THE BACTERIAL FLORA, TRIMETHYLAMINE AND TOTAL VOLATILE NITROGEN OF FISH MUSCLE AT 0° C. (IN ICE)

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Observations on the changes occurring in the bacterial flora and the trimethylamine and total volatile nitrogen content of fish muscle at 3° C. were reported previously (Velankar, 1956). The results of similar studies on fish kept in ice storage are presented in this paper and the bacterial flora, isolated during these investigations, is described.

Material and Methods

The fishes were obtained from shore-seine catches made at Dhanushkodi and Rameswaram Road on the Rameswaram Island and at places near Mandapam on the mainland. Some fish were also taken from perch-traps operated at Vedalai (near Mandapam). The fishes were transported in ice to the laboratory and kept in the round condition in ice storage in batches of ten, and one fish was removed at a time for sampling at appropriate intervals. The sampling procedure for the bacterial counts and the methods for the estimation of the trimethylamine and total volatile nitrogen were the same as described previously. When the bacterial colonies, appearing on the minimum dilution plates (1 : 100), were less than 10 the count was not recorded. Bacterial isolations were made from the colonies appearing on the counting plates. Each isolate was examined for its morphology, Gram stain and for certain biochemical reactions, particularly the reduction of nitrate, gelatin liquefaction, fermentation of dextrose, and the B.C.P. milk reaction. Representative strains, as differentiated on the basis of the preliminary examination, were studied in detail.

Results

The experimental results are shown in a tabular form (Table I). The series are divided into four groups on the basis of the place and mode of catching the fish.

Group 1

The fishes were obtained from shore-seine landings at Dhanushkodi and Rameswaram Road. The duration of storage in ice, till the TMA values* exceeded 4–6, usually regarded as the threshold values for fish in fresh condition, was 15 days for the mackerel, 7 days for the pomfret and 8 days for the

* TMA Value—Mg. trimethylamine nitrogen per 100 g. of fish muscle.

TABLE I

1 Series	2 Days in storage	3 Trimethylamine (mg. N per 100 g. of fish muscle)	4 T.V.N. (mg. N per 100 g. of fish muscle)	5 Bact. count (per g. of muscle)	6 No. of isolates	7 Bacterial types
			GROUP 1			
I Mackerel (*Rastrelliger* sp.)	3	4·77	21·95	7,000	8	*Bacillus* (2 types)
	6	1·96	18·27	..	..	
	10	5·8	18·58	19,000 (Sea-water agar) 17,000 (Fresh-water agar)	8	*Bacillus* (1 type)
	15	4·67	20·4	Plates crowded	6	*Bacillus* (2 types)
	20	11·09	27·73	do	5	*Bacillus* 4; Gram-negative asporogenous rod 1
II Pomfret (*Stromateous* sp.)	4	1·71	28·52	30,170 (F. w. agar) 17,040 (S. w. agar)	7	*Bacillus* 5; *Micrococcus* 1 Gram-negative asporogenous rod 1
	7	3·98	34·81	41,018 (F. w. agar) 9,000 (S. w. agar)	3	*Bacillus* 2; Gram-negative asporogenous rod 1
	10	22·26	38·95	244,000 (F. w. agar) 61,000 (S. w. agar)	2	*Bacillus* 1; Gram-negative asporogenous rod 1
	15	..	..	Plates crowded	5	Gram-negative asporogenous rods 4; *Micrococcus* 1

