Studies on the Chemical Control of Psychrophilic Bacterial Spoilage of Fish. iii - The Effect of Chemical Preservatives on the Growth of Psychrophilic Bacteria Isolated from Marine Fish

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Among the six chemical preservatives tried, propyl and methyl paraben were found to be very effective in inhibiting the growth of all the cultures. While propyl paraben could check the growth of all the cultures at 0.1% level, methyl paraben could do the same only at 0.2% level. The other preservatives namely, orthochlorobenzoic acid (upto 0.2%), sodium hypochlorite (upto 25 p.p.m.) ethoidin (upto 0.025%) and polyethylene glycol (upto 4 p.p.m.) had no inhibitory effect on any of the cultures tried.

Earlier attempts to use chemical preservatives to extend the storage life of ice-stored fish have not yielded any useful results. (Tarr & Deas, 1948; Tetsumoto & Yamada, 1948; Shewan, 1956). Recently a few attempts have been made to test the effectiveness of some of these preservatives on the spoilage organisms isolated from fish rather than testing them directly on fish (Heather & Vander Zant, 1958; Surendran & Iyer, 1971; Anand & Setty, 1977). In this paper the results of our studies with six of the various chemical preservatives tried on the representative cultures belonging to six genera are presented.

Materials and Methods

The chemical preservatives used were propyl-hydroxy-4-benzoate (E. Merck), methyl-para hydroxy benzoate (Societe. des Usiness Chemicques, France), o-chlorobenzoic acid (Veb Laborchemic Apolda, Germany), sodium hypochlorite (Chempure Ltd., India), polyethylene glycol 400 (Glaxo, India) and 6, 9-diamino-2-ethoxy acridine lactate (Sigma).

Cultures used in the study (Table 1) were selected from a large number of psychrophilic bacterial cultures isolated from marine fish belonging to six genera (Anand & Setty, 1977)

Table 1. *Cultures selected for the study*

Culture number	Identity
1	*Achromobacter aquamarinus*
2	*Achromobacter delicatulus*
3	*Achromobacter liquefaciens*
4	*Achromobacter superficialis*
5	*Alcaligenes bucheri*
6	*Alcaligenes faecalis*
7	*Flavobacterium diffusum*
8	*Flavovacterium halmephilum*
9	*Pseudomonas fragi*
10	*Pseudomonas* sp.
11	*Micrococcus conglomeratus*
12	*Micrococcus varians*
13	*Vibrio costicolus*

The medium used for testing the sensitivity of cultures consisted of glucose 0.1%, bactopeptone 0.5%, beef extract 0.3%, sodium chloride 3.0%, agar 1.5% prepared in distilled water. The pH of the medium was adjusted to 7.2 and sterilized for 20 min at 1.05 kg/cm² pressure. The chemicals used for the medium were either Difco or BDH make.

Plate culture technique is the same as described by Anand & Setty (1981).

Nutrient broth medium was distributed into 30 ml test tubes in 15 ml quantities and

sterilized at 1.05 kg/cm² pressure for 20 min. These tubes were inoculated with different test organisms and after growing them for 24 h the various preservatives at different concentrations were introduced. The optical density of the inoculum was always adjusted to 0.2. The tubes were incubated at 25–28°C for 72 h and the growth was recorded turbidimetrically every 12 h. Suitable controls were always included in all cases. Wherever the preservatives were not easily soluble in water, small amount of alkali was used to dissolve them and the pH of the medium was suitably adjusted after the addition of the preservatives.

Growth measurements were done turbidimetrically in a Klett-Summerson colorimeter using the green filter (no. 54). pH recorded with BDH pH indicator papers. As there was no difference in the effectiveness of preservatives on these cultures at 0–5°C and at 25–28°C, the plate and broth culture experiments were carried out at 25–28°C to obtain quick results.

Results and Discussion

Four concentrations of each chemical preservative were selected (Tables 2–5) for plate culture studies depending on the nature of the chemical and the concentrations used by earlier workers.

