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Selection of Bacterial Flora in the Chlortetracycline Treated Oil Sardine *(Sardinella longiceps)*, Indian Mackerel *(Rastrelliger kanagurta)* and Prawn *(Metapenaeus dobsoni)* During Ice Storage*

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The native flora of oil sardine and mackerel consisting of *Pseudomonas* spp; *Moraxella* spp; *Acinetobacter* spp. and *Vibrio* spp. underwent significant changes during ice storage. At the time of spoilage, *Pseudomonas* spp. were predominant. CTC treatment significantly reduced the *Pseudomonas* spp. in the initial stages of storage; but later *Pseudomonas* spp. reasserted and constituted the bulk of the spoilage flora. In prawn, the native flora was comprised of *Pseudomonas* spp; *Acinetobacter* spp; *Moraxella* spp. and *Vibrio* spp. At the time of spoilage a heterogeneous flora, consisting of *Pseudomonas* spp; *Moraxella* spp. and *Acinetobacter* spp. predominated. CTC treatment significantly changed the flora of prawns. During spoilage, *Pseudomonas* predominated in CTC treated prawns.

The chlortetracycline (CTC) treatment of fish and prawn enhances their shelf life under refrigerated storage. The CTC affects the growth of sensitive strains of the native flora of fish and prawn, thereby reducing bacterial proliferation and consequent spoilage. Although considerable information is available on the storage characteristics and spoilage of tetracycline treated fish, little effort has been made to examine the resultant microbial population. A knowledge of the effect of antibiotic treatment on the succession of bacterial flora is vital to the preservation of fish by antibiotics. Lee *et al.* (1967) observed no appreciable change in microbial population of CTC treated ocean perch from temperate waters excepting in Gram positive bacteria and yeasts. However, such information from tropical fish and prawn is lacking. The effect of CTC treatment on the bacterial groups at various stages of spoilage of fish and prawn under ice storage are reported in this communication.

Materials and Methods

Fresh oil sardine (*Sardinella longiceps*), mackerel (*Rastrelliger kanagurta*) and prawn (*Metapenaeus dobsoni*) were procured from fishing crafts operating off Cochin, brought to the laboratory within 2–4 h after they are caught. The fish/prawn were divided into three lots, the first dipped in 5 p.p.m. chlortetracycline (CTC) solution for 10 min, drained well and stored in ordinary crushed ice; the second stored in crushed 5 p.p.m. CTC ice and the third (control) was stored under ordinary crushed ice in thermocole insulated boxes in 1:1 fish to ice ratio. The storage continued for 20–30 days and the meltage of ice was compensated by the addition of respective ice every alternate days. The CTC solution and CTC ice were prepared as described by Surendran (1980). Fish/prawn samples were analysed at the beginning of the study and afterwards at 3–5 days interverval.

The total plate count (TPC) was determined using sea water agar (SWA) media. The plates were incubated at 28 ± 2°C for 3 days. Bacterial cultures were isolated from TPC plates of CTC treated and control samples during each sampling. The isolated cultures after purification by repeated streaking on SWA plates, were maintained on SWA slants for morphological and biochemical characterisation.

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Morphology and Gram staining (Anon, 1957) were observed in 16–24 h old cultures grown on SWA slants. Presence of spores was detected usually by staining 48–72 h cultures and examining microscopically or by the method of Lee & Pfeifer (1975). Motility was observed by the hanging drop method (Anon, 1957).

The biochemical reactions ot the cultures were determined by standard methods (Salle, 1954; Anon, 1957; FDA, 1973), mode of attack of glucose by cultures determined by using the 'ox-ferm' media of Hugh & Leitson (1953), presence of cytochrome oxidase in the cultures by the modified Kovacs' test (1956), catalase by observing under low power of the microscope the evolution of gas when a drop of 3% H_2O_2 (v/v) was mixed with a speck of young culture on a clean slide, pigmentation by observation on sea water–skimmed milk–peptone agar (Surendran, 1980) after a week's incubation at 28±2° C, luminescence by examining the cultures in a dark room daily for 4 days after incubation, sensitivity to penicillin (2.5 I.U.) by the pad-plate method and sensitivity of the cultures to CTC determined on peptone beaf extract glucose agar (PBGA) as described by Surendran & Iyer (1971).

