ISOLATION OF 01 AND NON-01 VIBRIO CHOLERAE FROM ESTUARIES AND BRACKISH WATER ENVIRONMENTS

R.R. Colwell, J. Kaper, R. Seidler, M.J. Voll, L.A. McNicol, S. Garges, H. Lockman, D. Maneval and E. Remmers

Department of Microbiology
University of Maryland
College Park, Maryland 20742

S.W. Joseph

Microbiology Department
Naval Medical Research Institute
Bethesda, Maryland 20014

H. Bradford and N. Roberts

Division of Laboratories
Office of Health Service and
Environmental Quality
New Orleans, Louisiana 70112

I. Huq and A. Huq

International Center for
Diarrheal Diseases Research, Bangladesh
Dacca 2, Bangladesh

Isolation of Vibrio cholerae at several locations in Chesapeake Bay in the fall of 1976 and spring 1977 was reported recently (Colwell et al., 1977). Isolations were made in late fall 1976 and early spring 1977, and we are continuing to isolate V. cholerae. Data gathered by Kaper et al. (1979) provide conclusive evidence that V. cholerae can be isolated throughout the Chesapeake Bay in a distinct, not random, pattern dependent chiefly upon salinity and inversely related to pollution. At locations in the upper Chesapeake Bay and in the Potomac and James Rivers, major tributaries of the Bay where the salinity of the water was very low, viz., < 1-3 °/oo, V. cholerae was not isolated from samples collected at these stations. Moderately saline regions of the Bay consistently yielded V. cholerae. Thus, the optimal salinity limits for this organism in Chesapeake Bay are concluded to lie in the range of 1-3 °/oo to 17 °/oo. Interestingly, the optimal salt concentration for V. cholerae in culture, i.e., approximately 1% (10 °/oo) is that which approximates the in situ salinity range for this organism in middle portions of the Chesapeake Bay.

Additional evidence of the autochthonous nature of V. cholerae in the Chesapeake Bay was obtained by examining water samples collected along a track line representative of a pollution gradient in the Upper Chesapeake Bay for presence of V. cholerae, to determine association,

if any, with recommended microbial indicators of pollution (Kaper et al., 1979). See Table 1. Results of correlation calculations indicated that the presence of Salmonella was indeed associated with elevated numbers of fecal coliforms ($P < 0.01$) but that V. cholerae was not. V. cholerae, as V. parahaemolyticus (Kaneko and Colwell, 1978), is an autochthonous species in the estuarine ecosystem.

The V. cholerae strains from Chesapeake Bay represented serotypes other than group 01, the so-called nonagglutinable vibrios, and were not recognized as a serious epidemic threat, although nonagglutinable V. cholerae have been associated with sporadic cases of cholera-like diarrhea. In principle, V. cholerae strains other than group 01 are more properly defined as V. cholerae non-01 since these are agglutinable, but not 01 serovar. Hence, non-agglutinable (NAG) and non cholera vibrio (NCV) are terms less preferable than V. cholerae non-01 (VCN).

Since the initial report of the isolation of V. cholerae from Chesapeake Bay (Colwell et al., 1977), we have isolated V. cholerae 01 serotype Inaba from Chesapeake Bay and from bayous in Louisiana.

In September, 1978, the first cholera outbreak in the United States since 1911 occurred in Louisiana (Morbid. Mortal. Weekly Rept., 1978). Eleven cases of clinical cholera were diagnosed. Southwestern Louisiana south of Interstate 10, where the cases of cholera occurred, is a marsh-land area of only a few feet above sea level. The marsh extends ca. 25 miles inland from the Gulf of Mexico, and is saline near the coast and brackish in the midportion. Association of the disease with eating improperly cooked or stored steamed or boiled crabs was noted (Morbid. Mortal. Weekly Rept., 1978).

V. cholerae Group 01, Inaba serotype, was isolated from water samples collected in Chesapeake Bay and from the Louisiana saltmarshes and we are continuing to isolate V. cholerae from these areas. Strains isolated from Louisiana and Chesapeake Bay, along with clinical isolates 4808 and 718, are listed in Table 2. Methods employed for isolation, characterization, and identification have been published (Colwell et al., 1977; Colwell and Kaper, 1979; Kaper et al., 1979). Confirmation of the 01 Inaba serotype was provided by Dr. R. Sakazaki, Tokyo, Japan, Dr. A. Furniss, Maidstone, England, and Dr. D. Brenner, Center for Disease Control, Atlanta, Georgia.

DNA/DNA homology studies have been done. For these studies, cultures of V. cholerae were shaken for 16 hr at 37C in Brain Heart Infusion (BHI) Broth (Difco Laboratories, Detroit, Michigan) with NaCl added to a final concentration of 1%. DNA was prepared and purified by a modification of the method of Marmur (1961). Percent guanine plus cytosine (% G+C) (over-all) DNA base composition was determined by the thermal denaturation method (Marmur and Doty, 1962; Mandel et al., 1970).

Measurement of reassociation was accomplished using the membrane filter technique (Denhardt, 1966; Sriranganathan et al., 1973; De Ley and Tijtgat, 1970). In addition, DNA renaturation was measured optically (Seidler and Mandel, 1971) and using S-1 endonuclease (Crosa et al., 1973).

