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Vibrio cincinnatiensis sp. nov., a New Human Pathogen

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A halophilic gram-negative rod was isolated from blood and cerebrospinal fluid collected from a 70-year-old male having no known contact with seafood or salt water. Positive biochemical tests included oxidase, sensitivity to 0/129, *O*-nitrophenyl-β-D-galactopyranoside, lysine decarboxylase and fermentation of glucose, salicin, *n*-inositol, sucrose, L-mannose, L-arabinose, and arbutin. Negative tests included indole, ornithine decarboxylase, arginine dihydrolase fermentation of lactose, and production of gelatinase and urease. The DNA base composition was 45.0 mol% guanine plus cytosine. Numerical taxonomy indicated 70% similarity with known reference *Vibrio* sp. strains. The 5S rRNA sequence for this strain has been determined: 5′-U G C C U G G C G A C C A U A G C G U U U U G G A C C C A C C U G A U U C C A U G C C G A A C U C A G U A G U G A A A C G A A A C A G C G U C G A U G G U A G U G U G G G G U C U C C C C A U G U G A G A G U A G A A C A U C G C C A G G C A U-3′. Based on the phenetic, molecular genetic, and nucleic acid sequencing data, it is concluded that *Vibrio cincinnatiensis* represents a new species of the genus *Vibrio sensu strictu* (as defined by 5S rRNA sequencing results). On a basis of 5S rRNA comparative sequence analysis, the organism appears to share a recent common ancestor with *V. gazogenes* (98% homology) and close ancestry with *V. mimicus*, *V. fluvialis*, and *V. metschnikovii*.

The species of the genus *Vibrio* comprise the majority of the family *Vibrionaceae*, as presently defined (1). These species are characteristically autochthonous, i.e., indigenous, to marine, estuarine, and brackish environments. Some strains have been implicated in fish disease (3), while others comprise the normal microbiota of aquatic vertebrates and invertebrates (4, 7a, 8). Human diseases of varying degrees of severity are frequently caused by *Vibrio* spp., most often transmitted via water and waterborne animals (6). This paper describes a new halophilic *Vibrio* species isolated from a case of meningitis. We propose the name *Vibrio cincinnatiensis* for the organism and designate ATCC 35912 as the type strain. Results of a polyphasic taxonomic analysis indicate that, although the organism can be identified as a member of the genus *Vibrio*, it is phenotypically and genetically distinct from all other *Vibrio* spp. characterized to date.

MATERIALS AND METHODS

Case history. The organism described was isolated from a 70-year-old male patient with bacteremia and meningitis at the University of Cincinnati Hospital. He had a 24-h history of lethargy, disorientation, and altered mental status. There was no history of diarrhea, rashes, exposure to seafood, or contact with salt water. Physical examination revealed a temperature of 103°F (39.4°C). Laboratory data reported normal hepatic enzymes, leukocytes of 13,200 cells per mm^3, hemoglobin of 14.5 g/dl, and a platelet count of 194,000/mm^3. Blood and cerebrospinal cultures were inoculated onto blood agar plates, and pure cultures of *V. cincinnatiensis* grew from both samples. Therapy was begun with ampicillin (day 1) and continued with moxalactam for the next 9 days. Recovery was uneventful, representing the first successful treatment of *Vibrio* sp. meningitis in an adult (R. B. Bode, P. R. Brayton, R. R. Colwell, R. Russo, and W. E. Bullock, Ann. Intern. Med., in press).

Morphology. Morphology was described for colonies growing on nutrient agar (Difco Laboratories, Detroit, Mich.) plus 1% (wt/vol) NaCl and on thiosulfate-citrate-bile salts-sucrose agar (Oxoid U.S.A. Inc., Columbia, Md.). An 18-h culture was Gram stained, using the method of Hucker (5), and the flagella staining procedure used has been described by Kodaka et al. (10).

