Vibrio aestuarianus: a New Species from Estuarine Waters and Shellfish†

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A group of phenotypically similar bacteria were isolated from estuarine waters and shellfish from the Oregon coast by procedures designed for the isolation of potentially pathogenic *Vibrio* species. Although these strains were typical of bacteria of the genus *Vibrio*, they could be distinguished from previously described species by their arginine dihydrolase activities, negative Voges-Proskauer reactions, fermentation of sucrose and lactose, and inability to grow in 0 or >5% added NaCl. The deoxyribonucleic acid base compositions of these strains ranged from 43 to 44 mol% guanine plus cytosine. There was >85% deoxyribonucleic acid relatedness among these strains (melting temperature –15°C; membrane filter competition method), whereas there was <25% relatedness with other *Vibrio* species, including *Vibria anguillarum*, *Vibrio nereis*, and *Vibrio splendidus*, which are also arginine dihydrolase positive and have similar guanine-plus-cytosine contents. Our results indicate that the strains which we studied are distinct from previously described species of the genus *Vibrio*, and we propose the name *Vibrio aestuarianus* for these strains, indicating their estuarine origin. The type strain is strain OY-0-002 (= ATCC 35048).

The recent realization that *Vibrio* species pathogenic to humans were endemic to United States coastal waters has led to a renaissance in the study of the ecology, pathogenicity, and taxonomy of these bacteria. This renewed effort has led to the recent reorganization of the genus *Vibrio* (2) and to descriptions of a number of new species (3–5, 7, 8, 10).

During the course of a survey to determine the distribution of pathogenic *Vibrio* spp. in estuarine waters and shellfish from the coast of Oregon (D. L. Tison, M. Nishibuchi, and R. J. Seidler, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, Q97, p. 216), phenotypically similar bacteria which were distinct from previously described *Vibrio* species were isolated. Comparisons with other lactose-fermenting *Vibrio* species indicated that the arginine dihydrolase-positive, lactose-positive strains isolated from Oregon estuaries were probably a new *Vibrio* species (11).

In this paper we describe the characteristics of these strains and show that these bacteria were genetically distinct from other phenotypically similar *Vibrio* species, thereby warranting their designation as a new species, *Vibrio aestuarianus* (formerly called “*Vibrio aestuarii*” [Tison and Seidler, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, 153, p. 148]).

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MATERIALS AND METHODS

Bacterial strains. Strains from water and shellfish from the Oregon coast were isolated by inoculating alkaline peptone broth and subsequently plating and isolating the organisms on thiosulfate-citrate-bile salts-sucrose agar (Oxoid) (6). The sources of *V. aestuarianus* and the reference strains used in this study are listed in Table 1.

Phenotypic and genetic characterization. The media and methods used in phenotypic characterizations, deoxyribonucleic acid (DNA) base composition determinations, and DNA-DNA hybridization studies were essentially as described previously (12, 13).

RESULTS AND DISCUSSION

***Vibrio aestuarianus* sp. nov.** The type strain of *V. aestuarianus* (aes.tu.a.ri.a'nus, L.n. *aestuarius* estuary; N.L. masc. adj. *aestuarianus* pertaining to an estuary) is strain OY-0-002 (= ATCC 35048). *V. aestuarianus* is a gram-negative, oxidase-positive, straight or curved, rod-shaped bacterium which is motile by means of a single polar flagellum. Cells are about 0.5 µm wide and 1.5 to 2.0 µm long when they are Gram stained. *V. aestuarianus* grows without swarming on Trypticase soy, blood, thiosulfate-citrate-bile salts-sucrose, and MacConkey agars containing 0.5% NaCl. No growth is observed in media lacking NaCl. The type strain and six of eight other strains failed to grow in media containing 7% NaCl. Growth is optimal at 20 to 25°C but also occurs at 5 and 37°C but not 42°C. *V. aestuarianus* is facultatively anaerobic and pro-

TABLE 1. DNA relatedness of *V. aestuarianus* OY-0-002^T to other *V. aestuarianus* strains and to other strains of *Vibrionaceae*