The differentiation of the bacterial cultures upto the generic level was done by the scheme (Surendran, 1980) outlined in Fig. 1.

Results and Discussion

Flora of oil sardine

Results of a typical study on the pattern of change in the bacterial flora of oil sardine during ice storage of control and CTC treated samples are given in Table 1.

The native flora of oil sardine underwent significant changes during ice storage. The initial flora consisted of *Pseudomonas* spp. (16%). *Moraxella* spp. (8%), *Acinetobacter* spp. (24%), *Vibrio* spp. (26%), *Flavobacter/Cytophaga* spp. (4%), *Micrococcus* spp. (8%) and others (14%). As the number of days of ice-storage increased, the percentage of *Pseudomonas* spp. increased progressively and after 21 days of ice-storage, 74% of the flora was constituted by *Pseudomonas* spp. alone. During the first few

Table 1. *Pattern of change in the bacterial flora of oil sardine (control and CTC treated) during ice storage*

	Percentage of microorganisms at different intervals in ice									
	Ordinary ice stored				Dipped in 5 p.p.m. CTC solution and stored in ordinary ice			5 p.p.m. CTC-ice stored		
Days	0	7	14	21	7	14	21	7	14	21
Pseudomonas	16	24	39	74	11	26	80	9	30	87
Moraxella	8	15	11	4	5	7	2	11	6	2
Acinetobacter	24	34	22	8	16	21	7	19	18	5
Vibrio	26	8	5	2	36	20	2	42	24	3
Flavobacter/Cytophaga	4	5	5	2	3	3	1	4	2	0
Micrococcus	8	6	7	4	11	6	3	6	8	1
Others including yeasts	14	8	11	6	18	17	5	9	12	2
Gelatin liquefiers	76	14	26	90	33	30	78	31	20	84
Putrefiers (fish media)	21	24	22	56	11	16	42	7	12	38
Capable of growth at 0°C	18	72	84	95	64	75	97	60	81	98
Sensitivity to 5 p.p.m. CTC	84	76	76	72	51	48	37	47	36	21
No. of cultures identified	82	71	66	72	65	52	57	70	62	67