III Horse-mackerel (*Caran. armatus*)	4	1·89	25·0		5	*Bacillus* (3 types)
	8	4·67	21·95	Plates crowded	2	*Bacillus* (2 types)
	13	17·05	28·05	465,000 (F. w. agar) 94,770 (S. w. agar)	5	*Bacillus* 3; Gram-negative asporogenous rod 1; *Sarcineæ* 1
	17	20·0	38·38	Plates crowded	2	*Bacillus* 1; Gram-negative asporogenous rod 1
IV Horse-mackerel (*C. leptolepis*)	4	1·15	18·4		6	*Bacillus* 5; Gram-negative asporogenous rod 1
	8	3·53	36·16	Plates crowded (spreading colonies)	5	*Bacillus* 5
	13	8·37	29·0	3,061,000 (F. w. agar) 1,377,000 (S. w. agar)	2	*Bacillus* 2
	17	50·03	63·54	Crowded plates	1	Gram-negative asporogenous rod sp.
			GROUP 2			
V Perch (*Lethrinus* sp.)	0	3·9	21·8	..	..	..
	7	1·14	19·95	..	..	..
	10	4·87	20·38	..	..	..
	15	2·17	15·8	..	..	..
	20	12·73	32·15	Plates crowded	8	Gram-negative asporogenous rods 6 belonging to 2 types *Bacillus* 2 (pink pigment producers)
VI Horse-mackerel (*Caranx* sp.)	14	3·33	16·63	8,500	7	Flavobacteria 5; *Bacillus* 1 Gram-negative achromic, asporogenous rod 1
	30	4·2	8·96	Less than 100 per g.	2	Gram-negative asporogenous rod
	45	14·57	21·13	Plates crowded	1	do
VII Perch (*Lutjanus* sp.)	14	2·04	8·24	..	..	..
	30	4·41	7·72	Less than 100 per g.	1	Gram-negative asporogenous rod
	45	4·05	26·81	Plates crowded	4	Gram-negative asporogenous rod 1; Coliforms 3

TABLE I (*Contd.*)

1	2	3	4	5	6	7
			GROUP 3			
VIII Perch (*Lutjanus* sp.)	12	10·74	32·7	4,800,000	3	Gram-negative asporogenous rod 1 " Coliforms 2
	18			Plates crowded	5	*Bacillus* 2; Coliforms 3
IX Perch (*Lethrinus* sp.)	12	18·65	30·3	2,700,000	2	Gram-negative asporogenous rod 1 Coliforms 1
	18	45·57	53·28	Very high	2	Gram-negative asporogenous rod 1 *Bacillus* 1
X Perch (*Lutjanus* sp.)	12	15·0	41·96	3,400,000	3	*Bacillus* 3
	18	26·64	..	Very high	4	*Bacillus* 2; Coliforms 2
* XI Perch (*Lethrinus* sp.)	2	2·41	38·62	3,000	7	Gram-negative asporogenous rods 7
	5	2·02	15·81	..	..	
	10	9·93	40·7	800,000	10	*Bacillus* 9 (2 types) Gram-negative asporogenous rod 1
			GROUP 4			
XII Seer (*Cybium* sp.)	2	..	10·37	..	..	
	4	0·48	10·17	..	..	Bacterial cultures were not isolated in this series
	6	4·16	31·42	110,000	..	
	9	4·81	37·41	115,000	..	

* Fish were purchased from the local market (previous history unknown).

two horse-mackerel series. In the mackerel the T.V.N.† showed no significant increase in 20 days, while in the pomfret the T.V.N. was significant on the 4th day. The bacterial count was significant on the 10th day in the mackerel, on the 4th day in the pomfret and in 8 days in the horse-mackerel. The bacterial flora consisted of *Bacillus* and Gram-negative achromic rods, the latter becoming more numerous in the later days of storage.

Group 2

These fishes were taken from shore-seine catches made near Mandapam. A comparatively longer duration in ice, till the TMA and T.V.N. values and the bacterial count became significant, is characteristic of these series. *Bacillus* was much less in evidence, compared with the last group. In series II (*Caranx* sp.) pigmented strains, *i.e.*, Flavobacteria, were present intially and were succeeded by achromic rods.

Group 3

These fishes were obtained from perch-traps operated at Vedalai. In view of the long keeping period for the perches in the last group, sampling in these series was commenced on the 12th day. In all the three series the TMA, T.V.N. and the bacterial count indicated considerable deterioration on the 12th day. The bacterial flora consisted of *Bacillus* and Gram-negative achromic rods; coliforms were present in large numbers in these fishes.

