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AUTECOLOGY OF VIBRIO VULNIFICUS AND VIBRIO PARAHAEMOLYTICUS IN TROPICAL WATERS

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Autecology of *Vibrio vulnificus* and *Vibrio parahaemolyticus* in Tropical Waters

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ABSTRACT

Water and shellfish samples collected from estuaries, mangroves, and beaches along the coast of Puerto Rico were examined for Vibrio vulnificus and Vibrio parahaemolyticus. An array of water quality parameters were also measured simultaneous with bacteria sampling. Both species of vibrio were associated with estuary and mangrove locations, and neither was isolated from sandy beaches. Densities of V. vulnificus were negatively correlated with salinity, 10 - 15 ppt being optimal. V. parahaemolyticus was isolated from sites with salinities between 20 and 35 ppt, the highest densities occurring at 20 ppt. Densities of Vibrio spp. and V. parahaemolyticus for a tropical estuary surpassed those reported for temperate estuaries by several orders of magnitude. Both densities of total Vibrio spp. and V. parahaemolyticus in the water were directly related to densities of fecal coliforms, unlike V. vulnificus. The incidence of ONPG(+) strains among sucrose(-) Vibrio spp. served as an indicator of the frequency of V. vulnificus in this group. More than 63% of the V. vulnificus isolated were pathogenic. V. vulnificus and V. parahaemolyticus occupy clearly separate niches within the tropical estuarine-marine ecosystem.

INTRODUCTION

Temperature and salinity seem to play important roles in regulating densities of V. vulnificus and V. parahaemolyticus (14, 16, 32). Increased water temperature appears to favor the survival and growth of V. vulnificus and V. parahaemolyticus in the environment. The peak recovery of V. vulnificus in sites around Galveston Island was in September when water temperature had been above 25°C for 4 months. Densities of V. vulnificus rapidly decreased as water temperature decreased in the fall (16). V. vulnificus was also isolated more often in two Florida estuaries when water temperature was greater than 17°C and in a greater proportion of samples over 29°C (32). Kaneko and Colwell (14) found the incidence of V. parahaemolyticus was directly related to season in the Chesapeake Bay. A temperature of 14° to 19°C was critical for the isolation of the organism from the water column and sediments. When water temperatures reached 28.5°C, V. parahaemolyticus counts up to 5.7 x 10^3 CFU/10 g sediment were observed. Yet, during the winter, counts of less than 1 CFU/g were typical (14).

Low salinities have also been positively correlated with increased densities of V. vulnificus and V. parahaemolyticus. Off Galveston Island, 47% of the samples from sites having salinities of < 16 ppt, yielded V. vulnificus (16). Negative correlations have also been observed between salinity and the incidence of lactose fermenting Vibrio spp. isolated from seawater, sediment, plankton, and animal samples (23,

24). Along the Louisiana coast, V. vulnificus was not recovered from freshwater nor brackish water when salinities were below 1 ppt (28). However, in a study conducted in two Florida estuaries, V. vulnificus was isolated more frequently in waters with a salinity > 17 ppt and in a higher proportion of samples with salinities > 23 ppt (32). Sayler et al. (30) also attributed a low frequency of V. parahaemolyticus isolation to the low salinity of their study sites. Colwell et al. (10) observed V. parahaemolyticus in waters with salinities from 8 to 20 ppt. Yet, Horie et. al. (13) reported densities as high as 1.5×10^5 cells/liter in water having a salinity of 5 ppt. Aiso et al. (1) showed that 5 to 10 ppt was the optimal salinity for V. parahaemolyticus growth at 20°C while 30 ppt was optimal for growth at 37°C.

The tropical climate of Puerto Rico, where year-round temperature averages 28°C, would seem ideal for Vibrio spp. and therefore Vibriosis. In temperate areas, 85% of V. vulnificus infections occurred in the warm months of the year (5). High evaporation rates and low rainfall increase estuary and coastal salinities in shellfish harvesting waters. Thus higher salinities and temperature should be optimal for V. vulnificus and V. parahaemolyticus growth in tropical areas. In addition, raw oysters are quite often consumed at road side stands in Puerto Rico where refrigeration is nonexistent. As observed by Oliver (22), the bacterium grows quite rapidly in unchilled raw oysters.

The importance of Vibrio spp. in recent seafood poisoning cases has been well established (5, 8). Blake et al. (5) reported that twenty-

four of 39 cases of disease caused by V. vulnificus were associated with food ingestion. Forty-six percent of these food ingestion cases were fatal. The source of contamination in 83% of these cases was identified as raw oysters. Outbreaks of gastroenteritis caused by V. parahaemolyticus are also invariably associated with the consumption of seafood (8). Considering that for 1986, Puerto Rico had 54,569 municipal clinic and hospital reported cases of gastroenteritis with a specific attack rate of more than 200/100,000 population (27), it is conceivable that Vibrio spp. are responsible for a many of these cases. This study examines the distribution, and pathogenicity of V. parahaemolyticus and V. vulnificus in shellfish and near shore coastal waters of Puerto Rico.

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MATERIALS AND METHODS

Study sites. Luquillo Beach (LB) and the Río Mameyes Estuary are on the northeast coast of the island at 18° 15' N, 65° 45' W, see Carrillo et al. (7) for details (Fig. 1). Torrecilla Lagoon (TL) (18° 20' N, 66° 00' W) is near San Juan, and is a recreational center and a shellfish harvesting area. It receives incoming currents from the Atlantic Ocean and is surrounded by mangroves. Palo Seco Channel (PSC) is on the northern coast of the island at 18° 20' N, 66° 10' W and drains into the Atlantic Ocean. Bayamon River Channel Estuary (BRC) has a total length of 6.9 km and drains into Ensenada de Boca Vieja cove. It is located on the northern coast of the island at 18° 25' N, 66° 09' W. This channel is a site of sewage effluent discharge. Ensenada de Boca Vieja (EBV) is a protected cove adjacent to San Juan Bay located at 18° 27' N, 66° 45' W, see Biamón and Hazen (3), and Valdés-Collazo et al., (33) for details. Bayamon River Estuary (BR) is on the northern coast of the island at 18° 25' N, 66° 10' W. It drains into San Juan Bay and is surrounded by mangroves. It is also a site of limited shellfish harvesting. Mandry Channel (MC) is near Humacao at 18° 9' N, 65° 46' W. It flows across low coastal lands receiving the running waters from farming and pasture lands.