With the exception of strains 718, a non-01 Vibrio sp. isolated from a patient in Louisiana hospitalized with severe diarrhea and V-11 from Chesapeake Bay, the strains isolated from Chesapeake Bay and the Louisiana Bayous showed species level DNA/DNA homology with the El Tor biotype of V. cholerae (Table 3). The phage typing and hemolytic reactions, which differ from those of the epidemic strain of V. cholerae, suggest that introduction of these strains by a cholera case or carrier is highly improbable (J. Lee and C. Parker, personal communication). The V. cholerae 01 Inaba strains, as well as several non-01 strains were found to be enterotoxigenic, as measured by the rabbit ligated loop and the Y-1 assay (Kaper et al., 1979). Strain 718 was negative in both tests. Strain 4808-78, isolated from a cholera patient in Louisiana, was tested in the rabbit ileal loop assay. Two separate assays were performed using 1 ml whole culture grown in BHI broth for 18-24 hr. Both assays were positive with 1.2 and 0.92 ml fluid accumulation/cm intestine, respectively. This strain also produced enterotoxin in several media detected at dilutions up to 1:256 of culture supernatants by the Y-1 assay. Strain V69, isolated from a water sample collected in Chesapeake Bay, gave positive reactions, 0.75, 0.75, and 1.0 ml/cm in ligated rabbit loops and was toxic to Y-1 adrenal cells at the 1:40 dilution.

Fifty-six strains of non-O Group I V. cholerae isolated from a tank and canal in the study area of Matlab Village and Dacca, Bangladesh were examined for presence of plasmids by agarose gel electrophoretic assay. The majority of the strains were examined by two procedures: 1) the method of Meyers et al. (1976), in which DNA extracted from cleared lysates is applied to the gels, and 2) a modification of the method of Eckhart (1978) adapted to horizontal gel electrophoresis (J.W. Newland, personal communication). The Eckhart method is a rapid procedure in which cells are directly lysed in the gel slots. Good agreement was obtained between the results of the two methods. CCC DNA was detected in 16 strains (28%). Toxicity in the Y-adrenal cell assay was observed for strains containing small molecular weight plasmids, but also in strains in which plasmid DNA was not detected. In general, strains containing large molecular weight plasmids did not show toxic activity. However, strain BV93, a toxigenic V. cholerae strain, appears to possess a large plasmid. The function of these plasmids in V. cholerae is under investigation.

Recently Rondle et al. (1978) presented the hypothesis that the source of some cases of cholera might be effluent discharge from

aircrafts. They point out that there is an apparent relationship between distribution of isolated outbreaks of cholera and the major airline routes. This hypothesis appears untenable since aircraft discharge only washbasin effluent, with sewage held in chemical tanks. *V. cholerae* in wash basin effluents would be subjected to drying and to lethal effects of ultra-violet irradiation. More important, however, is the fact that the flight paths of regular airline services from Calcutta, where cholera is endemic, to Europe cover the entire estuarine and brackish water areas of those countries where sporadic cases of cholera occur. The low incidence of cholera carriers on aircraft, coupled with the dilution factor of cholera aerosols, make it unlikely that this route of infection is a feasible explanation for the spread of cholera. In fact, the link between disease and planes carrying cholera-infected passengers is much less probable than that between the natural habitation of *V. cholerae* in estuarine and brackish water areas and occurrence of cholera when sanitation or proper food handling practices are lacking, a view also expressed by Bashford *et al.* (1979).

As indicated above, *V. cholerae* has been isolated from water samples collected in Chesapeake Bay and Louisiana in areas free of fecal contamination, based on fecal coliform determinations. Similar results were obtained by Bashford *et al.* (1979) who isolated *V. cholerae* 01 from marshes in England. The association of *Vibrio* spp. with estuarine and marine copepods (Sochard *et al.*, 1979) has been demonstrated. Interestingly, Nalin *et al.* (1978) found that association with chitin confers resistance to acid pH for *V. cholerae*. *V. cholerae* is, as other brackish water, estuarine, and marine vibrios (Colwell, 1965), capable of digesting chitin. The majority of the strains of *V. cholerae* isolated from Chesapeake Bay were found to be capable of digesting chitin (Kaper *et al.*, 1979).

During March, 1979, samples of plankton, aquatic plants, water and sediment were collected from canals near Matlab, Bangladesh and, in late June, from stations throughout Chesapeake Bay. Enrichment in alkaline peptone and, in general, other methods previously published (Kaper *et al.*, 1979) were employed. *V. cholerae* non-01 were isolated in large numbers. Association with plankton was particularly noted, both for the Bangladesh and Chesapeake Bay samples. In addition, five strains of Group F Vibrios were also isolated from the plankton samples collected in Chesapeake Bay during the June, 1979, cruise.

Plankton samples collected in Bangladesh were preserved in 1% formalin immediately upon retrieval of the plankton net and brought to the University of Maryland for examination by scanning electron microscopy (SEM). Plankton samples from Chesapeake Bay were fixed in gluteraldehyde solution on board ship and brought back to the laboratory for examination by SEM. Methods employed for SEM were essentially as described by Zachary *et al.* (1978). A variety of planktonic forms

were observed in the Bangladesh samples. See Figs. 1 and 2. Also, bacteria were found associated with the plankton. See Fig. 3. Bacteria attached to leaves of the aquatic plants were also observed. See Fig. 4. Association of V. cholerae with plankton, both in canal waters in Dacca, Bangladesh, and in Chesapeake Bay, appears to be a significant factor in the ecology of this species, although a great deal of work remains to be done before the questions of association and of seasonality can be answered.

The phenetic, molecular genetic, and ecological data obtained to date in our laboratory support the conclusion that V. cholerae is, indeed, a component of the autochthonous flora of brackish water, estuaries, and salt marshes of coastal area of the temperate zone. Work is in progress to determine the basis of the commensal relationship, if any, of V. cholerae and related vibrios to plankton species, including larvae of crustacea, as well as association of these microorganisms with adult shellfish (Sizemore et al., 1975). Work in our laboratory is now directed toward the hypothesis that the enterotoxin may play a role in salt tolerance and osmoregulation in chitinaceous plankton and shellfish of brackish and estuarine waters.

The epidemiology of cholera appears to be closely linked to the microbial ecology of the environment, especially in the association of V. cholerae with the macro- and micro-flora and fauna of coastal waters and estuaries.

