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Detection of Toxins Produced by *Vibrio fluvialis*

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The results of studies with cell-free extracts and culture supernatant fluids of *Vibrio fluvialis* (a recently recognized, potential enteric pathogen for humans) grown in the absence and presence of lincomycin indicated that the bacterium could produce (i) a factor which causes CHO cell elongation (CEF) similar to that elicited by *V. cholerae* enterotoxin and by the heat-labile enterotoxin of *Escherichia coli*, (ii) cytolysin(s) active against rabbit erythrocytes, (iii) nonhemolytic, CHO cell-killing factor(s), and (iv) protease(s) active against azocasein. The CEF was heat labile and ammonium sulfate precipitable, and it had an isoelectric point (estimated by sucrose density gradient electrofocusing) and molecular weight (estimated by gel filtration) of about 5.1 and 135,000, respectively.

Vibrio fluvialis is a halophilic bacterium which phenotypically resembles *Aeromonas* species and previously has been called group F *Vibrio* species (7, 11, 13; J. V. Lee, P. Shread, and A. L. Furniss, J. Appl. Bacteriol. **45**:ix, 1978) and group EF-6 *Vibrio* species [10; I. Huq, B. R. Davis, R. E. Weaver, D. G. Hollis, W. T. Martin, and D. J. Brenner, Abstr. 13th Joint Conf. Cholera U.S.-Japan Coop. Med. Sci. Prog. 1977, p. 82; D. J. Brenner, R. E. Weaver, D. G. Hollis, and B. R. Davis, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, B(H)14, p. 35]. The bacterium has been isolated from marine and estuarine environments and from numerous humans exhibiting severe diarrheal disease, including humans from whom previously described bacterial enteric pathogens could not be isolated (4, 7, 10, 18, 21–22). The bacterium may be differentiated from *Aeromonas* species by its (i) ability to grow in 6% NaCl, (ii) partial sensitivity to growth inhibition by the vibriocidal agent 0129, and (iii) DNA base composition of 49.3 to 50.6 mol% guanine plus cytosine (14). In addition, the bacterium may be differentiated from other *Vibrio* species by its (i) decarboxylase reactions, (ii) negative indole reaction, (iii) possession of a constitutive arginine dihydrolase system, (iv) production of acid from arabinose and D-galacturonate, and (v) lipopolysaccharide endotoxin sugar composition (9, 11, 13, 14, 21). Two biovars of the bacterium have been identified; biovar I includes anaerogenic strains of clinical and environmental origin, whereas biovar II consists of aerogenic strains found only in the environment (14).

Studies on the pathogenesis of enteric disease associated with isolation of the bacterium indicated that the clinical features of the disease closely resembled cholera, except that some patients had blood and mucus in their stools and some had abdominal pain and fever (10). The limited number of reports of tests for toxin production by *V. fluvialis* have given conflicting results. Blake et al. (4), McNicol et al. (18), and Spira et al. (22) reported negative results; however, other investigators reported that the bacterium killed mice when injected intraperitoneally and produced a heat-labile toxin that caused fluid accumulation in ligated rabbit ileal loops (M. I. Huq, M. U. Khan, K. M. S. Aziz, and D. J. Brenner, Abstr. 15th Joint Conf. Cholera U.S.-Japan Coop. Med. Sci. Prog. 1979, p. 68). In addition, Seidler et al. (21) found that anaerogenic strains of *V. fluvialis* elicited fluid accumulation in ligated rabbit ileal loops and caused cytotoxic and cytotonic responses when growing in Y-1 adrenal cell cultures. Also, Agarwal et al. (R. K. Agarwal, S.C. Sanyal, E. Annapurna, and J. V. Lee, Abstr. Suppl. 6th Int. Symp. Animal Plant Microb. Toxins 1979, p. 1) reported that the bacterium and its sterile culture filtrates caused fluid accumulation, comparable to that elicited by *V. cholerae* 569B, in ligated rabbit ileal loops. However, because of interfering cytotoxic factors present in the culture filtrates, the culture filtrates did not cause elongation of CHO cells, as is typically produced by known cytotonic enterotoxins.

