Short Paper

Bacterial Flora in the digestive tracts of File-Fish*

It has been reported¹⁾ that the dominant bacterial flora in the digestive tract of red sea bream, a fish with developed stomach, markedly differed from those in environmental sea water or diet in several properties, and was not influenced by the composition of diet. This fact suggested that the environmental condition in the digestive tract, especially in the stomach, limited the bacterial flora to only a few specific groups. The present investigation was undertaken to make clear whether or not such a selection of bacterial flora take place also in the digestive tract of file-fish (*Stephanolepis cirrhifer*), whose stomach is considerably undeveloped in morphology and thus in function.

The fish to be examined were caught at Maizuru Bay and reared in a glass tank at 20°C. The fish, which had been starved for 2 days prior to experiment, were killed after four hours feeding with shell-fish meat. Viable counts of bacteria in the digestive contents were made by the plate method reported previously¹⁾. Numbers of aerobic heterotrophs in contents of fore-half (from throat to the middle part of intestine) and post-half (from the middle to the anal part of intestine) of the digestive tracts (conventionally designed fore- and post-intestine, respectively) and in the diet (shell-fish meat) were 1.7×10^6 , 2.1×10^6 and 3.0×10^6 cells/g, respectively. The pH values of intestinal contents were 6.4-6.7 in the fore-half and 7.3-7.5 in the post-half, respectively. These pH values probably do not effect the bacterial

Table 1. Morphological and biochemical properties of 49 strains isolated from contents of digestive tract of file-fish and the diet (shell fish meat). (1971. Jan.)

Characteristics Examined	Number of Positive Strains		
	Source of Isolates		
	Fore-Intestine	Post-Intestine	Diet
Total Number of Bacteria	25	13	11
Gram Negative	25	13	10
Shape: Rod	25	13	10
Cocci	0	0	1
Motility	16	5	3
Single polar flagellum	13	5	3
Peritrichous flagella	3	0	0
Cytochrome Oxidase	25	12	10
NH ₃ production from peptone	25	13	11
H ₂ S production from cystine	25	13	7
NO ₃ reduction	21	13	4
Indol production	10	6	2
Hugh & Leifson's Test:			
Fermentation of Glucose	20	7	5
Oxidation of Glucose	1	0	1
Gas production	1	0	0
Fermentation of N-acetyl-glucosamine	20	7	4
Oxidation of N-acetylglucosamine	0	0	0
Gas production	1	0	0
Hydrolysis of Chitin	11	4	2
Starch	12	4	5
Casein	6	4	5

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Table 2. Effect of low pH value and bile salt on the growth of strains isolated from file-fish, and the diet (shell fish meat).

Source of Isolates	Total Strains Examined	Number of Strains			
		Bile Salt		H ⁺ Concentration	
		S.b. < 0.5	S.b. ≥ 0.5	S.h. < 0.5	S.h. ≥ 0.5
Fore-Intestine	25	21	4	11	14
Post-Intestine	13	7	6	7	6
Diet	11	10	1	6	5

S.b. = $\frac{\text{Turbidity of Basal Culture Containing Bile Salt (2\%)}}{\text{Turbidity of Bile Salt Free Basal Culture}}$

S.h. = $\frac{\text{Turbidity of Basal Culture at pH 5.5}}{\text{Turbidity of Basal Culture at pH 7.5}}$

survival, unlike the case of red sea bream.

Forty nine strains, in total, were isolated from contents of the fore- and post-half of the intestines and the diet. The morphological and biochemical properties of these strains were examined by the ordinary method²⁾ (Table 1). Most of the isolates were Gram-negative, asporogenous rods and were able to produce NH₃ from peptone, H₂S from cystine and to oxidize cytochrome C. In respects to the other properties, however, these isolates were not similar each other, in contrast to the case of red sea bream.

With respect of the resistance to bile salt and low pH value (pH 5.5), the bacteria were remarkably different from the intestinal bacteria isolated from red sea bream; in the former case (Table 2) more than 50% of the strains were inhibited by bile and low pH values (S.b. and S.h. values were below 0.5), but in the latter case most of the strains isolated were resistant to these factors¹⁾.

It has been clear that the bacterial flora in the intestine of file-fish was composed of various species, as was in the diet, in contrast to the case of a fish with developed stomach^{1,3,4)}. The difference of the flora between in file-fish and in red

sea bream may be attributed to the degree of development of stomach in function; the chemical and physical conditions in developed stomach (low pH etc.) may select the bacteria and give rise to an almost uniform bacterial flora. Thus, the bacterial flora in the fish with undeveloped stomach as file-fish may reflect almost directly the exotic flora.

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