A more complete understanding of the epidemiology of cholera requires recognition of the fact that V. cholerae occurs in the absence of fecal contamination and that sporadic outbreaks can be expected in coastal areas when proper food handling techniques are not used. Thus, public health surveillance of these areas for V. cholerae, as well as cholera-like disease, appears warranted, but should be done with an understanding of the natural history of the microorganism and its habitat.

SUMMARY

In the course of studies carried out at the University of Maryland and in collaboration with the Cholera Research Laboratory in Dacca, Bangladesh, isolations of Vibrio cholerae, including Vibrio cholerae 01, from the estuarine and brackish water environments of Chesapeake Bay and Louisiana have been accomplished. The isolates have proved, on the basis of DNA/DNA hybridization, to be highly related to the type strain of Vibrio cholerae ATCC 14033. From analyses of environmental variables, it has been found that salinity correlates with incidence of Vibrio cholerae but that incidence of fecal coliforms was not correlated with isolation of V. cholerae. Methods for isolation of Vibrio cholerae from the environment require adaptation of methods used in clinical

laboratories. However, isolation of V. cholerae requires an increased awareness of its ecological role in the estuarine environment.

Production of enterotoxin has been tested and fluid accumulation has been found in the rabbit ileal loop model. Both cytotoxin and enterotoxin-like effects have been observed in Y-1 adrenal cell assays.

The relationship between antibiotic resistance, enterotoxigenicity and plasmid carriage in the environmental vibrios has been examined. The plasmids observed are cryptic in function, and much more common in the Bangladesh isolates. Correlation between toxigenicity and plasmid carriage, if any, remains unclear.

The role of Vibrio cholerae in the natural environment is under study. The chitinoclastic nature of these vibrios and of other estuarine and aquatic vibrios appears to be important in the aquatic ecosystem. We conclude that V. cholerae is an autochthonous bacterial species in the estuarine environment and that the incidence of Vibrio cholerae requires an ecological explanation in order for epidemiological controls to be effective.

ACKNOWLEDGEMENTS

We gratefully acknowledge the assistance of Dr. R. Sakazaki, Dr. A. Furniss and Dr. D. Brenner in serotyping the Chesapeake Bay and Louisiana isolates of V. cholerae and Mr. M.E. Taylor in preparing the scanning electron micrographs. This investigation was supported by National Institute of Allergy and Infectious Diseases Grant No. 1 R22AI14242-01, by Sea Grant 04-3-158-7 from the National Oceanic and Atmospheric Administration, U.S. Department of Commerce, by NSF Grant No. GB-35261X, and by the Naval Medical Research and Development Command under Research Task ZF51524009 - 0057.

REFERENCES

1. Bashford, D.J., T.J. Donovan, A.L. Furniss and J.V. Lee. 1979. Vibrio cholerae in Kent. Lancet, Feb. 24, pp. 436-437.
2. Colwell, R.R. 1965. Proposal of a neotype, ATCC 15381, for Vibrio marinus (Russell 1891) Ford 1927 and request for an opinion. Internat. Bull. Bacteriol. Nomen. and Taxon 15: 165.
3. Colwell, R.R. and J. Kaper. 1979. Vibrio species as bacterial indicators of potential health hazards associated with water. In: Bacterial Indicators/Health Hazards Associated With Water, A.W. Hoadley and B.J. Dutka (Eds.). American Soc. for Testing Materials, pp. 115-125.
4. Colwell, R.R., J. Kaper, and S.W. Joseph. 1977. Vibrio cholerae, Vibrio parahaemolyticus, and other Vibrios: Occurrence and distribution in Chesapeake Bay. Science 198: 394-396.

5. Crosa, J.H., D.J. Brenner, and S. Falkow. 1973. Use of a single-strand specific nuclease for analysis of bacterial and plasmid deoxyribonucleic acid homo- and hetero-duplexes. J. Bacteriol. 115: 904-911.
6. DeLey, J., and R. Tijtgat. 1970. Evaluation of membrane filter methods for DNA-DNA hybridization. Anton. van Leewen. 36: 461-474.
7. Denhardt, D.T. 1966. A membrane filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Comm. 23: 641-646.
8. Eckhardt, T. 1978. A rapid method for the identification of plasmid deoxyribonucleic acid in bacteria. Plasmid 1: 584-588.
9. Kaneko, T., and R.R. Colwell. 1978. The annual cycle of Vibrio parahaemolyticus in Chesapeake Bay. Microbial Ecol. 4: 135-155.
10. Kaper, J. 1979. Isolation, ecology, and taxonomy of human pathogens in an estuary. Ph.D. Thesis, University of Maryland. 309 pp.
11. Kaper, J., H. Lockman, R.R. Colwell and S.W. Joseph. 1979. Ecology, serology, and enterotoxin production of Vibrio cholerae in Chesapeake Bay. Appl. Environ. Microbiol. 37: 91-103.
12. Mandel, M., L. Igambi, J. Bergendahl, M.L. Dodson, Jr., and E. Scheltgen. 1970. Correlation of melting temperature and cesium chloride buoyant density of bacterial deoxyribonucleic acid. J. Bacteriol. 101: 333-338.
13. Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. 3: 208-218.
14. Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. 5: 109-118.
15. Meyers, J.A., D. Sanchez, L.P. Elwell, and S. Falkow. 1976. Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. J. Bacteriol. 127: 1529-1537.
16. Morbidity and Mortality Weekly Report. 1978. Vibrio cholerae infection-Louisiana. 27: 367.
17. Nalin, D.R., A. Reid, and M.M. Levine. 1978. Cholera, stomach acid, and chitin. 18th Interscience Conf. on Antimicrobial Agents and Chemotherapy, Atlanta, Ga. Abstr. Meeting, p. 119.
18. Rondle, C.J.M., B. Ramesh, J.B. Krahn, and R. Sherriff. 1978. Cholera: possible infection from aircraft effluent. J. Hyg. 81: 361-371.
19. Seidler, R.J., and M. Mandel. 1971. Quantitative aspects of deoxyribonucleic acid renaturation: base composition, state of chromosome replication, and polynucleotide homologies. J. Bacteriol. 106: 608-614.
20. Sizemore, R.K., R.R. Colwell, H.S. Tubiash and T.E. Lovelace. 1975. Bacterial flora of the hemolymph of the blue crab, Callinectes sapidus: numerical taxonomy. Appl. Environ. Microbiol. 29: 393.

