Microflora in the Alimentary Tract of Gray Mullet—II.
A Comparison of the Mullet Intestinal Microflora in Fresh and Sea Water.

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(Received July 21, 1977)

The purpose of this study was to determine the changes in microflora in the intestine of the gray mullet Mugil cephalus, an amphidromous fish with an undeveloped stomach, under different conditions. Samples were taken from fishes kept sequentially in fresh water, then in a half fresh and half sea water mixture, then in sea water, again in half fresh and half sea water, and finally returned to fresh water. The intestinal bacterial flora of the mullet in fresh water were composed primarily of Enterobacter, Bacillus, and Micrococcus. When the mullets were transferred to sea water, however, the flora changed; the dominant genera were Vibrio, Pseudomonas, and Aeromonas. After the return to fresh water the microflora consisted of only one genus, Enterobacter, the predominant one usually found in fishes from fresh water. The other bacterial genera did not appear to survive the transition through the water changes used.

In the previous paper the authors have carried out the isolation and identification of the intestinal bacterial flora of gray mullet cultivated in fresh and sea water and it had been concluded that water conditions effected a change in the microflora from the predominant genera of Enterobacter and Bacillus to Pseudomonas, Vibrio and Aeromonas, when mullets were transplanted from fresh water to sea water. The present study was undertaken to determine the response of the representatives of the microflora in the digestive tract of mullet cultured in fresh and sea water and again transferred back to the original fresh water.

Materials and Methods

Fishes were kept in a culture tank overflowing with running tap water or with sea water supplied from the reservoir. Three days after establishment of the transfer into each culture tank fishes were used for test. The fishes were fed on a diet composed of protein (37%), fat (3%), fibre (3%), and ash (11%).

The methods for enumeration of bacteria in the intestinal tracts, procedures for isolation of the bacteria, compositions of the growth media, incubation temperatures, and the methods for morphological and biochemical examinations of bacteria were the same as described previously.

Considering an environmental condition which might be present in the intestinal tract, effects of temperature, pH and bile salt on the growth of isolates from intestinal contents and environmental water were examined. The effect of bile salt was tested by the method of SERA and ISHIDA, SERA et al. and SERA and KIMATA. Bacterial growth at various pH values and temperatures was estimated by following the change in optical density (OD) at 540 nm as measured by spectrophotometer, Model 1V, Tokyo photo-electric Co., Ltd.

Results

It was determined whether the amphidromous mullet with an undeveloped stomach possesses selective ability for microorganisms in marine and fresh water environments.

Fishes (gray mullet) used for this experiment were cultivated in an aerated tank overflowing with running tap water or with sea water supplied from the reservoir for 3 days in each tank under room temperature. Fishes were given with diet (composition stated in materials and methods) twice a day.

The morphological and physiological properties of the bacteria which were isolated from the alimentary tract, the diet, and both the environmental sea water and fresh water are shown in Table 1, where the numbers of Gram negative rods were significantly higher than Gram positive bacteria present in specimens. Bacteria posess-
ing gelatinolytic activity were comparatively more abundant than those possessing caseinolytic activity. There were only Gram negative bacteria in both the environmental water and no Vibrio in the environmental fresh water. No coccus was isolated either from the environmental water or from the diet given to the fish. However, only Gram positive bacteria were isolated from the diet.

In order to determine the changes in microflora in the intestine of fishes, which were supposed to come from the environmental water or diet, observations were carried out with mullet kept in different conditions of water as shown in Table 2. The genera Enterobacter, Bacillus, and Micrococcus represented the microflora in the intestine of fishes cultivated in fresh water for three days. There was no existence of other bacteria in the intestine of fishes living in fresh water.

Three days after establishment of the transfer into half fresh and half sea water mixture, the proportion of the genera Bacillus, Enterobacter and Micrococcus in the intestine decreased as the con-
concentration of sea water increased. On the other hand the genera *Vibrio*, *Acinetobacter*, *Pseudomonas* and *Aeromonas* gradually appeared with the increment of NaCl concentration and became the main microflora in the intestine.