Water quality. In situ measurements were taken of salinity and both air and water temperature. Salinity was measured by using a hand refractometer (model 10419, American Optical, Buffalo, N.Y.). Collected samples were analyzed in the laboratory for turbidity, pH,

chlorophyll *a*, nitrites plus nitrates, phosphates, total phosphorus, and dissolved oxygen. These were determined using Standard Methods for Water and Wastewater Analysis (2).

Bacteriological procedures. Sterile, one liter bottles were filled with water for bacteria counts. Collected shellfish were placed in sterile Whirl-Pak bags (Nasco International, Fort Wilkinson, Wis.). All samples were transported to the laboratory for analysis within 3-5 h. Total cell counts were determined by direct count (AODC) methods using acridine orange (12). Percent activity was established by calculating the ratio of red cells to the total cell number (18). Density of actively respiring cells was determined using the INT reduction technique of Zimmermann et al. (37). All techniques are as described previously (3, 7, 11, 17, 25). Densities of fecal coliforms were estimated by membrane filtration (2).

Densities of Vibrio spp. were determined by filtering with a 0.45 µm pore size, 47 mm diameter, HA-type membrane filter (Millipore Corp., Bedford, Mass.). After filtration, filters were placed on TCBS medium (Difco, Detroit, Mich.) in sterile tight fitting petri dishes and incubated at 35°C for 24 h. When incubation was completed, total Vibrio spp. were estimated by counting all colonies. Sucrose positive Vibrio spp. were counted as colonies appearing yellow. Sucrose negative Vibrio spp. were counted as colonies appearing blue or green. Random sucrose negative colonies were picked and transferred to marine agar medium (Difco). All isolates were tested for oxidase production using the API Oxidase kit (Analytab Products, Plainview,

N.Y.), and ONPG hydrolysis using ONPG diffusion disks (Difco) or API-20E strips (Analytab). All oxidase positive organisms were subjected to a battery of biochemical tests using API-20E strips (Analytab) with 20 ppt marine salts diluent (Instant Ocean, Aquarium Systems, Eastlake, Ohio) and incubation at 22°C (19). Isolates with typical reactions were identified as presumptive V. vulnificus and V. parahaemolyticus and subjected to further tests in order to confirm their identity. Sensitivity to 2-4 diamino 6-7 di-isopropyl pteridine phosphate (O/129) was determined using the disk diffusion method. Presumptive V. vulnificus sensitive to both 150 µg and 10 µg of O/129 were tested further as were presumptive V. parahaemolyticus isolates sensitive to 150 µg but resistant to 10 µg of O/129. Salt tolerance tests were conducted by adding 0%, 7%, and 10% NaCl to modified salt water yeast extract agar MSWYE (26). Isolates growing in 7% NaCl but not 10% NaCl, with typical biochemical reactions for V. parahaemolyticus were identified accordingly. Those isolates unable to grow in either 7% or 10% NaCl with typical biochemical reactions for V. vulnificus were tested for sensitivity to penicillin (10 U) and colistin (10 µg). Isolates resistant to colistin and sensitive to penicillin were identified as V. vulnificus. V. vulnificus (ATCC 27562) and V. parahaemolyticus (ATCC 17802) were used as controls for all tests and media.

Identification of V. parahaemolyticus and V. vulnificus was further confirmed with a slide flocculation procedure using core flagellar antiserum against V. vulnificus and, both flagellar and core

flagellar antiserum against V. parahaemolyticus donated by Dr. Ronald Siebeling, Louisiana State University (31).

Pathogenicity. Isolates positively identified as V. vulnificus were used to prepare an active inoculum containing 10^9 cells ml^{-1} grown in Brain Heart Infusion broth (Difco) 1.5% NaCl and incubated for 18 h at 35°C. One-half ml of this inoculum was injected intraperitoneally to 6-8 week old AKR/J female white mice to determine strain pathogenicity (26). Pathogenicity of V. parahaemolyticus isolates was determined by the Kanagawa test (20). Fresh human blood was used with Wagatsuma's agar (9, 34) to determine the isolates ability to cause ß-hemolysis of erithrocytes.

Data analysis. One factor analysis of variance (ANOVA) without replication was used to test differences between sites using programs developed for a Macintosh computer. Multiple correlation was used to determine relationships between density and water quality parameters. Any statistical probability less than 0.05 was considered significant (36).

RESULTS

Representative water quality data for each site is given in Table 1. A total of 409 sucrose negative isolates were examined (Table 2). The nine study sites examined ranged in AODC density from 9.6 x 10^5 to 1.7 x 10^7 cells ml^{-1} (Fig. 2). The AODC measurement correlated positively with viable count densities of both Vibrio spp. and fecal coliforms (Table 3). Total bacterial densities also held strong positive correlations with concentrations of phosphate and total phosphorus in the environment. The percent activity of the bacterial population at the various sites ranged from 14.4 to 74.7 (Fig. 2). Bacterial densities as measured by both direct count and all viable count methods were negatively correlated with percent activity (Table 3). Although the percentage of respiring cells in the bacterial community was much lower than the percent activity for all sites examined (Fig. 2), both measurements were significantly positively correlated.

