·8	6,700,000	1	1	4
	3 weeks	..	..	9,000,000	..	..	6
	4 weeks	..	..	Plates crowded	..	3	3
Perch (*Lethrinus* sp.) ..	1 week	6·04	18·6	8,700	..	..	2
	2 weeks	5·36	16·1	1,880,000	3	..	4
	3 weeks	11·8	66·4	2,100,000	..	2	5

TABLE III

Spoilage at room temperature

Sample	*Bacillus*	*Achromobacter* (No. of isolates)	*Micrococcus*	TMA (mg. N /100 g. muscle)	T.V.N. (mg. N /100 g. muscle)
Seer (*Cybium* sp.)					
24 hours ..	3	..	..	14·7	60·3
Dorab (*Chirocentrus* sp.)					
24 hours ..	4	..	..	16·9	53·0
Seer (*Cybium* sp.) ..					
12 hours ..	4	..	1 Sarcinæ	13·6	33·7
24 hours ..	4	..	..	17·8	52·7
Shark (*Scoliodon* sp.)					
12 hours ..	1	4	..	..	17·8
24 hours ..	..	3	..	22·5	77·5
Mullet (*Mugil* sp.) ..					
24 hours ..	2	..	..	16·4	47·9
Horsemackerel (*Caranx* sp.)					
24 hours ..	3	..	..	27·5	64·8

The bacterial flora at 3° C. consisted of *Achromobacter,** *Micrococcus* and *Bacillus* (Table II). In series I micrococci alone were present; at the end of 2 weeks the TMA† and T.V.N.‡ had not reached significantly high values. The odour of the fish was not offensive. Different workers have described the ranges of trimethylamine and total volatile nitrogen levels at which fish is " sea-fresh ", " stale " and "decisively spoilt " (Beatty and Gibbons, 1935; Dyer and Dyer, 1949). The latter workers found TMA values§ up to 4 for fish in very fresh condition, 4–15 for fish in the early stages of spoilage, and above 15 for decisively spoilt fish. In Denmark and Norway the amount of T.V.N.

* Used collectively for denoting "Gram-negative, non-sporing, achromic rods" which do not produce gas from sugars, as suggested by Wood (1940).

† Trimethylamine.

‡ Total volatile nitrogen.

§ Mg. trimethylamine N/100 g. muscle.

permissible for fresh fish appears to be 30 mg. N/100 g. of muscle (Petersen *et al.*, 1951). In series II, micrococci and, to a lesser extent, spore-forming rods were present in the first week; the count was low. After 2 weeks achromobacteria appeared and the count increased significantly; the TMA and T.V.N. had already passed the " threshold " levels. The fish gave ammoniacal odours. In series III *Bacillus* alone was present throughout 6 weeks. The TMA and T.V.N. levels were low, and organoleptic signs of spoilage were not discernible. In series IV *Micrococcus* was present in addition to *Bacillus*; the results were similar to those of series III. In series V, the TMA and T.V.N. were low during the first 20 days; the flora consisted of *Bacillus* alone. At the end of 4 weeks the TMA and T.V.N. increased, and the flora consisted of *Bacillus* and achromobacteria. The count had increased significantly. The results of series VI resembled those of series V except that *Micrococcus* was present in addition to *Bacillus*, the achromobacteria appeared late, but were not dominant; the TMA had not increased as much as in series V. In series VII achromobacteria appeared after 3 weeks. In series VIII and IX, achromobacteria were present throughout; the bacterial count increased earlier than in previous series.

B. subtilis, *B. pumilus*, *B. megatherium*, *B. lentus* and other spp. of *Bacillus* not identified yet, were present at 3° C. and also at room temperature. The micrococci consisted of strains which resembled *M. aurantiacus*, *M. caseolyticus*, *M. epidermidis*, *M. candidus*, *M. flavus* and *M. roseous* (*Bergey's Manual of Determinative Bacteriology*, 1948) but differed from the described species in some respects. Among the Gram-negative asporogenous rods some were pleomorphic, having both rod and coccoid forms, and these usually showed bipolar staining. The bacteria isolated in these studies will be described in detail in a subsequent paper.

Discussion

A succession occurs in the bacterial types during storage at 3° C., Gram-negative asporogenous rods succeeding micrococci and spore-forming rods. Sometimes a single type alone prevailed during the storage, *Micrococcus* in series I, *Bacillus* in series III. Generic succession in the prevailing bacterial flora during fish spoilage has been observed by various workers previously (Wood, 1940; Castell and Anderson, 1948; Shewan, 1949) who recorded micrococci and flavobacteria in the early stages, which later gave place to achromobacteria and pseudomonas. In the present investigation, flavobacteria were not encountered, but spore-forming rods occurred in large numbers. The presence of *Bacillus*, or *Micrococcus*, exclusively in the fish muscle, appears to be associated with low values of TMA (series I, III and IV, Table II).

Baird and Wood (1944) found that many well-known species of *Micrococcus* do not reduce trimethylamine oxide to trimethylamine, though some micrococci isolated from fish are known to do so. Castell (1949) recorded that *B. subtilis*, *B. mesentericus* and *B. mycoides* do not reduce the oxide to the amine. Gram-negative, achromic, non-sporeforming rods are associated with pronounced spoilage (Table II).

Bacillus was predominant in fish muscle spoiling at room temperature (Table III). Venkataraman and Sreenivasan (1952) also reported sporeformers to be predominant during the spoilage of mackerel at room temperature. Though *Bacillus* is not common in sea-water (Velankar, 1955), a selective enrichment of this genus might occur in fish muscle at temperatures prevailing in the tropical conditions, since this genus is essentially mesophilic. Its presence in large numbers in fish muscle at 3° C. is, however, noteworthy.

The bacterial count is significantly high, *i.e.*, over 100,000/g., when " threshold " values of TMA are reached. The TMA values are mostly insignificant during the first week of storage. The increase in TMA observed in these experiments is rather slow compared with the findings of other workers on fish stored at low temperatures (Shewan, 1949). The production of TMA is distinctly influenced by the nature of the prevailing bacterial flora.

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