Mem. Fac. Fish., Kagoshima Univ.
Vol. 28 pp. 35~40 (1979)

Microflora in the Alimentary Tract of Gray Mullet-VI

Identification of the species of Vibrio and Enterobacter

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Abstract

Bacterial isolates of *Vibrio* and *Enterobacter* isolated from the alimentary tract of gray mullet (*Mugil cephalus*), living in sea water and in fresh water were examined. Morphological and physiological recognizations of two predominant genera were subjected to calculate the similarity values. The data obtained in this study indicated that the isolates of *Vibrio* were similar to *Vibrio anguillarum* and the isolates of *Enterobacter* showed similarity to *Enterobacter aerogenes*. The characteristic features of these two isolates are presented.

This is an attempt to gain more specific details on the normal and major two types of intestinal bacteria, *Vibrio* and *Enterobacter*, since they were supposed to be important and useful for the nutrition of gray mullet[1-5], living in sea and fresh water. This study described the identity of the isolates of *Vibrio* and *Enterobacter*.

Materials and Methods

Bacteria and their growth conditions. Details of experimental plan, methods used, chemicals and media employed have been published[1-5] or are in press. Thirteen standard strains of *Vibrio* and three standard strains of *E. coli* were used from the stock cultures which were received from Hokkaido University.

Characterizations. The common morphological and physiological tests including Kovac's oxidase test, fermentation test in Hugh-Leifsons medium, reduction of nitrate, production of indole and hydrogen sulfide, reduction of methylene blue and hydrolysis of chitin, gelatin, casein and tween 80 were carried out according to the standard methods described by Harrigan et al.[6]. Growth at 42 C was determined in 1 % peptone water with 2 % NaCl. Growth at different concentrations of NaCl was tested using 1 % peptone solution at pH 7.2.

Biochemical tests used to study the isolates of *Vibrio* were as follows. Ferm-

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entation of sugars (glucose, sucrose, mannose, arabinose, maltose and fructose) and decomposition of alcohols (mannitol, inositol, sorbitol and adonitol), were investigated in Hugh-Leifson's medium.

Decarboxylation of three amino acids, i,e lysine, arginine and ornithine were examined by the method of Moeller[7].

Similarly the main biochemical characteristics of the isolates of *Enterobacter* were examined following the tests for sucrose, arabinose, adonitol, mannitol and decarboxylases for arginine, ornithine and lysine.

The similarity values were calculated by the following equation; $\%s = nsp/(nsp + nd) \times 100$, where $\%s$ = similarity coefficient ; nsp = number of similar positive matches and nd = number of dissimilar matches.

Results and Discussion

The characteristics of five isolates of *Vibrio* isolated from the alimentary tract of gray mullet were compared with thirteen standard strains of *Vibrio* including *Vibrio parahaemolyticus* (6 strains), *Vibrio anguillarum* (3 strains), *Vibrio alginolyticus*

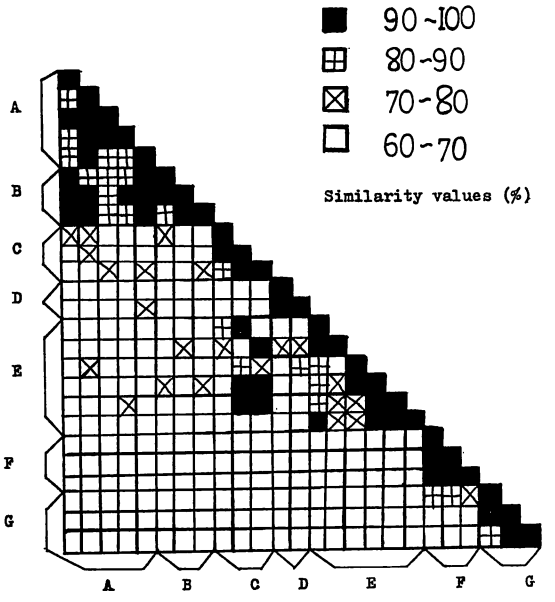

Fig. 1 Similarity diagram of *Vibrio*, *Enterobacter* and standard strains.

