APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1986, p. 1407–1411
0099-2240/86/121407-05$02.00/0

Vol. 52, No. 6

Occurrence and Distribution of Halophilic Vibrios in Subtropical Coastal Waters of Hong Kong

KWONG-YU CHAN,[1]* MAN LUEN WOO,[2] KWOK WAI LO,[1] AND GARY L. FRENCH[2]

Department of Biology[1] and Department of Microbiology,[2] The Chinese University of Hong Kong, Shatin, N.T., Hong Kong

Received 13 June 1986/Accepted 23 September 1986

The summer occurrence and distribution of halophilic vibrios in the subtropical coastal waters of Hong Kong were investigated. The density of vibrios in six sample sites ranged from 90 to 6,700 per ml, which made up 0.41 to 40% of the total bacterial populations of these sample sites. The sucrose-positive vibrios were found to be much more common (88% of total vibrios) than the sucrose-negative ones. A total of 48 strains belonging to six *Vibrio* species were fully characterized. Among these, *Vibrio alginolyticus* was the most frequently isolated, followed by *V. parahaemolyticus*, *V. harveyi*, *V. vulnificus*, *V. campbellii*, and *V. fluvialis*. The finding that eight of the nine strains of *V. harveyi* showed a positive Kanagawa reaction warrants further study.

Several marine species of the genus *Vibrio* are pathogenic for man, and some have caused fatal infections (6). Most of the infections with halophilic vibrios are known to be associated with either consumption of seafood or exposure to marine environments. Infection due to *Vibrio parahaemolyticus* is worldwide in distribution (6, 15) and is the main cause of summer diarrhea in Japan (42). *V. parahaemolyticus* is commonly found in the coastal waters of Hong Kong (2). *V. vulnificus* infection, which causes septicemia and soft-tissue necrosis, was first recognized in the United States (13), and sporadic cases have been reported in Japan (24), Australia (11), and Belgium (25). We have recently seen a case of necrotizing fasciitis caused by *V. vulnificus* in Hong Kong (41). Other halophilic vibrios such as *V. alginolyticus* and *V. fluvialis* may cause wound infection (6) and diarrhea (14), respectively, but infections due to these organisms have not been reported in Hong Kong.

Although the ecology and distribution of *V. parahaemolyticus* have been studied extensively (12, 15–17, 22, 38, 39), those of *V. vulnificus* and other marine vibrios are less well understood. Studies of the ecology and distribution of *V. vulnificus* have been limited to the Gulf coast (18, 23) and the eastern and southeastern coasts of the United States (3, 10, 29, 30). In addition, there is no information on the distribution of vibrios other than *V. parahaemolyticus* and *V. alginolyticus* in marine coastal waters of Southeast Asia (2, 9, 26, 28, 35). In view of the potential pathogenicity of these organisms, we have conducted a study of the occurrence and distribution of marine vibrios in the subtropical coastal waters of Hong Kong.

MATERIALS AND METHODS

Sampling protocol. Six coastal sites in Hong Kong, including four recreational beaches (Repulse Bay, Mid Bay, Stanley Bay, and Shek O) and two densely populated dockside areas (Aberdeen shelter and Jordan ferry pier), were sampled (Fig. 1). Samples were taken from two or three subsites at each sampling site during summer (May to October) 1984. Surface water samples were collected in sterile glass bottles. Each water sample was tested for salinity, temperature, pH, turbidity, levels of NO_3^- and PO_4^{3-}, fecal coliform counts, total bacterial counts, and total vibrio counts. The water temperature, salinity, and pH measurements were made on site with portable meters. Turbidity was measured spectrophotometrically at 450 nm. PO_4^{3-} and NO_3^- levels were measured by the method of Murphy and Riley (27) and the brucine method (1), respectively. After serial dilution of the water samples, fecal coliforms were counted on Levine eosin-methylene blue agar (Difco Laboratories, Detroit, Mich.) after incubation at 44.5°C for 24 h. Total bacterial counts were performed on 2216 marine agar (Difco) after incubation at 30°C for 48 h. Total vibrios were counted on TCBS (thiosulfate-citrate-bile salts-sucrose) agar (Difco) after incubation at 30°C for 24 h.