The observations cited above prompted us to investigate the possibility that *V. fluvialis* elaborates products which could contribute to the pathogenesis of the enteric disease attributed to the bacterium. In this paper, we report on the production by the bacterium of (i) CHO cell elongation factor (CEF), (ii) cytolysin(s) active against rabbit erythrocytes, (iii) nonhemolytic,

CHO cell-killing factor(s) (CKF), and (iv) protease(s).

MATERIALS AND METHODS

Bacteria. Table 1 shows the sources and habitats of the *V. fluvialis* strains examined. The prototype *V. fluvialis* strain 5439, kindly supplied by J. Wells of the Centers for Disease Control (Atlanta, Ga.), was isolated from a human with diarrhea in Bangladesh. Frozen specimens of the bacteria were prepared by growing the strains, with shaking at 37°C, to the mid-logarithmic phase of growth in heart infusion broth (HI; Difco Laboratories, Detroit, Mich.) and freezing portions of the cultures with sterile glycerin (40%, vol/vol, final concentration) at −70°C.

Assays. (i) Activity against CHO cells. The ability to kill or to cause a change in the morphology of CHO cells (elongation or rounding) was estimated by the general methodology of Guerrant et al. (8). Stock cultures of CHO cells were grown, at 37°C in a humidified atmosphere containing 5% CO_2, in Eagle minimal essential medium containing Hanks base salts (EMEM; GIBCO Laboratories, Grand Island, N.Y.) and supplemented with 10% fetal calf serum (Flow Laboratories, Inc., Rockville, Md.), 10% tryptose phosphate broth (Difco), 2 mM L-glutamine (North American Biologicals, Miami, Fla.), 0.22% $NaHCO_3$, penicillin (100 IU/ml), and streptomycin (100 μg/ml). The cells were harvested with buffered Versene (per liter, disodium EDTA, 0.5 g; NaCl, 8.5 g; Na_2HPO_4, 1 g; 0.5% phenol red solution, 2 ml, pH 8), and portions of the washed cell suspension (about 800 cells in 0.1 ml of supplemented EMEM without tryptose phosphate broth and containing 1% fetal calf serum) were added to wells of 96-well microtiter assay plates (Linbro, 76-003-05, Flow Laboratories). Serial twofold dilutions of the preparations to be tested were made in supplemented EMEM without fetal calf serum and tryptose phosphate broth, and 10 μl of each dilution was added to separate microtiter wells. The cells were examined (without staining) for necrosis, elongation, and rounding after incubation in 5% CO_2 for 18 h at 37°C. The CKF, CEF, and CHO cell-rounding titers of the tested preparations were expressed as the reciprocal of the highest dilution that killed >10% of the cells or that caused >10% of the cells to exhibit a change in their characteristic epithelioid morphology, respectively.

(ii) Hemolytic activity. Cytolytic activity against rabbit erythrocytes was determined by the method of Bernheimer and Schwartz (3). One hemolytic unit was defined as the reciprocal of the dilution that caused the release of 50% of the hemoglobin in the standardized (0.7%, vol/vol) erythrocyte suspension.

(iii) Protease activity. Protease activity was assayed by the azocasein substrate method described by Kreger and Gray (12). One unit of activity was defined as the reciprocal of the dilution that yielded, under the standard assay conditions, a reaction mixture containing enough trichloroacetic acid-soluble, diazotized peptides to give an absorbance of 1 at 440 nm.