21. Sochard, M.R., D.F. Wilson, B. Austin, and R.R. Colwell. 1979. Bacteria associated with the surface and gut of marine copepods. Appl. Environ. Microbiol. 37, 750-759.
22. Sriranganathan, N., R.J. Seidler, W.E. Sandine, and P.R. Elliker. 1973. Cytological and deoxyribonucleic acid-deoxyribonucleic acid hybridization studies on Lactobacillus isolates from San Francisco sourdough. Appl. Microbiol. 25: 461-470.
23. Zachary, A., M.E. Taylor, F.E. Scott, and R.R. Colwell. 1978. A method for rapid evaluation of materials for susceptibility to marine biofouling. Int. Biodeterior. Bull. 14: 111-118.

Table 1. Physical, chemical, and bacteriological data for Chesapeake Bay. Transect cruise, July 1977. Station numbers correspond to those of Kaper *et al.* (1979).

Station	Trans-parency (m)	Tempera-ture (°C)	Salinity (°/oo)	DO[a] (mg/1)	pH	Suspended particu-lates (mg/1)	Salmonella MPN[b]	Coliform MPN[b]	Fecal coliform MPN[b]	TVC[c] ($\times 10^5$)	Total Vibrio species	*V. parahae-molyticus* MPN[b]	*V. cholerae* MPN[d]
6	1.3	27.5	9.9	9.1	7.5	12.2	<.03	24	<.3	0.16	10	9.3	0.7
5	1.3	26.6	10.8	5.0	7.2	7.6	<.03	46	4.3	0.1	14	24.0	1.5
7	1.0	29.4	8.2	12.5	8.8	12.4	<.03	21	<.3	0.6	27	1.5	0.4
8	1.2	28.2	7.8	10.3	8.0	7.8	<.03	46	.4	1.2	48	24.0	0.4
9	1.0	28.5	6.7	6.2	6.9	10.8	<.03	150	<3	0.9	45	24.0	1.5
10	0.9	29.2	7.6	7.5	6.4	19.2	.23	1100	240	10.0	1500	>240.0	46.0
12	0.9	27.8	8.0	6.7	6.4	34.6	>240.0	110000	24000	15.0	3400	>240.0	0.7
13	1.3	27.0	8.0	0.1	6.3	12.4	4.6	>240000	46000	110.0	1200	46.0	0.3

[a] Dissolved oxygen.

[b] Most probable number per 100 ml of water.

[c] Total viable count per 1 ml of water.

[d] *V. cholerae* most probable number per 1 liter of water.

Table 2. Biochemical, serological, and toxigenic characteristics of *V. cholerae* isolates from Chesapeake Bay[a] (Kaper, 1979).

Strain	Source[b]	Date	Heiberg group[c]	Serotype[d] Smith	Serotype[d] Sakazaki	Toxin[e]	Phage[f] rxn	VP[g] rxn	Misc.[h]
V-2	Jones Falls	9/76	II	999	81	+	-	- (+)	Pig.
V-3	Jones Falls	9/76	I	999	45	++	-	- (+)	
V-4	Jones Falls	9/76	I	24	45	-	+	-	
V-5	Jones Falls	9/76	I	23	37	++	+	- (+)	
V-10	Jones Falls	9/76	II	14	6	++	-	- (+)	
V-11	Jones Falls	9/76	V	999	UK	+	-	-	
V-15	Jones Falls	9/76	V	999	UK	+	+	-	
V-19	Jones Falls	9/76	V	999	UK	++	+	-	
V-20	Jones Falls	9/76	V	999	UK	++	+	-	
V-24	Jones Falls	9/76	V	999	UK	++	+	-	
V-25	Jones Falls	9/76	V	999	UK	++	+	-	
V-26	Jones Falls	9/76	V	999	UK	++	+	-	
V-29	Jones Falls	9/76	V	999	UK	(+)	-	-	
V-31	Ft. McHenry	4/77	II	999	58	-	-	+	
V-33	Ft. McHenry	4/77	II	14	6	-	-	+	
V-35	Chester River	5/77	V	23	24	(+)	-	+	
V-36	Chester River	5/77	II (I)	999	6	++	-	+	
V-37	Chester River	5/77	II	999	6	+	-	+	
V-38	Chester River	5/77	II	17	2	++	-	+	
V-39	Chester River	6/77	II	343	2	++	-	- (+)	Man-
V-40	Chester River	6/77	V	94	66	++	-	+	
V-41	Ft. McHenry	6/77	I	23	37	++	-	+	
V-42	Colgate Creek	6/77	I	999	41	++	-	+	
V-43	Colgate Creek	6/77	V	94	66	++	-	+	
V-44	Colgate Creek	6/77	II	31	14	+	-	+	
V-48	Choptank River	6/77	V	999	UK	(+)	-	+ (-)	
V-50	Potomac River	6/77	II	33	12	ND[i]	-	+	
V-51	Patuxent River	6/77	I	14	6	(+)	-	+	
V-52	Patuxent River	6/77	I	14	6	++	-	+	
V-53	Patuxent River	6/77	II	19	23	++	+	+	
V-54	James River	6/77	I	14	6	+	-	+	man-
V-55	Bay Bush Point	7/77	II	19	23	++	+	- (+)	
V-56	Bay Bush Point	7/77	II	23	37	-	-	- (+)	
V-57	Bay Bush Point	7/77	I	999	39	++	-	- (+)	
V-58	Eastern Bay	7/77	II	999	10	++	-	- (+)	
V-60	Ft. McHenry	7/77	II	308	54	-	-	- (+)	
V-61'	Ft. McHenry	7/77	II	16	28	++	-	- (+)	
V-62	Hawkins Point	7/77	I	333	49	++	-	- (+)	
V-63	Hawkins Point	7/77	I	333	37	++	+	- (+)	
V-64	Hawkins Point	7/77	I	37	27	+	+	- (+)	
V-65	Hawkins Point	7/77	II	31	2	++	-	- (+)	