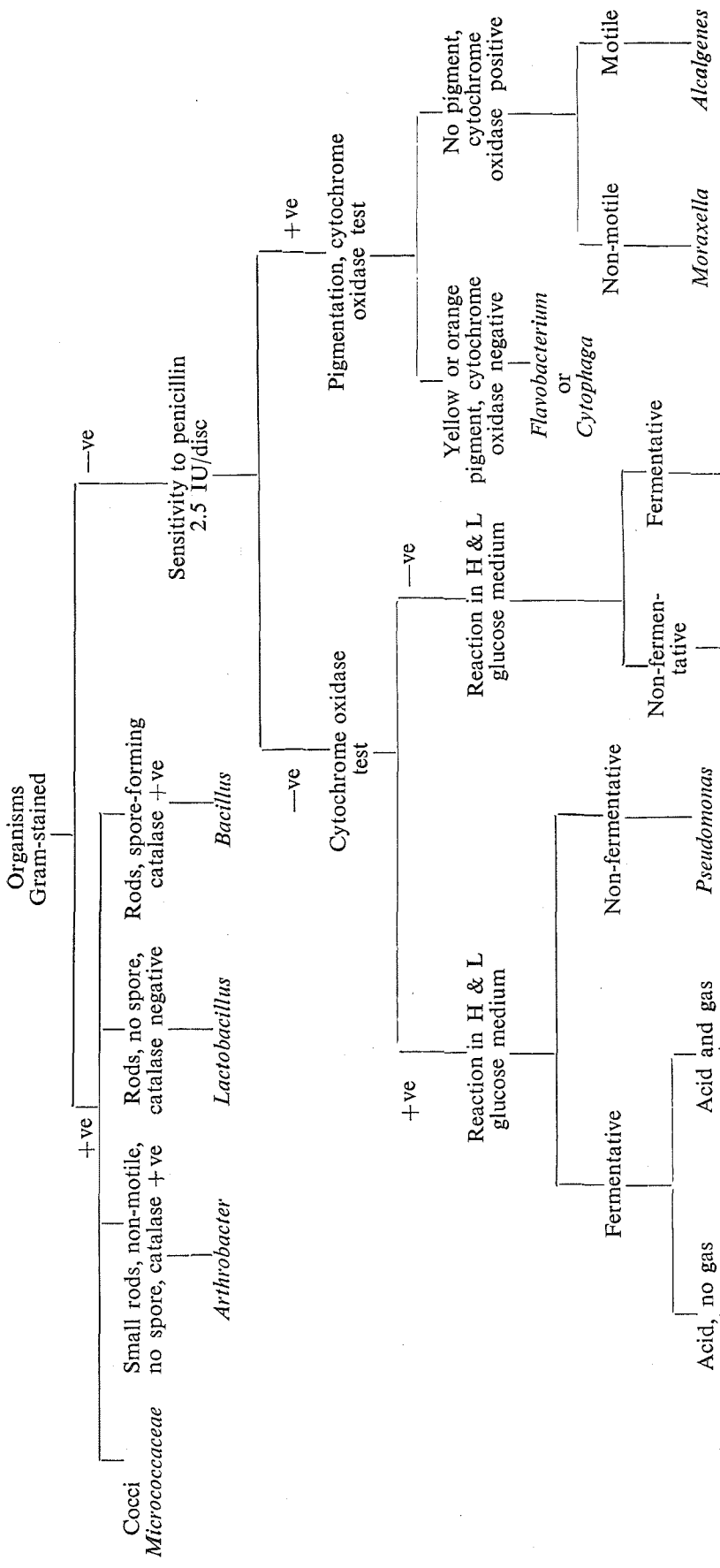

Fig. 1. Scheme used for classifying the cultures

Note:- (1) Gram negative, sensitive to 2.5 I. U. penicillin, no pigment, but cytochrome oxidase negative are *Acinetobacter*-like.
(2) Penicillin sensitive, Gram negative cytochrome oxidase positive, pigmented yellow or orange, are also grouped as *Cytophaga*/*Flavobacterium*.

days of ice-storage, there was some increase in the proportion of *Moraxella* and *Acinetobacter* spp. But later, their relative proportion decreased and at the 21st day of ice storage when the fish was spoilt, their share was only 4% and 8% respectively. *Vibrio* spp. which formed 26% of the initial flora, decreased rapidly during ice storage and by the 21st day, their share was only 2% of the total population. Changes in the proportions of *Flavobacter/ Cytophaga* and *Micrococcus* were not of much significance.

In fish treated with CTC, either in the form of dips or incorporated in ice, the pattern of change in the flora is quite different. Upto the 7th day of storage, the population is quite heterogeneous in character, the predominant groups being *Vibrios,* yeasts and *Micrococcus.* The *Pseudomonas,* and *Moraxella-Acinetobacter* group were found to decrease. During the next few days, however, the *Pseudomonas* group gradually predominated and by the end of 21 days in ice, more than 80% of the flora of the CTC treated samples were constituted by *Pseudomonas* group. All other groups particularly yeasts and *Vibrios* decreased drastically.

The changes in the flora of control and CTC treated oil sardine, so far as the biochemical groups are considered, were not much different. In the initial flora of fresh fish, 76% were gelatin liquefiers, which in the first few days of ice storage decreased. But later on, the proportion of the flora capable of gelatin hydrolysis increased and by 21st day, 90% of the flora were gelatin liquefiers. The trends in the succession of gelatin liquefiers in the CTC treated samples were more or less similar to that in the untreated control fish.

The percentage of flora capable of putrefaction of fish media, was 21% of the initial flora of fresh oil sardines, which then gradually increased during ice storage to 56% within 21 days in ice. In the case of antibiotic treated samples, there was rapid initial decrease in the proportion of putrefiers. By the 7th day of ice-storage, putrefiers constituted only about 10% in the treated samples, compared to 24% of the control. However, the percentage of putrefiers gradually increased and by 21 days, 42% of the total flora of the sample dipped in 5 p.p.m. CTC and stored in ordinary ice, and 38% of the flora of the sample kept in 5 p.p.m. CTC, were putrefiers.

