INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, July 1982, p. 350–357 Vol. 32, No. 3
0020-7713/82/030350-08$02.00/0

Vibrio diazotrophicus sp. nov., a Marine Nitrogen-Fixing Bacterium

M. L. GUERINOT,[1]† P. A. WEST,[1] J. V. LEE,[2] AND R. R. COLWELL[1]

Department of Microbiology, University of Maryland, College Park, Maryland 20742[1] and Public Health Laboratory, Preston Hall Hospital, Maidstone ME20 7NH, United Kingdom[2]

Facultatively anaerobic, nitrogen-fixing bacterial strains were isolated from sources as diverse as the gastrointestinal tracts of sea urchins collected in Nova Scotia, Canada, and the surfaces of reeds growing in a drainage ditch in Kent, England. These strains were placed in the genus *Vibrio* Pacini 1865 of the family *Vibrionaceae* on the basis of their morphological, physiological, and biochemical characteristics, as well as on the basis of the guanine-plus-cytosine contents of their deoxyribonucleic acids (45.9 to 47.2 mol%). They were clearly distinguished from strains of the currently recognized species in the genus *Vibrio* by a combination of diverse traits, including the production of nitrogenase, the inability to hydrolyze casein, deoxyribonucleic acid, gelatin, and Tween 80, the ability to ferment L-arabinose, cellobiose, salicin, and D-xylose, and the presence of an arginine dihydrolase system. Deoxyribonucleic acid homology studies supported recognition of these nitrogen-fixing strains as a new species, for which the name *Vibrio diazotrophicus* is proposed. Strain ATCC 33466 (= strain 1 = NS1) is the type strain.

Species of *Vibrio* are ubiquitous in estuarine and oceanic environments and are commonly found in association with marine animals. Recently, *Vibrio*-like organisms capable of fixing N_2 were isolated from the gastrointestinal tracts of temperate and tropical sea urchins (6, 7). These strains possessed certain characteristics that are unusual for vibrios, including the ability to ferment D-xylose and the inability to hydrolyze casein, deoxyribonucleic acid (DNA), gelatin, and Tween 80. These isolates were distinct from strains of any previously described *Vibrio* species and were classified as members of a marine *Vibrio* sp. pending the results of DNA homology studies.

Phenotypically similar organisms were identified during a numerical taxonomy study of group F vibrios (now designated *Vibrio fluvialis*) (11) and during a similar study of *Vibrio* species collected from natural waters in Kent, England (P. A. West, Ph.D. thesis, University of Kent at Canterbury, Kent, England, 1980). However, the ability of these strains to fix N_2 was not determined.

The objectives of this study were to establish the nitrogen-fixing capability of the strains described by Lee et al. (11) and West (Ph.D. thesis) and to define the taxonomic status of the N_2-fixing vibrios.

† Present address: Michigan State University-Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, MI 48824.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1, together with their sources.

Characterization tests. The methods of Guerinot and Patriquin (6) were used to identify strains 1 through 3, 14, and 21, and the methods of Lee et al. (11) were used to identify strains 4 through 13 and 15 through 20, with the exceptions and additional tests noted below. Acid production from various substrates was determined for strains 4 through 13 and 15 through 20 in phenol red base broth (Difco Laboratories, Detroit, Mich.). Sulfatase and *o*-nitrophenyl-β-D-galactopyranoside tests were performed by the methods of Cowan (3) for strains 1 through 4. Tests for hydrolysis of albumin, elastin, lecithin, and chrondroitinase and tests for growth on ethanol, 1-propanol, melezitose, propionic acid, and D-xylose were performed for strains 1 through 3 by using the methods of Lee et al. (11). The mode of flagellation of strains 5 through 13 was determined by electron microscopy, using the methods described of Lee et al. (11).