The percentage that Vibrio spp. represented in the total bacterial community was very small for all sites (Table 2). Yet, Vibrio spp. share with the entire bacterial community a significant positive correlation with phosphates and total phosphorus concentrations in the the water. When densities of fecal coliforms increased so did the density of Vibrio spp. as did the proportion of Vibrio spp. in the total bacterial community (Fig. 2). Densities of Vibrio spp. and the percentage of Vibrio spp. in the total bacterial community were negatively correlated with dissolved oxygen. Densities of Vibrio spp. by site ranged from 16.9

CFU ml^{-1} to 1.5 x 10^6 CFU ml^{-1} (Table 2). For shellfish, densities of Vibrio spp. by site, ranged from 5.2 x 10^3 CFU g^{-1} to 1.5 x 10^4 CFU g^{-1}. Densities of Vibrio spp. were not correlated with salinity, while both sucrose(-) Vibrio spp. ml^{-1} and the percentage of sucrose(-) Vibrio spp. were negatively correlated with salinity. The percentage of sucrose(-) Vibrio spp. making up the vibrio population decreased with increasing salinity of the sites (Table 2). The percentage of sucrose(+) Vibrio spp. was not correlated with salinity and was generally higher than that of sucrose(-) Vibrio spp. Densities of sucrose(-) Vibrio spp. at the various sites ranged from 3.24 CFU ml^{-1} to 12.76 x 10^5 CFU ml^{-1} (Table 2). In shellfish, densities of sucrose(-) Vibrio spp. by site ranged from 1.3 x 10^3 CFU g^{-1} to 2.7 x 10^3 CFU g^{-1}.

The densities of sucrose(-) Vibrio spp. showed a highly significant positive correlation with densities of ONPG(+) Vibrio spp. (Table 3). Both the density of ONPG(+) Vibrio spp. and the percentage of sucrose(-) Vibrio spp. made up of ONPG(+) vibrios were significantly negatively correlated with salinity (Table 3). A significant difference by site was observed for ONPG(+) Vibrio spp. ml^{-1}. Sites with increasing salinity showed decreasing percentages of ONPG(+) Vibrio spp. Densities of ONPG(+) Vibrio spp. for the various sites ranged from 0.83 CFU ml^{-1} to 5.94 x 10^5 CFU ml^{-1} (Table 2). Densities of ONPG(+) Vibrio spp. in shellfish ranged from 208 CFU g^{-1} to 449 CFU g^{-1} by site.

As shown in Table 2, densities of V. vulnificus by site ranged from 38 CFU 100 ml^{-1} to 4,124 CFU 100 ml^{-1}. Both the highest densities and the highest frequencies of isolation of V. vulnificus were obtained at

salinities of 10 and 15 ppt. V. vulnificus was never isolated from sandy beach, seawater samples (sites LB and EBV). Bayamon River estuary (BR) and the upper Río Mameyes estuary (URM) possess extreme salinities of 32.2 ppt and 1.7 ppt respectively (Table 1). At these sites the lowest frequencies of isolation were observed, representing less than 4% of sucrose negative Vibrio spp. In Torrecilla Lagoon (TL), for both water and shellfish, V. vulnificus was isolated only when salinities were between 20 and 25 ppt. The percentage of V. vulnificus isolates which proved lethal to mice showed an even higher significant negative correlation with salinity than did all V. vulnificus isolates (Table 3). At sites TL and BR where salinities were highest none of the V. vulnificus isolated proved pathogenic. Overall, 46% of V. vulnificus isolates were pathogenic. It is interesting to note that densities of fecal coliforms were not significantly correlated with densities of V. vulnificus. Significant negative correlations were observed between densities of V. vulnificus and; phosphates, total phosphorus, and pH.

The proportion of sucrose(-) Vibrio spp. were confirmed as V. parahaemolyticus, were significantly positively correlated with salinity. Sites yielding V. parahaemolyticus isolates ranged in salinity from a mean of 20.2 ppt to 35.0 ppt. V. parahaemolyticus, like V. vulnificus, was never isolated from LB or EBV which are coastal sites far removed from marsh lands and estuaries. The highest density of V. parahaemolyticus was observed in BRC with a mean salinity of 20.2 ppt, a site which never yielded V. vulnificus. The densities of V. parahaemolyticus for the various sites ranged from 315 CFU 100 ml-

^{1} to 3.2 x 10^{5} CFU 100 ml^{-1}. In shellfish the densities of V. parahaemolyticus for TL and BR were 37.4 CFU g^{-1} and 207.6 CFU g^{-1} respectively (Table 2). Significant positive correlations were observed between the percentage of V. parahaemolyticus among sucrose(-) Vibrio spp. and concentrations of phosphates and total phosphorus. Fecal coliform densities in the water column showed a significant positive correlation with densities of V. parahaemolyticus (Table 3). The Kanagawa pathogenicity test for 94% of V. parahaemolyticus isolates resulted in identical hemolysis as the Kanagawa(+) control (ATCC 17802). These results were confirmed on isolates sent to Charles A. Kaysner, Food and Drug Administration, Seattle. The percent V. vulnificus and percent V. parahaemolyticus among sucrose negative Vibrio spp. was negatively correlated (Table 3).

DISCUSSION

The density of fecal coliforms and Vibrio spp. in the water column was positively correlated with other pollution indicators in Puerto Rico waters, like phosphates, total phosphorus, and total bacterial counts, and significantly negatively correlated with dissolved oxygen, and salinity. A highly significant positive correlation was observed between densities of Vibrio spp. and densities of fecal coliforms. Oliver et al. (24) also observed a significant correlation between Vibrio spp. and fecal contamination in marine environments along the east coast of the United States.