Group 4

The seer-fish were obtained from shore-seine landings at Uchipalli. The results are similar to those of Group 1. The TMA, T.V.N. and bacterial count became significant on the 6th day.

In all the experimental series no marked fishy or spoilage odours developed even when the TMA and T.V.N. concentrations had reached significant levels. The flesh became softer progressively, the softness becoming apparent in about three days. The eyes showed shrinking in two days and the gills turned brownish in the same period. In the seer-fish there was reddening along the backbone after one week's storage.

The numerical distribution of the different bacterial types isolated at 0° C. and at 3° C. (in a previous investigation) respectively, is shown in Table II. Among the *Bacillus*, *B. subtlis*, *B. megatherium*, *B. pumilus* and *B. lentus* were identified and these species accounted for over 50% of the *Bacillus* strains. In addition, a number of strains, which show some variation from the related described types, were found and these are described in Table III. The asporogenous rods are shown in Table V and the micrococci and sarcinæ in

† T.V.N.—Total volatile nitrogen (mg. N per 100 g. of muscle).

TABLE II

Bacterial flora at 0° C. (in ice) and at 3° C.

Temperature	*Bacillus*	Gram-negative achromic rods	*Micrococcus*	*Sarcinæ*	*Flavobacterium*	Coliforms	Miscellaneous
0° C.	77 (4 produce pink pigment)	35 (5 fluorescent *Psuedomonas*; 4 produce gas from dextrose and no other sugar)	1	2	5 (Gram-negative)	11	Pink yeast 2
3° C.	72	57 (33 motile rods) (24 non-motile)	35	1	7 (Gram-positive)	3	..

TABLE III

Spore-forming rods

	Type 1	Type 2	Type 3	Type 4
Morphology ..	Gram-positive, motile stout rods, central, cylindrical spores; sporangia not bulged. Ends rounded. Size 1·1 $\mu \times 2{\cdot}8\ \mu$. Cells from glucose agar do not stain evenly	Gram-positive rods; short chains; waddling motility. Central cylindrical spores. Size 0·5 $\mu \times 1{\cdot}6\ \mu$. Sporangia not bulged. Cells from glucose agar stain evenly	Gram-positive, short, stout rods; motile; central spores; sporangia moderately bulged. Cells from glucose agar stain evenly	Gram-positive, actively motile rods of medium thickness. Central, oval spores; sporangia not bulged. Cells from glucose agar stain uniformly. Ends rounded
Growth on nutrient agar ..	Abundant white, smooth	Moist, moderate, dull white	Moist, dull, grey-white growth	Smooth, moderate, dull
Growth at pH 6·0 ..	+++	−	−	+
Peptone water ..	Pellicle	No pellicle	No pellicle	Pellicle
Nitrate reduction ..	+	+	+	−
Gelatin liquefaction ..	+ rapid (24 hours)	+ Slow (1 week)	+	+
Acetylmethyl carbinol production	−	−	−	−
Litmus milk ..	Peptonised	Peptonised	No change	Peptonised
Carbohydrate fermentation	Acid from glucose. Lactose, mannitol not fermented	Acid from glucose; slight acid from mannitol. Lactose not fermented	Slight acid from glucose, mannitol; trace of acid from lactose	Slight acid from glucose and mannitol; lactose not fermented
Starch hydrolysis ..	++	−	+ slight	−
Casein digestion ..	+	Slight	+	+
H_2S production ..	+++	−	−	−
Indol production ..	−	−	−	−
Growth on potato ..	Creamy, white, moist luxurious	Dark-brown, moist, abundant	No growth	Moist, brown, moderate

TABLE III (*Contd.*)