Nitrogenase activity. Nitrogenase activity was determined by the acetylene (C_2H_2) reduction assay (8). Cultures were inoculated into malate glucose medium (6) and incubated at 25°C. After 24 h, tubes were closed with serum stoppers, and 0.2 ml of C_2H_2 was injected into each tube. After incubation for 7 days, ethylene (C_2H_4) production was analyzed by gas chromatography with a Shimadzu model GC-4BM gas chromatograph equipped with a flame ionization detector and operated at a column temperature of 50°C;

TABLE 1. Bacterial strains used in this study

Strain no. or species	Original strain designation	Source	Site of isolation
1	NS1	M. L. Guerinot[a]	Sea urchin gastrointestinal tract, Nova Scotia, Canada
2	NS2	M. L. Guerinot[a]	Sea urchin gastrointestinal tract, Nova Scotia, Canada
3	CB42	M. L. Guerinot[a]	Water, Chesapeake Bay, Maryland
4	VL 6110	J. V. Lee[b]	Water, Humber River, England
5	P103	P. A. West	Ditch water, Chetney Marsh, England
6	P104	P. A. West	Ditch water, Chetney Marsh, England
7	P105	P. A. West	Ditch water, Chetney Marsh, England
8	P106	P. A. West	Ditch water, Chetney Marsh, England
9	P107	P. A. West	Ditch sediment, Chetney Marsh, England
10	P108	P. A. West	Ditch sediment, Chetney Marsh, England
11	P109	P. A. West	Reed surface, Chetney Marsh, England
12	P110	P. A. West	Ditch water, Chetney Marsh, England
13	P111	P. A. West	Ditch water, Chetney Marsh, England
14	Bar	M. L. Guerinot[a]	Sea urchin gastrointestinal tract, Barbados
15	VL 1491	J. V. Lee[b]	Oysters
16	VL 2610	J. V. Lee[b]	Cockles
17	VL 4780	J. V. Lee[b]	Oysters
18	VL 5802	J. V. Lee[b]	Cockles
19	VL 5815	J. V. Lee[b]	Cockles
20	VL 5970	J. V. Lee[b]	Whelks
21	31	C. R. McClung[c]	Roots of *Spartina alterniflora*, Chesapeake Bay salt marsh, Maryland
Culture collection strains			
Vibrio alginolyticus	ATCC 17749	ATCC[d]	
Vibrio anguillarum	ATCC 14181	ATCC	
Vibrio cholerae	ATCC 14035	ATCC	
Vibrio metschnikovii	NCTC 11170	NCTC[e]	
Vibrio parahaemolyticus	ATCC 17802	ATCC	
Oceanospirillum beijerenckii	ATCC 12754	ATCC	
Oceanospirillum linum	ATCC 11336	ATCC	
Oceanospirillum maris subsp. *maris*	ATCC 27509	ATCC	
Vibrio nereis	ATCC 25917	ATCC	
Vibrio fluvialis	NCTC 11327	NCTC	

[a] See reference 6.
[b] See reference 11.
[c] C. R. McClung, Plant Virology Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville Agricultural Research Center-West, Beltsville, Md.
[d] ATCC, American Type Culture Collection, Rockville, Md.
[e] NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England.

the carrier gas flow rate was 25 ml/min in a column (0.32 by 50 cm) packed with 80- to 100-mesh Porapak T. The retention times for C_2H_4 and C_2H_2 were 15 and 30 s, respectively.

DNA extraction. Cells were grown overnight in 1 liter of 1% tryptone–3% NaCl broth at 30°C with agitation. The cells were harvested by centrifugation, washed twice with TS buffer [0.05 M tris(hydroxymethyl)aminomethane (Tris), 0.1 M NaCl, pH 8.1], and the DNA was extracted by a modification of the method of Marmur (12). Cells suspended in 50 ml of TES buffer (0.05 M tris, 0.05 M ethylenedinitrilotetraacetic acid [disodium salt], 0.1 M NaCl, pH 8.1) were lysed by adding 5 ml of 20% sodium dodecyl sulfate, after which the suspension was incubated with 50 μg of pronase (75,000 U/g; Calbiochem, La Jolla, *Calif.*)

per ml for 2 h at 37°C. The suspension was then extracted three times with TES buffer-saturated phenol and then three times with chloroform-isoamyl alcohol (24:1). The DNA was precipitated with ethanol, spooled, and dissolved in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0). The DNA solution was adjusted to 1× SSC and then incubated with 50 μg of ribonuclease per ml for 2 h at 37°C. The DNA was deproteinized by two extractions with TES buffer-saturated phenol and chloroform-isoamyl alcohol, precipitated with ethanol, and dissolved in 0.1× SSC.