Prior to this study the maximum density of Vibrio spp. reported in natural waters was in a temperate estuary, 10^2 MPN ml^{-1} (14). The highest densities of Vibrio spp. observed in this study, a tropical estuary, were 1.5 x 10^6 CFU ml^{-1}. The constant optimum growth temperature offered by a tropical climate may allow Vibrio spp. to stabilize at higher densities. The periodic drastic reduction in Vibrio spp. densities caused by winter (14, 16, 32), would not be a regulating factor in a tropical estuary. The sucrose(-) vibrio population showed the same high density as did the total Vibrio spp. population. Kaneko and Colwell (15) report a sucrose(-) vibrio maximum density of 62.0 CFU ml^{-1} in Chesapeake Bay. Bayamon River Channel estuary (BRC) had mean densities of sucrose(-) Vibrio spp. of 1.3 x 10^6 CFU ml^{-1}. This could be a combination of both favorable temperature and a allochthonous source, eg., sewage. Fecal coliform densities at this site

averaged 3.0 x 10^3 CFU ml^{-1}. The significant positive correlation between Vibrio spp. and fecal coliforms observed for all sites would support this observation. Other studies by our laboratory. (3, 7, 11, 17, 18, 33) indicate that the survival of Vibrio spp. and other enteric bacteria in natural waters is much greater in the tropics.

Salinity appears to play a role in regulating sucrose(-) Vibrio spp. Densities of sucrose(-) Vibrio spp. and proportion of sucrose negative vibrios, were significantly negatively correlated with salinity. Oliver et al. (24) made similar observations for salinity and sucrose(-) vibrios from oysters. The negative effect that salinity has on densities of V. vulnificus follows the same pattern as that observed for sucrose(-) vibrios and sucrose(-) ONPG(+) vibrios. The frequency of ONPG(+) spp. among sucrose(-) Vibrio spp. may serve as an indicator of the presence of V. vulnificus. The highly significant negative correlation that this bacteria has with salinity is also suggested by a markedly reduced frequency of isolation from sites with increased salinity. Sites having salinities of 10 and 15 ppt had both the highest densities of V. vulnificus and the highest frequency of isolation among sucrose(-) vibrios. Kelly (16) also found that V. vulnificus was most frequently isolated from sites where salinities ranged between 7 and 16 ppt. Tamplin et al. (32) reported the isolation of V. vulnificus more frequently in waters with a salinity greater than 17 ppt and in a higher proportion of samples greater than 23 ppt. The results of the present study do not corroborate those findings. V. vulnificus was isolated only from estuaries, and mangroves. Sandy beaches such as LB and EBV did

not yield V. vulnificus. Bayamon River estuary and the upper Río Mameyes estuary which have extreme differences in salinities were sites of lowest isolation. Less than 4% of sucrose(-) isolates tested from these sites resulted in positive identification. Considering that in vitro experiments have shown the optimum salinity ranges for V. vulnificus to be between 10 and 20 ppt (16), it is understandable that these sites would not harbor this bacteria. The isolation of V. vulnificus in Torrecilla Lagoon from water and shellfish only when salinities ranged between 20 and 25 ppt also indicates it's low salinity requirements.

When estimating the frequency of isolation of V. vulnificus based on the number of sucrose(-) vibrio isolates which were also ONPG(+) the frequency of isolation increases. Estimated in this manner, the percentage of V. vulnificus obtained from all sites averaged 23%. These results are comparable to those of Oliver et al. (24) who found V. vulnificus represented 20% of all lactose(+) sucrose(-) vibrios.

The highest densities of V. vulnificus were obtained from Mandry Channel. Densities at this site averaged 4.1 x 10^3 CFU 100 ml^{-1} with 63% testing positive for pathogenicity. The detection of a V. vulnificus mean density of 225 CFU g^{-1} shellfish in Torrecilla Lagoon further suggests the importance of this bacteria as a probable agent of foodborne disease in Puerto Rico. In Torrecilla Lagoon, the percentage of sucrose(-) vibrios that were V. vulnificus was over three times greater in shellfish than in the over lying water column. The incidence of V. vulnificus; however, was not connected to sewage contamination, since no correlation was observed with fecal coliforms. This organism

appears to be an inhabitant of marine aquatic systems that are totally unaffected by sewage effluent. This lack of association between V. vulnificus and fecal coliforms has also been noted in temperate areas (23, 24).

V. parahaemolyticus, unlike sucrose(-) vibrios, was positively correlated with salinity. This bacteria was found at sites with salinities between 20 and 35 ppt and was never isolated from sites with salinities less than 20 ppt. This would indicate that in the tropics higher salinities favor V. parahaemolyticus; however, the highest density of this bacteria (3.2×10^3 CFU 100 ml^{-1}) was detected at BRC, a site with a salinity of only 20.2 ppt. In contrast, PSC, 35 ppt salinity, harbored only 433 CFU 100 ml^{-1}. An increase in salinity was also accompanied by a general decrease in the percentage of sucrose(-) vibrios that were confirmed as V. parahaemolyticus. Intermediate salinities appear more favorable to this bacteria The fact that the organism was never isolated from sandy beaches indicates that although it can tolerate high salinity environments, it is an estuary and marsh inhabitant.

On the coast of West Africa (6), the lagoon system proved to be the most important reservoir of V. parahaemolyticus. The seasonality observed in the incidence of this bacterium for West Africa was closely related to salinity. During the dry season, when isolation was most frequent, salinity of the lagoons was between 15 and 21 ppt. The rainy season, which rendered lagoon salinity between 1.6 and 4.2 ppt had the lowest incidence of V. parahaemolyticus. These findings are in close agreement to the present study.

Maximum densities of V. parahaemolyticus in this study were observed for Bayamon River Channel estuary (3.2 x 10^7 CFU 100 ml^{-1}). These elevated densities contrast markedly with those obtained for temperate estuaries. Kaneko and Colwell (14) report maximum densities of 400 CFU 100 ml^{-1} in Chesapeake Bay. They observed that V. parahaemolyticus were undetectable until early June, when the water temperature was 19°C. Watkins and Cabelli (35) also report far lower densities for Narragansett Bay, R.I. (495 CFU 100 ml^{-1}), than those recorded in this study. As in the case of total vibrios, high densities of V. parahaemolyticus may be due to the high constant temperature of a tropical climate and associated increased survival.

