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Microflora in the Alimentary Tract of *Tilapia*-*II*

Comparison among Microflora of Intestine, Sediment and Pond Water

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Abstract

Viabl counts and microflora were compared among the intestinal contents, sediments and water obtained from 4 different culture ponds of *Tilapia* (*Sarotherodon niloticus*).

Viable counts in the intestinal contents, sediments and water were 8.9, 8.3 and 5.1 (log No/g or ml), respectively. Obligate anaerobes were predominant over the aerobic growth bacteria in intestines, and both were almost the same level in sediments and vice versa in water.

Among the aerobic microflora, *Aeromonas*(0/129 risistant), *Vibrio* (0/129 sensitive isolates) and *Pseudomonas* spp became predominant in the intestines and sediments, whereas *Flavobacterium*, *Acinetobacter (Moraxella)*, *Aeromonas* and *Pseudomonas* constituted the dominant genera in water.

Most of the *Aeromonas* spp isolated from the intestines were distinctly different from *Vibrio* spp with respect to starch decompositions as well as 0/129 resistant and aerogenesis.

Aquaculture of *Tilapia* has been developed in Japan, because these tropical fresh water fish have a biological ability to grow successfully if only warm water over 20 C is supplied. Fecal materials including intestinal bacteria are considered to give a serious effect on microflora in water and sediments of intensive aquaculture ponds and drains.

Many investigators[1,2,3] reported that facultative anaerobic bacteria, mostly *Aeromonas* spp, become dominant in the intestinal microflora of general fresh water fishes, whereas we described the specrific obligate anaerobes exeeded facultative ones by 5-10 times in the intestines of *Tilapia* in previous papers[4,5].

In this paper, we examined and compared the microflora in fish intestines, water and sediments of *Tilapia* culture ponds.

Materials and Methods

Fish examined Fish (*Sarotherodon niloticus*), which had been fed with commercial pelleted diets, were obtained from 4 diffferent fish culture ponds in Hayato-cho and Ibusuki-shi, Kagoshima Prefecture, Japan. The fish samples were returned to the laboratory within about 2 hours for bacterial analysis, while fish containers were aerated by using air pumps. The

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gastrointestinal tracts of the fish were removed aseptically soon after arrival at the laboratory. The intestinal contents were squeezed out of the posterior of the fish intestine with a sterile pincette.

Bacterial enumeration Bacterial counts in intestinal contents were determined by the spread plate method as described previously[4]. Inoculated plates were incubated at 25 C aerobically and anaerobically. An anaerobic condition was attained by replacement of the atmosphere in an anaerobic jar with N_2 gas and steel wool. In this expreiment, the following agar media were employed for bacterial enumeration: EG[6], NBGT-1/3S[4], Z-AII[7], M-AII[7], P[6], BTB-teepol (Eiken) and DHL (Eiken).

Identification of aerobic isolates Among the bacteria grown on Z-AII plates aerobically, 30-35 strains were selected at random for each sample and identified according to the identification scheme proposed by SHEWAN et al[8] and SIMIDU[9]. Characteristics used for identification were as follows: cell form, motility, flagellation, gram-stain, spore-forming, pigmentation, oxidase, catalase, HUGH and LEIFSON test[10] and 0/129 sensitivity[11]. The genus *Vibrio* was differenciated from the genus *Aeromonas* tentatively on the basis of 0/129 sensitivity in this experiment among the gram-negative rods with glucose-fermentative and oxidase-positive.

Hydrolytic activities Hydrolytic activities of isolates against chitin, starch and casein were examined by using Z-AII agar containing 0.5% (w/v) of chitin (Nakarai, HCl-treated), starch (Nakarai) and casein (Merck, Hammarsten), respectively. A clear zone around the colony indicated hydrolysis, observing directly for chitin and casein decomposition and after flooding the plate with iodine solution in the case of starch decomposition.

Water analysis Chemical analysis for water samples from cultural ponds was performed according to the following standard methods described in the textbook of water analysis[12]: WINKLER method (DO), alkaline $KMnO_4$ method (COD), MOHR-KNUDSON titration method (Cl^-), chelate titration method (Ca^{++} and Mg^{++}), indo-phenol method (ammonium-N) and LORENZEN method (chlorophyll).