Toxin production by *V. fluvialis* strain 5439 in baffle flask culture. Seed cultures were obtained from a slant of modified yeast extract agar (per liter, Tris, 6.1 g; NH_4Cl, 1.02 g; NaCl, 11.7 g; $MgSO_4 \cdot 7H_2O$, 12.3 g; KCl, 0.746 g; $CaCl_2 \cdot 2H_2O$, 1.47 g; yeast extract [Difco], 5 g; glycerol, 3 ml; agar, 15 g, pH 7.4 to 7.6) streaked with one loopful of a rapidly thawed specimen of the bacterium and incubated for 6 h at 37°C. The bacteria were washed off the slant with HI (3 ml), and the suspension was adjusted to an optical density at 640 nm of about 6. The results of previous studies indicated that lincomycin increases the production of *V. cholerae* enterotoxin and the heat-labile enterotoxin of *Escherichia coli* (15, 16; D. E. Lockwood and S. H. Richardson, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, B33, p. 21). Therefore, portions (0.1 ml) of the suspension were added to HI (50 ml) not containing lincomycin, or supplemented with 50 μg of lincomycin hydrochloride (The Upjohn Co., Kalamazoo, Mich.) per ml, in 500-ml baffle flasks (Bellco Glass, Inc., Vineland, N.J.). The media were incubated at 30°C on a gyratory shaker-incubator (PsychroTherm; New Brunswick Scientific Co., New Brunswick, N.J.) operating at 220 cycles/min, the growth of the bacterium was monitored by plate counts every 2 h, and portions (2 ml) of the culture supernatant fluids were obtained by centrifugation (17,000 × *g*, 15 min, 4°C). In addition, cell-free extracts were prepared from the washed (5 ml of 0.85% NaCl) cell pellets suspended in 0.85% NaCl (5 ml) and sonically disrupted (Bronwell Biosonik; Bronwell Scientific Co., Rochester, N.Y.), at 4°C with maximum power, until the suspension was clear (two 30-s pulses). Debris was removed by centrifugation (27,000 × *g*, 15 min, 4°C). The culture supernatant fluids and cell-free extracts were stored at −60°C until assayed.

Screening of clinical and environmental isolates of *V. fluvialis* for activity against CHO cells. Cultures of *V. fluvialis* isolates examined for CEF activity and CKF activity were prepared by inoculating HI (5 ml in 50-ml Erlenmeyer flasks) with five colonies from Columbia agar (BBL Microbiology Systems, Cockeysville, Md.)–5% sheep blood cultures grown overnight at 37°C and incubating the broths at 30°C for 18 h with gyratory agitation (220 cycles/min). The culture supernatant fluids were obtained by centrifugation and were stored at −60°C until assayed. The results of preliminary experiments revealed that the polymyxin B extraction procedure of Cerny and Teuber (5), which previously had been used successfully to release the periplasmic-bound, heat-labile enterotoxin of *E. coli* (6), was a more efficient method than sonic disruption for obtaining biologically active cell-free extracts of *V. fluvialis*. Therefore, cell-free extracts of the washed (10 ml of 0.85% NaCl) bacteria were prepared by shaking the cells for 7 min at 37°C (150 reciprocating oscillations/min) in 1 ml of 0.005 M Tris-HCl-buffered saline (pH 7.1) containing 1 mg of polymyxin B (Calbiochem-Behring Corp., La Jolla, Calif.) per ml (PB buffer) and removing debris and remaining cells by centrifugation (27,000 × *g*, 15 min, 4°C). The extracts were stored at −60°C until assayed.

Partial purification and characterization of CEF. Unless otherwise noted, all steps were performed at about 4°C.

Stage 1: extraction with polymyxin B. Cells from 10 24-h baffle flask cultures of strain 5439, prepared as for the toxin production experiments (without lincomycin in the medium), were washed twice with 0.85% NaCl (200 ml) and were extracted by shaking for 7 min at 37°C in 50 ml of PB buffer. Debris and remaining cells were removed by centrifugation (17,000 × *g*, 15 min).

