

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

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Among the various antibiotics tried, tetracyclines particularly chlorotetracycline (CTC), chloramphenicol and chlorostrep were found to be fairly effective at 8 and 10 p.p.m. levels. The order of sensitivity to CTC among the six genera studied was found to be *Achromobacter* < *Flavobacterium* < *Pseudomonas* < *Micrococcus* < *Vibrio* < *Alcaligenes*. It was observed that species belonging to *Achromobacter*, *Flavobacterium* and *Pseudomonas* were generally more resistant to antibiotics than species belonging to other genera. Among the resistant organisms, *Achromobacter superficialis* was found to be most resistant and only CTC at 10 p.p.m. level could partially inhibit this culture. The useful combinations of antibiotics for commercial application are discussed.

A large number of antibiotics have been tried earlier as preservatives to check the growth of psychrophilic flora of fish stored in ice to extend its shelflife for a reasonably long period (Tarr *et al.*, 1952; Tomiyama *et al.*, 1955; Velankar & Sastri, 1958; Sult *et al.*, 1970; Surendran & Iyer, 1973). While most of these earlier studies have confined to direct testing of the preservatives on fish, only a few attempts have been made to understand the effect of these preservatives on psychrophilic cultures commonly encountered on marine fishes (Heather & Vanderzant, 1958; Velankar, 1958; Surendran & Iyer, 1971, 1976; Tunstall & Gowland, 1974). Even here no comprehensive studies have been done, so far. While working towards the above objective the authors have already reported on the isolation of psychrophilic cultures from marine fishes of South Kanara Coast of Karnataka (Anand & Setty, 1977). In this paper, the effect of various antibiotics on those psychrophilic cultures is presented.

Materials and Methods

The antibiotics tried were Aureomycin (Cyanamid India), Terramycin (Pfizer), Tetracycline (Indian Drugs and Pharmaceuticals), Chloramphenicol and Chlorostrep (Parke Davis), Dihydrostreptomycin (Sigma) and Bacitracin (NBC).

Six genera of psychrophilic bacterial cultures isolated from marine fish (Anand & Setty, 1977) and employed in this study are given in Table 1.

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 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.

The plate culture technique employed consisted of incorporating known concentration of preservative into the sterilized and cooled medium before pouring on to the plates. The plates were divided into four parts by marking on the plates with a glass mark and each part was inoculated with a different culture, thus covering four cultures per plate per concentration of the preservative. The inoculum consisted of freshly grown cells prepared from a 24 h old slant culture whose optical density was adjusted to 0.5 always and a loopful of these was placed at the centre of each part as a spot and slightly spread out. The plates were incubated at 25–28°C for 96 h and presence or absence of growth was recorded every 24 h. Depending on the extent of growth, grades were given as shown in the

Table 1. Cultures selected for the study

Culture 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 sp.</i>
11	<i>Micrococcus conglomeratus</i>
12	<i>Micrococcus varians</i>
13	<i>Vibrio costicolus</i>

tables. This type of grading was done as the work was qualitative rather than quantitative. Suitable control for each preservative was included.

Results and Discussion

The results of the seven antibiotics are shown in Tables 2 and 3. Four concentrations namely 4, 6, 8 and 10 p.p.m. have been tried on the 13 selected cultures drawn from different genera of psychrophiles mentioned in Table 1.

As seen from Table 2, chlorotetracycline (CTC) was found to be effective at 8 p.p.m. concentration for all the cultures except two species each of *Achromobacter* (culture nos. 3 and 4) and *Flavobacterium* (7 and 8) genera. CTC at 10 p.p.m. level could further inhibit the growth of cultures 3 and 7 and partially 4 and 8. Oxytetracycline (OTC) at 8 p.p.m. was found to be equally effective like CTC but could not inhibit two species of *Achromobacter* (3 and 4) and one species each of *Flavobacterium* (8), *Pseudomonas* (9) and *Vibrio* (13). However, OTC at 10 p.p.m. could inhibit *Vibrio* sp. Surprisingly enough, tetracycline (TC) was found to be even more effective than CTC and OTC against these cultures. However, 8 and 10 p.p.m. levels of TC were effective against most of the cultures but not against cultures 4, 8 and 10. It may be seen that one species each of *Achromobacter*, *Flavobacterium* and *Pseudomonas* are not inhibited

again by TC like the other two tetracycline derivatives.

As shown in Table 3, dihydrostreptomycin had very little effect in checking the growth of these cultures except that it could inhibit only one species each of *Achromobacter* (2), *Alcaligenes* (6) and *Pseudomonas* (10). Streptomycin in combination with chloramphenicol (Chlorostrep) could inhibit almost all cultures at 8 and 10 p. p. m. levels except culture 4. However chloramphenicol alone at 10 p.p.m. level was found to be even more effective than chlorostrep since it could inhibit all cultures, including culture 4 to some extent. Here again *Achromobacter superficialis* (4) stood out as the most resistant organism among these cultures.

Bacitracin, however was not found to be effective against these cultures at any of the concentrations tried in the study.