Isolation and identification of vibrios. Water samples were diluted, plated on TCBS agar, and incubated at 30°C for 24 h. The sucrose-negative (green) and sucrose-positive (yellow) colonies were picked and purified by being restreaked on TCBS agar. In addition, the sucrose-negative isolates were streaked onto an estuarine agar (Difco) containing 1% lactose at pH 7.4 (29) to induce β-galactosidase synthesis.

All isolates and the reference strains *V. vulnificus* ATCC 27562, *V. parahaemolyticus* 113 (obtained from P. Baumann), *V. harveyi* ATCC 14126, *V. alginolyticus* 118 (obtained from P. Baumann), *V. fischeri* 61 (obtained from P. Baumann), *V. marinus* ATCC 15381, and *V. campbellii* 40 (obtained from P. Baumann) were examined for 63 physiological, biochemical, and morphological features (see Table 3). Multiple isolates that were obtained from a single sample and that showed identical features were considered to belong to one strain. Some physiological and biochemical characteristics were tested by use of the API 20E system (API System S.A., Montalieu Vercieu, France) modified by emulsification of the organisms in 3% NaCl solution instead of distilled water. Tests for carbohydrate fermentation were also carried out in yeast extract broth (Difco) by the method of Baumann et al. (4), for which pH changes in the culture medium were measured with a pH meter after incubation. Tests for utilization of various carbon sources were performed as described previously (4). Each of the tested carbon compounds was added to basal medium agar (pH 7.4) without any carbon source to give a final concentration of 0.1% (or 0.2% for sugars). Salt tolerance was tested by using

* Corresponding author.

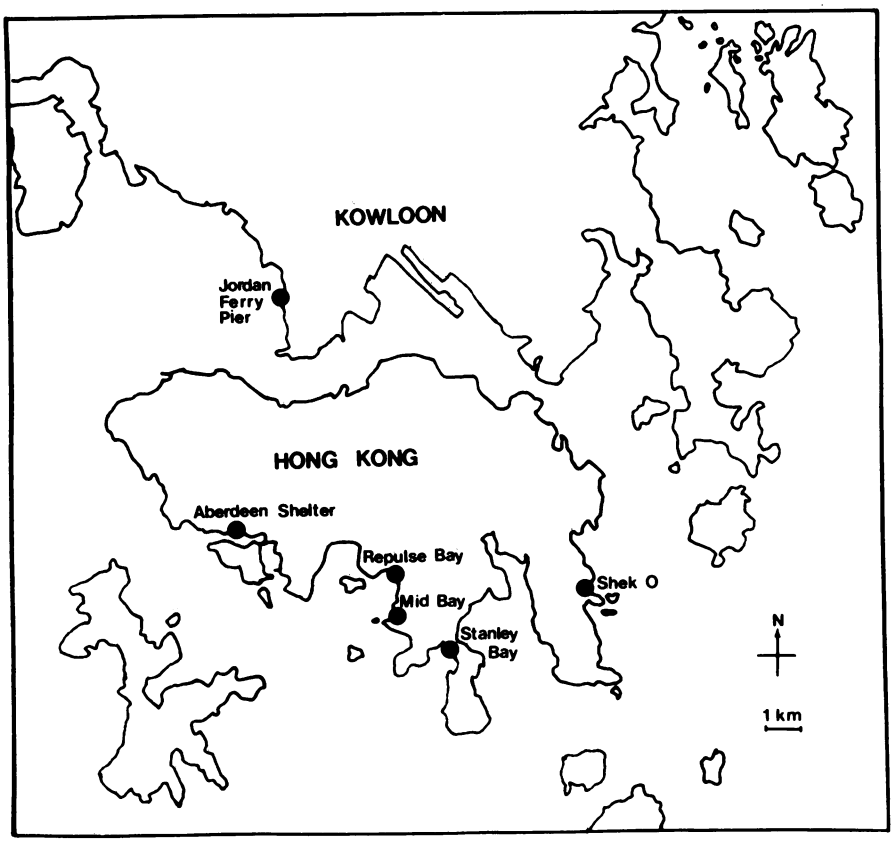

FIG. 1. Sample sites.

basal medium (4) supplemented with NaCl at final concentrations of 0, 3, 6, 8, and 10%. Except for the test for hemolytic reaction, which was carried out with Wagatsuma agar (37) at 37°C, all other tests involved incubation at 30°C. The *Vibrio* isolates were identified by the methods in the literature (5, 6, 15, 34, 40).