Although previous studies conducted in the tropics did not quantify V. parahaemolyticus, they did establish the presence of this bacterium in tropical waters and shellfish (21). In our study, densities in shellfish were found to be 37.4 and 207.6 CFU g^{-1}. Although these levels are low in terms of the 10^6 cell dose required to trigger gastroenteritis (29), they do bring to light the presence of this bacterium in shellfish harvested for local consumption. The possible health hazard that these shellfish may represent is aggravated by the typical handling they receive upon harvesting. The local practice of selling shellfish at road side stands where there is no refrigeration would favor a marked increase in numbers of V. parahaemolyticus present (4).

The results of the present study demonstrated a significant positive correlation between fecal coliform levels and density of

V. parahaemolyticus in the water column. Watkins and Cabelli (35) also reported a significant positive correlation between the level of fecal pollution and density of V. parahaemolyticus. These authors observed that it's densities decreased sharply with distance from the source of fecal pollution. Maximum density of E. coli recorded in their study was 2.3×10^3 100 ml^{-1}. Maximum density of fecal coliforms in the present study was recorded for Bayamon River Channel estuary, 3.0×10^5 CFU 100 ml^{-1}. Thus, the difference in densities of V. parahaemolyticus between the tropical and temperate estuaries may be attributed not only to temperature differences but also to differences in levels of fecal contamination.

The present study indicates that V. vulnificus and V. parahaemolyticus behave distinctly in tropical waters. While one species is strongly associated with fecal contamination the other is not. In addition, both species appear to be strongly influenced by some of the same environmental factors, but with opposite effects. While highest densities of V. vulnificus were obtained at low salinities, V. parahaemolyticus densities were greatest at high salinities. Phosphate and total phosphorus levels both were significantly correlated with densities of V. vulnificus and V. parahaemolyticus; however, like salinity these relationships were inverse. The differences observed indicate that these two organisms, although very similar, occupy clearly separate niches in the tropical aquatic ecosystems.