The bacteria capable of growth at 0°C, were only 18% of the initial flora of fresh oil sardine, but this fraction increased during storage in ice and by 21 days in ice, 95–98% of the flora of both control and treated fish were capable of growth at 0°C.

The proportion of cultures sensitive to 5 p.p.m. CTC remained more or less in the range of 84–72%, in the case of untreated oil sardine stored in ordinary ice. Whereas, in the case of treated samples, the CTC sensitive flora decreased during storage. This decrease was more pronounced in the case of the fish stored in 5 p.p.m. CTC ice. The flora sensitive of 5 p.p.m. CTC in the case of sardine dipped in 5 p.p.m. CTC solution and subsequently stored in ordinary ice was only 37% and in the case of fish stored in 5 p.p.m. CTC ice was only 21%, by 21 days of storage.

Flora of mackerel

Table 2 presents the typical pattern of change in the bacterial flora of mackerel during ice storage of control and CTC treated samples.

The pattern of the succession of the flora during storage resembled that of oil sardine. Only 16% of the initial flora of fresh mackerel was *Pseudomonas* spp. while 42% was comprised of by *Vibrio* spp. But, as storage in ice proceeded, the proportion of *Pseudomonas* group increased steadily and reached 78% of the total flora within 21 days in ice, whereas *Vibrio* spp. rapidly decreased to 4% by that period. Similarly, major portion (69–80%) of the flora of treated samples on the 21st day of ice storage was constituted by *Pseudomonas* group alone.

The changes in proportion of gelatin liquefiers, psychrophiles, putrefiers and flora sensitive to 5 p.p.m. CTC, were more or less similar to the corresponding changes in oil sardine during ice storage of control and treated samples.

Table 2. *Pattern of change in the bacterial flora of mackerel (control and CTC treated) during ice storage*

	Percentage of micro-organisms at different intervals in ice									
	Ordinary ice stored				Dipped in 5 p.p.m. CTC solution and stored in ordinary ice			5 p.p.m. CTC-ice stored		
Days	0	7	14	21	7	14	21	7	14	21
Pseudomonas	16	18	32	78	14	31	69	16	27	80
Moraxella	10	16	9	2	9	5	6	14	8	3
Acinetobacter	20	26	14	10	15	11	8	18	21	7
Vibrio	42	22	11	4	36	21	5	32	18	3
Flavobacter/Cytophaga	3	2	4	2	5	4	2	5	4	1
Micrococcus	4	5	8	2	7	11	4	7	8	2
Others including yeasts	5	11	22	2	14	17	6	8	14	4
Gelatin liquefiers	96	16	28	81	22	34	68	18	29	84
Putrefiers (fish media)	19	25	31	46	9	17	32	8	19	28
Capable of growth at 0°C	11	56	65	88	60	68	90	49	63	83
Sensitivity to 5 p.p.m. CTC	90	81	81	69	52	58	46	46	42	28
No. of cultures identified	64	50	60	48	70	40	55	65	61	52

Table 3. *Pattern of change in the bacterial flora of prawn (control and CTC treated) during ice storage*

	Percentage of micro-organisms at different intervals in ice									
	Ordinary ice stored				Dipped in 5 p.p.m. CTC solution and stored in ordinary ice			5 p.p.m. CTC-ice stored		
Days	0	5	15	25	5	15	25	5	15	25
Pseudomonas	14	16	16	24	9	19	48	6	22	74
Moraxella	20	31	42	40	18	14	12	12	12	6
Acinetobacter	14	22	26	20	11	10	11	14	8	7
Vibrio	18	10	5	2	32	22	9	46	25	6
Flavobacter/Cytophaga	12	6	2	2	6	7	3	5	4	2
Micrococcus	6	4	2	2	8	6	7	5	2	2
Others including yeasts	16	11	7	10	16	22	10	12	25	3
Gelatin liquefiers	80	28	39	85	18	26	69	14	30	91
Putrefiers (fish media)	14	17	37	52	6	15	36	6	22	39
Capable of growth at 0°C	8	46	72	81	24	65	90	30	71	92
Sensitivity to 5 p.p.m. CTC	75	72	81	76	42	47	41	33	19	14
No. of cultures identified	70	65	61	50	56	49	64	48	52	60

Flora of prawn (M. dobsoni)

Pattern of change in the bacterial flora of prawn (*M. dobsoni*) during ice storage of control and CTC-treated samples, is presented in Table 3.