Results

Fish and pond water examined Chemical analysis of water from each fish pond is summarized in Table 1. Culture pond H-A (Hayato-cho, Ichijo) and H-B (Hayato-cho, Ishizeki) showed higher chlorophyll contents in spring and summer seasons. It was found characteristically that the concentrations of Cl^-, Ca^{++} and Mg^{++} ions were relatively higher in water samples from H-C pond (Hayato-cho Shimazushinden) near the coast of Kinko Bay, indicating the water supplied from an underground source is influenced by sea water. Water samples from I-F (Ibusuki-shi) showed to be slightly higher in Cl^- ion compared with that of other pond water.

As shown in Table 2, adult fish, body weights of which were 119-1,625g, were used in this experiment. The intestinal contents looked like dark brown or dark green clay materials and

Table 1. Water analysis of *Tilapia* culture ponds

Pond	Temp. C	pH	DO ppm/l	COD ppm/l	NH_4–N μg-atom/l	Ca^{++} mg/l	Mg^{++} mg/l	Cl^- mg/l	Chl-a mg/m³	Pheo. mg/m³
H–A*1(2)*2	25.4	7.8	8.3	5.8	48.5	23.3*3 (±3.7)	19.9 (±0)	46.5 (±4.7)	166.8	69.1
H–B (1)	22.8	7.3	6.9	4.1	328.9	39.3	22.8	51.8	neg*4	neg
H–C (2)	27.0	8.4	11.9	8.4	44.0	102.0 (±1.7)	121.4 (±5.1)	1621.8 (±206.3)	100.7	43.5
I–F (4)	23.4	7.2	5.1	2.6	27.5	20.6 (±0.5)	5.4 (±0.3)	145.0 (±6.5)	1.1	7.5
L (2)	21.0	7.2	14.6	30.9	163.0	16.3 (±6.4)	7.5 (±2.5)	34.3 (±1.2)	neg	neg

*1 *Tilapia* culture ponds: H–A, Hayato-cho Ichijo; H–B, Hayato-cho Ishizeki; H–C, Hayato-cho Shimazushinden; I–F, Ibusuki-shi; L, laboratory tank.
*2 Sample numbers.
*3 Mean ± standard deviation.
*4 Negligible.

Table 2. *Tilapia* samples examined

Culture Place	Pond Extent (m²)	Date	Fish sample Body length (cm)	Body weight (g)	Intestine length (cm)
H–A	529 m²	'81, 5/26	20.7	173	156
		7/24	26.0	360	249
H–B	663 m²	5/26	39.4	1625	356
H–C	10,000 m²	5/26	18.7	119	141
		7/24	37.0	1200	273
I–F	32 m²	7/23	24.0	310	182
		10/20	24.0	260	123
		12/2	23.0	280	180
		'82, 2/17	28.0	450	250
L	50 ℓ	'81, 5/20	20.0	150	95
		12/22	19.0	140	107

contained many bacterial cells, skeltons of diatoms and other amorphous materials when observed by a light microscope.

Viable counts As shown in Fig.1, the mean of viable counts in the intestinal contents, sediments and water were obtained for various agar plates after the smeared plates were incubated aerobically and anaerobically. Viable counts on EG agar or NBGT-1/3S agar under anaerobic condition exceeded those on EG agar or Z-AII agar under aerobic condition by several times in the intestinal contents, and slightly in sediments. On the other hand, viable counts obtained on Z-AII agar aerobically were more than those on EG agar anaerobically in the case of water samples. Therefore,the colony counts recovered on anaerobic EG or aerobic Z-AII agar

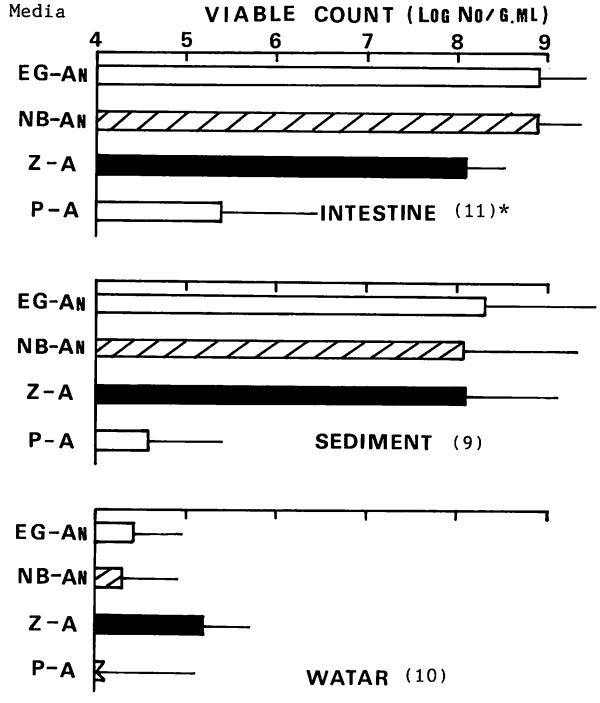

Fig. 1. Viable counts in three environments. Data shown are a mean ± standard deviation.
*; No. of Samples.
An: Anaerobically, A; Aerobically.

represented approximately the total viable counts in the intestinal contents (8.9) and sediments (8.3) or water (5.2, log No/ml), respectively.

