The Intestinal Microflora of Carp Cyprinus carpio, Grass Carp Ctenopharyngodon idella and Tilapia Sarotherodon niloticus*1

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Microflora in the contents of intestinal tract of carp, grass carp and tilapia was investigated using 7 different agar media. The intestinal microflora varied with the species of fish. The predominant bacteria in the intestinal tract of carp were Aeromonas hydrophila, Bacteroides type A, Citrobacter freundii, Pseudomonas and Micrococcus. The intestinal microflora of grass carp mainly consisted of A. hydrophila and Bacteroides type A. The intestinal microflora of tilapia was mainly composed of Bacteroides type A, Bacteroides type B, Plesiomonas shigelloides and A. hydrophila. The ratio of the obligate anaerobes to the facultative anaerobes plus aerobes of carp, grass carp and tilapia was 0.05 to 0.95, 0 to 0.49 and 32.4 to 111.1, respectively. It is suggested that the predominance of obligate anaerobes in the intestine of tilapia was due to their long intestine.

Since TRUST et al.1) reported the presence of non-sporing obligate anaerobes in the gastrointestinal tract of grass carp and goldfish, the gastrointestinal bacteria including obligate anaerobes were studied by several workers. SAKATA et al.2)–4) isolated two types of non-sporing gram-negative rods from the intestine of six fishes, tilapia Sarotherodon niloticus (synonym of Tilapia nilotica), carp, goldfish, ayu, rainbow trout and dace, and classified them into Bacteroides type A and Bacteroides type B. Although these bacteria were predominant in the gastrointestinal of ayu and tilapia as reported by SUGITA et al.5,6) they decreased and/or disappeared when the tilapia was acclimated into seawater.6) SUGITA et al.7) observed that Bacteroides type A was established in the gastrointestinal tract of tilapia Sarotherodon mossambicus (synonym of Tilapia mossambica) 20 to 60 days after hatching.

Facultative anaerobes also, were isolated from the gastrointestinal of freshwater cultured fishes and examined taxonomically. Aeromonas hydrophila were isolated from grass carp,1) salmonid fishes,5) ayu,1) and eel,9) Plesiomonas shigelloides were isolated from tilapia S. niloticus9) and eel,9) and Hafnia alvei were isolated from salmonid fishes.6)

Material and Methods

Fish

Carp Cyprinus carpio (121–154 g in body weight) and tilapia S. niloticus (26–43 g) were purchased from a commercial supplier. All fish were reared for about one month in plastic tanks which were filled with 800 l of tap water, aerated with air.
pumps at a rate of 2.4 l/min and circulated with water pumps at a rate of 25 l/min. Fish were fed pellet diet (Nissin Flour Milling) daily. During this period no mortalities and no symptoms of disease were observed. Temperature of tank when sampled was 14.5°C in a carp-rearing and 25.7°C in a tilapia-rearing tank.

Juvenile grass carp *Ctenopharyngodon idella* (20–24 g) were obtained from Saitama Prefectural Fisheries Experimental Station, Kazo, Saitama where the fish were cultured about one month after hatching, fed a pellet diet. Water temperature when sampled, was 27.0°C. The fish were killed, cooled on ice and transported into the laboratory within 3 h.

**Bacteriological Sampling**

Each fish was pithed and its abdominal cavity was opened aseptically. The intestine was removed to a sterile petri dish. After the intestinal length was measured, its content was weighed and placed in a test tube and a suitable amount of diluent was added to effect a tenfold dilution. The diluent solution is phosphate buffer solution (pH 7.6) containing 0.05% L-cysteine hydrochloride and 0.1% agar reported by MITSUOKA et al.¹⁷) The sample was then vigorously vibrated with a vortex mixer for 2 min, diluted serially and plated onto 7 different agar media. The media included Trypticase soy blood agar [TS] (BBL), Phenyl-ethyl alcohol blood agar [PEA] (BBL), MacConkey agar (Eiken), FM-CW blood agar [FM-CW] (Eiken), *Bacteroides* type A-selective media [AS]¹⁸) and *Bacteroides* type B-selective media [BS].¹⁸) The AS medium is NBGT-1/3S medium³) supplemented with 0.001% Erythromycin (Abbott) and the BS medium is NBGT-1/3S medium supplemented with 0.001% Colimycin (Kakenyaku). The inoculated TS, PEA and MacConkey agar plates were aerobically incubated and the EG, FM-CW, AS and BS agar plates were anaerobically incubated both at 25°C for 7–8 days. Anaerobiosis was established by evacuating the atmosphere of an anaerobic jar containing steel wool which was activated by an acidic cupric sulfate and replacing the atmosphere with CO₂ gas.¹⁷)