Source of unlabeled DNA ^a	Origin or source of strain ^a	% Relatedness ^b
<i>V. aestuarianus</i>		
OSU OY-0-002 ^{Tc}	Oyster; Yaquina Bay, Oreg.	100
OSU OY-0-003	Oyster; Puget Sound, Wash.	86
OSU OY-0-004	Oyster; Puget Sound, Wash.	100
OSU WA-0-034	Water; Netarts Bay, Oreg.	98
OSU WA-0-035	Water; Tillamook Bay, Oreg.	84
OSU OY-0-006	Oyster; Coos Bay, Oreg.	93
OSU CL-0-004	Clam; Coos Bay, Oreg.	87
OSU CR-0-002	Crab; Tillamook Bay, Oreg.	94
OSU CR-0-006	Crab; Tillamook Bay, Oreg.	ND ^d
<i>Vibrio</i> spp.		
UNCC 1077	J. Oliver	16
UNCC 1084	J. Oliver	27
UNCC 1085	J. Oliver	27
<i>V. alginolyticus</i> ATCC 17749 ^T	ATCC	19
<i>V. anguillarum</i> OSU LS174	J. Rohovec	19
<i>V. cholerae</i> CDC E7323	CDC	2
<i>V. damsela</i> CDC 2588-80 ^T	D. Fisher	14
<i>V. diazotrophicus</i> UMD NS1 ^T	P. West	0
<i>V. fluvialis</i> UMD 2386	R. Colwell	0
<i>V. nereis</i> ATCC 25917 ^T	ATCC	17
<i>V. ordalii</i> OSU 21	J. Rohovec	11
<i>V. parahaemolyticus</i> CDC A8659	J. Baross	10
<i>V. splendidus</i> ATCC 33125 ^T	ATCC	24
<i>V. vulnificus</i> ATCC 27562 ^T	ATCC	20
<i>Aeromonas hydrophila</i> NMRI 7	S. Joseph	0

^a OSU, Oregon State University culture collection; UNCC, University of North Carolina at Charlotte culture collection; ATCC, American Type Culture Collection; CDC, Centers for Disease Control culture collection; UMD, University of Maryland culture collection; NMRI, Naval Medical Research Institute culture collection.

^b Experiments were conducted under supraoptimal conditions (melting temperature - 15°C).

^c T = type strain.

^d ND, DNA relatedness was not determined (this strain was used in phenotypic studies only).

duces beta-hemolysis on sheep blood agar, indole, catalase, gelatinase, deoxyribonuclease, *ortho*-nitrophenyl- β -D-galactopyranosidase, and arginine dihydrolase. Lysine decarboxylase, ornithine decarboxylase, collagenase, and elastase are not produced. No strain tested produced luminescence on marine agar, acetoin (Voges-Proskauer test), or H₂S. All *V. aestuarianus* strains tested produced acid without gas in less than 72 h at 37°C from D-amylgalin, D-glucose, lactose, D-mannitol, D-mannose, sorbitol, sucrose, and trehalose. The type strain and most of the other strains tested produced acid from cellobiose (seven of nine strains), glycerol (eight of nine strains), and melibiose (seven of nine strains). No strain produced acid from adonitol, L-arabinose, dulcitol, raffinose, L-rhamnose, salicin, or xylose. The key characteristics of *V. aestuarianus* are shown in Table 2.

Antimicrobial susceptibility. All *V. aestuarianus* strains tested were susceptible to vibriostatic agent 0/129 (10- μ g disk) and to amikacin, ampicillin, carbenicillin, cephalothin, colistin, chloramphenicol, gentamicin, kanamycin, nitrofurantoin, tetracycline, tobramycin, and tri-

methoprim-sulfamethoxazole at standard concentrations with the disk diffusion method (1).

DNA base composition. The DNA base composition of the *V. aestuarianus* type strain is 43.2 mol% guanine plus cytosine. The DNA base compositions of the eight other strains tested range from 43 to 44 mol% guanine plus cytosine.