These results indicate that obligate anaerobes are predominant over the aerobic growth bacteria in fish intestines and that both groups of bacteria are much the same in sediments and vice versa in water. The obligate anaerobes grown on NBGT-1/3S agar selectively, which are considered to be indigenous in fish intestines, were always detectable in sediments and water of fish ponds.

Yeast colonies were also routinely obtained on P agar from most samples of intestinal contents, sediments and water in *Tilapia* culture ponds, although they were not so much.

Generic composition of aerobic microflora Obligate anaerobes isolated on NBGT-1/3S agar ware demonstrated to comprise of *Bacteroides* A and B groups, judging from morphological observation as described previously[5].

On the other hand, the comparison among generic compositions of aerobic and facultative microflora in intestinal contents, sediments and water obtained from *Tilapia* cluture ponds is illustrated in Fig.2. Generally, in the intestinal contents and sediments *Aeromonas, Vibrio* and *Pseudomonas* spp were the predominant genera, whereas in water *Flavobacterium, Acinetobacter*

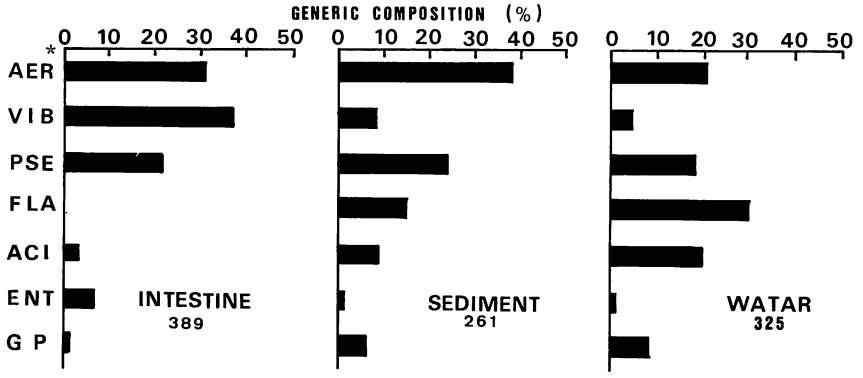

Fig. 2. Generic composition of microflora in three environments.
*; Aer; *Aeromonas*, Vib; *Vibrio*, Pse; *Pseudomonas*, Fla; *Flavobacterium*.
Aci; Acinetobacter (*Moraxella*), Ent; *Enterobacteriaceae*,
GP; Gram positive

(Moraxella), *Aeromonas* and *Pseudomonas* constituted the dominant genera. Microflora in sediments of *Tilapia* culture ponds was similar to that in intestinal contents, suggesting that sediments consisted mainly of fish fecal pellets in *Tilapia* culture ponds.

In comparison among generic compositions of fermentative isolates in fish intestines obtained from each culture ponds, *Aeromonas* spp occupied a higher percentage in H-A and H-B ponds but *Vibrio* spp became a major group in H-C pond, of which salinity was relatively higher under the influence of sea water. It is worth while to remark that *Aeromonas* as well as *Vibrio* represented a large portion of isolates in I-F pond and the laboratory tank (L), to which fish were introduced from I-F pond (Fig.3).