**Identification of Bacteria**

After incubation, the bacterial colonies were counted, then 20 colonies were isolated at random from each plate. Of aerobic and facultatively anaerobic bacteria isolated aerobically, gram-negative bacteria were identified by genus using the modified scheme¹⁹) of SHEWAN et al.,²⁰) and gram-positive bacteria were identified according to the procedure of COWAN.²¹) *Vibrio* a *ceae* was further examined on sensitivity to vibrio static compound (O/129), production of 2,3-butandiol dehydrogenase and reactions in AH medium.²²) *Enterobacteriaceae* and *Pseudomonas* were identified by species using Minitek system (BBL).

The obligate and facultative anaerobes isolated anaerobically were classified on the basis of gram reaction, cellular morphology and arrangement, spore formation and the ability to grow aerobically.

**Results**

**The Intestinal Length**

The intestinal and total length of sampled fishes is shown in Table 1. The ratio of intestinal to total length of carp, grass carp and tilapia is 1.37–1.96, 2.17–2.45 and 3.87–5.21, respectively.

**Viable Counts of Bacteria**

The number of viable bacteria recovered when the contents of the intestinal tract of five carp, five grass carp and four tilapia were sampled, was measured. The viable counts for each sample varied with the agar medium used as shown follows: The number of viable counts on TS agar was ranged from 10⁶ to 10⁸ g⁻¹; PEA agar, 10⁶ to 10⁷ g⁻¹; MacConkey agar, 10⁶ to 10⁸ g⁻¹; EG agar, 10⁷ to 10⁸ g⁻¹; FM-CW agar, <2×10⁸ to 10⁸ g⁻¹; AS agar, <2×10⁶ to 10⁸ g⁻¹; BS agar, <2×10⁵ to 10⁸ g⁻¹. No colonies appeared on BS agar inoculated with the grass carp samples.

**Bacteria Isolated**

A total of 722 strains of aerobic and facultative...
tively anaerobic bacteria was isolated aerobically. These were composed of Vibrionaceae (662 strains), Pseudomonas (50), Enterobacteriaceae (32), Micrococcus (24), Bacillus (2) and Streptococcus (2). Of the isolates belonging to Vibrionaceae, 527 strains were resistant to O/129, grew at 37°C, produced 2,3-butanediol dehydrogenase and indole, did not produce H₂S, and showed a yellow butt with a band of purple at the top of AH medium. All the other strains were sensitive to O/129, grew at 37°C, produced indole, did not produce H₂S and gave an alkaline reaction throughout the tube of AH medium. According with Kaper et al., Sakazaki and Balows, Yamamoto and Wakabayashi, Popoff, and Schubert, the former was presumptively identified as Aeromonas hydrophila, and the later was presumptively identified as Plesiomonas shigelloides. The isolates belonging to Enterobacteriaceae were divided into two species, Citrobacter freundii (31 strains) and Enterobacter agglomerans (1). The 38 strains of Pseudomonas isolated from the carp samples could not be identified by the Minitek system.

A total of 933 strains of anaerobic bacteria was isolated anaerobically. These were obligate anaerobes (594 strains) and facultative anaerobes (339). The obligate anaerobes consisted of Bacteroides type A (510) and Bacteroides type B (84).