Stage 2: acid precipitation. The stage 1 preparation

TABLE 1. Screening of clinical and environmental isolates of *V. fluvialis* for production of CHO CEF and CKF activities[a]

Strain	Received from[b]:	Isolated from:	CKF titer in culture supernatant fluids	Cell-free extracts	
				CEF titer[c]	CKF titer
715-77	1	Human (Bangladesh)	100	400	—
718-77	1	Human (Bangladesh)	3,200	800	—
807-77	1	Human (Bangladesh)	100	4,800	100
1272-78	1	Human (Indonesia)	—[d,e]	800	—
1273-78	1	Human (Indonesia)	200	400	50
9551-78	1	Human (United Kingdom)	—	400	50
9553-78	1	Human (United Kingdom)	800	300	—
9555-78	1	Human (United Kingdom)	100	1,200	100
291-80	1	Human (Peru)	—	800	—
292-80	1	Human (Peru)	—	800	—
5435	1	Human (Bangladesh)	50	300	200
5439	1	Human (Bangladesh)	50	1,600	200
5440	1	Human (Bangladesh)	6,400	—	6,400
F-6	2	Human (India)	3,200	800	50
2926	3	Human (United Kingdom)	200	300	—
7648	3	Human (India)	3,200	—	200
8096	3	Human (United Arab Emirates)	—[e]	600	—
8315	3	Human (United Kingdom)	—[e]	—	—
8760	3	Human (Yugoslavia)	—[e]	300	—
8761	3	Human (Yugoslavia)	800	—	—
8808	3	Human (United Kingdom)	—[e]	150	50
9049	3	Human (Bahrain)	—[e]	300	—
No. (%) with activity			13 (59)	18 (82)	10 (45)
1197-78	1	Sewage	—	—	—
1198-78	1	Sewage	—	—	—
F-14	2	Sewage	—[e]	200	—
F-18	2	Crab	4,800	600	100
F-21	2	Prawn	—[e]	600	50
F-23	2	Seawater	400	—	—
2386	3	River	200	300	—
7386	3	Seawater	50	—	—
7662	3	Crab	—[e]	—	—
SGM-364	4	Oyster	200	400	50
SGM-367	4	Top water	—[f]	1,600	100
SGM-371	4	Bottom water	—[e]	400	50
SGM-378	4	Oyster shell	—[e]	—	200
SGM-387	4	Sediment	400	200	50
SGM-388	4	Oyster shell	200	200	100
SGM-391	4	Bottom water	6,400	1,600	400
SGM-523	4	Top water	—[e]	400	50
SGM-539	4	Bottom water	—[e]	800	50
SGM-547	4	Top water	—[e]	3,200	800
SGM-557	4	Oyster	—[e]	800	200
H-5	4	Water	—[e]	200	100
No. (%) with activity			8 (38)	15 (71)	14 (67)

[a] Bacteria were grown without lincomycin as described in Materials and Methods. Activity was considered present if the titer was ≥ 50.

[b] 1, Enterobacteriaceae section of the Centers for Disease Control (Atlanta, Ga.); 2, S. C. Sanyal, Banaras Hindu University, Varanasi, India; 3, A. L. Furniss, Public Health Laboratory, Maidstone, United Kingdom; 4, Chesapeake Bay isolates received from R. R. Colwell, University of Maryland.

[c] CKF activity did not interfere with detection of CEF activity in the cell-free extracts because the former could be removed by dilution before reaching the titer of CEF activity.

[d] —, No activity.

[e] CHO cell-rounding activity was detected.

[f] CEF activity was detected (titer = 1,600).

