Enterotoxigenic Substance and Other Toxins Produced by *Vibrio fluvialis* and *Vibrio furnissii*

Masatsugu CHIKAHIRA and Kokichi HAMADA

Division of Microbiology, Public Health Institute of Hyogo Prefecture, 2-1, Arata-cho, Hyogo-ku, Kobe 652, Japan

(Received 27 July 1987/Accepted 12 May 1988)

ABSTRACT. Cultures of *Vibrio fluvialis* and *Vibrio furnissii* isolated from the environment (40 strains) and diarrheal humans (8 strains) were examined for enterotoxicity and other biological activities. The CHO-K1 cell-elongation factor (CEF) was found in 18 and the cell-killing factor (CKF) in 39 of the 48 strains. CEF produced by two strains of *V. fluvialis* was appreciably neutralized by anti-cholera toxin serum (ACT) but CKF was not. Live cultures of all the human strains tested (3/3) and some environmental strains (8/16) caused fluid accumulation in rabbit ileal loops; the activity shown with concentrated ($\times 10$) filtrate was not neutralized by ACT. Cholera toxin (CT)-like substance was detected by ELISA in 28 of 38 cell-free extracts of *V. fluvialis* and *V. furnissii*. The correlation between CEF and CKF was discussed. These results and ELISA suggest that CEF has an immunological relation to CT and is distinct from CKF. None of culture filtrates of the 48 strains caused fluid accumulation in suckling mice. Most strains had mouse-lethal and hemolytic activities.—**KEY WORDS:** CHO cell assay, cholera toxin, enterotoxicity, *Vibrio fluvialis*, *Vibrio furnissii*.

Jpn. J. Vet. Sci. 50(4): 865–873, 1988

Vibrio fluvialis is widely distributed in the marine environment and marine products [4, 10, 16]. The organism is a causative agent of clinical infections and frequently isolated from patients with diarrhea [6, 8, 9, 17]. We previously isolated the organism from shellfishes at a very high rate and suggested the possibility of food-borne poisoning by this organism [4].

Some authors have indicated the presence of diarrhea causing enterotoxin in cultures containing live cells [15, 16] or culture supernatant fluids of *V. fluvialis* [15] in the rabbit ileal loop model. As do such other diarrheal agents as *Vibrio cholerae* (01 [5, 7] and non-01 [18, 19, 20]) and *Aeromonas hydrophila* [1, 3], *V. fluvialis* produces several kinds of toxins including cell-elongation factor (CEF), cell-killing factor (CKF), hemolysin [9, 11], and a mouse lethal factor. None of these factors, however, has definitely been correlated with the diarrheal activity, and similarity and dissimilarity between the related substances and

the factors are also unclear. Lockwood *et al.* [11] showed the presence of such factors as CEF, CKF and hemolysin in the culture supernatant and cell free-extract of *V. fluvialis* and estimated that its enterotoxin is cholera toxin (CT)-like CEF; but they did not try to correlate CEF with the diarrheal activity.

To examine enterotoxin and other toxins for the roles in the pathogenicity of *V. fluvialis*, it is imperative to clarify the factor accounting for the diarrheal activity. This should be helpful also in establishing a rapid assay method for enterotoxin of *V. fluvialis*. In this paper, we report the various biological activities of *V. fluvialis* and *V. furnissii* isolated from marine products and diarrheal humans and the possible relation of these activities with the diarrheal function.

MATERIALS AND METHODS

Bacteria: Thirty-nine strains of *V. fluvialis* and nine of *V. furnissii* (40 from shellfishes

and eight from diarrheal humans) were used in this study. The two species were involved in the environmental isolates; whereas *V. fluvialis* only in the clinical isolates.

Culture of bacterial strains: Single colonies were transferred to tubes containing 5 ml of brain heart infusion broth (Difco) supplemented with 1.0% NaCl (BHI) or Casamino acid-Yeast extract broth (CAYE). They were incubated at 37°C for 6 hr. Of these cultures, 0.1 ml amounts were transferred to 20 ml test tubes containing 10 ml of medium (BHI or CAYE). They were incubated with shaking at 50 cycles per min at 30°C for 16 hr and assayed.

