The gastrointestinal microflora structure in the marine finfish

ZHOU Zhi-gang[1*], Bin YAO [1], SU Yong-quan[2], Wang Zhengnan[1]

([1] Feed Research Institute of Chinese Academy of Agricultural Sciences, Beijing 100081; [*]Email: zhouzhigang@caas.net.cn, yaobin@mail.caas.net.cn)

([2]Marine & Environ. College of Xiamen University, Xiamen, Fujian, 361005)

The marine finfish aquaculture in China, which is the vital part in the exploitation of marine resources, is confronted with two key problems: ① the shortage of fingerlings due to their higher mortality; ② the use of antibiotics for food safety issues of the products.

The supplement of exterior digestive enzyme in juvenile stage or alternatives of antibiotics in aquaculture are as one of the potential approaches to resolve the problems. Subsequently, the special, efficient, and indigestious prebiotics, which could excrete the functional enzymes or produce the anti-bacteria matter, are highlighted in marine finfish aquaculture because of the popular cage-cultured mode. However, the clarification of the gastrointestinal microflora structure in the marine finfish is the presupposition in search of the indigestious prebiotics, which is also the main research task of our lab in 2005-2006.

As we know, the usual TSA culture method in studying the microflora has shown many shortcomings, e.g., less than 5% bacteria in nature might be cultured in lab, therefore, the molecular identification of the gastrointestinal microflora structure in the marine finfish is urgent prior to the search of the indigestious prebiotics, in which the predominant bacteria or yeast might be the potential functional microflora to the juvenile survival or the health of the growing fish. We are using 16S rDNA PCR-DGGE (denaturant gradient gel electrophoresis) to compare the gene fingerprint of the gastrointestinal predominant bacterial community in *Lutjanus sebae, Ephippus orbis, Epinephelus awoara, Trachinotus Ovatus, Mycteroperca tigris, Lutjanus erythopterus,* and *Rachycentron canadum*, and then sequencing the 16S rDNA to identify the bacteria species in Genebank. Out of question, the indigestious prebiotics for the fingerlings or the cage-cultured marine finfish are the final aim of our research.

As part of the research results, the PCR-DGGE fingerprints of *Lutjanus sebae* are shown as below:

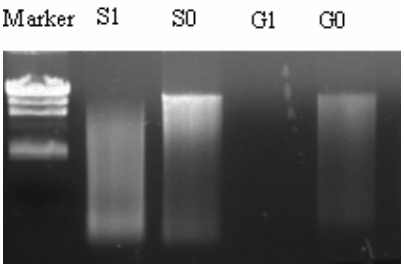

Fig. 1 Agarose gel electrophoresis of the community DNA extracted from the digestive tract of *Lutjanus sebae*

S1: the stomach content sample of *Lutjanus sebae*; S0: the stomach wall sample of *Lutjanus sebae*; G1: the intestinal content sample of *Lutjanus sebae*; G0: the intestinal wall sample of *Lutjanus sebae*; Marker: 3μl hind Ⅲ

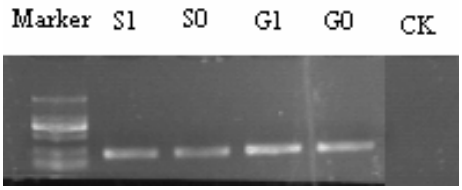

Fig. 2 Agarose gel electrophoresis of the community DNA extracted from the gastro-intestine of *Lutjanus sebae*

S1: the stomach content sample of *Lutjanus sebae*; S0: the stomach wall sample of *Lutjanus sebae*; G1: the intestinal content sample of *Lutjanus sebae*; G0: the intestinal wall sample of *Lutjanus sebae*; Marker: 3μl D200; CK: negative control

