Microflora in the Digestive Tract of Inshore Fish in Japan

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SUMMARY

The aerobic bacterial flora of the digestive tract of jackmackerel (*Trachurus japonicus*) was found to be composed almost exclusively of species of the genus *Vibrio*. Comparison of the flora of the stomachs with that of the intestines of these fish indicated that only vibrios were found in the intestines.

INTRODUCTION

The digestive tract of marine fish carries a heavy bacterial load which plays an important role in spoilage after the death of the fish. Workers have reported quite different generic compositions of the bacterial flora of the digestive tract (Shewan, 1961). The differences could be ascribed mainly to the following three factors: (1) environmental conditions under which the fish were caught; (2) changing views of microbial classification, especially of Gram-negative rods, which are the predominant organisms in the gut flora; (3) differences in methods of isolating the bacteria. Concerning the last factor, special consideration should be given to the constitution of the media used for isolating fish bacteria; this seems particularly true for the bacteria of the digestive tract. Liston (1957) suggested that some vibrios of the digestive tract have strict nutritional requirements and hence the isolation medium should contain higher amounts of organic nutrients. A distinctively halophilic nature of the flora of the digestive tract has been found in some cases (Simidu & Hasuo, 1968*a*). The present investigation was undertaken to determine the generic composition of the aerobic bacterial flora in the digestive tract of marine fish caught in Japanese waters. An improved medium which was developed to obtain the maximal viable counts with marine fish (Simidu & Hasuo, 1968*b*) was used to isolate the bacteria.

METHODS

Jackmackerel (*Trachurus japonicus*) used in this investigation were bought at Funabashi Fish Market and at Katakai Beach, Chiba Prefecture. When bought in the market the fish had been kept in ice-water for a day after being caught. At Katakai Beach, fresh fish were obtained immediately after landing. The fish were brought to the laboratory in an icebox, about 2 hr distant from the beach. Fish were immersed in 0.5% crystal violet solution for 3 min., then washed with 98% (v/v) ethanol in water. Whole digestive tracts or separated stomachs and intestines were removed aseptically and homogenized with a diluent solution of 9 times the weight of the sample. The diluent solution used consisted of 0.8% NaCl + 1.0% KCl. Plate counts

were made on two different media (see Table 1). Colonies were counted after 5 days of incubation at 24°. Before counting, about 30 colonies were transferred from an area chosen at random on each plate of medium B to a liquid medium of the same composition (B broth). The generic composition of the isolates was determined mainly according to the scheme of Shewan, Hobbs & Hodgkiss (1960).

Table 1. *Composition of the media used*

	A (g.)	B (g.)
Polypepton (Daigo Eiyo-Kagaku)	10.0	10.0
Lab-Lemco beef extract (Oxoid)	3.0	3.0
Yeast extract (Difco)	3.0	3.0
NaCl	1.6	8.0
KCl	2.0	10.0
MgSO ₄ ·7H ₂ O	0.6	3.0
CaCl ₂ ·2H ₂ O	0.3	1.5
Agar	12.0	12.0
Distilled water	1000 ml.	1000 ml.

Adjusted to pH 7.5

RESULTS AND DISCUSSION

Results with whole digestive tracts of fish from the market are shown in Table 2. The average weight of the fish used for the experiment was 59 g. The present results with jackmackerel confirm previous experimental results with plaice, namely, that halophilic vibrios were predominant in the gut flora (Simidu & Hasuo, 1968*a*). Although some isolates produced gas from glucose in Barsiekow medium containing 3 % NaCl, they were classified as members of the genus *Vibrio*, based on their sensitivity to the vibriostatic compound 2,4-diamino-6,7-di-isopropylpteridine.