A—tested strain (*Vibrio*)
B—*Vibrio anguillarum*
C—*Vibrio alginolyticus*
D—*Vibrio fischeri*
E—*Vibrio parahaemolyticus*
F—tested strain (*Enterobacter*)
G—*E. coli*

(2 strains) and *Vibrio fischeri* (2 strains). The *Vibrio* isolates from the fish were characterised by the standard confirmative test procedures. The comparison

Table 1. Some comperative properties of the species of genus *Vibrio* and tested isolates isolated from the alimentary tract of mullet.

Characteristic features	Tested isolates	*Vibrio parahaemolyticus*		*Vibrio anguillarum*		*Vibrio alginolyticus*		*Vibrio fischeri*	
Form	rod	5*	rod	6*	rod	3*	rod	2*	rod	2*
Gram stain	—	5	—	6	—	3	—	2	—	2
Motility	+	5	+	6	+	3	+	2	+	2
Flagellation	M	5	M	6	M	3	M	2	M	2
Oxidase	+	5	+	6	+	3	+	2	+	2
Catalase	+	5	+	6	+	3	+	2	+	2
Methyl red	+	5	+	6	+	3	+	2	+	2
Indole	+	5	+	6	+	3	+	2	—	2
Citrate utilization	—	5	+	6	—	3	+	2	—	2
Reduction of nitrate	+	5	+	6	+	3	+	2	+	2
H_2S production	—	5	—	6	—	3	—	3	—	2
0/129 sensitivity	+	5	+	6	+	3	+	3	+	2
Hydrolysis of										
gelatin	+	5	+	5	+	3	+	2	+	2
casein	+	5	+	5	+	3	+	2	+	2
lecithin	+	5	+	5	+	3	+	2	+	2
chitin	+	5	+	6	+	3	+	2	+	2
starch	+	5	+	6	+	3	+	2	+	2
tween 80	—	4	+	5	—	3	+	2	+	2
Acid from										
sucrose	+	4	—	5	+	3	+	2	—	2
mannose	—	4	+	5	+	3	+	2	+	2
arabinose	—	4	+	5	—	3	+	2	—	2
glucose	+	5	+	6	+	3	+	2	+	2
fructose	—	4	+	6	—	2	+	2	+	2
maltose	—	5	+	6	+	3	+	2	+	2
lactose	—	5	—	6	—	3	—	2	—	2
dextrin	—	5	+	6	—	3	+	2	+	2
adonitol	—	5	—	6	—	3	+	2	+	2
mannitol	—	5	+	6	+	2	+	2	+	2
inositol	—	5	—	6	—	2	—	2	—	2
glycerol	—	3	+	6	—	3	+	2	—	2
Decarboxylase										
lysine	—	5	+	6	—	3	+	2	+	2
arginine	+	5	—	6	+	3	—	2	—	2
ornithine	—	5	+	6	—	3	—	2	—	2
Growth without NaCl	+	5	—	6	+	3	—	2	—	2
Growth with 7% NaCl	—	5	+	6	+	3	+	2	+	2
Growth with 10% NaCl	—	5	—	6	—	3	—	2	—	2
Growth at 37C	+	5	+	6	—	3	—	2	—	2
Growth at 42C	—	5	—	6	—	3	—	2	—	2
Growth at pH										
4.5	+	5	—	6	—	3	—	2	—	2
5.5	+	5	+	6	+	3	+	2	+	2
9.5	+	5	+	6	+	3	+	2	+	2
Total strains		5		6		3		2		2

* Number of strain used for tests.

between *Vibrio* isolates and the standard strains based on the main characteristics is shown in the Table 1. The similarity values among them were calculated from these results.

The indole test together with other major biochemical characteristics, such as, abilities to ferment glucose, arabinose, sucrose and tolerance to sodium chloride were very important in identification of strains isolated from the intestine of fish, from related true marine *Vibrios*.

Table 2. Characteristics of the species of *Enterobacter* and tested isolates.