RESULTS AND DISCUSSION

The physical and chemical measurements at the sample sites are listed in Table 1. Vibrios were isolated from all 6 sample sites and from 14 of the 16 subsites. Table 2 shows the bacterial distribution among these six sample sites. Except in Stanley Bay, where vibrios made up a high percentage (40%) of the bacterial population, vibrios were a minority, ranging from 0.41 to 12% of the total bacterial populations. This differs from results reported for the coastal waters of the eastern and southeastern United States, where vibrios averaged 15 to 31% of the total bacterial population during the summer (29, 30).

Sucrose-positive vibrios predominated over sucrose-negative vibrios in water samples from all sample sites except those obtained from Mid Bay, where there was an almost equal distribution of these two types (Table 2). The fact that the sucrose-positive vibrios were found to be predominant (88% of total vibrios) proved the ubiquity of this group of bacteria in subtropical coastal waters, although they did not make up a major fraction of the total bacterial population in the water column. It has been found that an average of 52% of the vibrios isolated from coastal waters of the United States are sucrose negative (30). There were no significant differences in the results of physicochemical measurements in our study (Table 1) and those in the study of the coastal waters of the United States (30), except that water samples in our study were more acidic, which presumably was due to large quantities of terrestrial runoff, since the levels of rainfall were much higher than normal during summer 1984 (31). The pH of the water might therefore be one of the important environmental factors affecting the

TABLE 1. Environmental data for the sample sites

Sample site	Water temp (°C)	Salinity (‰)	pH	Turbidity at 450 nm	Amt of NO_3^- (μg/liter)	Amt of PO_4^{3-} (μg/liter)
Repulse Bay	27–28	26–27	6.3–6.6	0.013–0.02	112–134	207–229
Mid Bay	27–28	26–28	6.3–6.7	0.005–0.03	74–92	110–131
Aberdeen shelter	24–25	26–28	5.6–5.8	0.005–0.01	186–205	362–387
Shek O	28–29	29–30	6.1–6.2	0.005–0.02	91–111	174–198
Stanley Bay	29–29.5	25–26	6.0–6.2	0.005–0.01	77–99	183–201
Jordan ferry pier	23–24	26–27	5.8–6.0	0.01–0.04	168–185	306–324

TABLE 2. Bacterial densities at the sample sites

Sample site	Total no. of bacteria/ml	No. (%)[a] of fecal coliforms/ml	Total no. (%)[a] of vibrios/ml	No. (%)[b] of sucrose-negative vibrios/ml	No. (%)[b] of sucrose-positive vibrios/ml
Repulse Bay	1.0×10^3	56 (6.4)	90 (9)	16 (17)	74 (83)
Mid Bay	8.1×10^3	10 (0.1)	30 (0.41)	16 (53)	14 (47)
Aberdeen shelter	2.0×10^5	7.6×10^3 (3.8)	5.5×10^3 (2.7)	50 (0.9)	5.3×10^3 (99.1)
Shek O	2.0×10^3	94 (4.9)	2.0×10^2 (10)	44 (22)	1.6×10^2 (78)
Stanley Bay	7.2×10^2	34 (6.1)	2.9×10^2 (40)	32 (11)	2.6×10^2 (89)
Jordan ferry pier	5.5×10^4	5.2×10^2 (0.9)	6.7×10^3 (12)	1.4×10^3 (20)	5.3×10^3 (80)
Mean	4.5×10^4	1.4×10^3 (3.1)	2.1×10^3 (4.6)	2.6×10^2 (12)	1.9×10^3 (88)

[a] Figures represent percent total bacteria.
[b] Figures represent percent total vibrios.