Table 2. *Viable counts and generic composition of aerobic bacteria from the digestive tracts of jackmackerel (Trachurus japonicus)*

	Sample	
	1	2
	Viable counts	
Medium A	3.7×10^3	4.0×10^3
Medium B	1.2×10^6	9.7×10^4
	Number of strains	
<i>Vibrio</i> { Aerogenic	5 (5)*	4
{ Non-aerogenic	15	15 (3)*
<i>Micrococcus</i>	0	3
Total	20	22

* Number of luminescent strains in parentheses.

The composition of the bacterial flora of the digestive tracts differed clearly from that of the sea water of the coastal area (Simidu & Aiso, 1962) and that of the surface slime of the fish (T. Haraguchi, personal communication). Both in sea water and

in fish slime, large numbers of pseudomonad and achromobacters were found in addition to vibrios.

In the next experiment with fresh jackmackerel obtained at Katakai Beach, the flora of stomachs was compared with that of intestines. The average weight of the fish was 43 g. The digestive tracts of the fish were removed aseptically, and the stomachs and intestines separated. Materials from six fish whose stomachs did not contain food were mixed and homogenized with the diluent solution. The results of the plate counts given in Table 3 show that a peculiar flora which was different from that of the stomachs was found in the intestines.

Table 3. *Viable counts and generic composition of the aerobic bacteria in the stomachs and intestines of jackmackerel*

	Stomachs	Intestines	Intestines*
	Viable counts (no./g. sample)		
Medium A	2.4×10^5	2.9×10^5	1.2×10^4
Medium B	2.6×10^5	4.6×10^6	1.2×10^6
	Number of strains		
<i>Vibrio</i> { Aerogenic	2 (2)†	9 (2)†	13 (1)†
{ Non-aerogenic	6 (0)†	17 (6)†	16 (13)†
<i>Pseudomonas</i>	2	1	0
<i>Achromobacter</i>	7	0	0
<i>Flavobacterium</i>	2	0	0
<i>Sarcina</i>	7	0	0
<i>Corynebacterium</i>	3	0	0
<i>Bacillus</i>	1	0	0
Total	30	27	29

* Result with the fish stored at 2° for 21 hr.

† Number of luminescent strains in parentheses.

Other than anaerobic conditions, several factors which favour the selective growth of vibrios in the intestines might be considered. One of these factors is the resistance of vibrios to the effect of bile salts. Table 4 shows the results of an experiment on the effect of bile on the growth of isolates from stomachs and intestines. Test organisms were grown in B broth for 24 hr at 25°, and then streaked on plates of B agar to which different amounts of Oxgall (Difco) had been added. The growth of the organisms was examined after 5 days of incubation at 25°.

Table 4. *Sensitivity of isolates to bile*

Concentration of Oxgall (Difco) in the medium (%)	Isolates from	
	Stomachs	Intestines
	No. of strains grown at the given concentration of bile	
0.0	27	23
0.1	26	22
0.3	18	22
1.0	17	22
2.5	15	22
10.0	15	22

Thjøtta & Sømme (1943), in their survey of bacteria of marine and freshwater fish, observed that while vibrios predominated in the flora of both surface and gut of fish from the west coast of Norway, other genera such as *Bacterium*, *Achromobacter* and *Escherichia* were the main constituents of the gut flora of fish from Oslofjord. Most of the strains of the dominant genus, *Bacterium*, were described as having polar flagella and producing acid from glucose, which suggests that some of them may have been vibrios according to the present classification.

Liston (1957) showed that the digestive tract of lemon sole and skate from the North Sea contained large numbers of vibrios, which constituted about 35–74% of the total aerobic flora throughout his continuous 27-month period of observation. In our experiments, the gut floras of plaice and jackmackerel consisted almost exclusively of vibrios. The differences in composition of the media used for isolation as well as regional difference may have been responsible for the quantitative differences.

The present results strongly suggest that the indigenous bacterial flora of the intestine of marine fish consists only of vibrios. This fact has aetiological significance for fish diseases in fish-farming ponds, as well as in food poisoning caused by *Vibrio parahaemolyticus*, which account for about half the total number of food poisoning in Japan.

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