Biochemical tests. All media were supplemented with 1% (wt/vol) NaCl and incubated at 25°C, with a duplicate incubated at 35°C. The method of Billy (2) was used to determine alginase production. β-Galactosidase was detected by the method of Lowe (11). GasPak anaerobic systems (BBL Microbiology Systems, Cockeysville, Md.) were used to assess growth under anaerobic conditions. All other biochemical testing procedures have been described elsewhere (17).

Nucleic acid studies. The DNA base composition (guanine plus cytosine) was ascertained by thermal denaturation (14). 5S rRNA was purified by polyacrylamide gel electrophoresis and sequenced enzymatically, using the methods described by MacDonell and Colwell (12, 13). Branch lengths and clusters for an estimate of the phylogeny of *Vibrio* species were generated by using the difference matrix programs FITCH and KITSCH (PHYLIP: Phylogency Inference Package; J. Felsenstein, University of Washington) from evolutionary distance coefficients derived by the method of Kimura (9).

Numerical taxonomy. A numerical taxonomy analysis was run to compare the similarity of *V. cincinnatiensis* and 23 reference strains of the family *Vibrionaceae*. Each strain was characterized by using four quantitative multistate characters and 111 qualitative characters. The multistate charac-

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TABLE 1. Biochemical reactions of *V. cincinnatiensis*[a]

Test	Result[b]
Gram reaction	−[c]
Oxidase	+[d]
Voges-Proskauer	+
Motility	+
Indole	−
Lysine decarboxylase	+
Ornithine decarboxylase	−
Arginine dihydrolase	−
Nitrate reduction	+
H_2S production	−
Growth at	
0% NaCl	−
1% NaCl	+
3% NaCl	+
6% NaCl	+
8% NaCl	−
10% NaCl	−
4°C	−
25°C	+
35°C	+
42°C	−
pH 4.5	−
pH 10.0	+
o-Nitrophenyl-β-D-galactopyranoside	+
Luminescence	−
Production of	
Gas from glucose	−
Catalase	+
Urease	−
Alginase	−
Elastase	−
Amylase	+
Gelatinase	−
Chitinase	+
Lecithinase	−
DNase	+
Caseinase	−
Hydrolysis of	
Tween 20	−
Tween 40	−
Tween 60	−
Tween 80	−
Fermentation of	
Arbutin	+
Trehalose	+
Sucrose	+
D-Cellobiose	+
D-Mannose	+
m-Inositol	+
D-Mannitol	−
Sorbitol	−
Salicin	+
L-Arabinose	+
Lactose	−
Glucose	+
Susceptibility to 0/129 (μg/ml)	
10	−
50	−
150	+
Resistance to	
Ampicillin (10 μg)	−
Streptomycin (10 μg)	+
Penicillin (10 U)	+
Kanamycin (300 U)	−
Polymyxin B (50 μg)	−
Tetracycline (30 μg)	−
Erythromycin (15 μg)	−
Chloramphenicol (30 μg)	−
Gentamicin (10 μg)	−
Utilization as sole carbon source	
Acetate	+
Citrate	+
Fumarate	+
α-Ketoglutarate	+
D-Alanine	+
L-Alanine	+
α-Aminobutyric acid	+
L-Arginine	+
L-Aspartate	+
L-Citrulline	+
L-Glutamate	+
L-Leucine	−
L-Ornithine	−
L-Proline	+
L-Tyrosine	−
D-Amygdalin	−
L-Arabinose	+
D-Cellobiose	+
D-Gluconate	+
D-Glucose	+
Glycogen	+
Lactose	−
Salicin	+
Sucrose	+
Trehalose	+
D-Xylose	+
Ethanolamine	−
Putrescine	−
Adenine	−
D-Glucuronate	−
Sarcosine	−
Xanthine	−
Taurine	−
D-Arabitol	−
Dulcitol	−
Ethanol	−
D-Mannitol	+
Sorbitol	−
p-Hydroxybenzoic acid	−
Phenylacetate	−