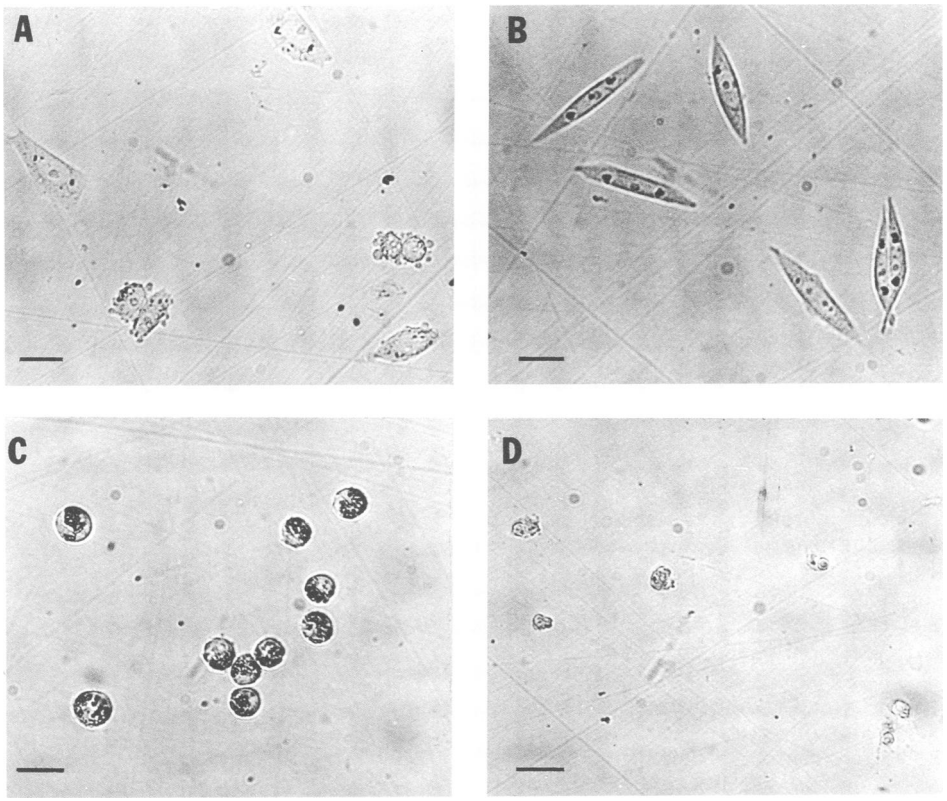

FIG. 1. Morphological changes elicited in CHO cells exposed to *V. fluvialis* strain 5439 products. Results shown are after exposure for about 18 h at 37°C. Line markers represent 20 μm. (A) Control cells. (B) Cells treated with cell-free extract containing CEF activity. (C) Cells treated with culture supernatant fluids containing protease and CHO cell-rounding activity. (D) Cells treated with culture supernatant fluids containing CKF activity.

was adjusted to pH 4.2 with concentrated HCl, the precipitate was sedimented by centrifugation and discarded, and the supernatant fluids were adjusted to pH 7.1 with NaOH.

Stage 3: ammonium sulfate precipitation. Ammonium sulfate (enzyme grade; Schwarz/Mann, Orangeburg, N.Y.) was dissolved slowly in the stage 2 preparation to a final concentration of about 85% saturation (610 g/liter). The precipitate was dissolved in 5 ml of 0.005 M Tris-HCl buffer (pH 7.5) and was dialyzed for about 16 to 18 h against the same buffer (4 liters). The dialyzed preparation was stored at −60°C until fractionated by isoelectric focusing.

Stage 4: sucrose density gradient isoelectric focusing. The stage 3 preparation (about 9 ml) was fractionated by high-speed electrofocusing (17, 23) in a pH 4 to 6 sucrose density gradient formed at 15 W for 18.5 h with an LKB 8100-1 column (LKB Instruments, Rockville, Md.). Fractions (4 ml) were assayed for absorbance at 280 nm and for CEF activity, and the active fractions were pooled and concentrated by ultrafiltration with a stirred cell unit equipped with a UM-10 membrane (Amicon Corp., Danvers, Mass.).

Stage 5: Sephadex G-200 gel filtration. A portion (5 ml) of the stage 4 preparation was applied to a calibrated column (1.6 by 64 cm) of Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) equilibrated with 0.1 M Tris-HCl buffer (pH 8) supplemented with 0.2 M NaCl and 0.002 M EDTA, and was eluted at a flow rate of about 20 ml/h. Fractions (4 ml) were assayed for absorbance at 280 nm and for CEF activity, and the apparent molecular weight of the CEF was estimated by the method of Andrews (1).