After transferring the fish into 100% sea water, as the environment of the fishes changed, the intestinal bacterial flora also underwent change. The genera *Bacillus* and *Micrococcus* completely disappeared as the concentration of sea water increased and the genera *Vibrio*, *Pseudomonas* and *Aeromonas* became more abundant than in the case of the fishes cultivated in fresh water.

The proportion of the genera *Pseudomonas*, *Vibrio* and *Aeromonas* were decreased with the decrease of the salt concentration, when the fishes were placed back into fresh and sea water mixture. However the genus *Enterobacter* increased in abundance over the other predominant intestinal bacteria in fresh water fishes.

Finally when the fishes were transferred back into fresh water, the mullet had only genus *Enterobacter*, the predominant one, which was positive in lactose fermentation. Other bacteria did not appear in the intestine of fishes in that condition. On the other hand, the genera *Acinetobacter*, *Corynebacterium*, and *Pseudomonas* were dominant in the environmental fresh water, while the main flora identified from the environmental sea water were the genera *Pseudomonas*, *Vibrio* and *Achromobacter*. Bacterial flora in the diet given to the fishes was mainly composed of the genus *Bacillus*.

From these data it was found that in the case of mullet, the growth of the bacteria which were derived from diet and the environmental water was inhibited in the intestine and only *Enterobacter* survived the condition in the intestine.

In order to know whether or not the isolated bacteria were resistant to the environmental conditions encountered in intestine the effects of pH and high concentration of bile salt and optimum temperature on the growth of the isolates were examined. Table 3, shows that only *Enterobacter* and *Vibrio* were resistant to high concentration of bile salt and low pH than those of the other isolates. *Pseudomonas*, *Micrococcus*, *Acinetobacter* and *Corynebacterium* were inhibited by the high concentration of bile and low pH. The optimum temperature was 30°C for *Enterobacter*, 37°C for *Bacillus*, *Micrococcus* and *Staphylococcus* at pH 8.0. However 25°C was the optimum temperature for other genera at pH 7.0, while the pH in the intestinal contents was 7.4.

**Discussion**

The present findings with the mullet kept in a fresh water tank show that the genera *Enterobacter*, *Bacillus* and *Micrococcus* could be detected in high percentage in the intestine, whereas the microflora of environmental fresh water contained various genera such as *Acinetobacter*, *Corynebacterium*, *Pseudomonas*, *Achromobacter*, *Aeromonas*, *Staphylococcus* and *Enterobacter*. The genus *Enterobacter* is thought probably to be released from fishes themselves into the surrounding water because it was not found in the tap water sample which was used in all the experiments. TRUST and SPARROW6) reported that the gastrointestinal microflora of salmon contained *Enterobacter*, *Acinetobacter* and *Aeromonas* as the predominant genera. MATTHEIS7—10) concluded that predominant genera are *Aeromonas*, *Pseudomonas* and *Vibrio*. Percentage of generic composition of isolates under different conditions.