	Type 5	Type 6	Type 7	Type 8
Morphology ..	Gram-positive rods in long chains. Motility not seen. Central oval spores. Cells from glucose agar stain uniformly. Ends rounded	Gram-positive, motile rods of medium thickness. Central spores. Sporangia not bulged. Cells from glucose agar stain uniformly	Gram-positive rods of medium thickness; Central cylindrical spores; sporangia not bulged. Sluggish motility. Cells from glucose agar stain uniformly	Gram-positive motile rods; chains not formed. Central spores; sporangia slightly bulged. Size 0·8 $\mu \times 2{\cdot}4$ μ. Cells from glucose agar stain uniformly
Growth on nutrient agar ..	Moist, moderate, white	Membranous, dry, dull growth	Moist, colourless and gummy	Moist, pink and butyrous
Growth at pH 6·0 ..	+	−	+	+
Peptone water ..	No pellicle	Granular pellicle	No pellicle	No pellicle
Nitrate reduction ..	−	−	+	−
Gelatin liquefaction ..	−	+	−	+ (1 week)
Acetylmethyl carbinol production	−	−	−	
Litmus milk ..	Alkaline	Peptonised	No change	Peptonised; alkaline
Carbohydrate fermentation ..	No acid from glucose, mannitol and lactose	Slight acid from glucose and mannitol; lactose not fermented	Slight acid from glucose. Mannitol, lactose not fermented	Slight acid from glucose; mannitol, lactose not fermented
Starch hydrolysis ..	+		−	+
Casein digestion ..	Slight	−	−	+
H_2S production ..	−	−	−	+
Indol production ..	−	−	−	−
Growth on potato ..	White, dry and abundant	White, dry and abundant	No growth	No growth

TABLE IV

Micrococci

GROUP I (Pigment: orange)

	Type 1	Type 2	Type 3	Type 4
Morphology ..	Gram-positive cocci in clusters. Size 1·0 μ diam. Orange, shining growth on agar	Gram-positive cocci in pairs and clusters. Size 0·8 μ diam. Orange shining growth on agar	Gram-positive cocci in pairs and clusters. Size 0·8 μ diam. Orange shining growth on agar	Gram-positive cocci in clusters. size 0·8 μ diam. Orange shining growth on agar
Nitrate reduction	+	+	−	+
Gelatin liquefaction ..	−	−	−	+
Litmus milk ..	Acid ; coagulated	No change	No change	Peptonised
Dextrose ..	Acid ; no gas	Acid ; no gas	Acid ; no gas	Acid ; no gas
Mannitol ..	Not fermented	Not fermented	Not fermented	Acid ; no gas
Urease ..	+++	−	−	+++
H_2S production ..	−	−	+++	−
Indol production ..	−	−	+	−
Utilisation of ammonium phosphate as sole source of N_2	++	Poor	Poor	Not utilised
Growth on potato ..	Slight colourless	Moist, dull-white, abundant	No growth	Moist, dull-white, abundant

TABLE IV (*Contd.*)

GROUP II (Pigment : Orange-yellow)

	Type 1	Type 2	Type 3
Morphology ..	Gram-positive cocci single and in pairs. Size 0·6 μ diam. Orange yellow, creamy growth on agar	Gram-positive cocci in pairs and clusters. Size 0·8 μ diam. Orange yellow, butyrous growth on agar	Gram-positive cocci in clusters. Size 0·7 μ diam. Orange-yellow growth on agar
Nitrate reduction ..	+	+	–
Gelatin liquefaction ..	–	–	–
Litmus milk ..	Acid, coagulated	No change	Slightly alkaline
Dextrose ..	Acid, no gas	Slight acidity	Not fermented
Mannitol ..	Slight acidity	Not fermented	,,
Urease ..	+	–	–
H_2S production ..	–	–	–
Indol production ..	–	–	–
Utilisation of ammonium phosphate as sole source of N_2	Not utilised	Poor	Not utilised
Growth on potato ..	Moist, abundant, creamy	No growth	Bright-yellow, abundant

TABLE IV (*Contd.*)