**Intestinal Microflora of Fishes**

The maximum viable counts of the various bacterial components, and total viable counts which were calculated by summing up the maximum viable counts in the contents of intestinal tract of carp are shown in Table 2. *A. hydrophila*, *Bacteroides* type A, *C. freundii*, *Pseudomonas* and *Micrococcus* were isolated from all five fish. These bacteria were predominant with densities of 10⁶ to 10⁹ g⁻¹. *E. agglomerans*, *Streptococcus*, *Bacillus* and coryneforms were detected in one or two samples with densities less than 10⁶ g⁻¹. Total viable counts in the intestine of carp were 1.3–4.3 x 10⁸ g⁻¹.

In the intestinal tract of grass carp, only *A. hydrophila* were isolated from all five fish and *Bacteroides* type A were isolated from four samples.

**Table 2. Viable counts (log No. g⁻¹) of different bacterial components in the contents of intestinal tracts of carp**

<table>
<thead>
<tr>
<th>Component</th>
<th>Specimen</th>
<th>Mean*¹</th>
<th>Occurrence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeromonas hydrophila</td>
<td></td>
<td>8.23±0.43</td>
<td>100</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td></td>
<td>7.22±0.39</td>
<td>100</td>
</tr>
<tr>
<td>Enterobacter agglomerans</td>
<td></td>
<td>7.20±0.20</td>
<td>20</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td></td>
<td>6.90±0.45</td>
<td>100</td>
</tr>
<tr>
<td>Micrococcus</td>
<td></td>
<td>6.85±0.21</td>
<td>100</td>
</tr>
<tr>
<td>Streptococcus</td>
<td></td>
<td>6.50±0.45</td>
<td>100</td>
</tr>
<tr>
<td>Bacillus</td>
<td></td>
<td>6.36±0.20</td>
<td>20</td>
</tr>
<tr>
<td>Coryneforms</td>
<td></td>
<td>6.74±1.23</td>
<td>40</td>
</tr>
<tr>
<td>Bacteroides type A</td>
<td></td>
<td>5.87±0.34</td>
<td>100</td>
</tr>
<tr>
<td>Total viable count</td>
<td></td>
<td>8.44±0.24</td>
<td>100</td>
</tr>
</tbody>
</table>

*¹ Mean±standard deviation of log viable counts when present.

**Table 3. Viable counts (log No. g⁻¹) of different bacterial components in the contents of intestinal tracts of grass carp**

<table>
<thead>
<tr>
<th>Component</th>
<th>Specimen</th>
<th>Mean*¹</th>
<th>Occurrence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeromonas hydrophila</td>
<td></td>
<td>7.71±0.26</td>
<td>100</td>
</tr>
<tr>
<td>Micrococcus</td>
<td></td>
<td>6.28±0.48</td>
<td>60</td>
</tr>
<tr>
<td>Bacillus</td>
<td></td>
<td>6.36±0.20</td>
<td>20</td>
</tr>
<tr>
<td>Bacteroides type A</td>
<td></td>
<td>7.00±0.42</td>
<td>80</td>
</tr>
<tr>
<td>Total viable count</td>
<td></td>
<td>7.78±0.31</td>
<td>100</td>
</tr>
</tbody>
</table>

*¹ Mean±standard deviation of log viable counts when present.

*² Not detected.
Table 4. Viable counts (log No. g\(^{-1}\)) of different bacterial components in the contents of intestinal tracts of tilapia fish

<table>
<thead>
<tr>
<th>Component</th>
<th>Specimen</th>
<th>Mean(^*1)</th>
<th>Occurrence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plesiomonas shigelloides</td>
<td>6.83</td>
<td>6.76±0.33</td>
<td>100</td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
<td>6.15</td>
<td>6.02±0.13</td>
<td>75</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>nd(^*2)</td>
<td>5.93±0.89</td>
<td>50</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>5.15</td>
<td>4.45±0.79</td>
<td>75</td>
</tr>
<tr>
<td>Bacteroides type A</td>
<td>8.53</td>
<td>8.60±0.13</td>
<td>100</td>
</tr>
<tr>
<td>Bacteroides type B</td>
<td>8.60</td>
<td>7.50±0.23</td>
<td>100</td>
</tr>
<tr>
<td>Total viable count</td>
<td>8.59</td>
<td>8.64±0.14</td>
<td></td>
</tr>
</tbody>
</table>

\(^*1\) Mean±standard deviation of log viable counts when present.