Preparation of assay samples: Bacterial cultures were centrifuged at $17,000\times g$ for 30 min at 4°C, and the supernatant fluids were filtrated through membrane filters (pore size 0.45 μm , Millipore Corp.). For the cell-free extracts, the cell pellets were washed with phosphate buffered saline (PBS), resuspended in 1.0 ml of PBS containing 10,000U polymyxin B sulfate (Taito Phizer), and disrupted by sonication for 30 sec. The cell debris was removed by centrifugation at $17,000\times g$ for 30 min at 4°C.

CHO tissue culture assay: The culture supernatant and cell-free extract were examined for cytotoxic and cytotoxic activities on an established cell line, CHO-K1 (Dainippon Seiyaku) [5, 11]. The harvested CHO cells were washed twice with MEM containing 1% fetal calf serum, resuspended in the same medium at a density of 10^4 cells/ml, and 0.1 ml aliquots were plated in the 96 wells of the tissue culture microplate (Corning), which were incubated for 6 hr at 37°C. After removal of the medium, 10 μl of each culture supernatant or bacteria-free extract was added to 5 wells. In 20 min at 37°C, 0.1 ml of MEM containing 0.5% fetal calf serum was added to each well. The cells were incubated for 16 hr at 37°C, Giemsa stained, and examined for elongation and necrosis.

Rabbit ileal loop test: The live bacteria suspended in 10% skim milk (about 10^9 cells/loop) were inoculated into 10 cm rabbit ileal loops [13, 15]. The inoculated rabbits were sacrificed 15 hr later and the loops were examined for fluid accumulation. FA ratio was defined as the mean volume of fluid/cm of the loop.

Preparation of antisera against purified CT: Rabbit (Japanese white) and guinea-pig (Hartley) antisera against purified CT (Sigma) were prepared by the method of Ristaino *et al.* [14]. IgG and IgM of the guinea-pig serum were partially purified by column chromatography on DEAE-Sephacel (Pharmacia Fine Chemicals) followed by salting out with ammonium sulfate.

Enzyme-linked immunosorbent assay (ELISA) for detection of CT [14]: Wells of a polystyrene 96-well multiplate (Dynatech) were coated each with 50 μl of guinea-pig anti-CT IgG or IgM (10 $\mu g/ml$) in 0.01M carbonate buffer (pH 9.5) at 4°C overnight. The plates were then washed with 0.1% Tween 20-containing PBS and unbonded reaction sites were blocked with 1.0% bovine serum albumin. The plates were washed and 50 μl of a test sample or purified CT (Sigma) was added to the well. After incubation for 60 min at 37°C and washing, 50 μl of rabbit anti-CT serum was added to each well. The plates were incubated for 60 min at 37°C. After washing, 50 μl of peroxidase-labeled goat anti-rabbit IgG (Kirkegaard & Perry Lab., 0.2 $\mu g/ml$) was added to each well and the plates were incubated under the same conditions. After final washing, 50 μl of o-phenylenediamine (12.5 mg/5 ml) was added to each well and the plates were incubated for 15 min at room temperature. The reaction was stopped by adding 50 μl of 1N H₂SO₄. Optical density (OD) of the reaction product was measured with a spectrophotometer at a wavelength of 495 nm. The net OD was

calculated by subtracting OD of control wells. The cut-off value was defined as follows: mean OD of negative samples $+0.15 \times$ mean OD of CT standard (20 ng/ml).

Hemolytic activity: For measurement of hemolytic activity, the reaction mixture contained about 0.5% rabbit or goose erythrocytes and 1.0 ml of culture filtrate. After incubation for 20 min at 37°C in a water bath, the mixture was centrifuged at 3,000 rpm for 5 min, and OD of the supernatant was measured for released hemoglobin with a spectrophotometer.

Suckling mouse assay: The procedure for assaying fluid accumulation in the intestine of the suckling mouse was similar to that of Baselski *et al.* [2]. The FA ratio of a sample was calculated from the weight of the pooled intestines and the body weight. The FA ratios were graded as follows: <0.05 as negative, 0.05–0.09 as gray, and >0.09 as positive.

