Mem. Fac. Fish., Kagoshima Univ.
Vol. 27, No. 1, pp. 73~78 (1978)

Microflora in the Digestive Tract of Marine Fish-II

Similarity between the Isolates and *Vibrio* Standard Strains

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Abstract

Vibrio species predominated in the intestines of marine fishes (26 strains) and *Vibrio* standard strains (15 strains) were examined using 120 bacteriological tests. The results were subjected to calculate the similarity values. There was a considerable number of differential characters between both groups. Phenotypic data obtained in this experiment indicated that the similarity among intestinal *Vibrio* species from different fishes was relatively higher than that between fish *Vibrio* and *Vibrio* standard strains.

It is well known that the indigenous microflora of marine fishes is made up of *Vibrio* species exclusively. *Vibrio* species are also commonly isolated from the coastal environment. In the Bergey's Manual[1] (8th ed.) are listed only 5 species for the genus *Vibrio*, nemely, *V. cholerae*, *V. parahaemolyticus*, *V. anguillarum*, *V. fischeri* and *V. costicola*. The objective of this study is to characterize the intestinal *Vibrios* from marine fishes and to determine the taxonomic relationship with *Vibrio* standard strains (type strains).

Materials and Methods

Bacterial strains. Bacterial strains used in this study are listed in Table 1. The fish strains were isolated from yellowtail (*Seriola*) cultured in Kinko Bay (1974, 1975), skipjack (*Katsuwonus*) and tuna (*Thunus*) caught in the southwest Pacific Ocean (1975, 1976). The representative strains were selected among the predominant groups in the intestines as being based on the main characteristics and employed in the subsequent experiments. *Vibrio* standard strains were supplied by Dr. TAKAGI, Hokkaido University.

Media used and cultural conditions. The compositions of basal media and cultural conditions were described previously[2]. All test cultures were inoculated from one day old culture in broth medium by use of a sterile needle.

Morphological and Physiological tests. The regular tests in bacteriology were

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Table 1. Isolation and Selection of Bacterial Strains

Fish sample	Year		Bacterial count	Isolated	Selected	
Yellowtail	2.0 kg	1974	5.9×10^6/g	50	Y I*	3 strains
Yellowtail	0.4	1975	1.0×10^7	54	Y I	4
			1.0×10^7	47	Y S	4
			1.6×10^6	38	Y F	3
Skipjack	4.3	1975	1.9×10^6	22	K I	2
Skipjack	2.3	1975	8.4×10^6	31	K I	4
Tuna	12.0	1976	2.0×10^6	28	T I	6
Vibrio standard strains						
	Vibrio fischeri					2
	Vibrio anguillarum					3
	Vibrio alginolyticus					3
	Vibrio parahaemolyticus					7
Total strains studied						41

* Y I, intestine of yellowtail; YS, stomach of yellowtail
Y F, food of yellowtail; KI, intestine of skipjack
T I, intestine of tuna.

carried out according to the standard methods described by HARRIGAN et al.[3].

Biochemical tests. Hydrolysis of macromolecules was tested using the methods described previously[2]. Zone of hydrolysis was detected after incubation for 3–7 days by flooding with mercuric chloride solution, Lugol solution and alcohol for gelatin, starch and alginate hydrolysis, respectively. Fermentation of carbohydrates was examined in Durham fermentation tubes and medium containing 1.0 % peptone (Daigo Eiyo), half strength ASW, 1.0 % carbohydrate and indicator (BTB). The ability to decarboxylate lysine, ornithine, arginine and glutamic acid was examined by using the method of MOELLER[4]. The ability to utilize nitrogen sources was determined by using the medium containing (g/*l*) glucose (10), KH_2PO_4 (1), $MgSO_4\cdot7H_2O$ (0.5) KCl (0.2), NaCl (15) and nitrogen source (1), pH 7.5. The sensitivity to various antibiotics was tested by a disc method using tri-discs (Eiken).