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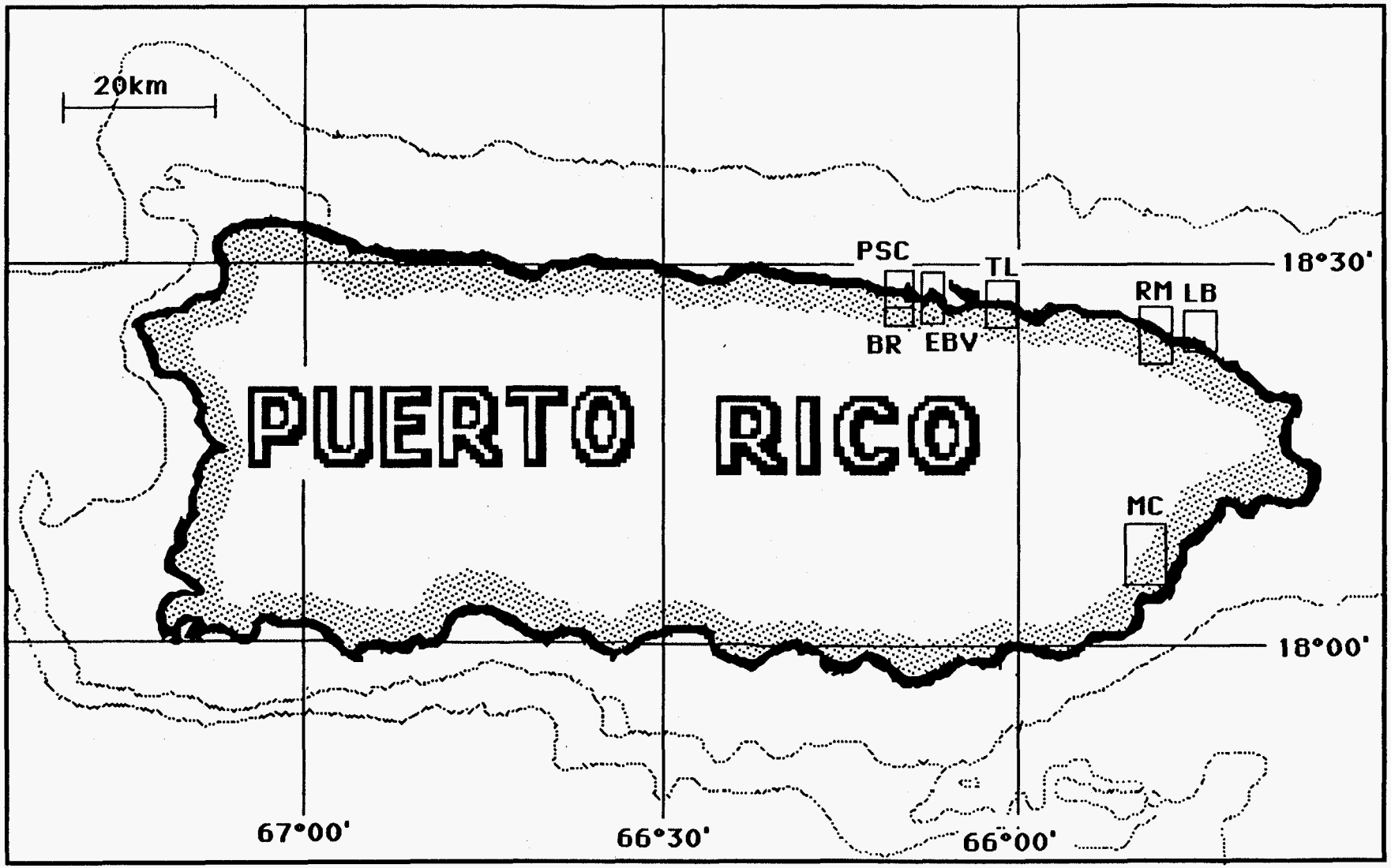
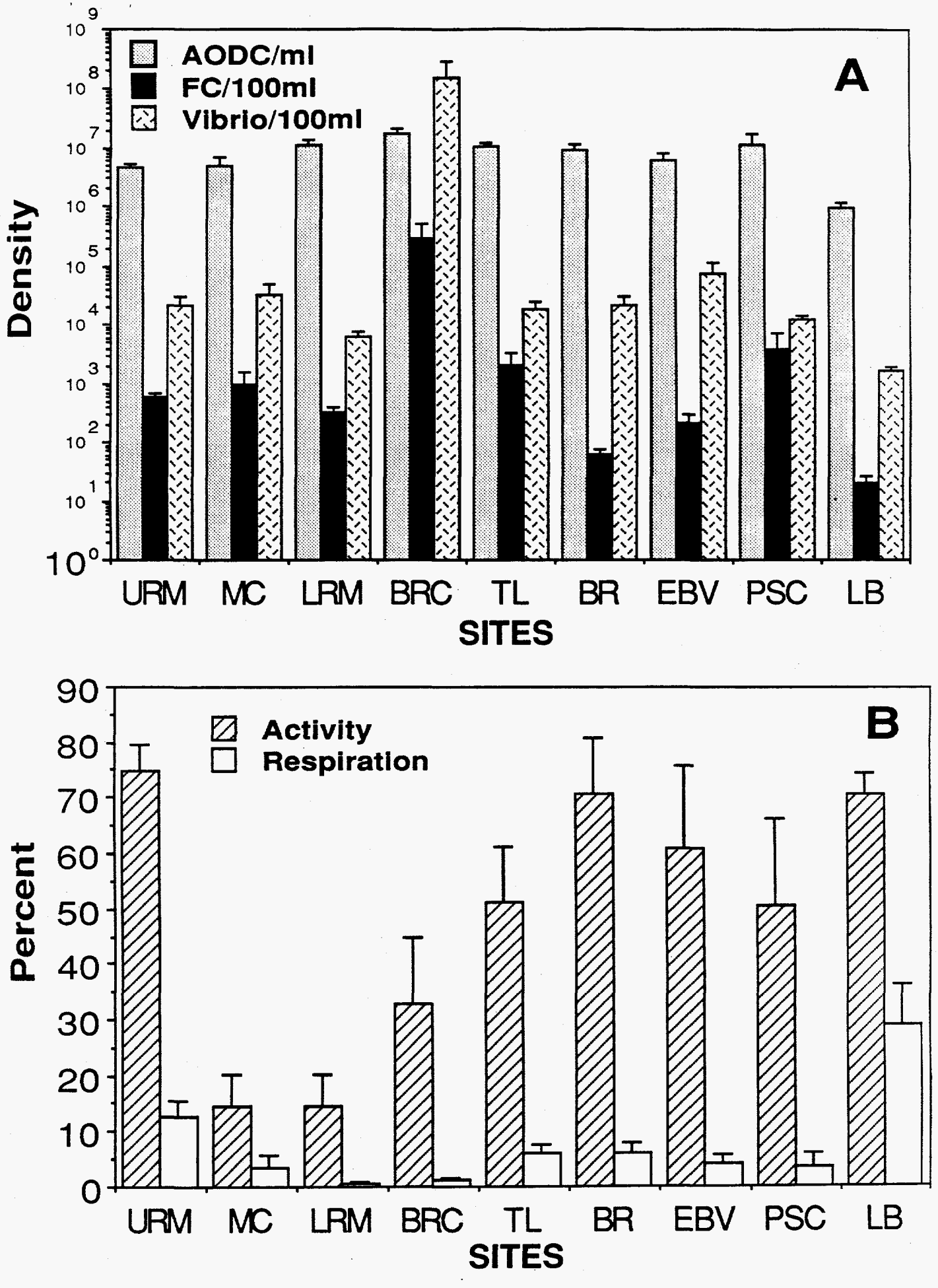
FIGURE LEGENDS

Figure 1. Map of study sites around Puerto Rico.

Figure 2. **A:** Density for Vibrio, fecal coliforms (FC), and total bacteria (AODC) by site (mean ± one standard error, n=7), **B:** Percent activity of total bacteria as measured by AODC (activity) and percent respiration as measured by INT (respiration) by site (mean ± one standard error, n=7).


20km
PSC
TL
18°30'
RM
LB
BR
EBV
PUERTO RICO
MC
18°00'
67°00'
66°30'
66°00'

F.5


A
AODC/ml
FC/100ml
Vibrio/100ml
Density
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
URM
MC
LRM
BRC
TL
BR
EBV
PSC
LB
SITES
B
Activity
Respiration
Percent
90
80
70
60
50
40
30
20
10
0
URM
MC
LRM
BRC
TL
BR
EBV
PSC
LB
SITES