[a] With the exception of those tests designated in the text under Materials and Methods, all other methods are described in West and Colwell (17).
[b] Identical results were obtained at 25 and 35°C incubations.
[c] −, Negative within 2 weeks.
[d] +, Positive within 24 h to 2 weeks.

ters, minimum and maximum salt concentrations, maximum growth temperature, and sensitivity to 2,4-diamino-6,7-diisopropyl pteridine (0/129) were scored as 0 (negative) or 1 (positive). A resemblance matrix was calculated using Euclidean distance and a phenogram was constructed by unweighted pair group arithmetic average sorting (15). The cophenetic correlation coefficient was calculated by the method of Sokal and Rohlf (16). Cluster analysis was performed by using the CLUSTAN package, version 2, release 2, on the University of Surry Prime computer systems (18).

RESULTS AND DISCUSSION

V. cincinnatiensis is a gram-negative, non-sporeforming rod, measuring approximately 0.7 by 2.0 μm. Overnight incubation at 25 and 35°C produces round, smooth, glossy colonies (1 to 2 mm in diameter) that are cream color on nutrient agar and yellow on thiosulfate-citrate-bile salts-sucrose agar. Slower growth is exhibited under anaerobic

TABLE 2. Characteristics useful in differentiation of *V. cincinnatiensis* from other members of the genus *Vibrio*[a]

Characteristic	*V. alginolyticus*	*V. anguillarum* biovar I	*V. campbellii*	*V. cholerae*	*V. cincinnatiensis*	*V. fischeri*	*V. fluvialis*	*V. furnissii*	*V. gazogenes*	*V. harveyi*	*V. metschnikovii*	*V. natriegens*	*V. nereis*	*V. nigripulchrituda*	*V. parahaemolyticus*	*V. pelagius* biovar I	*V. pelagius* biovar II	*V. proteolyticus*	*V. splendidus* biovar I	*V. splendidus* biovar II	*V. vulnificus*
Cytochrome oxidase	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+
0/129 sensitivity																					
10 μg	−	+	−	+	−	+	−	−	+	−	+	−	−	−	−	+	+	−	+	+	+
150 μg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Luminescence	−	−	−	v	−	+	−	−	−	v	−	−	−	−	−	−	−	−	+	−	−
Arginine dihydrolase	−	+	−	−	−	−	+	+	−	−	+	−	+	−	−	−	−	+	+	−	−
Lysine decarboxylase	+	−	+	+	+	+	−	−	−	+	v	−	−	−	+	−	−	+	−	−	+
Ornithine decarboxylase	+	−	−	+	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	+
Growth at 42°C	+	−	−	+	−	−	−	−	+	v	v	v	v	−	+	−	−	−	−	−	+
Growth at percent NaCl																					
0	−	v	−	+	−	−	v	v	−	−	v	−	−	−	−	−	−	+	−	−	−
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	v	+	+	+	+	+	+	+	+	+	−	+	+	+	+	v	v	+
8	+	v	v	−	−	+	v	v	−	v	v	v	v	−	+	v	v	+	−	−	−
10	+	−	−	−	−	−	−	−	−	v	−	−	v	−	−	−	−	−	−	−	−
Voges-Proskauer reaction	+	+	−	+	+	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−
Gas from glucose fermentation	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−
Fermentation of																					
L-Arabinose	−	v	−	−	+	−	+	+	+	−	−	+	−	−	v	−	−	−	−	−	−
m-Inositol	−	v	−	−	+	−	−	−	−	−	v	v	−	v	−	−	−	−	−	−	−
D-Mannose	+	+	+	v	+	+	+	+	+	+	v	−	−	−	+	v	v	+	+	−	+
Sucrose	+	+	−	+	+	−	+	+	+	v	+	+	+	−	−	+	+	−	v	−	−
Enzyme production																					
Alginase	−	−	−	−	−	−	−	−	−	v	−	−	−	−	−	+	+	−	v	−	−
Amylase	+	+	+	+	+	−	+	v	+	+	+	v	−	+	+	−	+	+	+	+	+
Chitinase	+	+	+	+	+	v	+	+	−	+	+	−	v	+	+	v	+	+	+	+	+
Gelatinase	+	+	+	+	−	−	+	+	+	+	+	+	v	+	+	−	+	+	+	+	+
Lipase	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+
Utilization as sole carbon source																					
γ-Aminobutyrate	−	−	−	−	+	−	+	+	−	−	−	+	+	−	−	−	+	−	−	−	−
Cellobiose	−	v	v	−	+	+	v	−	+	+	−	v	−	+	−	−	−	−	+	v	+
L-Citrulline	−	−	−	−	+	−	+	−	−	v	−	+	+	−	−	+	+	−	+	−	−
Ethanol	v	−	−	−	−	−	+	+	−	−	−	+	+	v	+	v	−	−	−	−	−
D-Gluconate	+	+	−	+	+	−	+	+	−	+	+	+	+	v	+	+	+	+	v	−	+
D-Glucuronate	−	−	−	−	−	−	+	−	−	+	−	v	−	+	v	−	−	−	+	−	+
L-Leucine	+	−	−	−	−	−	−	−	−	−	−	v	+	−	+	−	−	−	−	−	−
Putrescine	v	−	−	−	−	−	v	+	−	−	−	+	+	−	+	+	+	+	−	−	−
Sucrose	+	+	−	+	+	−	+	+	+	v	+	+	+	−	−	+	v	−	v	−	−
D-Xylose	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−