RESULTS

Detection of toxic products in baffle flask cultures. Uninoculated HI did not contain detectable activity against CHO cells (Fig. 1A), hemolytic activity against rabbit erythrocytes, or protease activity. However, the results of our studies with cell-free extracts and culture supernatant fluids of *V. fluvialis* strain 5439, grown in the absence and presence of lincomycin, indicated that the bacterium could produce at least four biologically active substances. A factor which caused CHO cell elongation (CEF) similar to that produced by *V. cholerae* enterotoxin and by the heat-labile enterotoxin of *E. coli* was detected in optimal amounts in cell-free extracts pre-

pared from cells grown without lincomycin (Fig. 1B and 2). In addition, cytolysin(s) active against rabbit erythrocytes and protease(s) were detected only in the supernatant fluids of cultures grown without lincomycin (Fig. 3A). The hemolytic activity of the cytolysin(s) appeared in the culture supernatant fluids before the protease activity and disappeared concomitant with the increase in protease activity. The kinetics of appearance of the protease activity (Fig. 3A) was similar to that of the appearance of CHO cell-rounding activity in the culture supernatant fluids (Fig. 1C and 3B), thus suggesting that the CHO cell-rounding activity was a function of the protease. Finally, CKF, which did not possess detectable cytolytic activity against rabbit erythrocytes, was found in the supernatant fluids of cultures grown with or without lincomycin (Fig. 1D and 3C).

Detection of morphological changes in CHO cells exposed to *V. fluvialis* toxic products. Figure 1 shows the morphological alterations of CHO cells after exposure to *V. fluvialis* toxic products for 18 h at 37°C. Control cells (Fig. 1A) exhibited a normal epithelioid shape and knoblike pseudopodal projections of the cytoplasmic membrane. Cells treated with cell-free extract (10 μl of a 1/32 dilution of a preparation having a CEF titer of 5,120) prepared by sonic disruption (Fig 1B) had elongated and lost the pseudopodal projections of the cytoplasmic membrane. The response was similar to that produced by the enterotoxin of *V. cholerae* and the heat-labile enterotoxin of *E. coli* (control observations not shown). Cells treated with culture supernatant fluids containing protease and CHO cell-rounding activity (Fig. 1C; 10 μl of a 1/32 dilution of a preparation having cell-rounding titer of 1,280) had become compact and granular in appearance and had lost the pseudopodal projections of the cytoplasmic membrane. Cells treated with culture supernatant fluids containing CKF activity (Fig. 1D; 10 μl of a 1/64 dilution of a preparation having a CKF titer of 2,560) either were necrotic or showed various degenerative changes.

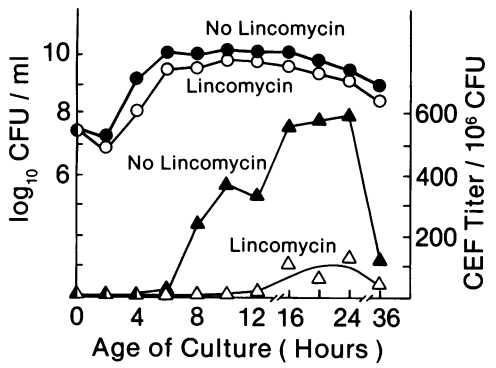

FIG. 2. Relationship between growth of *V. fluvialis* strain 5439 and the amount of intracellular CEF activity. The data presented are the means of values obtained from two closely agreeing experiments. Bacterial growth was followed by plate counts and expressed as colony-forming units (CFU) per milliliter (circles), and the cell-free extracts were assayed for CEF activity, which was expressed as CEF titer/10^6 CFU (triangles).