TABLE 3. Selected characteristics of halophilic *Vibrio* species isolated from coastal waters of Hong Kong[a]

Characteristic	No. of strains showing positive reaction[b]:					
	V. vulnificus (n = 8)	*V. harveyi* (n = 9)	*V. parahaemolyticus* (n = 10)	*V. alginolyticus* (n = 14)	*V. fluvialis* (n = 3)	*V. campbellii* (n = 4)
Swarming on solid complex media	0	0	0	14	0	0
Growth in the presence of NaCl						
3%	8	9	10	14	3	4
6%	8	9	10	14	3	4
8%	0	0	8	10	2	1
10%	0	0	1	10	0	0
Growth at						
20°C	8	9	10	14	3	4
37°C	8	9	10	14	3	4
42°C	7	2	9	14	0	0
ONPG hydrolysis	8	9	0	0	3	0
Arginine dihydrolase	0	0	0	0	3	0
Ornithine decarboxylase	8	9	9	13	0	0
Lysine decarboxylase	7	9	10	14	0	4
Citrate utilization	2	5	1	8	3	2
Indole production	8	9	10	14	3	4
Acetoin production (Voges-Proskauer reaction)	0	0	0	14	1	0
0/129 sensitivity						
10 μg	7	0	0	0	0	0
150 μg	8	8	9	11	3	4
Starch hydrolysis	7	8	10	12	1	4
Gelatinase	7	9	9	12	2	4
Lipase	7	6	7	11	1	3
Alginase	0	2	0	0	1	0
Chitinase	5	7	8	13	2	4
Acid from						
Lactose	8	9	0	0	3	0
Glucose	8	9	10	14	3	4
Mannitol	6	9	10	14	3	1
Inositol	0	0	0	0	1	0
Sucrose	0	9	0	14	3	0
Mannose	8	9	10	0	2	4
Salicin	8	2	4	7	0	0
Arabinose	8	1	6	0	2	0
Methyl red	8	8	10	2	1	3
Kanagawa reaction	7	8	3	3	0	0
Sucrose	0	9	0	14	3	0

[a] All strains were motile gram-negative rods or vibrios with a single polar flagellum; grew on TCBS agar; were glycerol, oxidase, catalase, and nitrate positive; fermented dextrose without gas; and grew in 1 to 6% but not 0% NaCl-peptone water. They were negative for H_2S production, urease, melibiose, and rhamnose fermentation, and utilization of D-xylose, glutarate, β-hydroxybutyrate, *p*-hydroxybenzoate, β-alanine, δ-aminovalerate, malonate, benzoate, spermine, betaine, sarcosine, hippurate, erythritol, and phenylacetate.
[b] n, Number of strains.

relative abundance of sucrose-negative and sucrose-positive vibrios in coastal waters.

Since the highest densities of vibrios were detected in water samples from Aberdeen shelter and Jordan ferry pier (Table 2), which also gave the highest counts of total bacteria, and since water samples from these two sites showed the highest levels of NO_3^- and PO_4^{3-} and the lowest pHs and temperatures, the abundance of vibrios in waters from these two sample sites may have been due to the combined effect of these environmental factors (Table 1). The high percentage of vibrios detected in water samples from Stanley Bay (Table 2) is unexplained at present, and further study is needed.

A total of 213 isolates were obtained from TCBS agar plates. After identification, 48 different strains were confirmed and identified to species level. Table 3 shows the selected characteristics of these strains.

Ten sucrose-negative, *o*-nitrophenyl-β-D-galactopyranoside (ONPG)-negative strains were identified as *V. parahaemolyticus*. This species was isolated from coastal waters of Hong Kong in 1967 (2). Only three strains showed a positive Kanagawa reaction on Wagatsuma agar, which is typical of environmental isolates of *V. parahaemolyticus* (7, 33, 36, 38). All 10 strains grew well in medium containing 6% NaCl, 8 strains could grow at 8% NaCl, but only 1 grew at 10% NaCl.

