Short Paper

Intestinal Microflora in the Fry of
*Tilapia mossambica**1

It was reported by SAKATA *et al.*¹⁾ and SUGITA *et al.*²⁾ that two anaerobic gram-negative rods, *Bacteroides* type A and type B, are predominantly present in the intestine of tilapia *Tilapia nilotica* and ayu *Plecoglossus altivelis*. These bacteria seem to be established in the intestine a certain period after hatching, because the embryo of normal animals is known to be sterile. YOSHIMIZU *et al.*³⁾ reported that normal flora in the intestine of adult salmonids is found after the stage of advanced fry. In this paper, we shall describe the development of microflora, especially anaerobes as observed in fry to young of *Tilapia mossambica*.

Sample fish were reared from egg stage in an aquarium equipped with a recirculating water system at 23.5 to 27.1°C. Fish were collected after 4 days (body weight, 11–22 mg), 20 days (53–80 mg), 2 months (106–275 mg), 4 months (1,060–1,890 mg), 6 months (19.8–29.5 g) and 8 months (39.0–39.4 g) of hatching. Fish were soaked in 70% ethanol to prevent the contamination from the body surface. In small fish, the operation to obtain the intestine was performed under a microscope with low magnification. Whole intestines of three to ten fish were aseptically homogenized and dilute quantitatively with diluent solution A. The plating media used in this experiment included Trypticase soy agar (BBL) with 5% FILDES solution (TSF), Cagar,⁴⁾ dEG-D agar,¹⁾ N-DF agar,⁵⁾ AS agar,²⁾ BS agar,²⁾ GAM agar (Nissui) and *Bacteroides* agar (Nissui). Inoculated TSF, C and dEG-D plates were in-

cubated aerobically, and dEG-D, N-DF, AS, BS, GAM and *Bacteroides* plates, anaerobically. Anaerobiosis was established by evacuating the atmosphere of a jar containing steel wool covered with reduced copper and replacing it with CO₂ gas. After incubation at 20°C for 5 days, colonies appearing on each plate were counted. 20 to 40 colonies were isolated from each plate at random and classified based on the characteristics of gram reaction, cell morphology, aerobic growth and spore formation. The maximum colony counts of bacteria belonging to different components in the intestine of tilapia fish are shown in Table 1.

Bacteria found in the intestine of *T. mossambica* were composed of *Bacteroides* type A, other *Bacteroidaceae*, clostridia, aerobic and facultatively anaerobic gram-negative rods, streptococci, other gram-positive cocci and coryneforms. Although the dominant components in the 4th- and 20th-day fishes consisted in aerobic and facultatively anaerobic gram-negative rods, gram-positive cocci and coryneforms, *Bacteroides* type A and aerobic and facultatively anaerobic gram-negative rods were predominant after 2 months. However, *Bacteroides* type B which could be selectively isolated from *T. nilotica* with BS and *Bacteroides* agars containing colistin and fradiomycin,²⁾ was not isolated from *T. mossambica* in this study. YOSHIMIZU *et al.*³⁾ found that the normal flora of adult was established after the stage of advanced fry in salmonid fish. Our results obtained, also show that the normal flora of adult tilapia may be established in the fish 20 to 60 days after hatching. It is assumed that the establishment of adult flora might be related with the structural and functional development of intestine, and that these bacterial components might originate from water, diets, sediment and/or feces. Further study concerning this problem should be performed.

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Table 1. Colony counts (log No. g⁻¹) of the bacteria belonging to different components in the intestine of *T. mossambica* 4th- and 20th-days, and 2nd-, 4th-, 6th- and 8th-months after hatching

Component	The duration after hatching					
	4-d*1	20-d	2-m*2	4-m	6-m	8-m
Obligate anaerobes						
<i>Bacteroides</i> type A	—*3	4.4	6.5	7.8	8.9	8.3
Other <i>Bacteroidaceae</i>	—	4.5	—	—	—	—
Clostridia	—	5.0	2.0	—	—	—
Aerobes and facultative anaerobes						
Gram-negative rods	8.3	7.1	7.3	9.0	9.1	8.8
Streptococci	—	2.1	2.9	—	—	—
Gram-positive cocci	7.3	5.4	—	—	—	—
Coryneforms	7.9	2.6	—	—	—	—

*1 Days. *2 Months. *3 Not detected.

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*1 ティラピア *Tilapia mossambica* の稚魚期における腸内細菌相。

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