Fig

Table 1. Physical-Chemical Water Quality by Site

Site	ATEMP	WTEMP	SAL	DO	PH	CHLA	TURB	NO_3	PO_4	TP
URM	27.0 ± 0.5	24.5 ± 0.6	1.7 ± 0.0	8.0 ± 0.2	7.2 ± 0.1	8.4 ± 2.2	96.3 ± 1.4	1.09 ± 0.14	0.030 ± 0.003	0.045 ± 0.006
MC	30.1 ± 0.0	29.2 ±0.4	8.0 ± 1.3	2.8 ± 0.4	7.3 ± 0.1	27.0 ± 12.6	95.9 ± 0.8	0.76 ± 0.55	0.029 ± 0.011	0.075 ± 0.003
LRM	29.3 ± 0.2	27.8 ± 0.5	15.0 ± 0.0	5.1 ± 0.7	7.7 ± 0.2	1.7 ± 0.4	97.3 ± 0.5	0.53 ± 0.12	0.045 ± 0.019	0.051 ± 0.019
BRC	30.7 ± 0.9	28.8 ± 0.4	20.2 ± 0.4	2.0 ± 0.7	7.9 ± 0.1	122.4 ± 117.9	96.0 ± 0.9	0.86 ± 0.29	0.423 ± 0.086	0.479 ± 0.078
TL	28.6 ± 0.6	26.9 ± 0.6	29.3 ± 1.3	7.7 ± 2.0	7.9 ± 0.2	30.1 ± 6.9	93.8 ± 0.9	0.59 ± 0.18	0.152 ± 0.017	0.219 ± 0.016
BR	28.3 ± 0.6	28.7 ± 0.4	32.2 ± 0.7	5.7 ± 0.6	7.8 ± 0.2	7.5 ± 1.2	95.3 ± 1.1	0.30 ± 0.06	0.052 ± 0.006	0.085 ± 0.012
EBV	25.7 ± 0.6	25.2 ± 1.4	34.8 ± 0.5	5.8 ± 0.8	7.0 ± 0.5	18.8 ± 12.4	94.5 ± 1.4	0.37 ± 0.03	0.048 ± 0.013	0.071 ± 0.015
PSC	28.2 ± 0.8	32.5 ± 1.0	35.0 ± 0.7	6.3 ± 0.5	7.4 ± 0.2	3.7 ± 1.1	96.1 ± 1.3	0.53 ± 1.25	0.038 ± 0.004	0.053 ± 0.004
LB	26.8 ± 0.7	25.7 ± 0.6	36.2 ± 0.7	6.9 ± 0.4	7.7 ± 0.0	11.8 ± 2.4	91.9 ± 1.9	1.75 ± 0.80	0.013 ± 0.003	0.018 ± 0.005

*All values are mean ± one standard error (n = 7), ATEMP = air temperature (°C), WTEMP = water temperature (°C), DO dissolved oxygen (mg/L), Sal = salinity (ppt), NO_{2+3} = nitrites plus nitrates (mg/L), PO_4 = orthophosphate (µg/L), TP = total phosphorus (µg/L), ChlA = chlorophyll *a* (mg/L), TURB = turbidity (% transmittance).

Table 2. Densities of bacteria by site.

Site	V	S(+)	S(-)	AODC
URM	213.3 ± 92.2	181.4 ± 88.9	27.00 ± 5.26	46.3 ± 7.9
MC	321.7 ± 161.7	235.9 ± 147.6	73.75 ± 21.44	49.6 ± 19.1
LRM	63.3 ± 13.6	40.5 ± 9.8	20.63 ± 4.67	111.0 ± 33.0
BRC	15.2 ± 14.4	2.4 ± 1.8	12.76 ± 12.62	173.0 ± 39.6
TL	189.0 ± 56.5	175.9 ± 54.1	10.51 ± 4.61	103.0 ± 20.9
TL Shellfish	5,169 ± 1,521	4,011 ± 1,506	1,271 ± 725	ND
BR	210.0 ± 103.6	173.9 ± 78.3	35.82 ± 28.66	90.3 ± 26.1
BR - Shell fish	14,625 ± 25	6,550 ± 4,550	2,700 ± 800	ND
EBV	746.3 ± 386.5	735.0 ± 392.6	16.67 ± 8.70	63.3 ± 19.2
PSC	126.7 ± 14.5	85.0 ± 14.4	32.50 ± 6.29	115.0 ± 59.7
LB	16.9 ± 2.6	13.8 ± 2.7	3.24 ± 0.72	9.6 ± 2.1

All units are in CFU ml^{-1}, except AODC which is x 10^5 cells ml^{-1}, shellfish values are CFU total Vibrio spp., S(+) = sucrose positive, S(-) = sucrose negative, AODC = Acridine Oran Counts, FC = fecal coliforms, Vp = Vibrio parahaemolyticus, O(+) = ONPG positive, Vv = vulnificus, VvP = pathogenic Vibrio vulnificus.

Table 2. continued

Site	FC	Vp	O(+)	Vv	VvP
URM	5.98 ± 1.15	0.00	20.54 ± 4.00	0.38 ± 0.07	0.38 ± 0.07
MC	10.03 ± 5.27	0.00	65.12 ± 18.92	41.24 ± 11.98	26.06 ± 7.57
LRM	3.20 ± 0.78	0.00	14.14 ± 3.21	3.23 ± 0.73	1.21 ± 0.27
BRC	0.03 ± 0.02	3.20 ± 3.16	5.94 ± 5.86	0.00	0.00
TL	21.16 ± 12.60	3.15 ± 1.38	1.58 ± 0.69	0.53 ± 0.23	0.00
Tl Shell fish	205.3 ± 125.3	37.4 ± 21.3	449 ± 256	225 ± 128	37.6 ± 21.4
BR	0.62 ± 0.12	6.39 ± 5.13	10.23 ± 8.19	1.28 ± 1.02	0.00
BR Shellfish	18.5 ± 5.5	207.6 ± 61.5	208 ± 62	0.00	0.00
EBV	2.13 ± 0.97	0.00	3.85 ± 2.01	0.00	0.00
PSC	39.70 ± 30.14	4.33 ± 0.84	2.16 ± 0.42	0.00	0.00
LB	0.20 ± 0.07	0.00	0.83 ± 0.19	0.00	0.00