Eight strains were classified as *V. vulnificus*, since they were sucrose negative and ONPG positive and produced acid from lactose in yeast extract broth. However, when phenol red lactose broth was used for detecting lactose fermentation, several of these ONPG-positive strains were found to be lactose negative even after 4 days of incubation. It has been proposed that a false-negative result for lactose fermentation may be due to an extremely rapid fermentation of lactose by some *V. vulnificus* strains followed by a reversion of the pH indicator to the neutral or alkaline state (29), but this is unlikely to be the case in the present study, since negative results were obtained as early as 0.5 h after incubation. Seven of the eight strains were found to produce a hemolysin, giving a positive Kanagawa reaction. All eight strains grew in medium containing 6 but not 8% NaCl.

Fourteen sucrose-positive, Voges-Proskauer-positive, ONPG-negative strains showed swarming colonies on solid complex media and were classified as *V. alginolyticus*. All 14 strains grew at 42°C, and 10 of them could grow in medium containing 10% NaCl.

Three ONPG-positive, lactose-positive strains were classified as *V. fluvialis*. These differed from *V. vulnificus* in being negative for ornithine decarboxylase and lysine decarboxylase and positive for arginine dihydrolase and sucrose fermentation. *V. fluvialis* has long been considered lactose negative, but recent evidence strongly suggests that some isolates may ferment this sugar (29).

Nine strains phenotypically similar to *V. vulnificus*, except that they could ferment sucrose, were identified as *V. harveyi*. Another four strains which were negative for ONPG, arginine dihydrolase, and ornithine decarboxylase and positive for lysine decarboxylase were classified as *V. campbellii*.

In contrast to previous reports (16, 30), the results of the present study indicate that vibrios do not make up a very high percentage of the marine coastal bacterial populations in the subtropical waters of Hong Kong. This is in agreement with other studies in Hong Kong in which few vibrios were isolated from marine waters (8) or seaweeds (19). During the enumeration of vibrios in the present study, it was found that a number of nonvibrios such as *Aeromonas hydrophila* could also grow on TCBS agar, and the true numbers of vibrios in the water samples may therefore be even lower than those reported here. The fact that most of the sucrose-positive vibrios detected were *V. alginolyticus* supports the finding that this species appears to be present in seawater in larger numbers than other *Vibrio* species (20, 26, 32).

Recently, it was reported that vibrios are part of the common flora of mussels, clams, and oysters in Hong Kong (21). Although marine vibrios make up only a small proportion of the bacterial populations of coastal waters in Hong Kong, they may accumulate in bivalve shellfish and cause a public health hazard, since shellfish in Hong Kong, especially oysters, are commonly eaten uncooked or only partially cooked. The finding that eight of the nine strains of *V. harveyi* were Kanagawa positive and were therefore a potential health hazard warrants further investigation. To the best of our knowledge, this is the first report of the isolation of *V. vulnificus* and *V. campbellii* from coastal waters in Southeast Asia.

ACKNOWLEDGMENT

This work was partially supported by a grant from the Ming Yu Research & Visiting Programme, New Asia College, The Chinese University of Hong Kong, to K.-Y.C.