Neutralization with antisera against CT: Neutralization tests were carried out by CHO cell assay or the rabbit ileal loop test. Serially diluted antisera were incubated for 1 hr at 37°C with either CT or a sample prepared from bacterial culture. The reaction mixture was transferred to a microtiter plate containing CHO cells or to rabbit ileal loops. The cells were then processed and observed by the conventional method described under Materials and Methods.

Mouse lethality test: A 1.0 ml dose of culture filtrate of *V. fluvialis* or *V. furnissii* was injected into the tail vein of mice (ddY male, weighing 19–21g). The lethal activity was defined as that killing the inoculated mouse within 24 hr.

RESULTS

A total of 48 strains of *V. fluvialis* and *V. furnissii* were examined for virulence by CHO cell assay (Table 1). Two types of

morphological changes (cell elongation and cell condensation) were elicited by CHO cells exposed to culture filtrate or cell-free extract (Fig. 1).

CEF changed the shape of CHO cells from round to spindle (Fig. 1D). Positive cell elongation was seen with 17 of the 48 strains (Table 1); whereas uninoculated BHI or CAYE contained no detectable CEF activity (10–15%). A similar phenomenon was observed in the CHO cells treated with CT, and such transformed cells could not be distinguished morphologically from those treated with a *V. fluvialis* or *V. furnissii* sample. As with CT (Fig. 2A), a dose response curves for CHO cell elongation was clearly demonstrated with either cell-free extract or culture supernatant (Fig. 2B). The morphological change of the cells to spindle shape was more strongly demonstrated with cell-free extract than with culture filtrate (Table 1). In the CKF-weak (E75) or negative strains (E99, E125, E170, E176, H3, H9, H10), samples (both culture filtrate and cell-free extract) from 16 hr cultures gave more transformed CHO cells than did those from 6 hr cultures.

Another change in the cell shape was caused by CKF. The cells were condensed and lost cytoplasm (Fig. 1C). Thirty-nine of the 48 strains had CKF activity, and the cell-killing phenomenon appeared more extensively in the cells treated with the culture filtrate than those with cell-free extract (Table 1). Of the 39 CKF-positive strains, only 10 had CEF. In some strains, both CKF and CEF were found in culture filtrate (E75 and E192) or cell-free extract (E70). It should be added that the CKF activity was not necessarily associated with either species, *V. fluvialis* or *V. furnissii*.

Eighty percent of CHO cells were changed from circular to spindle by purified CT (Fig. 1B, 2A). This activity was completely neutralized with diluted anti-CT (Fig. 3A). Neutralization tests of CEF and

Table 1. Cell-elongation and cell-killing factor in cultures of *Vibrio fluvialis* and *Vibrio furnissii*^{a)}

Strain	Source	Cell elongation (%) ^{b)} (elongated/counted)		ELISA ^{c)}	Cell killing	
		Filtrate	Cell extract	Cell extract	Filtrate	Cell extract
<i>V. fluvialis</i>						
E34	Environ- ment	—	—	+	+	+
E36		—	—	+	+	+
E41		—	—	+	+	+
E49		—	—	n.t. ^{d)}	+	+
E52		—	—	n.t.	+	+
E58		—	—	+	+	+
E62		—	—	+	+	+
E66		—	—	+	+	+
E67		—	88	+	+	—
E70		—	42	+	+	+
E75		—	95 94	+	—	—
E82		—	47	+	+	—
E86		—	—	—	+	—
E89		—	36	+	+	—
E90		—	43	+	+	—
E92		—	—	—	+	+
E99		—	43 48	+	—	+
E105		—	39	+	+	—
E115		—	—	—	+	+
E125	—	26 21	+	—	—	
E155	—	20	—	+	—	
E161	—	—	—	+	+	
E170	—	37 22	+	—	—	
E176	—	38 40	+	—	—	
E181	—	—	—	+	+	
E190	—	—	—	+	+	
E192	—	19 53	+	+	—	
E207	—	—	n.t.	+	+	
E235	—	—	—	+	+	
H1	Human	—	—	+	+	+
H2		—	—	+	+	+
H3		—	—	n.t.	+	+
H4		—	38	—	n.t.	—
H7		—	—	n.t.	+	+
H8		—	—	n.t.	n.t.	+
H9		—	—	n.t.	n.t.	+
H10		—	39	n.t.	n.t.	—
T1		—	37	—	n.t.	—
T15		—	—	—	+	+
<i>V. furnissii</i>						
E39	Environ- ment	—	—	+	+	+
E43		—	—	+	+	+
E47		—	—	n.t.	+	+
E55		—	—	—	+	+
E74		—	41	+	+	—
E83		—	—	+	+	+
E222		—	—	+	+	+
E233		—	—	+	+	+
T13		—	—	n.t.	+	+