	GROUP 3 (Pigment: apricot)		GROUP 4 (Pigment: lemon yellow)	GROUP 5 (Pigment: rose-pink)
	Type 1	Type 2	Type 1	Type 1
Morphology ..	Gram-positive cocci in singles and clusters. Size 0·6 μ diam. Apricot-coloured growth on agar	Gram-positive cocci in pairs and clusters. Size 0·6 μ diam. Apricot-coloured growth on agar	Gram-positive cocci in clusters. Size 0·9 μ diam. Lemon-yellow, abundant growth on agar	Gram-positive cocci in clusters. Size 1·1 μ diam. Rose-coloured, moist, butyrous growth on agar
Nitrate reduction	+	−	−	+
Gelatin liquefaction ..	−	−	−	−
Litmus milk ..	Alkaline	No change	No change	Decolourised
Dextrose ..	Slight acidity	Not fermented	Not fermented	Slight acidity
Mannitol ..	Not fermented	"	"	Not fermented
Urease activity ..	−	−	−	−
H_2S production ..	−	+ (slight)	++	−
Indol production ..	−	−	−	−
Utilisation of ammonium phosphate as sole source of N_2	Not utilised	Not utilised	Utilised	Poor utilisation
Growth on potato ..	No growth	Slight, colourless	Moist, yellow, abundant	No growth

TABLE IV (*Contd.*)

GROUP 6. (Pigment not produced)

	Type 1	Type 2	Type 3	Type 4
Morphology ..	Gram-positive cocci in clusters. Size 0·8 μ diam. Translucent colourless growth on agar	Gram-positive cocci in clusters. Size 0·8 μ diam. Translucent colourless growth on agar	Gram-positive cocci in pairs and clusters. Size 0·7 μ diam. Dull white, butyrous growth on agar	Gram-positive cocci in pairs and clusters. Size 0·8 μ diam. Dull-white, with a faint yellow tinge, butyrous growth on agar
Nitrate reduction	+	+	−	+
Gelatin liquefaction ..	−	+	−	+
Litmus milk ..	No change	Peptonised ; acid	No change	Acid coagulation
Dextrose ..	Acid, no gas	Acid; no gas	Acid; no gas	Acid; no gas
Mannitol ..	Not fermented	Not fermented	Not fermented	Not fermented
Urease activity ..	−	+ (slight)	−	+ + (24 hrs.)
H_2S production ..	−	−	+ + + (24 hrs.)	−
Indol production ..	−	−	−	−
Utilisation of ammonium phosphate as sole source of N_2	Poor	Poor	−	−
Growth on potato ..	Brown, moist, abundant	No growth	Moist, abundant, creamy	No growth

TABLE IV (*Contd.*)

GROUP 7 (*Sarcina*)

	Type 1	Type 2	Type 3
Morphology ..	Spherical cells in cubical packets. Strongly Gram-positive. Sulphur-yellow, raised, abundant moist growth on agar	Same as Type 1. Bright yellow, moist, raised growth on agar	Same as Type 1. Bright yellow, moist, raised, abundant growth on agar
Nitrate reduction ..	−	−	+
Gelatin liquefaction ..	+	+	−
Litmus milk ..	No change	Alkaline	No change
Carbohydrate fermentation ..	Dextrose, mannitol, lactose, sucrose, arabinose not fermented	Same as Type 1	Same as Type 1
Urease activity ..	−	+++	−
H_2S production ..	−	−	−
Indol production ..	−	−	−
Utilisation of ammonium phosphate as sole source of nitrogen	+	Poor	Poor
Growth on potato ..	Slight yellow growth	Yellow, dry, moderate	Slight, colourless