LITERATURE CITED

1. **American Public Health Association.** 1971. Standard methods for the examination of water and wastewater, 13th ed. American Public Health Association, Washington, D.C.
2. **Aoki, Y., S. T. Hsu, and D. Chun.** 1967. Distribution of *Vibrio parahaemolyticus* in the sea and harbors in Southeast Asia and Central Pacific. Endem. Dis. Bull. Nagasaki Univ. **8**:191–202.
3. **Barbay, J. R., H. B. Bradford, Jr., and N. C. Roberts.** 1984. The occurrence of halophilic vibrios in Louisiana coastal waters, p. 511–520. *In* R. R. Colwell (ed.), Vibrios in the environment. John Wiley & Sons, Inc., New York.
4. **Baumann, P., L. Baumann, and M. Mandel.** 1971. Taxonomy of marine bacteria: the genus *Beneckea*. J. Bacteriol. **107**:268–294.
5. **Baumann, P., and R. H. W. Schubert.** 1984. *Vibrionaceae*, p. 516–528. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
6. **Blake, P. A., R. E. Weaver, and D. G. Hollis.** 1981. Diseases of humans (other than cholera) caused by vibrios. Annu. Rev. Microbiol. **34**:341–367.
7. **Bockemuhl, J., and A. Triemer.** 1974. Ecology and epidemiology of *Vibrio parahaemolyticus* on the coast of Togo. Bull. W.H.O. **51**:353–360.
8. **Chan, K.-Y., and C. S. W. Kueh.** 1976. Distribution of heterotrophic bacteria related to some environmental factors in Tolo Harbour. Int. J. Ecol. Environ. Sci. **1**:47–57.
9. **Chun, D., J. K. Chung, S. Y. Seol, and R. Tak.** 1974. *Vibrio parahaemolyticus* in the Republic of Korea. Am. J. Trop. Med. Hyg. **23**:1125–1130.
10. **Felsenfeld, O., and H. B. Cabirac.** 1977. A study of the ecology of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* in southeast Louisiana, USA, with special consideration of seafood consumption. J. Appl. Nutr. **29**:17–24.
11. **Ghosh, H. K., and T. E. Bowen.** 1980. Halophilic vibrios from human tissue infections on the Pacific coast of Australia. Pathology **12**:397–402.
12. **Golten, C., and W. A. Scheffers.** 1975. Marine vibrios isolated from water along the Dutch coast. Neth. J. Sea Res. **9**:351–357.
13. **Hollis, D. G., R. E. Weaver, C. N. Baker, and C. Thornsberry.** 1976. Halophilic *Vibrio* species isolated from blood cultures. J. Clin. Microbiol. **3**:425–431.
14. **Huq, M. I., A. K. M. J. Alam, D. J. Brenner, and G. K. Morris.**