a) Samples were prepared from 16 hr cultures.

b) CHO cell-elongation or killing activity was regarded as negative, if elongated or killed cells accounted for 15% or less in counted cells.

c) ELISA for detection of cholera toxin. Sera of rabbits and guinea-pigs immunized with purified cholera toxin were used for the assay.

d) Not tested.

Fig. 1. CHO cell appearance treated with *Vibrio fluvialis* samples and purified cholera toxin. A) Control cells, B) Cells treated with cholera toxin (5 ng/ml), C) Cells treated with culture filtrate (E181), D) Cells treated with bacteria-free extract (E75).

Fig. 2. Dose-response curves for the CHO cell elongation with *V. fluvialis* samples and purified cholera toxin. Morphological changes of CHO cells were examined after incubation for 16 hr. A) Purified cholera toxin, B) Culture filtrate of E75 (△—△) and bacteria-free extract of E67 (▲—▲) by serial two-fold dilutions.

Fig. 3. Neutralization of CHO-cell elongation factor in *V. fluvialis* samples with anti-cholera toxin. CHO cells were treated with samples neutralized by anti-cholera toxin and incubated for 16 hr at 37°C CO₂ incubator. A) Neutralization of cholera toxin, B) Neutralization of *V. fluvialis* samples (E75: △—△, E99: ▲—▲).

CKF with anti-CT were carried out. In these tests, CKF was not inactivated; on the contrary, CEF in culture filtrates was appreciably neutralized (Fig. 3B). A similar

neutralization pattern was observed with *V. fluvialis* samples, in which about 20% of the activity remained unneutralized although CEF of CT was neutralized completely with

10^0 to 10^2 -fold diluted anti-CT serum.

In ELISA, the mean OD values of the five CT-negative strains and CT standard (purified CT, 20 ng/ml) were 0.065 and 0.580, respectively. Therefore, the reaction was regarded as positive if the net OD was greater than the cut-off value, 0.152. Cell-free extracts of 26 strains of *V. fluvialis* and *V. furnissii* gave positive and 12 negative reactions. Net OD of positive samples varied from 0.153 to 0.512.

Live bacteria obtained from the 4-hr culture of 11 of 19 strains of *V. fluvialis* and *V. furnissii* caused fluid accumulation in rabbit ileal loops (Table 2). All the human strains gave high FA ratios of 0.5–1.3 (average 1.01), whereas environmental strains relatively low ratios of 0–1.23 (average 0.34). FA ratio of the positive control (0.5 μ g of purified CT) was 2.4. Of the 11 strains with the fluid accumulation activity, 8 had CKF activity, 10 only CEF, and only one (E75) both CEF and CKF in the culture filtrate. These results show that the ileal loop test is not necessarily compatible with that of CEF or CKF.

When culture filtrate of the strains with high FA ratios in the initial loop test with live organisms were concentrated 10 times by ultrafiltration, two of 17 strains gave positive results (FA ratio were 0.6–2.4). The FA activity of these strains was not neutralized by diluted anti-CT; the positive control of CT (0.5 μ g/ml) was completely deactivated.

No fluid accumulation in suckling mice given culture filtrate was caused by all the samples tested (FA ratios were 0.037–0.060). The highest FA ratio was 0.053 for BHI and CAYE medium controls.

Culture filtrates of most strains of *V. fluvialis* and *V. furnissii* had lethal activity (Table 3), and the mice injected died within 20 min. Blank medium (CAYE) had no lethal activity.