The initial flora of fresh prawn consisted in *Pseudomonas* spp. (14%). *Moraxella* spp. (20%), *Acinetobactor* spp. (14%), *Vibrio* spp. (18%), *Flavobacter/Cytophaga* spp. (12%), *Micrococcus* spp. (6%) and others including yeasts (16%). The changes in the flora during ice storage was quite different from those of oil sardine or mackerel. While the *Vibrios* decreased rapidly during ice storage to 2% by the 25th day of ice storage, *Pseudomonas, Moraxella* and *Acinetobacter* spp. steadily increased and by 25 days in ice, together they constituted 84%

of the flora, the *Pseudomonas* sharing 24%, *Moraxella* 40% and *Acinetobacter* 20%. The results indicated that the spoilage flora of prawn *(M. dobsoni)* stored in ordinary ice, was a mixed group of bacteria, comprising of *Moraxella, Pseudomonas* and *Acinetobacter* spp.

But, the patterns of change in the flora of the CTC treated samples were quite different. In the case of prawn dipped in 5 p.p.m. CTC solution, followed by storage in ordinary ice, there was the initial preponderance of *Vibrio* spp., while *Pseudomonas, Moraxella* and *Acinetobacter* spp. decreased. Later, *Pseudomonas* spp. began to reassert and by the 25th day of storage they constituted 48% of the flora. Also, *Moraxella* and *Acinetobacter* spp. respectively comprised of 12% and 11% of the flora, the share of *Vibrio* being only 9%.

In the case of prawn stored in 5 p.p.m. CTC ice, the pattern of change of flora was different. In the initial stage of storage in CTC ice, there was a decline in the percentage of *Pseudomonas* spp; accompanied by an increase in the proportion of *Vibrio spp.* Within the first 5 days in CTC ice, *Vibrio* increased to 46% of the flora, while *Pseudomonas* was only 6%; the share of *Acinetobacter* and *Moraxella* spp. being 14% and 12% respectively. By the 25th day in CTC ice, the pattern was considerably different. *Pseudomonas* group formed 74% of the flora, the rest being *Moraxella* spp. (6%), *Acinetobacter* spp. (7%) and *Vibrio* spp. (6%).

It is quite interesting to note that while the normal spoilage flora of prawn *(M. dobsoni)* stored in ordinary ice, consisted predominantly of non pseudomonads namely *Moraxella* spp. (40%) and *Acinetobacter* spp. (20%), *Pseudomonas* spp. constituting only 24%, the spoilage flora of CTC treated prawn were predominantly *Pseudomonas* spp. namely 48% in the CTC dipped and then stored in ordinary ice sample and 74% in CTC iced sample. This meant that CTC treatment brought about a qualitative change in the spoilage flora of prawn, whereas such qualitative changes in the spoilage flora of oil sardine and mackerel were not at all resulted from CTC treatment.

The pattern of change in the proportions of gelatin liquefiers during different intervals of ice storage was similar in the control and treated samples of prawn. However, the incidence of putrefiers in the control and treated samples was different. In the former sample, the putrefiers formed 52% of the flora on the 25th day of ice storage, while the corresponding percentages of putrefiers in the treated samples were 36 to 39%. The incidence of psychrophiles steadily increased during ice storage of control and treated samples. While more than 70% of the flora was sensitive to 5 p.p.m. CTC throughout the entire storage period of the control sample, the proportion of the flora sensitive to 5 p.p.m. CTC steadily decreased in the CTC treated samples. Only 14% of the entire flora of the 5 p.p.m. CTC iced prawn by 25th day, was sensitive to 5 p.p.m. CTC.