1980. Isolation of *Vibrio*-like group, EF-6, from patients with diarrhea. J. Clin. Microbiol. **11**:621–624.
15. **Joseph, S. W., R. R. Colwell, and J. B. Kaper.** 1982. *Vibrio parahaemolyticus* and related halophilic vibrios. Crit. Rev. Microbiol. **10**:77–124.
16. **Kaneko, T., and R. R. Colwell.** 1973. Ecology of *Vibrio parahaemolyticus* in Chesapeake Bay. J. Bacteriol. **113**:24–31.
17. **Kaneko, T., and R. R. Colwell.** 1978. The annual cycle of *Vibrio parahaemolyticus* in Chesapeake Bay. Microb. Ecol. **4**:135–155.
18. **Kelly, M.** 1982. Effect of temperature and salinity on *Vibrio* (*Beneckea*) *vulnificus* occurrence in a gulf coast environment. Appl. Environ. Microbiol. **44**:820–824.
19. **Kong, M. K., and K.-Y. Chan.** 1979. A study on the bacterial flora isolated from marine algae. Bot. Mar. **22**:83–97.
20. **Kristensen, K. K.** 1974. The occurrence of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* in the Sound. Nord. Vet. Med. **26**:188–196.
21. **Kueh, C. S. W., and K.-Y. Chan.** 1985. Bacteria in bivalve shellfish with special reference to the oyster. J. Appl. Bacteriol. **59**:41–47.
22. **Liston, J., and J. Baross.** 1973. Distribution of *Vibrio parahaemolyticus* in the natural environment. J. Milk Food Technol. **36**:113–117.
23. **MacDonell, M. T., and M. A. Hood.** 1984. Ultramicrovibrios in the gulf coast estuarine waters: isolation, characterization and incidence, p. 551–562. *In* R. R. Colwell (ed.), Vibrios in the environment. John Wiley & Sons, Inc., New York.
24. **Matsuo, T., S. Kohno, T. Ikeda, K. Saruwatari, and H. Ninomiya.** 1978. Fulminating lactose positive vibrio septicaemia. Acta Pathol. Jpn. **28**:937–948.
25. **Mertens, A., J. Nagler, W. Hansen, and E. Gepts-Friedenreich.** 1979. Halophilic, lactose-positive *Vibrio* in a case of fatal septicemia. J. Clin. Microbiol. **9**:233–235.
26. **Molitoris, E., S. W. Joseph, M. I. Krichevsky, W. Sinhuhardia, and R. R. Colwell.** 1985. Characterization and distribution of *Vibrio alginolyticus* and *Vibrio parahaemolyticus* isolated in Indonesia. Appl. Environ. Microbiol. **50**:1388–1394.
27. **Murphy, J., and J. R. Riley.** 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta **27**:31–47.
28. **Neumann, D. A., M. W. Benenson, E. Hubster, T. N. T. Nguyen, and T. V. Le.** 1972. *Vibrio parahaemolyticus* in the Republic of Vietnam. Am. J. Trop. Med. Hyg. **21**:464–466.
29. **Oliver, J. D., R. A. Warner, and D. R. Cleland.** 1982. Distribution and ecology of *Vibrio vulnificus* and other lactose-fermenting marine vibrios in coastal waters of the southeastern United States. Appl. Environ. Microbiol. **44**:1404–1414.
30. **Oliver, J. D., R. A. Warner, and D. R. Cleland.** 1983. Distribution of *Vibrio vulnificus* and other lactose-fermenting vibrios in the marine environment. Appl. Environ. Microbiol. **45**:985–998.
31. **Royal Observatory.** 1984. Annual weather report, Hong Kong. Royal Observatory, Hong Kong.
32. **Ruby, E. G., and K. H. Nealson.** 1978. Seasonal changes in the species composition of luminous bacteria in nearshore sea water. Limnol. Oceanogr. **25**:530–533.
33. **Sakazaki, R., K. Tamura, T. Kato, Y. Obara, S. Yamai, and K. Hool.** 1968. Studies on the enteropathogenic, facultatively halophilic bacteria, *Vibrio parahaemolyticus*. III. Enteropathogenicity. Jpn. J. Med. Sci. Biol. **21**:325–331.
34. **Seidler, R. J., D. A. Allen, R. R. Colwell, S. W. Joseph, and O. P. Daily.** 1980. Biochemical characteristics and virulence of environmental group F bacteria isolated in the United States. Appl. Environ. Microbiol. **40**:715–720.
35. **Takahira, Y.** 1965. Distribution of *V. parahaemolyticus* on the open sea and in harbors of southeast Asia. Endem. Dis. Bull. Nagasaki Univ. **7**:245–256.
36. **Thompson, C. A., Jr., and C. Vanderzant.** 1976. Serological and hemolytic characteristics of *Vibrio parahaemolyticus* from marine sources. J. Food Sci. **41**:204–205.
37. **Wagatsuma, S.** 1968. On a medium for hemolytic reaction. Media Circle **13**:159–162.
38. **Wagatsuma, S.** 1974. Ecological studies on Kanagawa phenomenon positive strains of *Vibrio parahaemolyticus*, p. 91–96. *In* T. Fugino, G. Sakaguchi, R. Sakazaki, and Y. Takeda (ed.), International Symposium on *Vibrio parahaemolyticus*. Saikon Publishing Co., Tokyo.
39. **Watkins, W. D., and V. J. Cabelli.** 1985. Effect of fecal pollution on *Vibrio parahaemolyticus* densities in an estuarine environment. Appl. Environ. Microbiol. **49**:1307–1313.
40. **West, D. A., and R. R. Colwell.** 1984. Identification and classification of Vibrionaceae—an overview, p. 285–363. *In* R. R. Colwell (ed.), Vibrios in the environment. John Wiley & Sons, Inc., New York.
41. **Woo, M. L., W. G. D. Patrick, M. T. P. Simon, and G. L. French.** 1984. Necrotising fasciitis caused by *Vibrio vulnificus*. J. Clin. Pathol. **37**:1301–1304.
42. **Zen-Yoji, H., S. Sakai, T. Terayama, Y. Kudo, T. Ito, M. Benoki, and M. Nagasaki.** 1965. Epidemiology, enteropathogenicity, and classification of *Vibrio parahaemolyticus*. J. Infect. Dis. **115**:436–444.