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Microflora in the alimentary tract of gray mullet–I Isolation and identification of bacteria

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Abstract

Comparison of the bacterial flora of the alimentary tract of gray mullet (*Mugil cephalus*) from fresh water with that of the fish cultured in sea water indicated that water condition results in changing the microflora from the predominant species of *Enterobacter* and *Bacillus* to mainly *Pseudomonas, Vibrios* and *Aeromonas*. Microscopic examinations of the alimentary tract samples show that the intestine of fresh water gray mullet contains a wide variety of bacterial flora. The change of bacterial flora in gray mullet can be directly related to the water quality. Indeed two or three genera appeared to dominate the microflora of grey mullet. These are consisted of *Enterobacter, Bacillus* and *Pseudomonas*.

This is an attempt to gain more information on the normal intestinal microflora of grey mullet with special references to the fresh and marine environment. In this study fresh water grey mullet was examined. The fish were taken from small river and selected to cultivate for reasonably a wide time period replacing fresh water by sea water. This initial paper reports on the bacterial flora found in the intestine of fresh water grey mullet and suddenly changed when it was cultivated in sea water. Experiments were carried out into three different conditions of water. In first experiment fish were dissected just immediately after catching from the fresh water. In the next experiment fish were cultured in water in which ratio of fresh and marine water was 1 : 1 and the last experiment was carried out with fish cultured in sea water. The fish take up sea water under the situation from hypotonic to hypertonic (F. LAGLER, John E. BARDACH and Robert R. MILLER, 1962) specially when they are placed in sea water. Fish are probably only the transient carriers of these bacterial species (TRUST and SPARROW, 1974).

Materials and Methods

Grey mullet caught from the small river situated on 600 m south of Faculty of Fisheries, Kagoshima University. This river has several houses along its shore. River provided fish with very low depth and muddy water. Samples were obtained by gill nets, during the sampling time the water temperature varied from 20C to 25C. The samples were cultured and maintained by weekly transfer of water.

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Strongly aseptic procedures were used during the dissection period. The ventral surface of the fish was thoroughly scrubbed from vent to the mouth to obtain the alimentary tract except undesirable content samples. Whole digestive tracts were cut into small pieces and transfer into test tube and diluted with 0.9% sodium chloride solution of 9 times the weight of the samples. The solution was homogenized at high speed six times, for 30′ each time (SUTTER, et al 1972). The diluted samples were cooled in water bath during homogenization to avoid over heating.

Five different types of media which were recommended by AISO et al (1968) were compared in this experiment for the microflora isolated from fresh water fish and six media including these five and ZoBell 2216E medium for fish cultured in sea water. The colonies developed on the plates were also compared and counted after incubation for 3–5 days at 30C and 25C respectively. The composition of these five media were 1.0% polypeptone (Daigo Eiyo Co.), 0.3% beef extract (Kyokuto Seiyaku Kogyo Co.), 0.3% Yeast extract (Nakarai Chemicals Co.), and 1.5% Agar (Nakarai Chemicals Co.). Among five media A, B, and C media were dissolved in distilled water containing various amounts of mineral salts and 5% glucose was supplemented in case of medium C. On the other hand medium D was dissolved in distilled water only and medium E was dissolved in the ratio of 1/6 Artificial Sea Water. Similarly ZoBell 2216E medium was used for the isolation of microflora from the fish intestine of sea water cultivated fish. The composition used in these media is given in Table 1. The pH values of the media were adjusted to 7.5 in the experiment. Identification was fascilitated by examination of colonial morphology, pigmentation, shape, staining characteristics, motility of cells and flagella arrangement, as well as examination of the ability of the isolates to produce catalase and oxidase. Other tests were of H_2S production, of indole production, of methyl red reaction, and of arginine de-

Table 1. Composition of different media used in the experiment (SIMIDU, U. and K. HASUO, 1968a and b).

	Media (gm)					
	A	B	C	D	E	ZoBell 2216E
Polypeptone	10.0	10.0	10.0	10.0	10.0	5.0
Beef extract	3.0	3.0	3.0	3.0	3.0	
Yeast extract	3.0	3.0	3.0	3.0	3.0	1.0
NaCl	1.6	8.0	1.6	—	—	—
KCl	2.0	10.0	2.0	—	—	—
$MgSO_4 \cdot 7H_2O$	0.6	3.0	0.6	—	—	—
$CaCl_2 \cdot 2H_2O$	0.3	1.5	0.3	—	—	—
Glucose	—	—	5.0	—	—	—
Distilled water	1000	1000	1000	1000	1000†	1000+

All media are adjusted to pH 7.5

† 1/6 Artificial sea water of Herbst's type

+ 1/2 Artificial sea water of Herbst's type

Table 2. Viable counts of the intestinal bacteria of fresh water mullet.