Chracteristics	Tested isolates		*E. coli*		*Enterobacter cloaca*	*Enterobacter aerogenes*
Form	rod	3*	rod	3*	rod	rod
Gram stain	−	3	−	3	−	−
Motility	+	3	+	3	+	+
Flagellation	p	3	p	3	p	p
Oxidase	−	3	−	3	−	−
Catalase	+	3	+	3	+	+
Nitrate reduction	+	3	+	3	+	+
H_2S production	−	3	−	3	−	−
V. P.	+	3	−	3	−	−
M. R.	−	3	+	3	−	+
Citrate urilization	+	3	−	3	+	+
Indole production	−	3	+	3	−	−
Hydrolysis of						
starch	+	3	−	3	−	+
casein	+	3	−	3	+	+
gelatin	+	3	−	3	+	+
lecithin	+	3	−	3	+	+
Gas from glucose	−	3	+	3	+	−
Acid from						
glycerol	+	3	+	3	+	+
lactose	+	3	+	3	+	+
sucrose	−	3	+	3	+	+
mannose	−	3	−	3	+	+
arabinose	+	3	+	3	+	+
fructose	−	3	+	3	+	−
galactose	−	3	+	3	+	+
inositol	+	3	−	3	−	+
adonitol	+	3	−	3	+	+
Decarboxylase						
arginine	−	3	−	3	+	−
lysine	+	3	+	3	−	+
ornithine	+	3	−	3	+	+

* Number of strain used for tests.

The bacteria sometimes showed different behaviour in major characteristics, including salt tolerance, growth at pH 4.5, growth at 37 C, gas production from carbohydrates. Differentiations of isolates of *Vibrio* in salt tolerance, temperature and low pH (4.5) indicate the characteristics of intestinal bacteria of fish living in fresh water and sea water, like gray mullet.

Some other biochemical characteristics, such as, decarboxylases for arginine, lysine and ornithine of *Vibrio* isolates were much similar to those of *Vibrio anguillarum*, used for standard strain, similarity diagram shown in Fig. 1, indicates that there is a relatively higher similarity with *Vibrio anguillarum*, a standard strain with the isolates of *Vibrio*. Thus there was a similarity agreement between *Vibrio* isolates and the strains of *Vibrio anguillarum*, which comprised the examinations of biochemical, physiological, morphological and staining properties.

From the characteristics described above, it was evident, therefore, that the *Vibrio* isolates, which carried out the characteristics features were similar to *Vibrio anguillarum*.

Table 2, shows the characteristics of the isolates of *Enterobacter* and *E. coli*. The described characteristics of *Enterobacter cloaca* and *Enterobacter aerogens* are also shown in the table only for the comparison. The study showed that all the isolates of *Enterobacter* isolated from the intestine were gram negative rods. All cultures were motile, negative for cytochrome oxidase and did not grow in peptone water with 2 % NaCl. H_2S and indole production were negative. Gas production from glycerol and adonitol were positive. Lysine and ornithine decarboxylase were produced in Moller's decarboxylase medium. Other characteristics were positive for all *Enterobacter* isolates.

It can be concluded from the data and the similarity diagram obtained in the study and that appearing in the literatures that the isolates of *Enterobacter* were similar to *Enterobacter aerogens* and the isolates of *Vibrio* were similar to *Vibrio anguillarum*, which supposed to be the important and helpful for the nutrition of gray mullet. Most recently, intestinal *Vibrio* isolated from the king salmon (*Oncorhyncus tschawytscha*) and some strains of family *Enterobacteriaceae* from the masou salmon (*Oncorhynchus masou*) were identified as *Vibrio fischeri* and *Hafnia alvei* respectively[8]. This discripancy is may be due to the different fish obtained from a muddy shallow waterstream following through the city, carries the polluted city drainage water during the tropic season of the year.

Additional studies are needed to identify the isolates of *Vibrio* and *Enterobacter* in more details. Ultra microscopic observation and pathogenic characteristics on these two types of isolates are in progress.

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