DNA relatedness. The results of DNA-DNA competition experiments conducted under supraoptimal conditions (melting temperature -15°C) (Table 1) show that the nine estuarine isolates represent a genetic species with $\geq 84\%$ relatedness to *V. aestuarianus* type strain OY-0-002. The *V. aestuarianus* type strain was found to be genetically distinct (<25% relatedness) from other *Vibrio* species with similar DNA base compositions and the type or reference strains of phenotypically similar species (*Vibrio anguillarum*, *Vibrio damsela*, *Vibrio diazotrophicus*, *Vibrio nereis* and *Vibrio splendidus*). Generally, DNAs from strains of the same species are $\geq 60\%$ related under supraoptimal reassociation conditions (melting temperature -15°C in this case), whereas species within the same genus are usually <30% related under supraoptimal

TABLE 2. Characteristics useful for distinguishing *V. aestuarianus* from other arginine dihydrolase-positive *Vibrio* species^a

Species	Oxidase	Luminescence	Voges-Proskauer test	Indole	Citrate	Gelatin hydrolysis	Gas from glucose	Acid from:			Guanine-plus-cytosine content (mol%)
								Arabinose	Lactose	Sucrose	
<i>V. aestuarianus</i>	+	—	—	+	+	+	—	—	+	+	43–44
<i>V. anguillarum</i>	+	—	+	v	—	+	—	v	—	+	44–46
<i>V. costicola</i>	+	—	—	—	—	v	—	—	—	+	50
<i>V. damsela</i>	+	—	+	—	—	—	+	—	—	—	44
<i>V. diazotrophicus</i>	+	—	—	+	+	—	—	+	+	+	45–47
<i>V. fluvialis</i>	+	—	—	—	v	+	v	+	—	+	50–51
<i>V. metschnikovii</i>	—	—	+	v	v	+	—	—	v	+	44–47
<i>V. nereis</i>	+	—	—	+	—	+	—	—	—	+	46–47
<i>V. proteolyticus</i>	+	—	+	+	+	+	—	—	—	—	50
<i>V. splendidus</i>	+	+	—	+	+	+	—	—	—	v	45–46

^a The characteristics of *V. aestuarianus*, *V. anguillarum*, and *V. fluvialis* are based on data from this study. Literature descriptions were used for *V. damsela* (8), *V. diazotrophicus* (4), and *V. costicola*, *V. metschnikovii*, *V. nereis*, *V. proteolyticus*, and *V. splendidus* (P. Baumann, A. L. Furniss, and J. V. Lee, manuscript in preparation).

^b +, Positive; —, negative; v, variable.

reassociation conditions (3). Under optimal conditions for hybridization (melting temperature -25°C), the relatedness value between species (e.g., between *V. aestuarianus* and *Vibrio alginolyticus*) may increase to as much as 50 to 60%, but it would still be less than that observed between strains within a species.

Vibrio sp. strains UNCC 1077, UNCC 1084, and UNCC 1085, which were isolated from the southeastern United States coast, were included in the DNA homology studies because these strains were phenotypically similar to *V. aestuarianus* and to strains grouped in cluster III in the numerical taxonomy study of lactose-fermenting marine vibrios by Oliver et al. (9). Strains UNCC 1077, UNCC 1084, and UNCC 1085 were not genetically related to *V. aestuarianus* at the species level (Table 1). An extensive phenotypic characterization of these strains and the strains in cluster III described by Oliver et al. (9) indicated that the strains isolated from the southeastern United States coast are probably *V. diazotrophicus*, a recently described species capable of nitrogen fixation (4).

V. aestuarianus is easily distinguished from pathogenic lactose-fermenting *Vibrio* spp. by its arginine dihydrolase activity and lack of ornithine and lysine decarboxylases. *Vibrio cholerae* and *Vibrio vulnificus* may also ferment lactose; however, these species lack arginine dihydrolase activity and usually decarboxylate lysine and ornithine. Characteristics which are useful in distinguishing *V. aestuarianus* from other arginine dihydrolase-positive *Vibrio* species are given in Table 2.

The public health significance of *V. aestuarianus* remains to be determined. However, the association of this species with crabs, clams, and oysters warrants an investigation into the

potential of *V. aestuarianus* strains as not only shellfish pathogens but also human pathogens since raw or improperly cooked shellfish serve as vehicles of infection by other pathogenic *Vibrio* species.

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