

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

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Dehydroacetic acid and ammonia were found to be very effective in checking the growth of all the cultures at all concentrations tried. The two nitrofurans namely, semicarbazone and AF-2 were fairly effective, semicarbazone being more effective than AF-2. Sodium nitrite was found to be totally ineffective against all the cultures at all concentrations tried.

The objective of these studies and a review of earlier works by others have already been reported (Anand & Setty, 1977, 1981 a, b). In this paper the results of five more preservatives tried on the various groups of psychrophilic bacteria isolated from fish are reported.

Materials and Methods

The preservatives used were dehydroacetic acid (Sigma), ammonia (BDH), 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide or AF-2 (Ueno Drug Co., Japan), 5-nitro-2-furfurylidene semicarbazone (Koch-Light Laboratories) and sodium nitrite (Sarabai Chemicals, India).

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

The medium used for testing the sensitivity of cultures consisted of glucose 0.1%; bacto-peptone 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 sterilised for 20 min at 1.05 kg/cm² pressure. The chemicals used for the medium were either Difco or BDH make. The plate culture technique is the same as described by Anand & Setty (1981 b).

Table 1. Cultures selected for the study

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

Results and Discussion

The results of four chemical preservatives are shown in Table 2. Four concentrations of each were selected depending on the nature of the chemical and the concentrations used by earlier workers. The criteria for using the agar plate technique for testing the effectiveness of preservative has been given in the previous paper (Anand & Setty 1981 a).

While the dehydroacetic acid and ammonia (Table 2) were very effective in checking the growth of all the cultures at all concentrations tried, sodium nitrite was totally ineffective at all levels of concentrations. The effectiveness of dehydro-acetic acid

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

Preservative	Dehydroacetic acid				Ammonia				Semicarbazone				AF-2									
	48 h	96 h	48 h	96 h	48 h	96 h	48 h	96 h	48 h	96 h	48 h	96 h	48 h	96 h	48 h	96 h						
Time of incubation h	48	96	48	96	48	96	48	96	48	96	48	96	48	96	48	96						
Concn. of preservative	0.25%	0.5%	0.75%	1.00%	0.24N	0.58N	1.05N	1.47N	0.05%	0.10%	0.15%	0.20%	0.05%	0.10%	0.15%	0.20%						
Culture numbers																						
1	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	≠	+	—	—	—	—	
2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	
3	—	—	—	—	—	—	—	—	—	—	—	—	—	≠	+	—	≠	—	≠	—	≠	
4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	≠	+
5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	≠	+	—	—	—	—
6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	≠	—	≠	—	≠	—	—
9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	≠	+	≠	+
10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	≠	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	≠	+	≠	+
13	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	≠	—	≠	—	≠
Initial pH	6.0	5.0	4.5	4.010.5.....			7.0.....			7.0.....									

Note: All cultures showed good growth in 24 h in control plates

Key : + good growth; — no growth; ≠ slight growth; ≠ very slight growth

and ammonia observed in the present study appears to be mainly a function of their effective pH in the medium. While the dehydroacetic acid reduces the pH of the medium from 6 to 4 depending on the concentrations used, ammonia on the other hand, increases the pH beyond 10.5. Tarr *et al.* (1950) however, found that 0.1 to 0.5% of dehydroacetic acid has no significant effect in inhibiting bacterial spoilage of fish. Ammonia has been tried earlier for preservation of fish by Subramanyan *et al.* (1963) and Mandal & Mukerjee (1974). Though ammonia appears to be a good preservative for storing fish over a period of 3 to 4 months, its use as a commercial preservative is limited, since it can bring about mild changes in texture and flavour of fish meat. In addition to this, the fact that the pH of the fish meat (6.2 to 6.5) is used as a criterion to judge the freshness of fish and use of ammonia for preservation may lead to adulteration of fresh fish with spoiled ones by the traders.

Sodium nitrite has been tried by many workers as a preservative for fish at various concentrations both alone and in combination with sodium chloride with varied results. It has also been conclusively shown to be very effective chiefly at pH 6, but has no effect at pH 7 (Tarr & Sunderland 1940). The negative results observed in the present study may be due to either low concentrations (50–200 p.p.m.) employed or due to the neutral pH provided in the medium. Since sodium nitrite is not allowed for use in foods at concentrations exceeding 200 p.p.m. higher concentrations were not tried in this study and results not shown in Table 2.

The effects of the two nitrofurans namely, semicarbazone and AF-2 on the growth of various cultures are shown in Table 2. Semicarbazone could inhibit the growth of all the cultures except culture no. 4 at all levels of concentrations tried. AF-2, on the other hand, was not effective at 0.1% level as it could inhibit only 5 out of 15 cultures and even at the highest concentration (0.2%), it could only inhibit 8 cultures. Here again culture no. 4 was not inhibited at all at any concentrations.

Nitrofurans derivatives, in general, according to Japanese investigations are effective

against many of the Gram-positive and Gram-negative bacteria and in particular, they are very effective against spore forming bacilli (Obatake, 1965; Matsuda, 1966). However, Obatake & Matsuda (1965) have found that AF-2 and AF-5 had almost the same preservative effect as that of CTC in the preservation of fish. In the present study, though AF-2 was effective at higher concentrations it could not inhibit cultures of *Achromobacter*, *Pseudomonas*, *Micrococcus* and *Vibrio* at lower temperatures. However, other nitrofurans derivative namely semicarbazone appears to be a potent preservative for fish, since even the lowest concentration tried could inhibit all the cultures except no. 4 which showed only slight growth at all concentrations.

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References

- Anand, C. P. & Setty, T. M. R. (1977) *Fish. Technol.* 14, 98
- Anand, C. P. & Setty, T. M. R. (1981a) *Fish. Technol.* 18, 45
- Anand, C. P. & Setty, T. M. R. (1981b) *Fish. Technol.* 18, 47
- Mandal, S. K. & Mukerjee, S. K. (1974) *J. Agri. Fd Chem.* 22, 832
- Matsuda, T. (1966) *J. Ferment. Technol.* 43, 106
- Obatake, A. (1965) *Fd Technol. Champaign.* 14, 585
- Obatake, A. & Matsuda, T. (1965) *Bull. Jap. Soc. Sci. Fish.* 31, 138
- Subramanyan, V., Lahiry, N. L., Moorjani, M. N., Balakrishnan Nair, R. & Krishna Swamy, M. A. (1963) *Science.* 142, 233
- Tarr, H. L. A. & Sunderland, P. A. (1940) *J. Fish. Res. Bd Can.* 5, 36
- Tarr, H. L. A., Southcott, B. A. & Bissett, H. M. (1950) *Fish. Res. Bd Can. Progress Rep. Pacific Coast Stas.* 83, 35