Some strains of *V. fluvialis* and *V. furnis-*

Table 2. Fluid accumulation in rabbit ileal loops caused by live culture and culture filtrate of *Vibrio fluvialis* and *Vibrio furnissii*^{a)}

Strain	Live cells		Concentrated filtrate	
	Positive/ tested	FA ratio of posi- tive loops	Positives/ tested	FA ratio of posi- tive loops
<i>V. fluvialis</i>				
E34	1/2	0.58	0/2	—
E36	0/2	—	0/2	—
E41	1/2	1.23	2/2	1.05
E49	2/2	1.07	n.t. ^{b)}	—
E62	0/2	—	0/2	—
E66	0/2	—	1/2	0.5
E67	0/2	—	0/2	—
E70	0/2	—	0/2	—
E75	1/2	0.53	0/2	—
E99	0/2	—	0/2	—
E115	2/2	1.05	2/3	1.53
E181	2/2	0.83	0/3	—
H1	3/3	1.19	0/3	—
H2	3/3	0.92	0/3	—
H4	3/3	0.92	0/3	—
<i>V. furnissii</i>				
E39	0/2	—	0/2	—
E47	0/2	—	n.t.	—
E83	1/2	0.5	0/2	—
T13	1/2	1.17	n.t.	—

a) Samples were made by concentration of 16 hr culture filtrate.

b) Not tested.

sii produced hemolysin, but the hemolytic activity on rabbit erythrocytes and that on goose erythrocytes were inconsistent (Table 3). The hemolysin-positive strains were not necessarily fluid accumulation-positive.

DISCUSSION

We demonstrated that some *V. fluvialis* and *V. furnissii* strains isolated from marine environment and marine products had diarrheal activity. Of the two species, most strains of *V. fluvialis* caused fluid accumulation in rabbit ileal loops. All the isolates from humans with diarrhea were *V. fluvialis* and two of them were tested and shown to

Table 3. Fluid accumulation in the intestine of suckling mouse, mouse lethality and hemolysis of RBCs caused by culture supernatant of *Vibrio fluvialis* and *Vibrio furnissii*^{a)}

Strain	Mouse lethality (Positive/ tested)	Suckling mouse assay (FA ratio)	Hemolysis of RBCs (OD)	
			Rabbit	Goose
<i>V. fluvialis</i>				
E34	2/2	0.060	0.054	0.038
E36	2/2	0.055	0.050	0.035
E41	2/2	0.054	0.088	0.067
E49	2/2	0.055	0.750	0.047
E62	1/2	0.055	0.946	0.040
E66	2/2	0.057	0.103	0.039
E67	1/2	0.054	0.060	0.038
E70	2/2	0.060	0.076	0.044
E75	2/2	0.040	0.068	0.067
E99	1/2	0.037	0.059	0.045
E115	2/2	0.037	0.111	0.053
E171	2/2	n.t. ^{b)}	n.t.	n.t.
E181	1/2	0.038	0.052	0.035
H1	2/2	0.059	0.717	0.038
H2	0/2	0.059	0.079	0.039
H4	1/2	0.061	0.057	0.039
<i>V. furnissii</i>				
E39	2/2	0.055	0.964	0.991
E47	2/2	0.056	0.992	0.050
E83	2/2	n.t.	0.976	0.509
T13	2/2	0.061	0.058	0.041

a) Samples were prepared from cultures for 16 hr.

b) Not tested.

be fluid-accumulation positive. Huq *et al.* [8] and Seidler *et al.* [16] attempted to detect enterotoxin of *V. fluvialis* by CHO cell assay; they discussed the morphological changes of CHO cells evoked by enterotoxin without distinction between CEF and CKF. Lockwood *et al.* [11] characterized partially purified CEF and stated that CEF was distinct from CKF against CHO cells, but they did not ascertain the direct enterotoxigenicity of the substances. Neutralization tests with anti-CT in CHO cell assay clearly showed that CEF differed from CKF and CEF had an immunological relation to CT. The results of ELISA did not agree with the cell elongation activity on CHO cells. Most strains with CEF and without

CKF appeared to be ELISA positive, and all the CKF and ELISA positive strains were assumed to be CEF negative. So we think that CKF masks CEF in CHO cells, because CHO cells would be broken down by CKF before the cells elongate. This view is supported by Lockwood *et al.* [11]. In our experiments, the diarrheal activity of *V. fluvialis* was not neutralized by anti-CT. So it is not clear whether fluid accumulation factor is a CT-like enterotoxin or not.