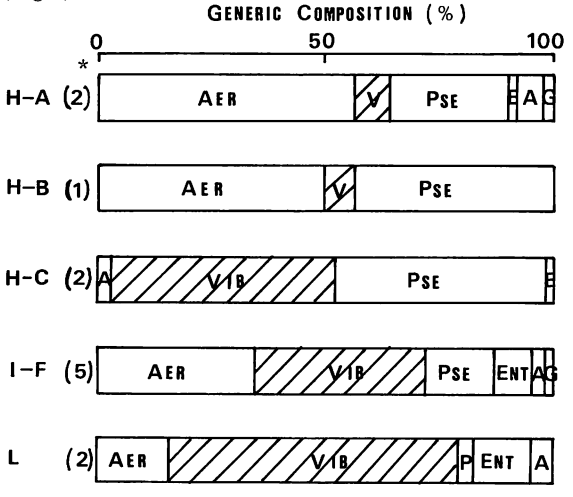

Fig. 3. Generic composition in *Tilapia* intestines
*; No. of fish samples

Macromolecule hydrolytic activities Fig.4 shows hydrolytic activities against chitin, starch and casein of the dominant genera isolates in fish intestines, sediments and water. A high proportion of *Aeromonas* spp decomposed chitin (98.9%) and starch (90.0%) but *Vibrio* spp showed a high percentage in only chitin decomposition (67.5%). Both of these genera became dominant in aerobic flora of the intestines and sediments. On the contrary, *Flavobacterium* spp were predominant in water, and contained positive strains in casein (76.4%) decomposition in relatively higher percentage but could not attack chitin at all. *Pseudomonas* spp showed lower percentage in decomposition of chitin, casein and starch.

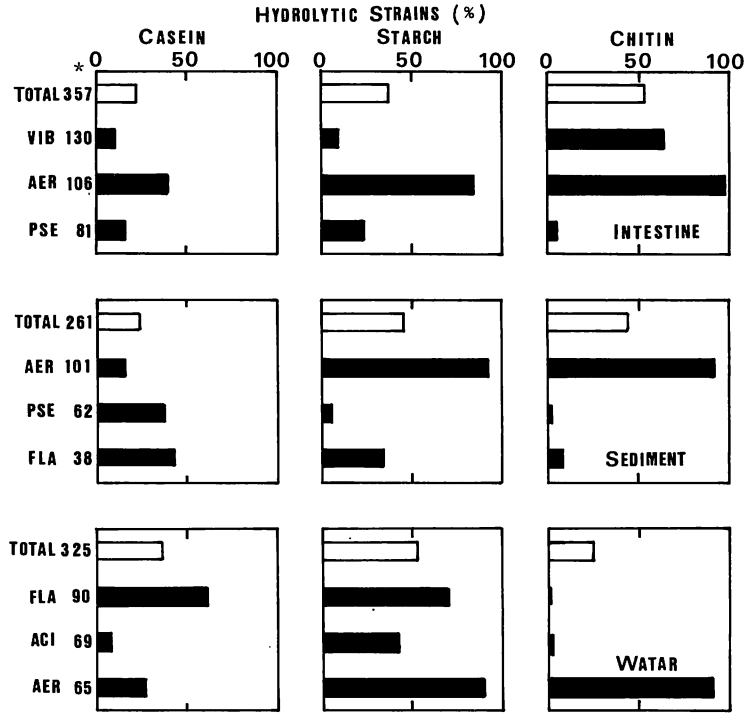

Fig. 4. Macromolecules hydrolytic activities of microflora
*; No. of test strains

Discussion

In pervious papers[5,6], we described that the specific obligate anaerobic bacteria including *Bacteroides* A and B groups distributed in various fresh water fish and that they specially become bacteria in the intestinal flora of *Tilapia* and *Ayu*.

TRUST and BARTLETT[13] tried to detect anaerobes in the tropical fish water though qualitative examination was not performed. SUGITA et al[14] reported that *Bacteroides* A group bacteria were found in water containing carp but that they disappeared in water after all the fish were taken out

of the aquarium. In intensive *Tilapia* culture ponds, these specific anaerobes were usually detectable in sediments and water as well as fish intesines. Especially, sediments contained the similar levels of obligate and facultative anaerobes compared with water, in which aerobic growth bacteria exceeded obligate anaerobes considerably. These specific anaerobes are considered to spread over surroundings by way of fecal pellets excreted by fish constantly.

As the predominant bacteria in various fresh water fishes, *Aeromonas* in common and *Enterobacteriaceae* in some cases were isolated by many workers. In the present study, we found *Tilapia* samples which comprised *Aeromonas* or *Vibrio* as dominant facultative bacteria, reflecting fish culture ponds. The *Aeromonas* spp isolated in this study were distinctly different from *Vibrio* spp with respect to starch decomposition. However, we differentiated *Vibrio* from *Aeromonas* on the basis of 0/129 sensitivity and gas production from glucose.

Further experiments are required to confirm whether the isolates assigned to *Vibrio* in this study should be within the genus *Vibrio* according to the acceptable definition in bacterial systematics.

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