TABLE V

Asporogenous Rods

	GROUP I. Motile, Gram-negative (Polar flagellation; nitrate reduced to nitrite; gelatin liquefied. No acid from lactose)				GROUP 2. Motile, Gram-negative (Polar flagellation; nitrate may or may not be reduced; gelatin may or may not be liquefied; no acid from lactose)				
	Type 1	Type 2	Type 3	Type 4	Type 1	Type 2	Type 3	Type 4	Type 5
Nitrate reduction	+	+	+	+	+	+	+	−	−
Gelatin liquefaction	+	+	+	+	−	−	−	−	+
B.C.P. milk ..	No change	No change	Peptonised	Peptonised	No change	No change	Peptonised	No change	No change
Dextrose ..	Acid; no gas	Acid; no gas	Acid; no gas	Acid ; no gas	Acid; no gas	Acid; no gas	Acid; no gas	Acid; no gas	No acid or gas
Lactose ..	No acid or gas	No acid or gas	No acid or gas	No acid or gas	No acid or gas	No acid or gas	No acid or gas	No acid or gas	No acid or gas
Mannitol ..	Acid only	Acid only	Acid only	Acid only	Acid only	No acid or gas	Acid only	No acid or gas	No acid or gas
Starch ..	+	−	−	+	−	+	+	−	+
H_2S production ..	slight	++	++	−	−	−	−	+++	slight
Indol production ..	−	+	+	−	−	−	slight	−	−

TABLE V (*Contd.*)

	GROUP 3. Motile, Gram-negative (Polar flagellation; produce acid but no gas from lactose)			GROUP 4. Motile, Gram-negative. (Produce greenish fluoresence; Polar flagellation)		
	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
Nitrate reduction	+	+	−	−	−	+
Gelatin liquefaction ..	+	+	+ (slow)	+	−	+
B.C.P. milk ..	Slight acid	Acid; peptonised	Acid; curdled	Peptonised	Peptonised	Peptonised
Dextrose ..	Acid; no gas	Acid; no gas	Acid; no gas	Slight acid	No acid or gas	Acid and gas
Lactose ..	,,	Acid (slight) no gas	,,	No acid or gas	,,	No acid or gas
Mannitol ..	,,	,,	,,	,,	,,	,,
Starch hydrolysis ..	−	−	+			
H_2S production ..	−	+++	Slight			
Indol production ..	+	+	+	−	−	+++
						V.P. and M.R.–Negative Citrate utilised as sole source of carbon

TABLE V (*Contd.*)

GROUP 5. (*Non-motile asporogenous rods; Gram-negative. Stain unevenly; cells are often coccoid; bipolar staining often found*)

	Type 1	Type 2	Type 3	Type 4
Morphology ..	Gram-negative, n. m. short rods and coccoid cells. Dull-white, moist growth on agar. Cloudy growth in broth and pellicle is formed.	Same as Type I	Same as Type 1. No pellicle in broth	Same as Type 1. No pellicle in broth
Nitrate reduction	–	–	–	–
Gelatin liquefaction ..	–	–	–	+
B.C.P. milk ..	No change	No change	Slight peptonisation	No change
Carbohydrate fermentation	Dextrose, mannitol, lactose, sucrose, arabinose not fermented	Acid from dextrose and mannitol; slight acid from arabinose. Lactose and sucrose not fermented	Slight acid from dextrose and arabinose. Lactose, mannitol and sucrose not fermented	Slight acid from dextrose and mannitol. Lactose, sucrose and arabinose not fermented
Starch hydrolysis ..	–	–	Slight hydrolysis	Slight hydrolysis
H_2S production ..	+++	+++	+++	+++
Indol production ..	–	–	–	–
V.P. test ..	–	–	–	–
M.R. test ..	–	–	–	–

TABLE V (*Contd.*)

GROUP 5. (*Contd.*)

	Type 5	Type 6	Type 7
Morphology ..	Same as Type 1. No pellicle in broth	Same as Type 1. No pellicle in broth	Same as Type 1. Pellicle produced in broth
Nitrate reduction ..	+	+	+
Gelatin liquefaction ..	−	+	+
B.C.P. milk ..	No change	Peptonised; slight acid	alkaline
Carbohydrate fermentation ..	Acid from dextrose and sucrose. Mannitol, arabinose and lactose not fermented	Acid from dextrose and sucrose. Slight acid from lactose and mannitol. Arabinose not fermented	Acid from dextrose. Mannitol, lactose, sucrose and arabinose not fermented
Starch hydrolysis ..	Slight hydrolysis	−	−
H_2S production ..	+ (Slight)	−	++
Indol production ..	+	−	−
V.P. test ..	+ (slight)	−	−
M.R. test ..	−	−	−