Several authors attempted to detect fluid accumulation activities of *V. fluvialis* in suckling mice, but they did not obtain consensus on the results of this test. Huq *et al.* [8] reported that infant mouse assay did not detect enterotoxin produced by *V.*

fluvialis isolated from patients with diarrhea. On the contrary, Kobayasi *et al.* [9] and Nishibuchi *et al.* [12] reported positive fluid accumulation by cultures grown in BHI containing 1.0% or 0.5% NaCl. When we employed the medium with 1.0% NaCl (BHI), none of *V. fluvialis* cultures accumulated fluid above the upper limit of control medium. The reason for the discrepancy between their results and ours is unclear.

The hemolysins produced by *E. coli*, *A. hydrophila*, and *V. cholerae* 01 and non-01 are different from diarrhea-causing cytotoxic enterotoxins, but the hemolysins of the latter two species possess also diarrhea-causing activities (in *A. hydrophila* a controversy exists in this matter [1, 3]). These irreconcilable notions have made us perplexed in explanation of the pathogenic roles of hemolysin in diarrhea. We indicated in this report that most strains of *V. fluvialis*, regardless of the origin, liberated both hemolytic and mouse lethal toxins into the medium. Our results suggest also that the hemolysin is not so closely correlated with the cell-killing and mouse lethal activities. We demonstrated that CEF had an immunological relation to CT and was distinct from CKF. This relation among the other factors showing cell-killing activity, hemolysis and mouse lethality and their roles in diarrheal pathogenesis have remained to be solved.

REFERENCES

1. Asao, T., Kinoshita, Y., Kozaki, S., Uemura, T., and Sakaguchi, G. 1984. Purification and some properties of *Aeromonas hydrophila* hemolysin. *Infect. Immun.* 46: 122-127.
2. Baselski, V., Briggs, R., and Parker, C. 1977. Intestinal fluid accumulation induced by oral challenge with *Vibrio cholerae* or cholera toxin in infant mice. *Infect. Immun.* 15: 704-712.
3. Chakraborty, T., Montenegro, M. A., Sanyal, S. C., Helmuth, R., Bulling, E., and Timmis, K. N. 1984. Cloning of enterotoxin gene from *Aeromonas hydrophila* provides conclusive evidence of production of a cytotoxic enterotoxin. *Infect. Immun.* 46: 435-441.
4. Chikahira, M., and Hamada, K. 1984. Isolation and characterization of *Vibrio fluvialis*. *Bull. Publ. Health Institute Hyogo Pref.* 19: 7-12. [in Japanese].
5. Guerrant R. L., Brunton, L. L., Schnaitman, T. C., Rebhum, L. I., and Gilman, A. G. 1974. Cyclic adenosine monophosphate and alternation of chinese hamster ovary cell morphology: a rapid, sensitive in vitro assay for the enterotoxins of *Vibrio cholerae* and *Escherichia coli*. *Infect. Immun.* 10: 320-327.
6. Hickman-Brenner, F. W., Brenner, D. J., Steigerwalt, A. G., Schreiber, M., Holmberg, S. D., Baldy, L. M., Lewis, C. S., Pickens, N. M., and Farmer III, J. J. 1984. *Vibrio fluvialis* and *Vibrio furnissii* isolated from a stool sample of one patient. *J. Clin. Microbiol.* 20: 125-127.
7. Honda, T., and Finkelstein, R. A. 1979. Purification and characterization of hemolysin produced by *Vibrio cholerae* biotype El Tor: another toxic substance produced by cholera vibrios. *Infect. Immun.* 26: 1020-1027.
8. Huq, M. I., Alam, A. K. M. J., Brenner, D. J., and Morris, G. K. 1980. Isolation of *Vibrio*-like group, EF-6, from patients with diarrhea. *J. Clin. Microbiol.* 11: 621-624.
9. Kobayashi, K., Taguchi, M., Shimada, T., and Sakazaki, R. 1983. Diarrhea associated with *Vibrio fluvialis* and its enterotoxicity. *J. Japan. Assoc. Infect. Dis.* 57: 375-381. [in Japanese].
10. Lee, J. V., Shread, P., and Furniss, A. L. 1981. Taxonomy and description of *Vibrio fluvialis* sp. nov. (synonym group F vibrios, group EF6). *J. Appl. Bacteriol.* 50: 73-94.
11. Lockwood, D. E., Kreger, A. S., and Richardson, S. H. 1982. Detection of toxins produced by *Vibrio fluvialis*. *Infect. Immun.* 35: 702-708.
12. Nishibuchi, M., and Seidler, R. J. 1983. Medium-dependent production of extracellular enterotoxins by non-O-1 *Vibrio cholerae*, *Vibrio mimicus*, and *Vibrio fluvialis*. *Appl. Environ. Microbiol.* 45: 228-231.
13. Ohasi, M., Shimada, T., and Fukumi, H. 1972. In vitro production of enterotoxin and hemorrhagic principle by *Vibrio cholerae*, NAG. *Japan. J. Med. Sci. Biol.* 25: 179-194.
14. Ristanio, P. A., Levine, M. M., and Young, C. R. 1983. Improved GM1-enzyme-linked immunosorbent assay for detection of *Escherichia coli* heat-labile enterotoxin. *J. Clin. Microbiol.* 18: 808-815.
15. Saynal, S. C., Agarwal, R. K., Annapurna, E., and Lee, J. V. 1980. Enterotoxicity of group F vibrios. *Japan. J. Med. Sci. Biol.* 33: 217-222.
16. Seidler, R. J., Allen, D. A., Colwell, R. R.