\(^*2\) Not detected.

(Table 3). The *A. hydrophila* and *Bacteroides* type A were predominant with densities of 10\(^6\) to 10\(^7\) g\(^{-1}\). *Micrococcus* and *Bacillus* were detected in one to three samples with densities of 10\(^5\) to 10\(^6\) g\(^{-1}\). Total viable counts in the intestine of grass carp were 1.8×10\(^7\) to 1.3×10\(^8\) g\(^{-1}\).

In the intestinal tract of tilapia, *Bacteroides* type A, *Bacteroides* type B, *P. shigelloides*, *A. hydrophila* and *Pseudomonas* were isolated with occurrence frequency more than 75\% (Table 4). The former 4 components were predominant with densities of 10\(^5\) to 10\(^8\) g\(^{-1}\). The total number of obligate anaerobes were 3.4-6.8×10\(^8\) g\(^{-1}\) while that of facultative anaerobes plus aerobes were 3.6×10\(^6\) to 2.1×10\(^7\) g\(^{-1}\). Total viable counts in the intestine of tilapia were 3.4-7.0×10\(^8\) g\(^{-1}\).

### Discussion

In the present paper four to five fish which were reared in the same conditions were simultaneously examined. From the results of the viable counts of various bacterial components in each individual, it is shown that the bacterial components which possessed the high occurrence frequency (more than 75\%) maintained high viable counts with relatively low values of standard deviation (Tables 2, 3 and 4). This result suggests that most of indigenous bacteria are predominant in the intestinal tract of freshwater culture fish. Moreover, the relatively low value of standard deviation may mean that the gastrointestinal microflora is stable in the fish population reared in the defined condition.

The intestinal microflora varied with the species of fish. The predominant bacteria in the intestinal tract of carp were *A. hydrophila*, *Bacteroides* type A, *C. freundii*, *Pseudomonas* and *Micrococcus* (Table 2). The intestinal microflora of the carp are characterized by the predominance of *C. freundii*, *Pseudomonas* and *Micrococcus* as reported by MATTHEIS.\(^23\) The *Pseudomonas* from the carp were not identified because the system is mainly intended to identify the clinical bacteria.

The intestinal microflora of grass carp mainly consisted of *A. hydrophila* and *Bacteroides* type A (Table 3). The simple composition of the intestinal bacteria constituted a character feature of this fish. As YOSHIMIZU et al.\(^27\) and SUGITA et al.\(^7\) reported that the intestinal microflora of salmonid fishes and tilapia changes with the development of fish, the possibility that the microflora of adult grass carp differs from the juvenile remains.

The intestinal microflora of tilapia was mainly composed of *Bacteroides* type A, *Bacteroides* types B, *P. shigelloides* and *A. hydrophila* (Table 4). The predominance of *Bacteroides* type B and *P. shigelloides* is a character of the microflora of tilapia.\(^10\) As suggested by SAKATA et al.,\(^28\) *A. hydrophila* was detected in the tilapia samples with densities up to 10\(^6\) g\(^{-1}\). It is notable that the obligate anaerobes outnumber the facultative anaerobes plus aerobes by 32.4 to 111.1:1 in tilapia, whereas the ratio of the former to the later is 0.05 to 0.95 in carp and 0 to 0.49 in grass carp. It is well known that the lower intestine of human appears to contain the larger number of bacteria than the upper.\(^29\) Therefore, the dominance of obligate anaerobes in the intestinal tract of tilapia seems to reflect the high ratio of intestinal to total length (3.87-5.21), compared with carp (1.37-1.96) and grass carp (2.17-2.45).

### Acknowledgement

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mental Station, Kazo, Saitama for supplying the specimen.

References