[a] Source of data is reference 17. Symbols: +, positive trait for at least 90% of the strains; −, negative trait for at least 90% of the strains; v, trait differs for strains within the species.

(GasPak) conditions. Single polar flagella are observed attached to cells grown on solid and in liquid media.

Biochemical reactions for *V. cincinnatiensis* are listed in Table 1. It is halophilic and tolerates 6%, but not 8%, NaCl supplement to the growth medium. It is positive for lysine decarboxylase, Voges-Proskauer reaction, and *o*-nitrophenyl-β-D-galactopyranoside, and is sensitive to the vibriostatic agent 0/129. It produces catalase, amylase chitinase, and DNase and ferments arbutin, trehalose, sucrose, D-cellobiose, D-mannose, *m*-inositol, salicin, L-arabinose, and glucose. It is capable of utilizing citrate, γ-amino butyric acid, L-citrulline, D-cellobiose, salicin, and D-xylose as sole carbon sources. Negative tests include indole, ornithine decarboxylase, arginine dihydrolase, and growth at 42°C. It is unable to hydrolyze Tween 80 or produce gelatinase, urease, alginase, caseinase, lecithinase, or elastase. Characteristics useful for distinguishing *V. cincinnatiensis* from other *Vibrio* spp. are presented in Table 2.

A phenogram was constructed by numerical taxonomy, incorporating *V. cincinnatiensis* and 23 reference strains (Fig. 1). Each strain was examined for 99 characters. Traits that were uniformly positive or negative for every strain were eliminated from the calculation of the similarity coef-