Among the two parabens tried (Table 2), propyl paraben was found to be very effective for all the cultures at 0.1% and above.. Methyl paraben was however not effective to many of these cultures upto 0.15% level and only at 0.2% was effective against all the cultures. The fact that all the four species of *Achromobacter,* particularly culture no. 4 *(Achromobacter superficialis),* which was not inhibited by most of the antibiotics tried (Anand & Setty, 1981) are inhibited by propyl paraben, even at 0.1% level is noteworthy for future considerations. Though methyl paraben is effective at higher concentration, its use as a commercial preservative for fish at such high concentrations is rather doubtful.

The two chloro compounds namely, o-chlorobenzoic acid and sodium hypochlorite were found to be not effective to all the cultures at the concentrations used in the study (Table 2). It may be seen from the tables that sodium hypochlorite had some amount of inhibitory effect on majority of the cultures even at 15 and 20 p.p.m. levels (as available chlorine), whereas o-chlorobenzoic acid had no effect at all on any of the culture. Sodium hypochlorite, however, could not inhibit the growth of cultures 6, 8, 9, 12 and 13 even at the highest concentrations.

The preservatives polyethylene glycol and ethoidin were found to be totally ineffective, since they could not inhibit any of the cultures at any of the concentrations tried and the results are not shown in the table.

Only three preservatives namely, propyl paraben, methyl-paraben and o-chlorobenzoic acid were tried using broth culture technique. While only four concentrations of preservatives were tried in the plate culture technique, one more higher concentration was included under broth culture technique.

As evident from the results shown in Table 3, propyl-paraben was effective on all the cultures at all levels of concentrations except the first concentration (0.05%). The cultures that were inhibited at 0.05% were *Achromobacter delicatulus* (culture no.2) and *Alcaligenes faecalis* (culture no. 6), which were also inhibited in plate culture studies at the above concentration (Table 2). In effect the results of plate cultures and broth culture techniques were extactly similar.

The results with methyl paraben (Table 4) were almost similar to that obtained with the plate culture technique. Most of the cultures were inhibited at concentrations of 0.2% and above, while at lower concentrations higher percentage of cultures survived.

The effect of o-chlorobenzoic acid (Table 5) on various cultures was as good as that observed with plate culture studies (Table 2). While this preservative was not effective on any of the cultures upto 0.2%, it could prevent the growth of only two cultures (4 and 12) at 0.25% concentration. For testing the effectiveness of preservatives, the agar plate technique was followed, as it was handy, less time consuming and

Table 2. *Effect of chemical preservatives on selected cultures*

Preservative	Propyl paraben %				Methyl paraben %				Ortho-chlorobenzoic acid %				Sodium hypochlorite p.p.m.			
Concn. of preservative	0.5	0.10	0.15	0.20	0.05	0.10	0.15	0.20	0.05	0.10	0.15	0.20	10	15	20	25
Time of incubation h	48 96	48 96	48 96	48 96	48 96	48 96	48 96	48 96	48 96	48 96	48 96	48 96	48 96	48 96	48 96	48 96
Culture number																
1	+ +	— —	— —	— —	+ +	+ +	± ±	— —	+ +	+ +	+ +	+ +	+ +	+ +	± ±	— ±
2	— —	— —	— —	— —	— —	— —	— —	— —	+ +	+ +	— +	+ +	— +	± +	± +	— —
3	+ +	— —	— —	— —	+ +	— —	— —	— —	+ +	+ +	+ +	+ +	± ±	± ±	± ±	± ±
4	+ +	— —	— —	— —	+ +	— +	— —	— —	+ +	+ +	+ +	+ +	± ±	± ±	± ±	± ±
5	+ +	— —	— —	— —	+ +	+ +	+ ±	— —	+ +	+ +	+ +	+ +	+ +	+ ±	± ±	± ±
6	— —	— —	— —	— —	+ +	— —	— —	— —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
7	+ +	— —	— —	— —	+ +	— —	— —	— —	+ +	+ +	+ +	+ +	± ±	± ±	± ±	± ±
8	+ +	— —	— —	— —	+ +	± +	± +	— —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
9	+ +	— —	— —	— —	+ +	— ∓	— ∓	— —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
10	— ∓	— —	— —	— —	+ +	— —	— —	— —	+ +	+ +	— +	+ +	+ +	± +	± +	— —
11	+ +	— —	— —	— —	+ +	— —	— —	— —	+ +	+ +	+ +	+ +	± ±	± ±	± ±	± ±
12	+ +	— —	— —	— —	+ +	± +	— ∓	— —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
13	+ +	— —	— —	— —	+ +	— +	— —	— —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +

Key: + good growth; — no growth; ± slight growth; ∓ very slight growth. Initial pH 7, all cultures showed good growth in control.