TABLE V (*Contd.*)

	GROUP 6 (Gram-negative, motile, peritrichous rods)	GROUP 7 (*Coliforms*) (Acid and gas from lactose)	GROUP 8 (Flavobacteria)	GROUP 9 (Flavobacteria)
Morphology ..	Gram-negative, rods of medium size; stain uniformly. Motile, with peritrichous flagella	Gram-negative rods of medium size. Stain uniformly. Motile with peritrichous flagella	Gram-negative, motile rods of medium size. Coccoid cells are also seen. Bipolar staining is present. Peritrichous flagella.	Gram-positive, motile peritrichous rods. Spores not produced. Size 0·6×1·6 μ
	Dull-white, moist, fairly abundant growth on agar.	Dull, nondescript growth on agar	Shining, moist, yellow, moderate growth on agar	Shining, moist, yellow, limited growth on agar
Peptone broth ..	Uniform cloudy growth. No pellicle	Uniform cloudy growth. No pellicle	Granular pellicle	Uniform growth, moderate cloudiness
Nitrate reduction	+	+	+	–
Gelatin liquefaction ..	–	–	–	–
B.C.P. milk ..	Coagulated; acid	Coagulated; acid	alkaline	No change
Carbohydrate fermentation ..				Dextrose, mannitol, sucrose, arabinose and lactose not fermented
Dextrose ..	Acid only	Acid and gas (24 hours)	Dextrose, mannitol, lactose, arabinose and sucrose not fermented.	
Lactose ..	"	"		
Mannitol ..	"	"		
Sucrose ..	Not fermented	Not fermented		
Arabinose ..	Acid only	Acid and gas		
H_2S production ..	+ (Slight)	+ (Slight)	–	++ (3 days)
Indol production ..	+	–	–	–
V.P. test ..	–	–	+	–
M.R. test ..	+	+	–	–
Citrate utilisation ..	–	–	+	

Table IV. The division into groups and types shown here is based on arbitrary considerations and no systematic classification is attempted (see following).

Discussion

The duration of storage in ice till the TMA, T.V.N. levels and the bacterial count become significant, varied considerably in the different series; however, the variation among the series in any one group was comparatively slight. It would appear, therefore, that the bacterial count and the TMA and T.V.N. values reflect the age of the fish in storage where the fish have the same previous history. Shewan (1949), describing the course of spoilage (of gadoids) in ice storage, defined 0–6 days as the period of absolute freshness. Judging by the TMA level as well as by bacteriological standards, the period of freshness is of a similar duration in Group 1 and Group 4 series in our experiments, the mackerel series alone showing a longer duration. In the case of the mackerel the flora consisted of *Bacillus* alone over a fairly long period.

A very long period elapses before deterioration sets in in Group 2 series. The reason for this is not clear. The bacterial flora found in the three groups (1–3) showed qualitative differences, and these differences probably reflect the environmental differences in the fish in the living condition. This might account, to some extent, for the different rates of deterioration of the fish in storage. The presence of coliforms in considerable numbers in fish taken from near the shore at Vedalai and their absence in the fish obtained from Dhanushkodi and Rameswaram Road are noteworthy; the latter two fishing areas are located near uninhabited, exposed shores, free from sewage pollution.

Deterioration of fish in storage at 0° C. and also at 3° C. (Velankar, 1956), by bacteriological standards and also by the TMA and T.V.N. levels, appears to be often slower and less pronounced, compared with the findings of Shewan (1949). It is possible that the bacterial flora, autochthonous to the temperate regions, might adapt itself more easily to growth at refrigeration temperatures than the flora autochthonous to tropical regions. The fact that only spore-formers were often found initially in the fish may then be explained, since these are more resistant to adverse conditions, and the more sensitive types, mainly Gram-negative asporogenous rods, may be expected to dominate after a lag period during which these adjust to growing at or near 0° C.