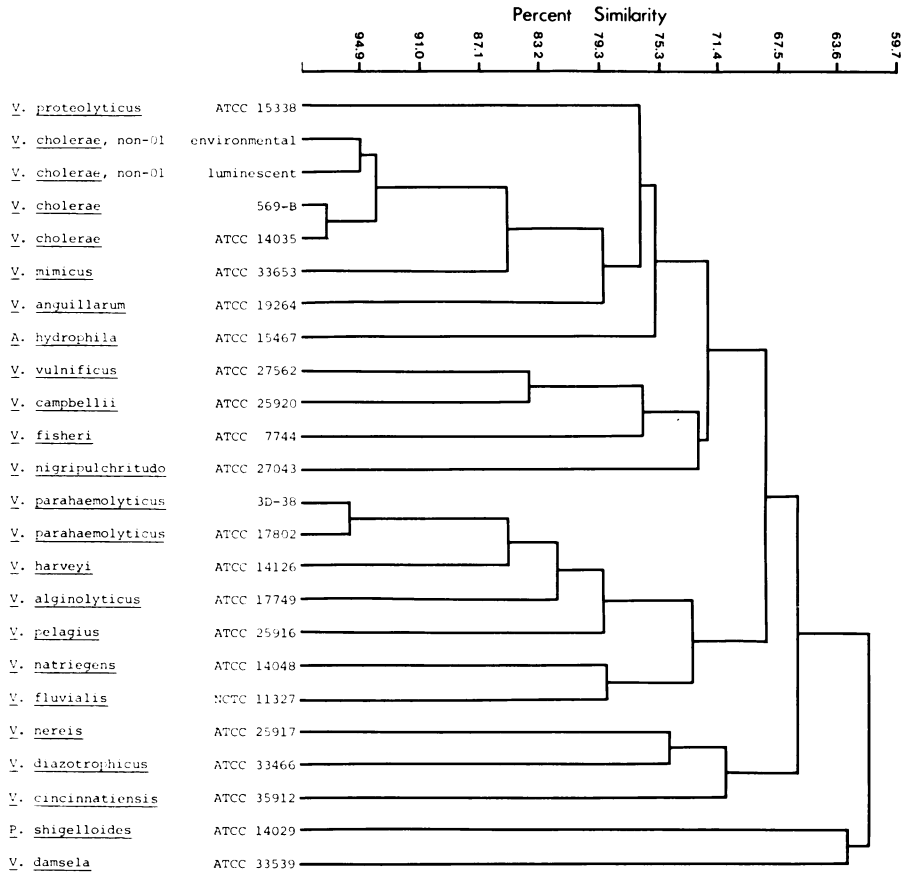

FIG. 1. Phenogram based on unweighted pair group arithmetic average sorting.

ficient. The relationships among the 24 strains were based on Euclidean distances calculated by the unweighted pair group arithmetic average method. The cophenetic correlation coefficient for the phenogram was 0.75. Results showed that, of all strains examined, *V. cincinnatiensis* possessed closest relationships, i.e., ≥70% similarity, with *V. diazotrophicus* and *V. nereis*. All three organisms required NaCl for growth, were positive for cytochrome oxidase, reduced nitrate, fermented sucrose, trehalose, and cellobiose, and were sensitive to 150 μg of 0/129. All were gelatinase negative. A 65% similarity was observed between *V. cincinnatiensis* and the majority of the other reference strains used in this study.

The base composition of the DNA was determined to be 45 mol% (standard deviation = 0.769), based on a total of six different spectrophotometric readings of separate DNA preparations. This value is well within the range described for *Vibrio* spp. (1).

The nucleotide base sequence of the 5S rRNA of *V. cincinnatiensis* was determined to be 5′-U G C C U G G C G A C C A U A G C G U U U U G G A C C C A C C U G A U U C C A U G C C G A A C U C A G U A G U G A A A C G A A A C A G C G U C G A U G G U A G U G U G G G G U C U C C C C A U G U G A G A G U A G A G A A C A U C G C C A G G C A U-3′. Cluster analysis (7) of evolutionary distance coefficients (9) derived from this and other *Vibrio* sp. 5S rRNA sequences indicates that *V. cincinnatiensis* shares a recent common ancestor with *V. gazogenes* (98.3% sequence homology; 13a); these in turn share a common ancestor with *V. mimicus*, *V. fluvialis*, and *V. metschnikovii* (Fig. 2). In fact, 5S rRNA sequences have been determined for some 50 species of the vibrio-enteric group, i.e., rRNA Superfamily I. Comparisons among these indicate that the *V. cincinnatiensis* 5S rRNA sequence is unique. The reader is referred to MacDonell and Colwell (13a) for an in-depth analysis.