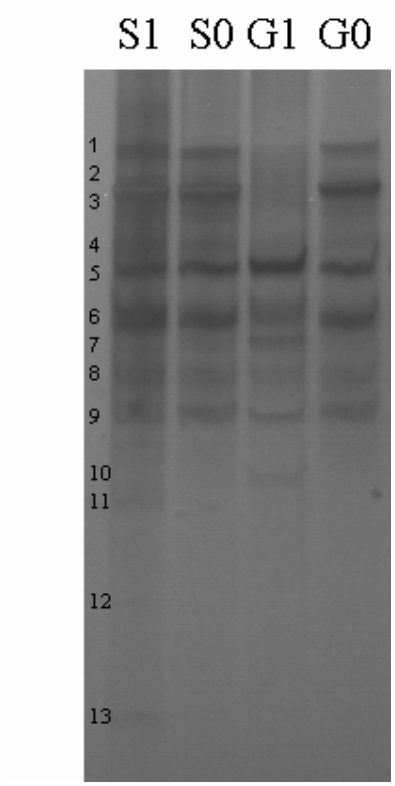

Fig. 3 The PCR-DGGE fingerprint of the V3 region of 16S rDNA of the bacteria from the gastro-intestine of *Lutjanus sebae*

S1: the stomach content sample of *Lutjanus sebae*; S0: the stomach wall sample of *Lutjanus sebae*; G1: the intestinal content sample of *Lutjanus sebae*; G0: the intestinal wall sample of *Lutjanus sebae*; 1-13: the band position

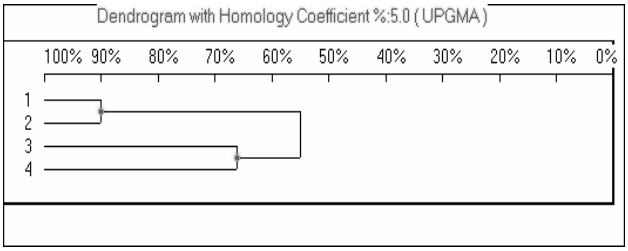

Fig. 4 The dendrogram of the PCR-DGGE fingerprint of the V3 region of 16S rDNA of the bacteria from the gastrointestine of *Lutjanus sebae*

1: the stomach content sample of *Lutjanus sebae*； 2: the stomach wall sample of *Lutjanus sebae*； 3: the intestinal content sample of *Lutjanus sebae*； 4: the intestinal wall sample of *Lutjanus sebae*

Tab.1 The relative abundance of predominant band in the PCR-DGGE fingerprint of the bacteria from the gastro-intestine of *Lutjanus sebae* (%)*

Band position	**S1**	**S0**	**G1**	**G0**
1	11.1	10.9	-	8.1
2	6.1	7.0	-	-
3	9.6	13.9	-	20.5
4	8.4	7.7	-	9.6
5	15.4	17.5	27.7	17.6
6	20.6	21.2	27.4	21.5
7	-	-	17.2	-
8	11.6	12.7	12.3	11.1
9	9.2	9.1	9.2	11.6
10	-	-	6.2	-
11	3.2	-	-	-
12	0.9	-	-	-
13	3.7	-	-	-
c.v.	59.7	36.9	50.3	35.4

Note: S1: the stomach content sample of *Lutjanus sebae*； S0: the stomach wall sample of *Lutjanus sebae*； G1: the intestinal content sample of *Lutjanus sebae*; G0: the intestinal wall sample of *Lutjanus sebae*; Band position: all the bands listed as the order from the top to bottom

The results showed those abundant bacteria were existed in the digestive tract of *Lutjanus sebae*, including the stomach wall, the stomach content, the intestine wall, and the intestinal content. The similarity was above 55% in the community structures in the gastrointestinal tract with the highest similarity of 90% in the bacteria ingredients between the stomach wall and the stomach content, which might reflect the diet fed processed from the stomach to the intestine. However, the difference of the relative abundance in the bacterium between the stomach wall and the intestinal wall might due to their different physical environment. The establishment and comparison of the 16S rDNA-DGGE fingerprint in the stomach and intestine of the finfish do help to elucidate the microflora structure in the gastro-intestine in *Lutjanus sebae*.