- Joseph, S. W., and Daily, O. P. 1980. Biochemical characteristics and virulence of environmental group F bacteria isolated in the United States. *Appl. Environ. Microbiol.* 40: 715-720.
17. Tacket, C. O., Hickman, F., Pierce, G. V., and Mendoza, L. F. 1982. Diarrhea associated with *Vibrio fluvialis* in the United States. *J. Clin. Microbiol.* 16: 991-992.
18. Yamamoto, K., AL-Omani, M., Honda, T., Takeda, Y., and Miwatani, T. 1984. Non-01 *Vibrio cholerae* hemolysin: purification, partial characterization, and immunological relatedness to E1 Tor hemolysin. *Infect. Immun.* 45: 192-196.
19. Yamamoto, K., Takeda, Y., Miwatani, T., and Craig, J. P. 1983. Purification and some properties of a non-01 *Vibrio cholerae* enterotoxin that is identical to cholera enterotoxin. *Infect. Immun.* 39: 1128-1135.
20. Yamamoto, K., Takeda, Y., Miwatani, T., and Craig, J. P. 1983. Evidence that a non-01 *Vibrio cholerae* produces enterotoxin that is similar but not identical to cholera enterotoxin. *Infect. Immun.* 41: 896-901.

要 約

環境由来 *Vibrio fluvialis* および *Vibrio furnissii* の下痢原性およびその他の生物活性について：近平雅嗣・浜田耕吉（兵庫県立衛生研究所・微生物部）—— *Vibrio fluvialis* および *Vibrio furnissii* の環境由来株（40株）およびヒト下痢症由来株（8株）について、兔結紮腸管ループ試験，株化細胞（CHO-K1）の伸長（CEF）・致死作用（CKF），その他の生物活性を調べた．下痢症由来株のすべては *V. fluvialis* で，ループ試験では液体貯留を示した．環境株は両菌種ともに陽性数は少なく，また FA 値も低かった．CHO 試験では，48株中39株が CKF を有し，18株が CEF を有し，一部の CEF は抗コレラ毒素血清（ACT）により中和されたが，CKF は中和されなかった．また，38株のポリミキシン B による抽出物中26株において，ELISA で CT 陽性反応が認められたが，CEF と下痢原性の関連性については確認できなかった．マウス致死作用及び赤血球溶血作用は株の由来，菌種による区別なく多くの株で認められた．