The effect of various antibiotics on different species belonging to six genera was, however, highly varying. It was generally seen that species belonging to *Achromobacter*, *Flavobacterium* and *Pseudomonas* were more resistant than species belonging to other groups. Among the resistant organisms, *Achromobacter superficialis* was found to be most resistant to antibiotics. Earlier studies by Kawabata *et al.* (1962) showed that CTC resistant bacteria isolated from CTC treated fish belonged to the above three genera and they were mainly responsible for putrefaction of fish stored under ice.

Among the resistant organisms, *Achromobacter superficialis* (culture no. 4) was found to be more resistant to tetracyclines than others and only CTC at 10 p.p.m. level could partially inhibit this culture. While *Achromobacter liquefaciens* (culture no. 3) was resistant to CTC even at 10 p.p.m. level, it was, however, easily susceptible to OTC and TC. The susceptibility of *Pseudomonas* sp. though varied with respect to OTC and TC, they were inhibited by CTC. *Alcaligenes*, *Micrococcus* and *Vibrio* were, however, susceptible to all the three tetracyclines.

Comparing the effect of other antibiotics, chloramphenicol was found to be more potent than others in checking the growth

Table 2. *Effect of tetracyclines on the growth of selected cultures*

Preservatives Concn. of preservative p.p.m.	Chlorotetracycline								Oxytetracycline								Tetracycline							
	4		6		8		10		4		6		8		10		4		6		8		10	
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
Culture number																								
1	—	—	—	—	—	—	—	—	±	±	—	—	—	—	—	—	±	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3	±	+	—	+	—	+	—	+	—	±	—	±	—	±	—	—	—	+	—	±	—	—	—	—
4	±	+	±	+	±	±	±	±	±	+	±	—	±	+	±	—	+	+	+	+	+	+	+	+
5	—	—	—	—	—	—	—	—	±	±	—	—	—	—	—	—	±	±	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7	±	+	—	±	—	±	—	—	—	—	—	—	—	—	—	—	—	+	—	±	—	—	—	—
8	—	+	—	±	—	±	—	±	±	+	±	±	—	±	—	±	±	±	±	±	—	±	—	±
9	—	±	—	—	—	—	—	—	—	+	—	+	—	±	—	±	—	±	—	—	—	—	—	—
10	±	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	±	—	±	—	±
11	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12	—	±	—	—	—	—	—	—	—	+	—	+	—	—	—	—	—	±	—	—	—	—	—	—
13	—	±	—	—	—	—	—	—	—	±	—	±	—	±	—	—	—	—	—	—	—	—	—	—

All cultures showed good growth in 24 h in control plates. Initial pH 7

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

Table 3. *Effect of other antibiotics on the growth of selected cultures*

Preservatives	Dihydrostreptomycin				Chlorostrep				Chloramphenicol			
Concn. of preservative p.p.m.	4	6	8	10	4	6	8	10	4	6	8	10
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
Culture number												
1	+	+	+	+	+	+	+	+	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—	—	—
3	—	+	—	—	—	—	—	—	±	+	—	—
4	±	±	±	±	±	±	±	±	+	+	+	+
5	+	+	+	+	+	+	+	+	—	—	—	—
6	—	—	—	—	—	—	—	—	—	—	—	—
7	—	±	—	—	—	—	—	—	±	—	—	—
8	+	+	+	+	+	+	+	+	—	—	—	—
9	—	+	±	+	±	+	±	±	—	—	—	—
10	—	±	—	±	—	—	—	—	+	+	—	—
11	—	+	—	±	—	±	±	±	—	—	—	—
12	+	+	±	+	±	+	±	±	—	—	—	—
13	+	+	+	+	+	+	+	+	—	—	—	—

All the cultures showed good growth in control plates. Initial pH 7

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

of all cultures except *Achromobacter superficialis* which could show slight growth even at 10p.p.m. concentration. Higher levels of these antibiotics may be effective against this culture. The use of chloramphenicol as a preservative for fish appears to be rather doubtful as it has not been allowed for use by any country so far. From this point of view, a combination of CTC and OTC or CTC and TC appears to be more practicable. With regard to the sensitivity of various groups of organisms to CTC, Surendran & Iyer (1971) found that the order of sensitivity was *Pseudomonas* < *Vibrio* < *Achromobacter* < *Flavobacter* at 5 and 20 p.p.m. levels. However the present study indicates that the order to be < *Achromobacter* < *Flavobacter* < *Pseudomonas* < *Micrococcus* < *Vibrio* < *Alcaligenes*. The *Pseudomonas* group which forms one of the major groups among psychrophiles commonly found in fish and which is responsible for rapid spoilage during cooler storage, has been found to be quite sensitive to many of the antibiotics like CTC, OTC and TC (Heather & Vanderzant, 1958; Velankar, 1958), Streptomycin, Kanamycin, colistin, neomycin, polymyxin and thiosporin (Tunstall & Gowland, 1974). However, some species of *Pseudomonas* like *P. fluorescens* have been found to be quite resistant to antibiotics (Kawabata *et al.* 1962).

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