see above

Table 3. Water Quality and Bacteria Correlations

	WTEMP	ATEMP	SAL	DO	PH	CHLA	TURB	NO_3	PO_4	TP	V	S(-)
WTEMP	1.000											
ATEMP	0.465	1.000										
SAL	0.227	-0.138	1.000									
DO	-0.052	0.138	0.288	1.000								
PH	-0.036	0.020	0.243	-0.301	1.000							
CHLA	-0.125	-0.097	0.120	0.323	-0.088	1.000						
TURB	-0.211	-0.233	-0.488	-0.216	-0.113	-0.365	1.000					
NO_3	-0.012	0.098	-0.137	-0.252	-0.028	-0.047	0.386	1.000				
PO_4	-0.318	0.046	0.368	-0.096	0.322	-0.048	-0.186	0.196	1.000			
TP	-0.256	0.132	0.299	-0.193	0.396	0.232	-0.403	0.095	0.885	1.000		
V	-0.029	0.150	-0.072	-0.552	0.226	-0.179	-0.134	0.215	0.511	0.542	1.000	
S(-)	0.018	-0.112	-0.358	-0.211	-0.088	-0.047	0.184	-0.155	-0.125	-0.077	0.467	1.000
S(+)	-0.040	0.143	0.028	-0.546	0.250	-0.234	-0.125	0.296	0.617	0.601	0.979	0.321
FC	-0.168	0.064	-0.191	-0.476	0.410	0.131	-0.134	0.022	0.330	0.460	0.766	0.501
AODC	-0.174	-0.215	0.384	0.021	0.184	-0.047	-0.479	-0.387	0.140	0.127	0.161	-0.003
Vv	0.046	0.108	-0.761	-0.217	-0.412	0.026	0.488	0.212	-0.536	-0.420	-0.165	0.326
Vp	0.059	-0.110	0.111	-0.169	0.153	-0.020	-0.167	-0.258	0.217	0.245	0.646	0.821
O(+)	-0.048	-0.056	-0.471	-0.269	-0.114	-0.105	0.200	-0.171	-0.156	-0.080	0.494	0.974
%S(-)	0.062	0.041	-0.598	0.021	-0.152	0.148	0.174	-0.313	-0.594	-0.439	-0.005	0.745
%V	-0.001	0.081	-0.060	-0.376	0.212	-0.132	-0.137	-0.050	0.368	0.419	0.889	0.720
%A	-0.246	-0.528	0.234	0.120	-0.040	0.332	0.343	0.236	0.147	0.008	-0.240	-0.236
%R	0.166	-0.088	0.050	0.265	-0.578	0.246	0.090	0.188	-0.094	-0.123	-0.181	-0.075
%O(+)	-0.136	0.269	-0.823	-0.150	-0.044	-0.239	0.454	-0.054	-0.359	-0.291	0.023	0.389
%Vv	0.026	0.066	-0.722	-0.235	-0.257	-0.011	0.537	0.122	-0.565	-0.435	-0.240	0.271
%VvP	-0.119	-0.025	-0.838	-0.086	-0.391	-0.084	0.476	0.101	-0.614	-0.602	-0.198	0.270
%Vp	0.138	0.136	0.656	0.192	0.092	0.251	-0.561	-0.089	0.472	0.467	0.176	-0.195

where $p < 0.05$ when $r > 0.381$, underlined values are significant, see previous tables for abbreviations.

Table 3. continued...

	S(+)	FC	AODC	Vv	Vp	O(+)	%S(-)	%V	%A	%R	%0(+)	%Vv	%VvP
S(+)	1.000												
FC	0.686	1.000											
AODC	0.150	0.299	1.000										
Vv	-0.248	-0.100	-0.492	1.000									
Vp	0.548	0.613	0.278	-0.241	1.000								
0(+)	0.344	0.508	-0.013	0.400	0.759	1.000							
%S(-)	-0.199	0.278	-0.075	0.545	0.401	0.755	1.000						
%V	0.811	0.742	0.164	-0.175	0.882	0.722	0.286	1.000					
%A	-0.175	-0.151	-0.185	-0.210	-0.147	-0.345	-0.290	-0.259	1.000				
%R	-0.156	-0.445	-0.493	0.079	-0.110	-0.112	-0.100	-0.147	0.446	1.000			
%O(+)	-0.175	0.101	-0.412	0.724	0.064	0.519	0.616	0.102	-0.425	-0.206	1.000		
%Vv	-0.320	-0.125	-0.548	0.942	-0.269	0.352	0.529	-0.216	-0.207	0.005	0.759	1.000	
%VvP	-0.282	-0.130	-0.378	0.752	-0.210	0.353	0.558	-0.156	-0.077	0.159	0.683	0.668	1.000
%Vp	0.229	0.074	0.364	-0.689	0.284	-0.289	-0.405	0.180	0.226	0.179	-0.762	-0.767	-0.603

where $p < 0.05$ when $r > 0.381$, underlined values are significant, see previous tables for abbreviations.

Table 4. Isolate Identification by Site

Site	S(-)	O(+)	Vv	VvP	Vp
URM (water)	26.6(71)*	76.1(54)	1.41(1)	100(1)	0(0)
MC (water)	46.4(34)	88.2(30)	55.9(19)	63.2(12)	0(0)
LRM (water)	46.5(51)	68.6(35)	15.7(8)	37.5(3)	0(0)
BRC (water)	37.1(56)	46.4(26)	0(0)	0(0)	25.0(14)
TL (water)	8.0(40)	15.0(6)	5.0(2)	0(0)	30.0(12)
(shellfish)	30.6(34)	35.3(12)	17.7(6)	16.7(1)	2.9(1)
BR (water)	16.3(28)	28.6(8)	3.6(1)	0(0)	17.9(5)
(shellfish)	18.5(13)	7.7(1)	0(0)	0(0)	7.7(1)
EBV (water)	9.9(13)	23.1(3)	0(0)	0(0)	0(0)
PSC (water)	26.8(15)	6.7(1)	0(0)	0(0)	13.3(2)
LB (water)	21.8(54)	25.9(14)	0(0)	0(0)	0(0)
TOTAL	409	190	37	17	35

* Percent positive (No. of positive isolates), see previous tables for abbreviations.