We conclude *V. cincinnatiensis* to be a member of the genus *Vibrio sensu strictu* and a species separate from other known *Vibrio* spp. A description of *V. cincinnatiensis* is here provided.

Description of the species. *Vibrio cincinnatiensis* sp. nov. (Latin adj., derived from the Society of Cincinnati from which the city of Cincinnati, Ohio, was named). Gram-negative rod, 0.7 by 2.0 μm. Cells are motile by a single polar flagellum; lateral flagella are not formed. Colonies on nutrient agar are cream color, round, smooth, and glossy. Yellow colonies are produced on thiosulfate-citrate-bile salts-sucrose agar. Facultatively anaerobic. Sodium chloride is required for growth. Ferments glucose, arbutin, trehalose, sucrose, D-cellobiose, D-mannose, *m*-inositol, salicin, and L-arabinose. Catalase, oxidase, amylase, chitinase, and DNase are produced. Gelatinase, urease, alginase, caseinase, lecithinase, and elastase are not produced. Positive for lysine decarboxylase, *o*-nitrophenyl-β-D-galactopyranoside, and Voges-Proskauer. Negative for ornithine decarboxylase, arginine dihydrolase, and indole production. Sensitive to 150 μg of vibriostatic agent 0/129. The DNA base composition is 45 mol% guanine plus cytosine. The base sequence of the 5S rRNA has been determined to be 5′-U G C C U G G C G A C C A U A G C G U U U U G G A C C C A C C U G A U U C C A U G C C G

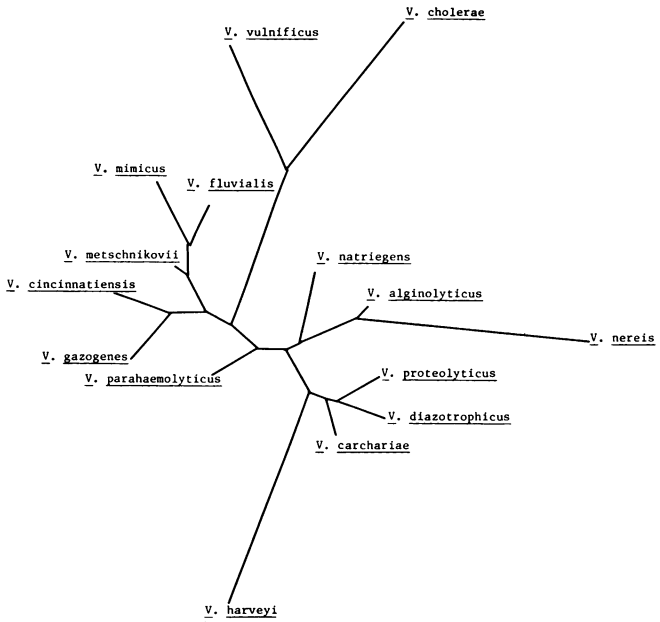

FIG. 2. Unrooted tree representation of evolutionary relationships among *Vibrio* species based on comparative sequence analysis of 5S rRNAs. Estimate of phylogeny was based on the treatment of evolutionary distance coefficients (9) by the clustering programs FITCH and KITCH (see reference 7) (PHYLIP program package; J. Felsenstein). Relative branch lengths may be taken as reasonable estimates of evolutionary distances along lineages. For a discussion of the use of the phylogeny of 5S rRNA molecules to estimate phylogenies of procaryotic species, see MacDonell and Colwell (13a).

A A C U C A G U A G U G A A A C G A A A C A G C G U C G A U G G U A G U G U G G G G U C U C C C C A U G U G A G A G U A G A A C A U C G C C A G G C A U-3′. The type strain is ATCC 35912. The description of the type strain is the same as that given above for the species.

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