Shewan *et al.* (1960) found that the initial flora of North Sea and Faroes cod, mainly consisting of *Pseudomonas, Archromobacter, Flavobacterium, Coryneforms* and *Micrococcus* spp. underwent significant change during storage in ice. *Pseudomonas-Achromobacter* group steadily increased during the initial stages, at the expense of the others and later, by about 15 days in ice, when the fish was in the early stages of spoilage, *Pseudomonas* group emerged as the predominant group, constituting 80–90% of the flora. Similar observations on the succession of genera during ice storage of codling and haddock had been made by Shewan & Stewart (1958), who reported that by 14 days in ice, the *Pseudomonas* spp. constituted 90% of the flora and that most of the active spoilers of fish muscle were members of the *Pseudomonas* group. Shewan (1971) reported that qualitatively, there was little change over the first few days in the bacterial flora of marine fishes such as cod during ice storage, but later, the *Pseudomonas* group particularly III/IV groups gradually took over and by the 12th day constituted 90% of the total flora.

The pattern of changes in the flora of oil sardine and mackerel during ice storage appears to be similar to those of the fishes of temperate waters. In the initial flora, the *Moraxella-Acinetobacter-Vibrio* groups accounted for 50–70% in these fishes, the share of *Pseudomonas* spp. being less than

20%. As the days of ice storage progressed, *Pseudomonas* spp. emerged as the dominant group, accounting for 80–90% of the total flora, at the time of spoilage in ice. This finding is in full agreement with the observation made by Shewan (1977) that irrespective of the initial flora of fish, *Pseudomonas* and *Alteromonas* groups emerged as the predominant genera during spoilage of fish in ice-storage. This has been attributed to the shorter generation times of these *Pseudomonas* and *Alteromonas* spp. at chill temperatures (Harrison-Church, quoted by Shewan, 1977) compared to other groups of bacteria.

In the case of codling from North Sea, stored in 5 p.p.m. CTC ice, the bacterial population upto the 8th day was quite heterogeneous in character, the predomiant groups being *Vibrios* and yeasts, *Pseudomonas* and *Achromobacter* groups constituting less than 25% of the total flora. However, the *Pseudomonas* group gradually predominated in the next few days and by the 16th day, the normal type of spoilage flora comprising of 79% of *Pseudomonas* spp. was established (Shewan & Stewart, 1958; Shewan, 1962 a). The findings reported in this paper regarding the changes in the flora of oil sardine and mackerel during storage in 5 p.p.m. CTC ice, are in agreement with the above findings. While 24% of the flora of the untreated sardine on the 7th day of ice storage was *Pseudomonas* spp. only 90% of the flora of the sample kept in 5 p.p.m. CTC ice was *Pseudomonas* spp. (Table 1). CTC treatment had brought about a significant reduction in the number of *Pseudomonas* spp. which comprised most of the active spoilers of fish. But, of course, the *Pseudomonas* spp. reasserted later and after a lapse of 7–10 days emerged as the dominant group constituting about 80–90% of the total flora. Hence, as observed by Shewan (1961, 1962 a) and Shewan & Stewart (1958), the extension of shelf life of 7–10 days obtained by CTC treatment, appeared to be the time required for the normal flora to recover from the effects of the antibiotic. But this advantage of the effect of CTC on bacterial flora, was of no avail for the preservation of oil sardine and mackerel, since their high fat content contributed to chemical spoilage of fat leading to rancidity of the muscle.

The changes in the bacterial flora of Gulf of Mexico shrimp during storage in crushed ice had been reported by Campbell & Williams (1952), who showed that irrespective of the composition of the initial flora, being heterogeneous in character, the flora of the shrimp after 16 days in ice, was composed of 82% of *Achrombacter* spp. and 16.5% of *Pseudomonas* spp. Walker *et al.* (1970) have found that eventhough 80% of the initial flora of the Dublin Bay prawn–Scampi (*Nephrops norvegicus*), was constituted by 'coryneforms' during ice storage the flora changed and finally consisted of 70% *Achrombacter* spp. and 8% *Pseudomonas* spp. In the case of tropical prawns from Mosambique, the spoilage flora after 12 days in ice, consisted of 67% *Achromobacter* spp., 19% *Coryneforms* and 8% *Flavobacterium/Cytophaga,* while the flora of prawns from Malayasia after 12 days in ice, comprised of 48% *Achromobacter* spp. and 41% *Coryneforms,* the *Pseudomonas* forming only 8% (Cann, 1974).