Medium	Number $\times 10^7$ per ml
A	2.6
B	2.2
C	3.2
D	1.6
E	2.5
ZoBell 2216E	1.6

carboxylase. In addition, the ability to liquify gelatine and the hydrolysis of both casein and starch, reduction of nitrate were tested. To ascertain the existence of *Escherichia coli*, the observation of the bacterial growth on the deoxycholate agar plate was examined. Final identification of the isolates was based on the schemes of GIBBS B. M. and F. A. SKINNER (1966) and JACOBS, M. B. and M. J. GERSTEIN (1960).

Results

According to the investigation of the first experiment, the bacteria from alimentary tract of fresh water fish were classified as *Bacillus* by reason of gram positive, rods with endospore, and *Enterobacter* with gram negative rods, peritrichous flagella, and no spore. Some of *Staphylococcus* were also found. The same experiment was carried out by using the fish cultured in 1 : 1 ratio of fresh and sea water. The flora isolated were mainly gram negative rods with monotrichous flagellum. A numbers of predominant species appeared in the first examination was markedly smaller than those of rods and monotrichous flagellum. Of the cultured species a large number of *Pseudomonas*, and small number of *Vibrio*, *Achromobacter*, and *Aeromonas* were identified. In the last experiment, it was found that the intestinal flora from the fish cultured in sea water differed from the former two experiments, that is, the predominant organisms once appeared in the former experiments were completely disappeared. This results suggested that a large proportions of the intestinal flora of the fresh water fish might be changed when they were cultured in sea water. The experiments were also carried out to compare the microflora in food and water with those of intestine. The microflora isolated from food was mainly *Bacillus*, whereas *Pseudomonas*, *Vibrio*, *Aeromonas* were isolated from sea water in which fish was cultured for a certain period.

Discussions

The present work demonstrated that the intestinal microflora of fresh water mullet contains the predominant species of genera *Enterobacter* and *Bacillus*. Species of *Lactobacillus* and *Staphylococcus*, as well as *Escherichia coli* type I and *Clostridium perfringens* are all commonly found in gastrointestinal tract of homothermic species (MUSHIN

Table 3. The characteristics of identified bacteria from fresh water mullet.

Strain	Form	Gram stain	Motility	Flagellation	Spore	Oxidase	Catalase	Nitrate reduction	H_2S production	Indole production	Hydrolysis of Starch	Hydrolysis of Casein	Gelatin	Hugh & Leifson	Bacterial genus identified
S–1	R	+	+	P	+	−	+	+	−	−	+	+	+	O	*Bacillus*
S–2	R	+	+	P	+	−	+	+	−	−	+	+	+	O	*Bacillus*
S–3	R	+	+	P	+	−	+	+	−	−	+	+	+	O	*Bacillus*
S–4	C	+	−	A	−	−	+	−	−	−	−	−	−	F	*Staphylococcus*
S–5	R	−	+	P	−	−	+	+	−	−	+	+	+	F	*Enterobacter*
S–6	R	−	+	P	−	−	+	+	−	−	+	+	+	F	*Enterobacter*
S–7	R	+	+	P	+	−	+	+	−	−	+	+	+	O	*Bacillus*
S–8	R	+	+	P	+	−	+	+	−	−	+	+	+	O	*Bacillus*
S–9	C	+	−	A	−	−	+	−	−	−	−	−	−	F	*Staphylococcus*
S–10	C	+	−	A	−	−	+	−	−	−	−	−	−	F	*Staphylococcus*
S–11	R	−	+	P	−	−	+	+	−	−	+	+	+	F	*Enterobacter*
S–12	R	−	+	P	−	−	+	+	−	−	+	+	+	F	*Enterobacter*
S–13	R	+	+	P	+	−	+	+	−	−	+	+	+	O	*Bacillus*
S–14	R	−	+	P	−	−	+	+	−	−	+	+	+	F	*Enterobacter*
S–15	R	+	+	P	+	−	+	+	−	−	+	+	+	O	*Bacillus*
S–16	R	−	+	P	−	−	+	+	−	−	+	+	+	F	*Enterobacter*
S–17	R	−	+	P	−	−	+	+	−	−	+	+	+	F	*Enterobacter*
S–18	C	+	−	A	−	−	+	−	−	−	−	−	−	F	*Staphylococcus*

Table 4. The characteristics of intestinal bacteria isolated from fishes cultured in fresh and sea water in 1: 1.