Table 2 (continued)

Strain	Source	Date	Heiberg group[c]	Serotype[d] Smith	Sakazaki	Toxin[e]	Phage[f] rxn	VP[g] rxn	Misc.[h]
V-66	Hawkins Point	7/77	II	999	6	(+)	-	- (+)	
V-67	Hawkins Point	7/77	I	94	53	++	-	- (+)	man-
V-68	Chester River	7/77	I	104	11	+	-	- (+)	
V-69	North Point	7/77	I	333	O-1, Inaba	+	++	- (+)	
V-70	Bodkin Point	7/77	II	43	32	++	-	- (+)	man-
V-71	Eastern Bay	7/77	I (II)	999	28	(+)	-	+	
V-72	Magothy River	7/77	I (II)	106	41	++	-	- (+)	
V-73	Hawkins Point	7/77	I (II)	14	6	+	-	- (+)	
V-74	Chester River	7/77	I (II)	102	11	(+)	-	- (+)	
V-75	North Point	7/77	I (II)	999	11	++	-	+	
V-76	North Point	7/77	II	333	2	++	+	+	man-
V-77	Chester River	9/77	II	94	10	+	-	+	
V-78	Chester River	9/77	II	999	10	++	-	+	
V-79	Chester River	9/77	II	19	23	++	+	+	man-
V-80	Chester River	10/77	II	333	43	++	+	+	man-
V-81	Chester River	10/77	I (II)	19	23	++	+	+	man-
V-82'	Chester River	10/77	I	94	53	++	-	+	man-
V-83	Plankton filtrate	10/77	I	115	53	++	+	+	man-
V-84	Chester River	10/77	II	999	19	+	-	-	
V-85	Chester River	12/77	I	999	UK	-	-	+	
V-86	Chester River	1/78	I	999	UK	-	ND	+	
V-87	Chester River	1/78	I	999	UK	-	ND	- (+)	
V-88	Chester River	1/78	I	999	26	+	ND	+	
V-89	Chester River	1/78	I	999	UK	-	ND	- (+)	
V-90	Chester River	3/78	II	999	UK	ND	ND	+	
V-91	St. Georges Island	6/78	II	999	19	ND	ND	+	
V-92	St. Georges Island	6/78	II	999	28	ND	ND	+	
V-93	St. Georges Island	6/78	I	999	49	ND	ND	+	
V-94	St. Georges Island	6/78	II	17	2	ND	ND	+	
V-95	Point Lookout	6/78	II	22	8	ND	ND	+	
V-96	Ft. McHenry Sed.	8/78	I	999	38	ND	ND	-	
V-97	Ft. McHenry Sed.	8/78	I	76	38	ND	ND	-	
V-98	Turkey Point	7/78	V	999	41	ND	ND	-	

[a] All isolates, unless otherwise noted, were positive for oxidase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, indole production, gelatin hydrolysis, growth in 0% NaCl, β-galactosidase, hemolysis and fermentation of glucose and mannitol. All isolates were negative for arginine dihydrolase, H_2S production, urease, and acid from arabinose, melibiose, rhamnose, sorbitol, inositol and amygdalin.

[b] All strains were isolated from water samples, unless otherwise noted.

[c] Heiberg group I indicates fermentation of both sucrose and mannose; group II indicates sucrose +, mannose -; group V indicates sucrose -, mannose +. Numbers in parentheses ( ) indicate a discrepancy between results obtained by Dr. H. L. Smith, Jr., Vibrio Reference Laboratory, Philadelphia, and Dr. R. Sakazaki, National Institute of Health, Tokyo. Results received from Sakazaki are noted in parentheses.

Table 2. Footnotes (continued)

[d]Strains were serotyped by both serotyping schemes commonly used, i.e., the scheme of Dr. H. L. Smith, Jr. and Dr. R. Sakazaki. Types "999" and "UK" indicate that the strain did not agglutinate in any antisera currently available in the laboratories of Dr. Smith and Dr. Sakazaki, respectively.

[e]Toxin assay using Y-1 Adrenal cells. ++ = positive within 10 hours, + = positive within 24 hours, (+) = weak positive within 24 hours, - = negative at 24 hours. Toxic response reported includes reaction of cytotoxin and enterotoxin.

[f]+ = susceptible to Mukherjee type III phage. ++ = susceptible to both type I and II.

[g]Voges-Proskauer rxn. Where two reactions are given, the first reaction is that obtained using the API 20 test strip and the second reaction (in parenthesis) is the result reported by Dr. Sakazaki.

[h]Miscellaneous characteristics: Pig. = extracellular pigment produced; man- = mannitol not fermented.

[i]ND = not determined.

Table 3. Results of DNA hybridization of fresh isolates of O Group I and non O Group I Vibrio cholerae from U.S. Coastal areas with V. cholerae Biotype El Tor strain 14033.[1]

V. cholerae Strain	Serotype[5]	DNA Base Composition[2] (% Guanine + Cytosine)	% Hybridization Optical Renaturation	% Hybridization Membrane Filter	% Hybridization S1 Endonuclease
Biotype El Tor					
ATCC 14033	01	47.0, 48.0	100	100	100
ATCC 14035 (neotype strain)	01	48.0, 47.0	ND	89	ND
CB V-37[3]	999, 6	47.0, 47.6	84	92	98
CB V-69	01	46.1, 46.6	96	88	ND
CB V-40	94, 66	47.4, 48.2	ND	98	75
CB V-43	94, 66	47.8, 48.4	ND	84	75
CB V-48	999, UK	47.6, 47.4	ND	80	ND
CB V-35	23, 24		ND	ND	92
CB V-98	999, 41	47.0, 46.8	ND	74	58
CB V-11	999, UK	46.4, 46.6	ND	35	36
LA 4808-78	01 Inaba	46.8, 46.8	96	95	ND
LA 6049-3	01 Inaba	48.6, 48.0	ND	88	ND
LA 718	14, 8[4]	46.4, 46.0	8	12	ND

[1] See text for details of methods. Strain 14033 was obtained from the American Type Culture Collection and is the proposed neotype for V. cholerae biotype El Tor. ND = Not Done.