Qualitatively the flora isolated during our studies differs from the fish spoilage flora described by other workers (Shewan, 1949; Wood, 1940). The ubiquitous presence of *Bacillus* and a paucity of flavobacteria and micrococci are characteristic of the flora isolated by us at 0° C. It is interesting

that at 3° C. micrococci were fairly abundant (Table II). In common with the findings of previous workers our observations indicate the presence of Gram-negative, achromic non-sporing rods to be particularly deleterious in causing spoilage. *Bacillus*, though encountered frequently, does not appear to be particularly significant in spoilage, at least from its association with low TMA values, at refrigeration temperatures.

Generic succession, more or less of a common pattern, in the prevailing bacteria during the course of spoilage has been noted by several workers. But since the bacterial flora of the fresh fish is known to vary considerably, depending upon environmental factors, generic succession of a particular pattern may not always occur (Tarr, 1953). Our observations lend support to this view. In one series alone (Series II, Group 2) in our experiments, a generic succession, analogous to that described by workers elsewhere, was encountered, *i.e.*, flavobacteria were present initially and were succeeded by Gram-negative achromic rods, *Bacillus* being insignificant.

Table II indicates that spore-formers abound at both 0° C. and 3° C., and micrococci are present in considerable numbers at 3° C., but not at 0° C. Green fluorescence producing *Pseudomonas* were present only in the iced fish and these are probably derived from the ice used in the experiments. Pink *Bacillus* spp. were also found only at 0° C. It may be recorded here that a few pink yeasts and *Nocardia* spp. were isolated during these investigations, mainly at 0° C.

Some of the *Bacillus* strains in Table III could no doubt be regarded as strains of well-known species varying slightly. A large number among the Gram-negative achromic rods showed polar flagellation and these belong therefore to the Pseudomonadaceæ. The coliforms appear to be atypical *E. coli* since indol is not produced by these strains. An interesting group is the Gram-negative, non-motile, asporogenous rods which often show coccoid forms and stain unevenly; many of these were inert in their biochemical reactions. These were usually associated with fairly high TMA values and are probably important in causing spoilage. These organisms and also some of the polar-flagellated rods resemble, to some extent, *Mycoplana* defined by Wood (1950). The classification of bacteria from marine sources, particularly the asporogenous Gram-negative rods, is uncertain and a considerably difficult procedure (Wood, 1950; Shewan *et al.*, 1954).

A majority of the micrococci showed orange or orange-yellow pigmentation; non-pigment producing cocci were relatively few. Unlike the lemon-yellow, yellow, or rose-pigmented types the orange strains often lost the capacity to produce pigment after a few subcultures. This peculiarity of the orange

micrococci isolated from marine sources has also been mentioned by Wood (1952).

Some of the micrococci are obviously related to, and are probably identical with, well-known described species; the same is also true of some of the asporogenous rods. However, since, in the opinion of experienced marine bacteriologists such as Wood (1950), the value of some of the biochemical reactions as the sole basis for differentiating into species is doubtful, it is felt that a description of the main characters of the isolated bacteria would be appropriate and probably more useful at present than an attempt at classification.

Summary

Studies were carried out on the spoilage, during storage in ice, of pomfrets, mackerel, horse-mackerel, seer-fish and perches. The duration of storage till the TMA, T.V.N. levels and/or the bacterial count became significant, varied considerably even in fish belonging to the same group; the variation was relatively less where fish with the same previous history were used. The bacterial flora varied considerably, depending, to some extent, on the environment from which the fish were caught. The flora of the muscle at 0° C. consisted mainly of Gram-negative asporogenous rods and Gram-positive spore-formers; the significance of the latter in causing spoilage at this temperature is doubtful. The paucity of flavobacteria and micrococci at 0° C. in this study is noteworthy. The different bacterial types isolated from fish muscle at 0° C. (in ice) and also at 3° C. are described.

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