Strain	Form	Pigment	Lumi-nescence	Gram stain	Motility	Fragellation	Spore	Oxidase	Catalase	Hugh & Leifson	Methyl red	Arginine decarboxylase	Genus name identified
S–19	R	–	–	–	+	M	–	+	+	O	–	+	*Pseudomonas*
S–20	R	–	–	–	+	M	–	+	+	O	–	+	*Pseudomonas*
S–21	R	–	–	+	+	P	+	–	+	O	–	–	*Bacillus*
S–22	R	–	–	–	+	M	–	+	+	O	–	+	*Pseudomonas*
S–23	R	–	–	+	+	P	+	–	+	O	–	–	*Bacillus*
S–24	R	–	–	–	+	M	–	+	+	O	–	+	*Pseudomonas*
S–25	R	–	–	+	+	P	+	–	+	O	–	–	*Bacillus*
S–26	R	–	–	–	+	M	–	+	+	F	–	–	*Vibrio*
S–27	R	–	–	–	+	M	–	+	+	F	+	–	*Vibrio*
S–28	R	–	–	–	+	M	–	+	+	F	–	–	*Vibrio*
S–29	R	–	–	–	+	P	–	+	+	O	–	–	*Achromobacter*
S–30	R	–	–	–	+	M	–	+	+	F	–	+	*Aeromonas*

Table 5. The characteristics of intestinal bacteria isolated from fishes cultured in sea water.

Strain	Form	Pigment	Lumi-nescence	Gram stain	Motility	Fragellation	Spore	Oxidase	Catalase	Hugh & Leifson	Methyl red	Arginine decarboxylase	Genus name identified
S–31	R	–	–	–	+	M	–	+	+	F	–	–	*Vibrio*
S–32	R	–	–	–	+	M	–	+	+	F	–	–	*Vibrio*
S–33	R	–	–	–	+	M	–	+	+	F	–	+	*Aeromonas*
S–34	R	–	–	–	+	M	–	+	+	O	–	+	*Pseudomonas*
S–35	R	–	–	–	+	M	–	+	+	O	–	+	*Pseudomonas*
S–36	R	–	–	–	+	M	–	+	+	O	–	+	*Pseudomonas*
S–37	R	–	–	–	+	M	–	+	+	O	–	+	*Pseudomonas*
S–38	R	–	–	–	+	M	–	+	+	F	–	+	*Aeromonas*
S–39	R	–	–	–	+	M	–	+	+	O	–	+	*Pseudomonas*
S–40	R	–	–	–	+	M	–	+	+	F	–	+	*Aeromonas*
S–41	R	–	–	–	+	M	–	+	+	F	–	+	*Aeromonas*
S–42	R	–	–	–	+	M	–	+	+	O	–	+	*Pseudomonas*

Table 6. Bacteria isolated from food.

Strain	Form	Gram stain	Motility	Flagellation	Spore	Oxidase	Catalase	Nitrate reduction	H_2S production	Indole production	Hydrolysis of Starch	Hydrolysis of Casein	Gelatin	Hugh & Leifson	Bacterial genus identified
S-43	R	+	+	P	+	−	+	+	−	−	+	+	+	O	*Bacillus*
S-44	R	+	+	P	+	−	+	+	−	−	+	+	+	O	*Bacillus*
S-45	R	+	+	P	+	−	+	+	−	−	+	+	+	O	*Bacillus*

Table 7. Bacteria isolated from sea water.

Strain	Form	Pigment	Luminescence	Gram stain	Motility	Fragellation	Spore	Oxidase	Catalase	Hugh & Leifson	Methyl red	Arginine decarboxylase	Genus name identified
S-46	R	−	−	−	+	M	−	+	+	O	−	+	*Pseudomonas*
S-47	R	−	−	−	+	M	−	+	+	O	−	+	*Pseudomonas*
S-48	R	−	−	−	+	M	−	+	+	F	−	+	*Vibrio*
S-49	R	−	−	−	+	M	−	+	+	F	−	−	*Vibrio*
S-50	R	−	−	−	+	M	−	+	+	F	−	−	*Vibrio*
S-51	R	−	−	−	+	M	−	+	+	F	−	+	*Aeromonas*
S-52	R	−	−	−	+	M	−	+	+	F	−	+	*Aeromonas*

and ASHBURNER, 1964; ROSEBURY, 1962; WOOD and TRUST, 1972), but not specifically found in the mullet, except *Staphylococcii.*

The bacterial species isolated from fresh water fish appear to differ from the normal microflora of fish cultured in sea water, where *Pseudomonas, Vibrio* and *Aeromonas* have been shown to predominant. It should be noted that these predominant species manifested as a progressive loss of ability to survive in the condition of sea water. The ability can probably be directly related to an increase in sensitivity to sodium chloride concentration. It seems likely that the gastrointestinal microflora have a role in nutrition, growth, and disease susceptibility of the fish. It is apparent that greater attention must be given to gastrointestinal microflora of fish (TRUST and SPARROW, 1974). SEKI (1972), has suggested that bacteria may even represent a source of nutritions for the fish. As far as we are aware, little work on bacterial flora of fish has been done using fresh and marine isolates. The practical implication of this work may be significant particularly on the nutrition. Further investigations of the role of these bacteria in the nutrition are in progress.

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