[2] Results of two separate determinations.

[3] CB = Chesapeake Bay isolates; LA = Louisiana isolates.

[4] Associated with severe diarrheal disease, but not identified as V. cholerae.

[5] First number given is from H. Smith, second from R. Sakazaki.

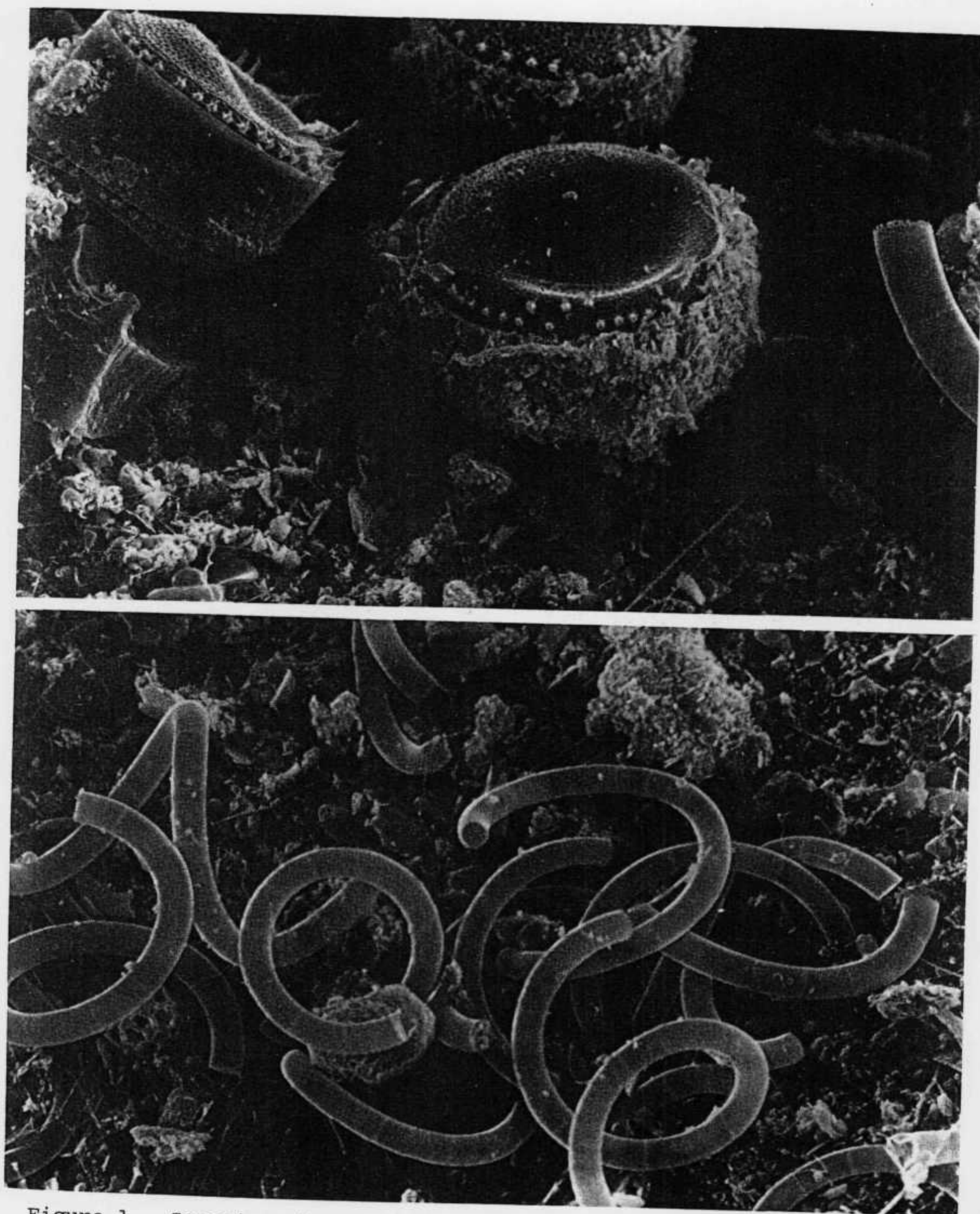
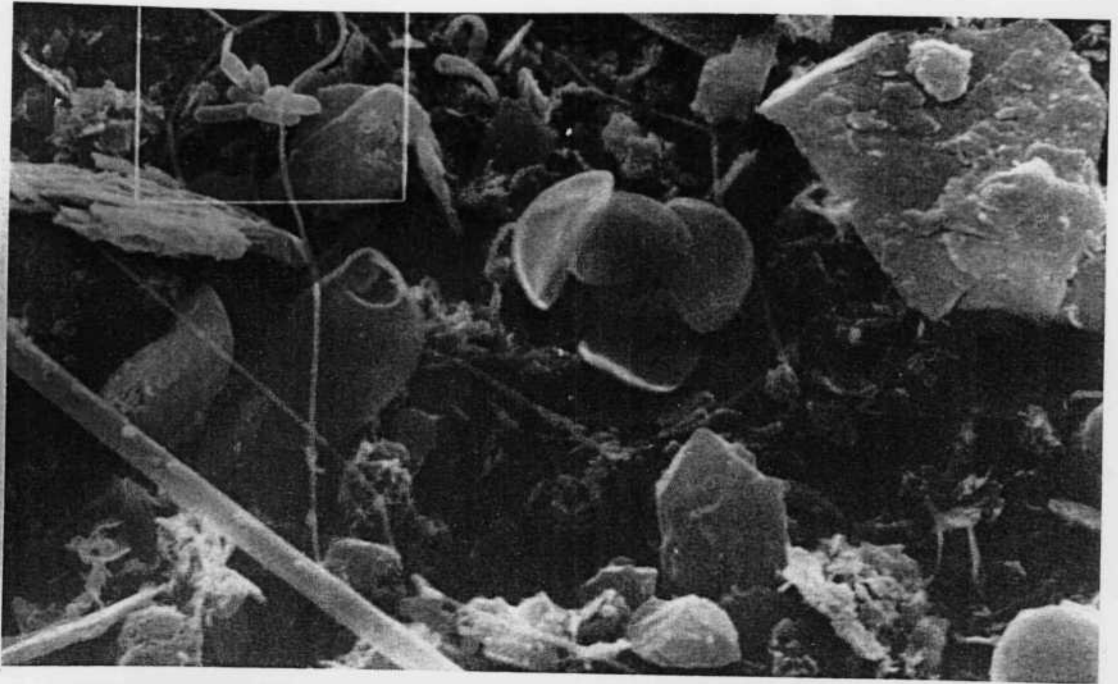