Results and Discussion

The predominant *Vibrio* species (26 strains) isolated from 3 kinds of marine fishes were compared with *Vibrio* standard strains including *V. parahaemolyticus* (7 strains), *V. alginolyticus* (3), *V. fischeri* (2) and *V. anguillarum* (3). The similarity values among them were calculated from the results obtained with 120 bacteriological tests. They were identical with respect to acid production from glucose, 0/129 sensitivity and chitin hydrolysis. These tests, however, are characteristics of the genus *Vibrio*.

The comparison between *Vibrio* isolates and standard strains based on main

Table 2. Physiological Chacteristics

Character		Fish isolates	*V.* standards
Colony form	Rough	10	0
	Smooth	14	12
	Swarm	2	3
Growth at pH	4.5	14	0
	5.5	26	14
	9.5	26	15
Growth at 42 C		10	0
Oxidase		22	15
Catalase		17	13
Indole		2	9
Nitrate reduction		23	15
V. P.		16	3
M. R.		24	15
Hugh & Leifson	F+G*	12	0
	F	14	15
0/129 sensitivity		25	15
Urease		6	0
Total strains		26	15

F+G, acid and gas production, F, acid production.

Table 3. Fermentation of Carbohydrates

Carbohydrates	Isolates		*V.* stand.	
	F+G*	F*	F+G*	F
Glucose	14	11	0	15
Xylose	1	3	0	0
Arabinose	0	0	0	0
Rhamnose	0	1	0	0
Fructose	15	9	0	15
Galactose	16	6	0	8
Mannose	15	11	0	15
Cellobiose	4	10	0	12
Sucrose	2	5	0	11
Maltose	11	7	0	8
Lactose	0	1	0	1
Trehalose	11	3	0	15
Raffinose	0	0	0	0
Dextrin	9	6	0	12
Starch	8	6	0	12
Glycogen	19	0	2	10
Inulin	4	18	0	8
Adonitol	0	0	0	0
Mannitol	1	3	0	15
Sorbitol	0	0	0	3
Inositol	0	0	0	0
Salicin	0	0	0	0
Total strains	26		15	

F+G, acid and gas production
F, acid production from carbohydrates.

Table 4. Nitrogen Source Utilization

Compounds	Isolates	*V.* standards
KNO_3	1	2
NH_4Cl	13	12
NH_4NO_3	13	12
Casamino acid	25	13
Phe	2	7
Tyr	6	9
Trp	2	9
Asp	7	10
Asn	20	12
Lys	9	13
Met	4	10
Thr	12	12
Ilu	2	9
Glu	9	10
Gln	3	10
Pro	2	8
Arg	15	12
Gly	9	9
Ser	11	12
Cys	14	10
Ala	5	10
Leu	6	11
Val	11	12
His	14	10
Total strains	26	15

Table 5. Hydrolysis of Macromolecules

Macromolecules	Fish isolates	*Vibrio* standards
Casein	9	13
Gelatin	11	15
Starch	1	13
Glycogen	1	13
Tributyrin	26	15
Tween80	12	14
Chitin	23	13
Cellulose	0	0
alginate	0	0
Total strains	26	15

characteristics is shown in Table 2, 3, 4, and 5. There was a considerable number of differential characters between fish *Vibrio* and *Vibrio* standard strains including colony form on agar plate, growth at pH 4.5, growth at 42 C, gas production from carbohydrates, ability to utilize amino acids, ability to hydrolyze casein and starch and so on. *Vibrio* species predominated in fish intestine had the tendency to be resistant to low pH (pH 4.5) and high temperature (42 C). Moreover, most of them were unable to hydrolyze casein, starch and glycogen and unable to utilize some kinds of amino acids as sole nitrogen source