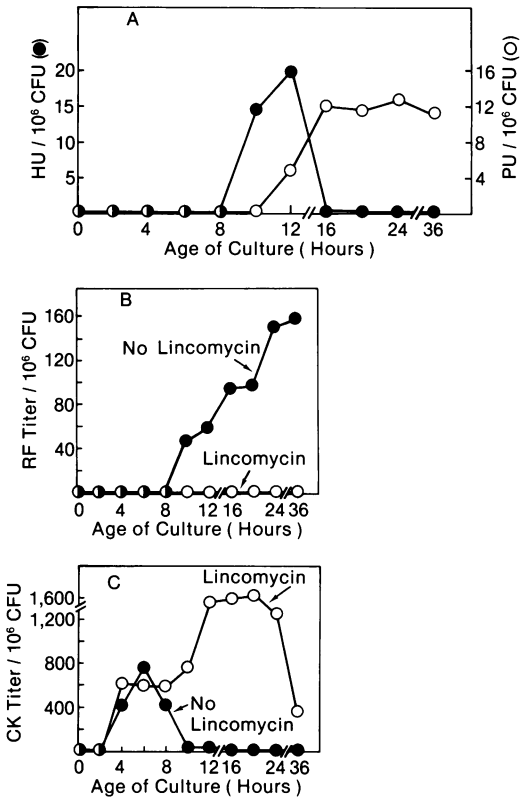

FIG. 3. Detection of extracellular hemolytic, protease, CHO cell-rounding, and CKF activity in cultures of *V. fluvialis* strain 5439. The data presented are the means of values obtained from two closely agreeing experiments. (A) Production of hemolysin(s) (hemolytic units [HU] per 10^6 CFU) and protease(s) (proteolytic units [PU] per 10^6 CFU) in cultures without lincomycin. (B) Production of CHO cell-rounding factor(s) (RF) in cultures with and without lincomycin. (C) Production of CKF in cultures with and without lincomycin.

Screening of clinical and environmental isolates of *V. fluvialis* for activity against CHO cells. No significant difference was noted in the production of the CEF and the CKF by the clinical and environmental isolates of *V. fluvialis* (Table 1). Approximately 75% of the 22 clinical isolates and of the 21 environmental isolates produced CEF, and about 60% of the isolates produced

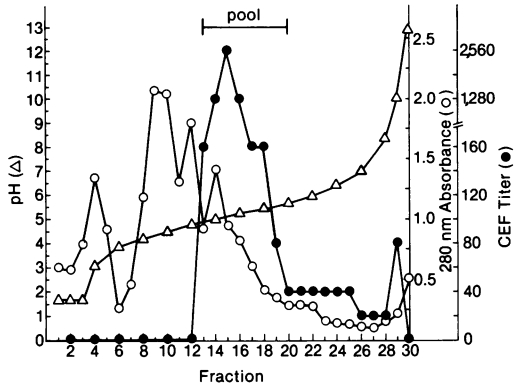

FIG. 4. Isoelectric focusing of a stage 3 CEF preparation of *V. fluvialis* strain 5439. The pH (△) of the fractions (4 ml) was determined at 4 C, and the fractions were assayed for absorbance at 280 nm (○) and for CEF activity (●). The active fractions which were pooled, concentrated, and subjected to gel filtration with Sephadex G-200 (Fig. 5) are indicated in the figure.

CKF. Strain SGM-367, which was isolated from the bottom water of Chesapeake Bay, was unique in that CEF activity was detected both extracellularly and intracellularly.

Partial characterization of the CEF obtained from *V. fluvialis* strain 5439. The stage 3 CEF preparation (purified by sequential acid precipitation and ammonium sulfate precipitation) was stable at 4, 22, and 37°C for 30 min; however, activity was lost after exposure to 60 or 100°C. The CEF had an isoelectric point of about 5.1 (Fig. 4) and an apparent molecular weight of about 135,000 (Fig. 5).

DISCUSSION

V. fluvialis recently has been incriminated epidemiologically as an etiological agent of enteric disease because of its isolation from many humans exhibiting cholera-like diarrhea, but from whom previously described bacterial enteric pathogens could not be isolated (4, 7, 10, 22). In addition, Seidler et al. (21) reported that anaerogenic, environmental isolates of the bacterium elicited fluid accumulation after inoculation of ligated rabbit ileal loops. Furthermore, we recently have found that oral challenge of infant mice (2) with *V. fluvialis* results in intestinal fluid accumulation, diarrhea, and death. The results of our studies on the virulence of the bacterium for the infant mouse model of Baselski et al. (2) will be the subject of a future communication.