Figure 1. Scanning electron micrograph of plankton samples collected in Dacca, Bangladesh, March, 1979. Magnification of the upper micrograph is 1100X and of the lower 6,600X.



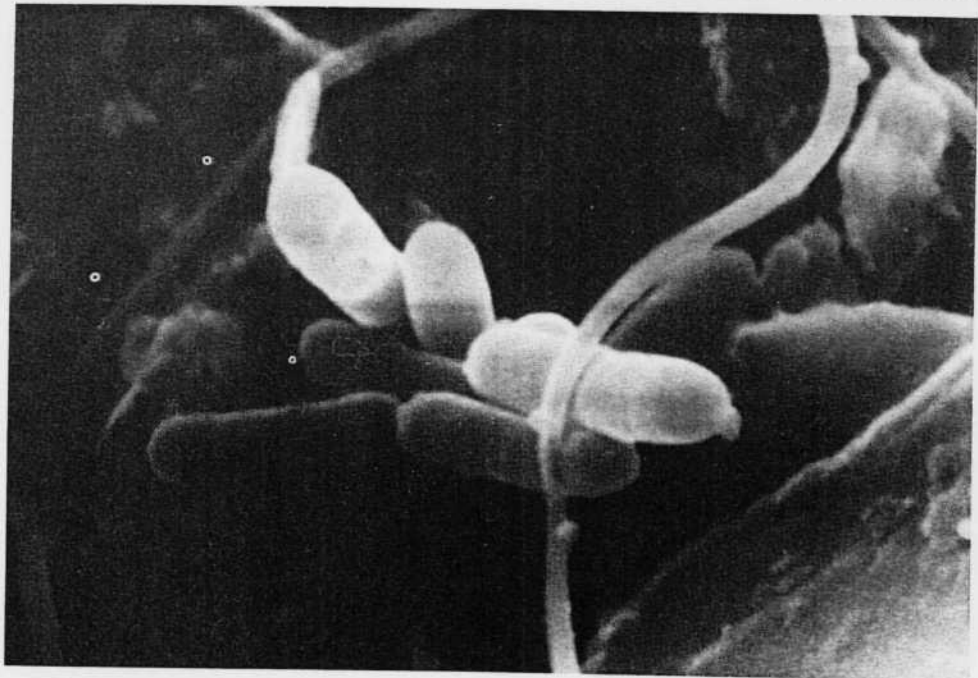
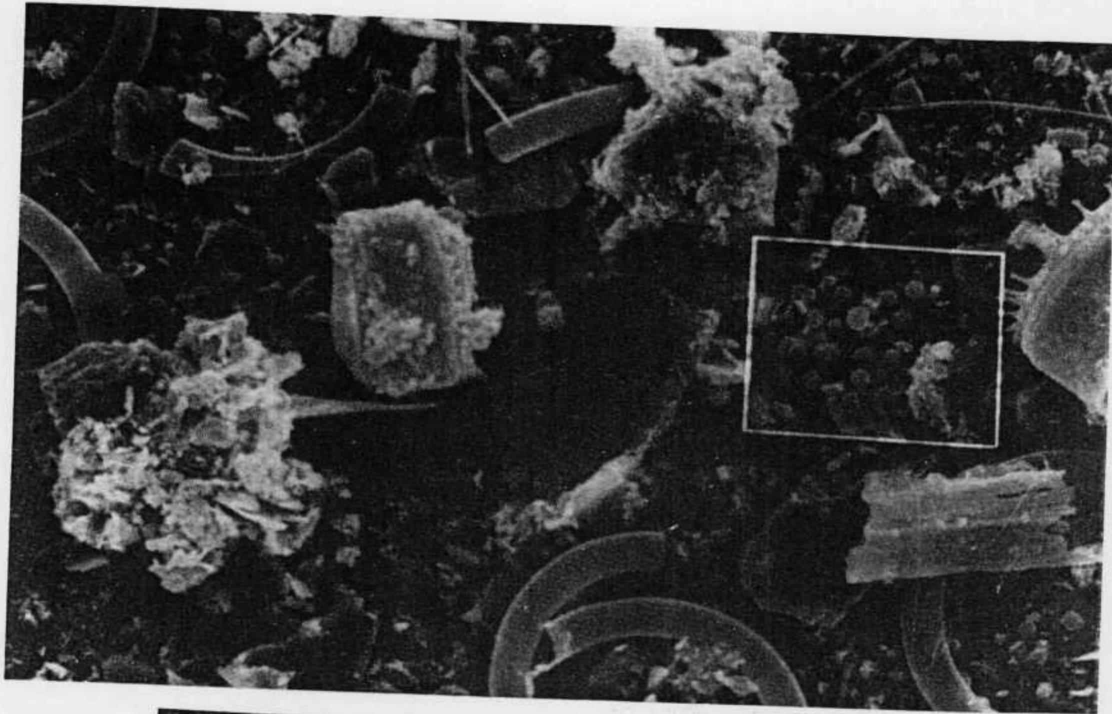


Figure 2. Scanning electron micrograph of Bangladesh plankton samples. Magnification of the upper micrograph is 3900X and the lower, an enlargement of the area outlined in the upper, is 20,000X. Bacteria can be seen in the lower micrograph.