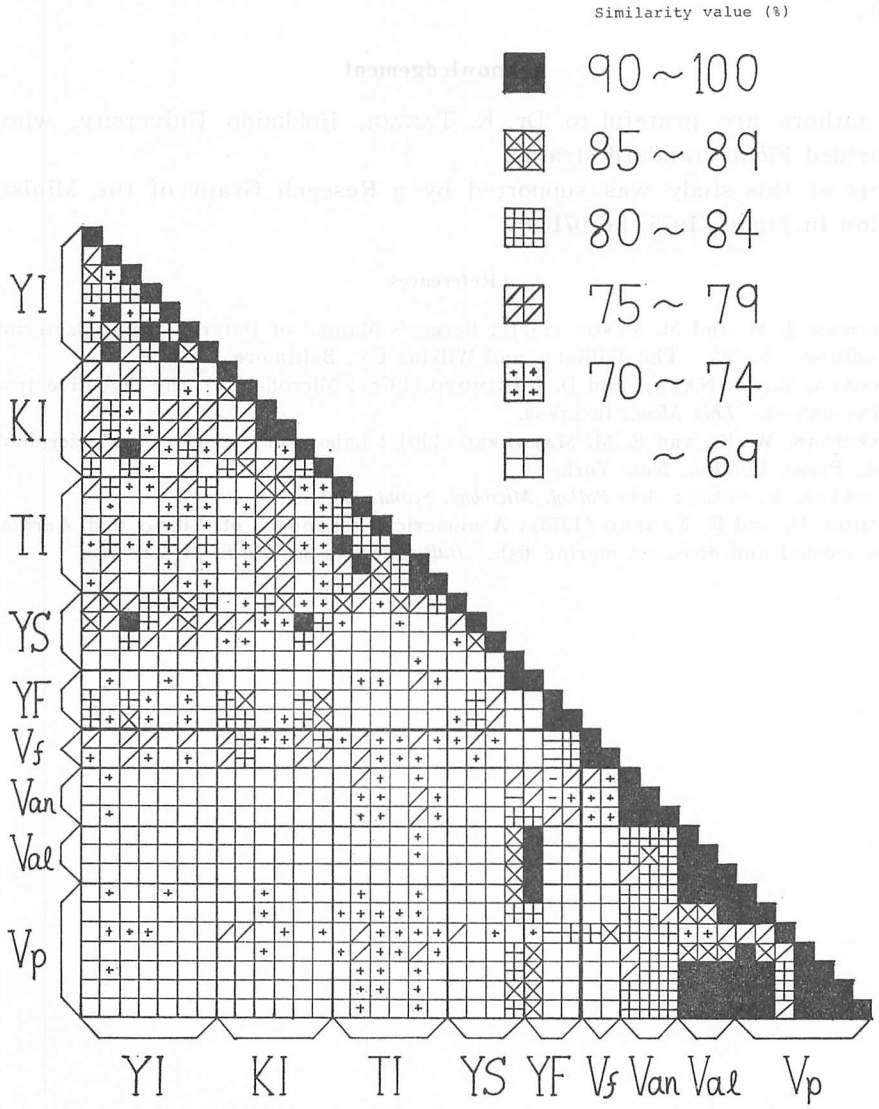

Fig. 1. Similarity Diagram of *Vibrio* isolates and Standards.

in contrast to *Vibrio* standard strains. Similarity diagram shown in Fig. 1 indicates that there is relatively higher similarity among the predominant *Vibrio* strains in the intestine of marine fishes than that between *Vibrio* species isolated from fishes and *Vibrio* standard strains.

SIMIDU et al.[5] analyzed 114 strains of *Vibrio* and allied genera isolated from sea water, normal marine fish and diseased fish and divided them into two large, distinct groups (phenons). The *Vibrio* species isolated from the marine fish intestines in this study showed a considerable similarity with phenon 1 proposed by SIMIZU et al.[5] in spite of diversity in casein, gelatin and starch hydrolysis.

Acknowledgement

The authors are grateful to Dr. K. TAKAGI, Hokkaido University, who kindly provided *Vibrio* standard strains.

A part of this study was supported by a Reseach Grant of the Ministry of Education in Japan (1975, no 07169).

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