Concentration of sodium hypochlorite is as available chlorine

Table 3. *Effect of propyl paraben on selected cultures**

Concn. of preservative %	0.00			0.05			0.10			0.15			0.20			0.25			pH		
Time of incubation h	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72
Culture number																					
1	20	45	53	20	30	46	20	15	10	20	13	10	20	10	10	23	10	5	7.0	6.5	6.5
2	15	38	43	28	28	15	25	20	15	18	10	15	15	15	15	15	15	10	7.0	6.5	6.5
3	63	83	95	63	40	30	50	40	35	68	60	50	63	40	40	63	45	40	7.0	6.5	6.5
4	30	40	45	35	45	45	35	28	28	35	25	20	35	25	20	35	25	20	7.0	7.0	6.5
5	20	43	55	30	35	40	20	15	13	20	10	15	20	15	15	28	13	0	7.0	7.0	6.5
6	10	28	43	20	20	18	28	23	25	10	10	10	15	15	15	18	8	0	7.0	7.0	7.0
7	45	70	73	60	60	70	53	43	40	65	30	40	40	25	40	43	28	40	7.0	7.0	6.5
8	35	60	65	45	40	43	30	23	20	33	23	25	35	33	28	35	30	28	7.0	7.0	6.5
9	45	88	98	53	63	95	55	48	40	45	40	40	40	28	28	40	35	30	7.0	7.0	6.5
10	43	78	95	43	40	40	40	33	33	43	33	30	43	30	30	43	40	30	6.5	7.0	6.5
11	13	28	40	13	13	15	13	13	10	13	10	10	13	10	10	15	10	8	7.0	7.0	6.5
12	20	43	63	15	18	20	20	13	10	25	15	13	15	13	10	20	13	10	7.0	6.5	6.5
13	14	68	75	35	35	40	40	38	35	40	35	30	30	25	20	50	43	35	6.5	7.0	7.0

*Growth in Klett units

Table 4. *Effect of methyl paraben on selected cultures**

Conc. of preservative %	0.00			0.05			0.10			0.15			0.20			0.25			pH		
Time of incubation h	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72
Culture number																					
1	13	30	55	10	28	50	13	28	35	11	23	25	15	15	15	15	15	12	7.0	7.0	7.0
2	23	45	60	45	40	33	33	30	25	20	20	20	23	20	20	28	20	20	7.0	6.5	6.0
3	53	75	103	50	28	25	50	28	20	53	18	10	53	28	10	50	28	8	7.0	7.0	6.5
4	15	43	60	15	18	19	15	18	20	20	10	8	20	10	10	20	8	8	7.0	7.0	6.0
5	10	38	55	5	58	65	10	25	45	10	25	45	13	5	8	13	5	5	7.0	7.0	7.0
6	15	38	63	25	25	25	18	12	10	15	15	15	15	15	13	15	15	13	7.0	7.0	5.5
7	33	68	78	65	78	65	50	50	42	38	32	20	30	22	25	25	25	20	7.0	7.0	6.0
8	10	38	65	35	43	60	18	40	45	10	15	30	17	10	10	35	25	23	6.5	7.0	7.0
9	25	68	85	30	27	55	25	30	37	20	20	29	35	20	33	30	18	17	7.0	7.0	7.0
10	45	78	73	45	55	50	38	41	38	38	38	33	45	45	33	43	30	28	7.0	6.5	5.5
11	10	25	48	18	25	55	20	15	20	20	15	15	17	12	14	15	12	12	7.0	6.5	5.5
12	13	43	68	13	33	53	11	13	45	11	13	18	11	10	8	11	10	5	7.0	7.0	6.5
13	18	38	53	18	30	50	18	35	35	20	20	20	20	15	15	28	23	23	6.5	7.0	7.0

