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Clara H. Bartley
University of New Hampshire, Durham

L. W. Slanetz
University of New Hampshire, Durham

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Occurrence of *Vibrio parahaemolyticus* in Estuarine Waters and Oysters of New Hampshire

CLARA H. BARTLEY AND L. W. SLANETZ
Department of Microbiology, University of New Hampshire, Durham, New Hampshire 03824

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Vibrio parahaemolyticus was isolated from water and oysters collected from seven different sampling stations in the Great Bay and Little Bay estuarine areas of New Hampshire. The morphological and biochemical characteristics of 50 isolates conformed in general to those described for this organism in the literature. All isolates produced hemolysis on blood-agar. To date, there have been no reports of *V. parahaemolyticus* food poisoning outbreaks due to the consumption of fish or shellfish harvested from this estuarine region.

Widespread distribution of *Vibrio parahaemolyticus* has been reported in the Sea of Japan where it has caused outbreaks of fish-borne food poisoning (7, 10). This organism has also been isolated in the United States from shrimp in the Gulf Coast (9), from blue crabs from the Chesapeake Bay (2, 4), and from the marine environments of Washington State (1). It was reported to be very infrequently encountered in fish sold in the Netherlands (3). In the fall of 1970, a survey was initiated to establish the occurrence of *V. parahaemolyticus* in the seawater and oysters of the Great Bay and Little Bay areas of New Hampshire.

Samples of oysters and overlying waters were collected from seven sampling stations in estuarine areas which have been used for other microbiological studies by Slanetz et al. (8). The samples were immediately transported to the laboratory and processed within 1 hr after collection. (These studies were conducted at the University of New Hampshire Jackson Estuarine Laboratory located at Adam's Point in the bay area.) Bromothymol blue-teepol-salt-agar with 0.02% lauryl sulfate substituted for the teepol and thiosulfate-citrate-bile salt-sucrose-agar (3, 5) were employed for the isolation of *V. parahaemolyticus*. Salt-glucose-teepol broth (4) with 0.04% lauryl sulfate substituted for the teepol was used as an enrichment medium. Corn starch-agar plates (R. M. Twedt et al., Bacteriol. Proc., p.6, 1970) were used to determine the anaerobic hydrolysis of starch with incubation at 43 C. Blood-agar plates for determining hemolysis (6) were prepared without heat sterilization using washed rabbit erythrocytes. Gelatin liquefaction was determined on the maintenance medium of Baross and Liston (1), and cultures were maintained either on this medium or 3% NaCl-Trypticase soy agar slants. Chitin hydrolysis was determined by inoculating the isolates into buffered 3% saline to which a piece of sterile chitin (3 to 4 mm^2) had been added. Evidence of hydrolysis was based on the disappearance of the chitin after incubation for 1- to 3-week periods. All media used for biochemical tests, unless otherwise indicated, were prepared with 3% NaCl. *V. parahaemolyticus* ATCC 17802 was used as a control for all tests.

Isolation of *V. parahaemolyticus* from water was accomplished by passing 1 liter through a membrane filter (0.45-μm pore size) on which approximately 0.05 g of Celite had been sprinkled. The membrane was then placed in a test tube (22 by 150 mm) containing 10 ml of sterile buffered saline, and the tube was shaken vigorously to wash off the filtered material. This was considered a 1:10 dilution of the material filtered from 1 liter of the water sample. One tenth to 0.3 ml of this dilution was plated by smearing on each of the two isolation media contained in 100-cm petri dishes. Serial dilutions were made by inoculating 1 ml directly into tubes containing 9 ml of enrichment medium. Samples from each dilution tube were plated on the isolation media before the tubes were incubated at 35 C. After overnight incubation, samples from all enrichment tubes showing growth were again plated on the isolation media.

Isolation of organisms from oysters was accomplished in the same manner as above, except that 0.1 to 0.3 ml of undiluted homogenate was spread directly on the isolation media before serial dilution of the sample.

Colonies on the isolation media with light blue

or green centers, which showed no evidence of sucrose fermentation and were composed of gram-negative, motile, pleomorphic rods, were selected for further identification. Growth from these colonies was inoculated onto the surface of well-dried starch-agar plates which were then incubated anaerobically in jars for 18 to 24 hr at 43 C. Cultures isolated from nonspreading colonies showing clear zones of starch hydrolysis on these plates were then tested for the following: reactions in triple sugar-iron-agar; salt tolerance; oxidation reaction according to Kovacs; indole, methyl red, Voges-Proskauer, citrate reactions; anaerogenic fermentation of glucose; production of catalase, reduction of nitrates; liquefaction of gelatin; hemolysis; carbohydrate fermentations; and chitin hydrolysis.

Mice were injected intraperitoneally with 0.5 ml of washed 24-hr cultures from Trypticase soy agar slants or were fed with pellets soaked in the washed cultures.

V. parahaemolyticus was isolated from the water and oysters collected from all seven sampling stations tested in this estuarine region. Samples collected from certain stations during the month of September contained up to 1,000 *V. parahaemolyticus* organisms per liter of water and 500 organisms per g of oysters. The numbers of this organism present in the samples decreased appreciably during October and particularly November as the temperature of the water decreased from 14 to 8 C. No *V. parahaemolyticus* organisms were isolated from oysters maintained in tanks receiving water with temperatures of 0 to 9 C pumped into the laboratory from the immediate estuarine area. However, cultures in tubes held beside the oysters in the tanks were viable 1 month later when the experiment was discontinued.

The morphological and biochemical characteristics of the 50 *V. parahaemolyticus* isolates tested conformed to those described for this organism in the literature (1, 2, 4, 7). It may be of interest to note that the strains fermented sorbitol anaerobically, fermented trehalose, and gave variable reactions in cellobiose. All strains were hemolytic (positive Kanagawa phenomenon; reference 6), some producing wider beta-hemolytic bands than others. Ninety per cent of the strains attacked chitin. Four strains were selected for serological typing. Three of these strains were typed as O antigen VI, K antigen 19, and one was untypable. Fifteen strains were tested for pathogenicity in mice. All strains killed mice within 12 hr after intraperitoneal injections of 3×10^8 organisms. *V. parahaemolyticus* was isolated from the heart blood and livers of these mice. The stomachs were greatly distended with undigested food, whereas the remainder of the gut was empty. The mice had a scruffy appearance and paralysis of the hind legs before dying. No mice died after feeding experiments. They passed through a stage of lethargy and diarrhea and then recovered.

It is evident from these studies that *V. parahaemolyticus* is commonly present in the estuarine waters and oysters of this northern New England region. No epidemiological evidence is available that oysters or fish harvested from these waters by private individuals have caused cases of food poisoning due to this organism.

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