

Marine Vibrios Associated with Bacillary Necrosis, a Disease of Larval and Juvenile Bivalve Mollusks

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The etiological agents of bacillary necrosis, a disease of larval and juvenile bivalve mollusks, are designated members of the genus *Vibrio*.

Five antigenically different strains of marine bacteria isolated from moribund and dead hatchery-reared hard clam (*Mercentaria mercenaria*) and American oyster (*Crassostrea virginica*) larvae and juveniles were shown to be the etiological agents of a disease termed bacillary necrosis (10). These strains, ATCC 19105, 19106, 19107, 19108, and 19109, have been included in taxonomic studies of the genus *Vibrio* (2, 8).

These studies, which included numerical taxonomic analysis by computer using 210 coded features, showed very high over-all similarity of the marine molluscan pathogens with strains of marine bacteria previously identified as members of the genus *Vibrio*. Methods employed in these studies have been reported elsewhere (1, 3, 7). The strains deposited with the ATCC were isolated from hard-clam larvae at Milford, Conn. (Long Island Sound), and from hard-clam juveniles from a seaside embayment on Virginia's Eastern Shore (10). Two recent isolates, strains CB4 and CB25 originating, respectively, from the mantle fluid of an overtly normal Chesapeake Bay adult oyster and from Chesapeake Bay mud (6), were included in the study because they killed oyster larvae on experimental exposure following procedures described elsewhere (10).

The bacillary necrosis organisms grouped together at a $\geq 75\%$ similarity level, except ATCC strain 19108, which clustered at a $\geq 75\%$ similarity level with *V. alginolyticus* (strain 5403). Relationships between the mollusk pathogens and *V. cholerae* (*V. comma*) and *V. parahaemolyticus* were $\leq 65\%$ S on the basis of the same set of features (4; L. Wang et al., *Bacteriol. Proc.*, p. 1, 1969). For the present, we are designating all seven strains as members of the genus *Vibrio*, although, on the basis of numerical taxonomy and deoxyribonucleic acid/ribonucleic acid homology data, we believe that the marine

vibrios may well belong and eventually be assigned to a separate new genus (2, 8).

According to previously published methods (1, 3, 7), the following features are held in common by the mollusk pathogens.

Cell morphology: Gram-negative rods, 0.6 to 1.0 μm , motile with polar monotrichous flagella.

Colony morphology: Smooth, off-white to gray, flat to convex, translucent when young, becoming opaque, entire edge, 1 to 4 mm diameter; no pigment, either diffusible or nondiffusible produced.

Physiology and biochemistry: Growth in NaCl-free broth, -; growth in broth with 3% NaCl, +; growth in 6% NaCl, \pm ; growth in 8% NaCl, -; growth at 25 C, +; growth at 37 C, \pm ; growth at 42 C, -; anaerogenic fermentation of glucose, +; sucrose fermentation, +; maltose fermentation, +; starch hydrolysis, +; lactose fermentation, -; xylose fermentation, -; citrate utilization, -; oxidase production, +; catalase production, \pm ; lipase production, +; arginine decarboxylase production, +; lysine decarboxylase production, -; ornithine decarboxylase production, -; indole production + or -; gelatin liquefaction, +; gluconate \rightarrow ketogluconate, -.

The mollusk pathogens can be divided into three groups on the basis of differentiating features (Table 1). ATCC 19108 comprises group I and is identified as *V. alginolyticus*. ATCC 19105, 19106, 19109, and CB4 comprise group II. Since group II strains share high over-all similarity with *V. anguillarum*, we are placing these strains within that species. Group III consists of ATCC 19107 and CB25. For the time being we are designating the two group III strains as *Vibrio* sp. "mollusk pathogens."

The necessity to cultivate food from the sea (aquaculture) requires knowledge of the normal commensal flora and the obligate and facultative

TABLE 1. Selected features differentiating strains of *Vibrio* spp. associated with bacillary necrosis

Group	Acid in glucose + iodoacetate	Leci-thinase	VP ^a	Sensitive to 0/129	Sensitive to penicillin	Sensitive to streptomycin	Sensitive to aureomycin
I (ATCC 19108)	+	-	+	-	-	+	-
II (ATCC 19105, 19106, 19109, CB4)	-	+	-	+	+	-	+
III (ATCC 19107, GB25)	-	+	-	+	+	+	+

^a Voges-Proskauer.

pathogens of those aquatic animals, such as bivalve mollusks, which may prove economically amenable to aquaculture. The studies reported here and elsewhere (6, 10) indicate that a group of bacteria, identified as *Vibrio* spp., can be isolated from overtly healthy, diseased, or moribund bivalve mollusks, or their environments, in highly productive commercial shell-fishery areas. Oyster setting occurs in Chesapeake Bay from mid-June through August and in Long Island Sound from mid-July through August, concomitant with increasing air and water temperatures (5, 9). We can, therefore, speculate that, during midsummer, when conditions favor both molluscan spawning and bacterial proliferation, natural epizootics of bacillary necrosis may limit the recruitment of commercially valuable bivalve mollusks.

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