*Growth in Klett units

Table 5. *Effect of Ortho-chlorobenzoic acid on selected cultures**

Concn. of preservative %	0.00			0.05			0.10			0.15			0.20			0.25			pH		
Time of incubation h	24	48	60	24	48	60	24	48	60	24	48	60	24	48	60	24	48	60	24	48	60
Culture number																					
1	18	43	63	20	38	65	18	45	45	18	50	48	30	38	35	20	20	23	7.0	7.0	7.0
2	40	60	60	40	58	60	40	55	58	30	58	63	30	48	58	25	38	35	7.0	7.0	7.0
3	55	83	110	50	75	80	40	65	75	40	70	83	50	73	75	55	75	80	7.0	6.5	6.5
4	25	43	45	25	45	45	20	35	38	25	35	35	25	30	30	25	25	25	7.0	6.5	6.5
5	20	53	60	25	53	48	10	40	38	20	40	38	20	35	28	18	18	23	7.0	6.5	6.5
6	20	45	50	15	35	50	20	38	43	20	40	43	20	35	43	20	20	23	7.0	7.0	7.0
7	40	73	78	40	70	75	45	73	75	50	73	85	45	75	78	45	50	50	7.0	6.5	6.5
8	10	53	60	15	40	55	18	40	48	15	40	45	20	30	33	20	40	33	7.0	6.5	6.5
9	30	55	78	20	58	60	25	65	70	25	60	68	30	68	75	28	45	48	7.0	7.0	7.0
10	45	55	60	40	53	58	35	48	53	40	53	50	45	55	60	45	53	53	7.0	6.5	6.5
11	10	28	35	10	23	48	10	30	35	15	25	28	10	10	18	10	15	20	7.0	7.0	7.0
12	15	40	55	10	40	50	10	48	50	10	45	55	15	45	50	15	15	13	7.0	7.0	7.0
13	25	68	85	30	63	65	30	45	45	35	63	63	38	55	60	38	63	65	7.0	6.5	6.5

*Growth in Klett units

equally efficient as that of other techniques. Also, this method facilitates screening large number of preservatives to various cultures in a short period. However, for comparison, broth culture technique was also done for three of the preservatives which in fact gave similar results as obtained under plate culture.

While the literature on the use of propyl and methyl parabens as preservatives for fish is very scanty, recently Shiralkar (1971) has tried the above preservatives on the cultures isolated from poultry meat for their effectiveness. The results of his study showed that propyl paraben was more effective than methyl paraben to all the cultures tried and there was no difference in the susceptibility of Gram positive and Gram negative bacteria to parabens. In the present study also the results of both plate and broth culture studies are in agreement with the findings of Shiralkar (1971).

The chloro compounds as a class of preservatives have been, generally, found to be either slightly effective or not, in the preservation of fish (Gibbs, 1923; Chen & Fellers 1926; Tarr 1948). These compounds apart from being unstable, generally, bring out undesirable changes in colour and flavour of the meat (Tetsumoto & Yamada, 1950). In the present investigation also, of the two chloro compounds tried, sodium hypochlorite was only marginally effective, whereas o-chlorobenzoic acid was totally ineffective as seen from plate and broth culture studies, in preventing the growth of bacteria belonging to different genera isolated from fish.

The earlier work on ethoidin by Tarr (1946), showed no significant effect in inhibiting the spoilage bacteria of fish when it was incorporated in ice at 0.0067%. Though higher concentrations (upto 0.025%) have been tried in the present study, it appears to be quite ineffective in preventing the growth of these bacteria.

It appears that polyethelene glycol has not been tried earlier as a preservative, although polypropyleneglycol has been tried at different concentrations by Wessells *et al.* (1972). Since propylene glycol could not be obtained in time, polyethylene glycol was tested on the assumption that ethylene moiety may have better effect than propylene moiety. However, it was not found to be of any use at the concentration tried.

The authors wish to thank Professor H.P.C. Shetty, Director of Instruction, College of Fisheries, Mangalore for his interest and also for providing facilities for the work.

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