<table>
<thead>
<tr>
<th>Genus name of isolates</th>
<th>Resistance to bile $^{*S.b}$ value</th>
<th>Resistance to low pH $^{*S.h}$ value</th>
<th>Optimum temperature (C)</th>
<th>Optimum pH</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus</em></td>
<td>0.1–0.3 ±</td>
<td>0.1–0.4 ±</td>
<td>37.0</td>
<td>8.0</td>
</tr>
<tr>
<td><em>Enterobacter</em></td>
<td>0.7–1.1 +</td>
<td>0.9–1.2 +</td>
<td>30.0</td>
<td>8.0</td>
</tr>
<tr>
<td><em>Micrococcus</em></td>
<td>&lt;0.1 −</td>
<td>&lt;0.1 −</td>
<td>37.0</td>
<td>8.0</td>
</tr>
<tr>
<td><em>Acinetobacter</em></td>
<td>0.3–0.5 ±</td>
<td>0.5–0.6 ±</td>
<td>25.0</td>
<td>7.0</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>&lt;0.1 −</td>
<td>&lt;0.1 −</td>
<td>25.0</td>
<td>7.0</td>
</tr>
<tr>
<td><em>Vibrio</em></td>
<td>0.5–0.8 +</td>
<td>0.4–0.7 +</td>
<td>25.0</td>
<td>7.0</td>
</tr>
<tr>
<td><em>Aeromonas</em></td>
<td>0.1–0.4 ±</td>
<td>0.2–0.3 ±</td>
<td>25.0</td>
<td>7.0</td>
</tr>
<tr>
<td><em>Achromobacter</em></td>
<td>&lt;0.1 −</td>
<td>&lt;0.1 −</td>
<td>25.0</td>
<td>7.0</td>
</tr>
<tr>
<td><em>Corynebacterium</em></td>
<td>&lt;0.1 −</td>
<td>&lt;0.1 −</td>
<td>25.0</td>
<td>7.0</td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>0.1–0.2 ±</td>
<td>0.1–0.3 ±</td>
<td>37.0</td>
<td>8.0</td>
</tr>
</tbody>
</table>

$^{*}$ S.b = Optical density of basal culture containing bile
$^{*}$ S.h = Optical density of bile-free basal culture

+: Resistant. ±: Weak resistant. −: Sensitive.
and the members of the family Enterobacteriaceae, in the intestine of fresh water fishes. This suggests that the genus Aeromonas which can be frequently detected in the intestinal microflora of fresh water fishes could be due to salmon with developed stomach used by the worker.

The predominant genera of intestinal microflora of the fish cultivated in fresh water were replaced with the genera Pseudomonas Vibrio, and Aeromonas when the fishes were transferred to 1:1 sea water and fresh water mixture. This flora was subsequently changed to one with a high percentage of the genus Vibrio, when fishes moved into 100% sea water. The environmental sea water contained bacteria belonging to the genera Pseudomonas, Achromobacter, Vibrio, Aeromonas, Acinetobacter, and Corynebacterium. The above findings resemble those of SERA and ISHIDA2-3), SERA et al.4), SERA and KIMATA5), and TRUST and SPARROW6) which indicated the predominance of genus Vibrio in the microflora of the intestine of marine fish. The results also agree with that of COLWELL11) and NEWMAN et al.12) which indicated a higher percentage of the genus Vibrio in the intestine of fishes than in the environmental water. It was found that when mullets were kept in 100% sea water, varieties of microflora were introduced from the environmental sea water into the intestine. Again when the fishes were placed to fresh water and sea water mixture (1:1) the proportion of the genera decreased with the decrease of sea water concentration.

Again when fishes were transferred back to the original fresh water via 1:1 sea water and fresh water mixture from sea water, almost all dominant bacterial genera gradually decreased in number and Enterobacter was the only survivor in the alimentary tract of gray mullet. This findings supported the report of YOSHIMIZU and KIMURA13) indicating that salmonids had the ability to select microorganisms in their digestive tract. Although bile and low pH were thought to be some of the factors which may select microflora of intestine, it is clear that there may be some other important factors in the alimentary tract which contribute to the stability of Enterobacter, specially in a fresh water condition. The importance of further studies on this point was taken into consideration. Thus it may be indicated that the digestive tract of gray mullet with an undeveloped stomach possessed an ability to select microorganisms, such as Vibrio in a marine environment, Enterobacter in a fresh water environment.

Acknowledgements

The authors wish to express their deep gratitude to Prof. ROY M. JOHNSON, Department of Botany and Microbiology, Arizona State University, who was in this Laboratory on a sabbatical leave, for the time he devoted to discussion, suggestion and reading the manuscript. Dr. SADAHIKO IMAI, Professor of Kagoshima University is also thanked for helpful discussion and suggestion during the progress of this work.

References