The authors' finding that 60% of the flora of Indian prawn (*M. dobsoni*) after 25 days in ordinary ice, was constituted by *Moraxella-Acinetobacter* group (the erstwhile *Achromobacter* group), very well agrees with the findings of Campbell & Williams (1952), Walker *et al.* (1970) and Cann (1974). But, it differs from the spoilage flora of Gulf of Thailand prawn (*Peneaus* spp.) where 90% of the spoilage flora was composed of *Pseudomonas* spp. and only 4% by *Achromobacter* spp. However, 24% of the flora of Indian prawns (*M. dobsoni*) (Table 3) at the time of spoilage in ice was comprised of *Pseudomonas* spp. also.

The observation that the spoilage flora of prawn in ice storage, comprised of 60% *Moraxella-Acinetobacter* group and only 24% *Pseudomonas* spp. clearly shows the heterogeneous nature of the spoilage of flora of prawn. In this respect, it is significantly different from the spoilage flora of fishes, both tropical (Tables 1 and 2) and temperate water fishes, in which cases, the spoilage of ice stored fishes, is brought about by predominantly *Pseudomonas* spp., the contribution by other groups, if at all, being insignificant.

The finding that the spoilage flora in the CTC iced prawn was, mainly comprised of *Pseudomonas* spp. is very significant. It evidently shows that there is a very important qualitative difference in the spoilage flora between the prawn stored in ordinary ice and antibiotic ice. As evident from earlier discussions, *Pseudomonas* spp. constituted the major spoilage flora of fishes. Hence, the emergence of *Pseudomonas* as the major spoilage flora of CTC stored prawn indicated that, there might be some difference in the spoilage of antibiotic treated and untreated prawn. The end products of spoilage in both cases may be different, both qualitatively and quantitatively.

CTC sensitivity of cultures from CTC treated fish and prawn

The CTC sensitivities of the bacterial types present on fish and prawn underwent appreciable changes during the storage of the fish and prawn in the antibiotic ice. Typical results on the CTC sensitivities of bacterial cultures isolated from CTC ice stored oil sardine are presented in Table 4.

In the case of the cultures isolated from oil sardine just before CTC storage, majority of the cultures belonging to *Pseudomonas, Moraxella, Acinetobacter, Micrococcus, Flavobacter/Cytophaga* and *Alcaligenes* spp. were sensitive to 5 p.p.m. CTC and 100% of these cultures were sensitive to 25 p.p.m. CTC. But, only 32% each of *Vibrio* and *Archrobacter* strains were sensitive to 5 p.p.m. CTC.

When the number of days ot storage in CTC-ice increased, there was evidently an increase in the percentage of insensitive flora. After 7 days in CTC-ice only 47% of *Pseudomonas* spp., 65% of *Moraxella* spp., 50% of *Acinetobacter* spp. and 49% of *Micrococcus* spp. were sensitive to 5 p.p.m. CTC.

Sensitivity to CTC further decreased in the case of cultures isolated from fish stored in CTC ice for 21 days. Only 21% of *Pseudomonas* spp., 32% of *Moraxella* spp., 26% of *Acinetobacter* spp. and 39% of *Micrococcus* spp. were then sensitive to 5 p.p.m. CTC.

Development of insensitive flora during the course of CTC storage of fish is very significant so far as the prospect of CTC being used as a preservative is concerned. Shewan & Stewart (1958) and Shewan (1962 b) also observed that, while only 1% of the original flora of codling and haddock, was insensitive to 5 p.p.m. CTC, by the 16th day of storage in CTC ice, about 90% of the total

Table 4. *CTC sensitivity of bacterial cultures isolated from CTC ice stored oil sardine*

Bacterial genus	Cultures from sardine before treatment				Cultures from sardine 7 days in 5 p.p.m. CTC ice				Cultures from sardine 21 days in 5 p.p.m. CTC ice			
		Percentage of cultures sensitive to the given CTC levels										
	No. of cultures	CTC p.p.m. 1	5	25	No. of cultures	CTC p.p.m. 1	5	25	No. of cultures	CTC p.p.m. 1	5	25
Pseudomonas	60	61	76	100	64	24	47	100	52	4	21	68
Moraxella	71	54	82	100	65	51	65	100	62	16	32	74
Acinetobacter	48	55	78	100	50	20	50	100	54	10	26	70
Vibrio	80	16	32	80	66	12	25	60	50	4	16	44
Micrococcus	24	50	96	100	30	33	49	100	32	18	39	82
Flavobacter/ Cytophaga	16	42	72	100	20	25	75	100	20	10	60	100
Alcaligenes	10	40	100	100	16	24	72	100	12	16	48	80
Arthrobacter	12	8	32	64	16	6	24	66	10	0	20	50