Three preliminary reports have appeared on the production of toxic products which might contribute to the pathogenesis of the enteric disease attributed to *V. fluvialis* (Huq et al., Abstr. 15th Joint Conf. Cholera U.S.-Jpn. Coop. Med. Sci. Prog. 1979, p. 68; Agarwal et al., Abstr. Suppl. 6th Int. Symp. Animal Plant Microb. Toxins 1979, p. 1; D. E. Lockwood, S. H. Richardson, and A. S. Kreger, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, B120, p. 37); however, to the best of our knowledge, this paper is the first full-length publication on the subject. At the present time, it is not possible to state definitively that the *V. fluvialis* products

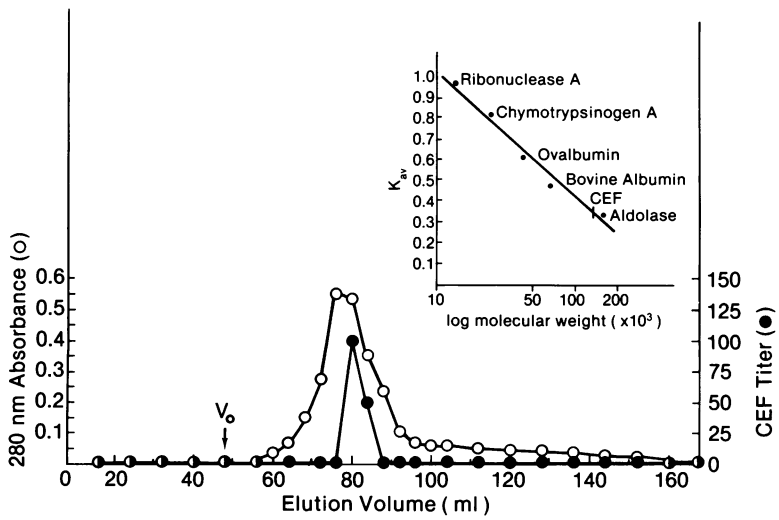

FIG. 5. Sephadex-G-200 gel filtration of a stage 4 CEF preparation of *V. fluvialis* strain 5439. The column void volume (V_0) was about 48 ml. Fractions (4 ml) were assayed for absorbance at 280 nm (○) and for CEF activity (●). The insert contains the data used to estimate the apparent molecular weight of the CEF by the method of Andrews (1).

described in this communication (CEF, cytolysin[s] with hemolytic activity, CKF, and protease[s]) have a role in the pathogenesis of enteric disease; however, at least three observations suggest that such is the case and prompt further investigation of the subject. First, CEFs produced by known enteric pathogens, such as *V. cholerae* and *E. coli*, are enterotoxins which play key roles in causing enteric disease. Second, the Kanagawa phenomenon-associated cytolysin of *V. parahaemolyticus* and the proteases of *Pseudomonas aeruginosa* (both known enteric pathogens) have been reported to be enterotoxic for experimental animals (19, 20). Third, we recently have found that oral challenge of infant mice (2) with crude or partially purified preparations of the various bacterial products elicits intestinal fluid accumulation, diarrhea, and death (D. E. Lockwood, A. S. Kreger, and S. H. Richardson, unpublished data). Studies are currently in progress to obtain homogeneous preparations of the products, so that we may commence studies to rigorously evaluate their role(s) in the pathogenesis of enteric disease caused by the bacterium.

The observation that CEF activity usually was detectable in cell-free extracts, but not in the supernatant fluids of *V. fluvialis* cultures, should not be interpreted as indicating that the toxin usually is not released into the bacterium's environment. The presence of high titers of CHO cell-killing and CHO cell-rounding factors in the culture supernatant fluids may interfere with the detection of CEF activity. This idea is supported by the observation that the only culture supernatant fluids in which we were able to detect CEF activity (strain SGM-367) did not possess detectable CHO cell-killing or CHO cell-rounding activity.

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