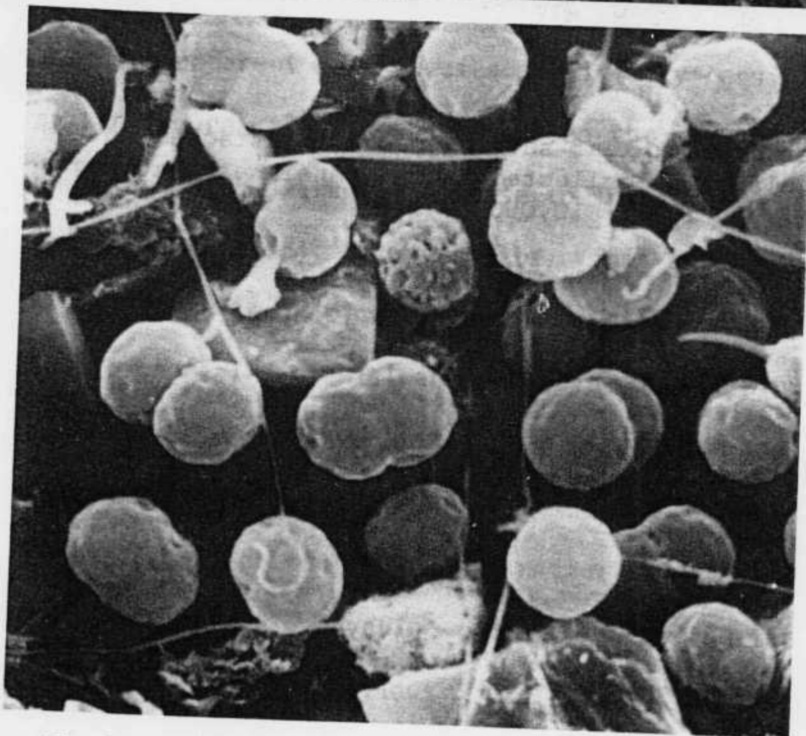


Figure 3. Plankton samples from Bangladesh. Magnification of the upper micrograph is 700X and the lower, an enlargement of the area outlined in the upper, is 4200X.

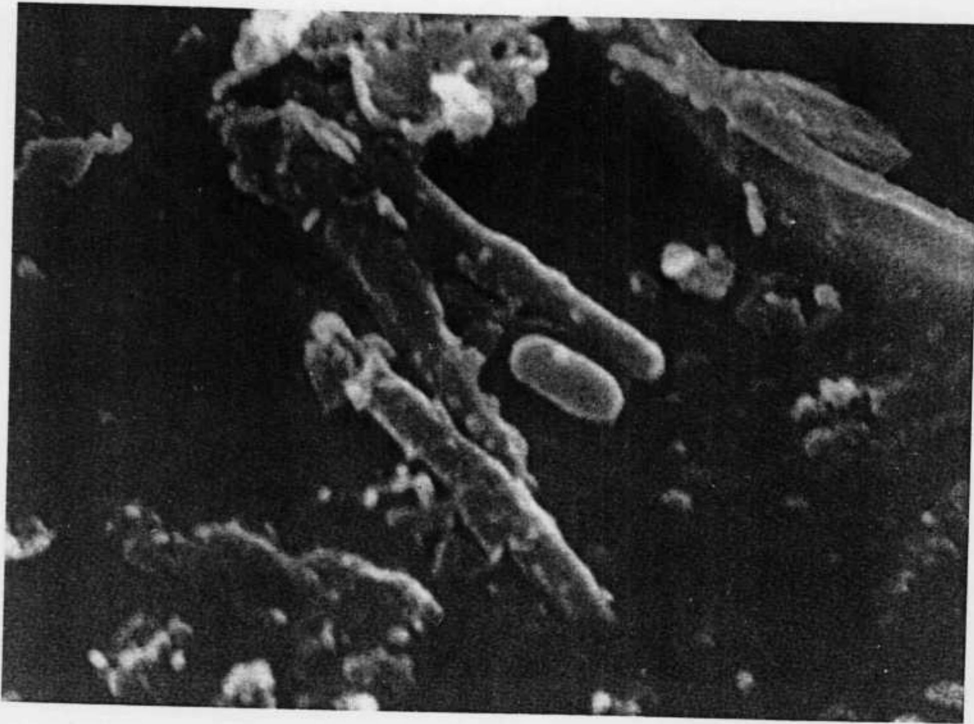


Figure 4. Aquatic plant leaf showing bacteria attached to the surface. The plant was collected from a canal near Matlab, Bangladesh in March, 1979. 10,000X.

ICDDR,B Library
Dacca—12

M15747

PROCEEDINGS OF THE FIFTEENTH JOINT CONFERENCE ON CHOLERA

SPONSORED BY THE U.S.-JAPAN COOPERATIVE MEDICAL SCIENCE PROGRAM
MICROBIOLOGY AND INFECTIOUS DISEASES PROGRAM
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
NATIONAL INSTITUTES OF HEALTH

BETHESDA, MARYLAND
JULY 23, 24, 25, 1979

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service National Institutes of Health
NIH Publication No. 80-2003
February 1980

29 OCT 1980