flora and 100% o *Pseudomonas* spp. were insensitive. Lee & Sinnhuber (1967) have found that the proportion of CTC resistant species increased with higher CTC concentration and the length of storage at 7°C. They found that among individual generic groups isolated from CTC treated ocean perch, 'coryneforms' and yeasts were more resistant to CTC and that *Pseudomonas, Achromobacter, Flavobacterium, Bacillus* and *Lactobacillus* all contained species either resistant or sensitive to CTC. More CTC resistant species in these genera accumulated with the increased CTC concentrations and with the length of storage in presence of CTC. The authors' findings that *Arthrobacter* spp. (formerly grouped as 'coryneforms') constituted the most insensitive group and that, the CTC resistance of other groups of bacteria increased with the length of storage in presence of CTC, are in full agreement with the observations of Lee & Sinnhuber (1967).

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References

Anon (1957) *Manual of Microbiological Methods. Society of American Bacteriologists.* McGraw Hill Book Co. Inc. New York

Campbell, L. L. (Jr) & Williams, O. B. (1952) *Fd Technol.* **6**, 125

Cann, D. C. (1974) *Bacteriological Aspects of Tropical Shrimp. in Fishery Products* (Kreuzer, R., Ed,), p. 338, Fishing News Books, Surrey.

F. D. A. (1973) *Bacteriological Analytical Manual for Foods* - Chapter XIX. *Examinations of Shell Fish and Meats. Division of Microbiology, Bureau of Foods; Food and Drug Administration, U. S. A.*

Hugh, R. & Leifson, E. (1953). *J. Bact.* **66**, 24

Kovacs, N. (1956) *Nature,* **178**, 703

Lee, J. S. & Pfeifer, D. K. (1975) *Appl. Microbiol.* **30**, 72

Lee, J. S. & Sinnhuber, R. O. (1967). *Appl. Microbiol.* **15**, 543

Lee, J. S., Willet, C. L., Robinson, S. M. & Sinnhuber, R. O. (1967) *Appl. Microbiol.* **15**, 368

Salle, A. J. (1954) *Laboratory Manual on Fundamental Principles of Bacteriology.* 4th edn. McGraw Hill Book Co. Inc. New York

Shewan, J. M. (1961). *The Microbiology of Seawater Fish.* in *Fish as Food.* (Borgstrom, G., Ed.) Vol. 1, P 487, Academic Press, New York

Shewan, J. M. (1962 a) *The Bacteriology of Fresh and Spoiling Fish and Some Related Chemical Changes.* in *Recent Advances in Food Science* (Hawthron & Muil Leitch eds.) p. 167, Butterworth, London

Shewan, J. M. (1962 b) *The Use of Antibiotics in the Preservation of Fish.* in *Antibiotics in Agriculture.* (Woodbine M., Ed) p. 289, Butterworths, London.

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

Shewan, J. M. (1977) *The Bacteriology of Fresh and Spoiling Fish and the Biochemical Changes Induced by Bacterial Action in "Proc. Trop. Prod, Inst. Conf. Handl. Process. Mark. Trop. Fish."* 1977, 511, London, July 1976.

Shewan, J. M. & Stewart, J. (1958). *Experiments in the Use of Antibiotics in fish Preservation. Proc. Intern. Inst. Refrig.* (Moscow). *DSIR Food Investigation Memoir no.* 1203. 15 *pp.*

Shewan, J. M., Hobbs, G. & Hodgkiss, W. (1960) *J. Appl. Bact.* **23**, 463

Surendran, P. K. (1980) *Chemical Preservatives in Relation to Control of Microbial Changes in Fishery Products.* Ph.D. Thesis, University of Kerala, Trivandrum

Surendran, P. K. & Iyer, K. M. (1971) *Fish. Technol.* **8**, 128

Walker, P., Cann, D. & Shewan, J. M. (1970) *J. Fd Technol.* **5**, 375