G+C content of the DNA. The guanine-plus-cytosine (G+C) content of the DNA was determined by the buoyant density method (16) or was calculated from the thermal denaturation temperature (13), as determined with a Gilford model 2400 spectrophotometer. DNA having a buoyant density 1.731 g/ml prepared from *Micrococcus lysodeikticus* (Sigma Chemical Co., London, England) and DNA from *Escherichia coli* strain WP2, which contained 51.0 mol% G+C (15), were used as the reference DNAs in the buoyant density and thermal denaturation procedures, respectively.

Preparation of radioactively labeled DNA. DNA was labeled in vitro with [α-^{32}P]deoxycytosine triphosphate (specific activity, >400 Ci/mmol) by the nick-translation method according to the instructions of the manufacturer (Amersham Corp., Arlington Heights, Ill.), with the following modification. The nick-translation reaction was stopped by loading the reaction mixture directly onto a 8-cm column of Bio-Gel P60 suspended in buffer containing 0.05 M Tris base, 0.05 M ethylenedinitrilotetraacetic acid (disodium salt), and 0.1 M NaCl, pH 8.1. Fractions (200 μl) were collected and monitored for radioactivity. The specific activities of the labeled DNA preparations were approximately 2 × 10^7 cpm/μg of DNA.

DNA-DNA hybridization. Type strains of both *Vibrio* and *Oceanospirillum* species were used in the DNA-DNA hybridization studies. The genetic relatedness of strains was determined by DNA-DNA hybridization on membrane filters. Native DNA (10 μg/ml in 0.1× SSC) was denatured by adding 0.1 volume of 1 N NaOH. After incubation for 10 min at room temperature, the DNA solution was neutralized by adding 0.1 volume 1.8 M Tris-hydrochloride–0.2 M Tris base. The concentration of DNA was adjusted to 5 μg/ml in 6× SSC. Samples (10 ml) of these DNA solutions were passed by gravity filtration through nitrocellulose filters (type BA85; diameter, 25 mm; pore size, 0.45 μm; Schleicher & Schuell Co., Keene, N.H.) that had previously been soaked in 6× SSC and washed with 20 ml of the same solution. Retention of DNA was estimated by monitoring the optical density of the filtrates at 260 nm. The filters were washed with 20 ml of 6× SSC, air dried at room temperature, and subsequently placed in a vacuum oven at 80°C for 2 h. Loaded filters were preincubated in Denhardt solution (4) to which 0.01% sodium dodecyl sulfate had been added. The filters were then transferred to vials containing 10^4 to 10^5 cpm of denatured labeled DNA in 2 ml of Denhardt solution supplemented with 0.01% sodium dodecyl sulfate. The vials were incubated at 65°C for 24 h. After incubation, each filter was washed three times with 20 ml of 3× SSC, air dried, and counted in 10 ml of scintillation fluid with a Beckman model LS-3155T or LS-7500 liquid scintillation counter.

RESULTS AND DISCUSSION

All of the strains in phenon 4 of West (strains 5 through 13) (West, Ph.D. thesis) and two of the strains in phenon 5 of Lee et al. (11) (strains 4 and 15) demonstrated nitrogenase activity. Of the two strains in phenon 5 which fixed N_2, only strain 4 was positive for arginine dihydrolase and phenotypically indistinguishable from strains 1 and 2. Indeed, strain 4 showed 95% homology to strain 1 when these strains were examined by DNA-DNA hybridization (Table 2). The other nitrogenase-positive strain in phenon 5 was more similar to strain 14; it was not able to decarboxylate the amino acids arginine, lysine, and ornithine and had the ability to hydrolyze gelatin. Strains 14 and 15 showed only 4 and 13% homology, respectively, to strain 1 (Table 2).

The strains in phenon 5 of Lee et al. (11) which did not fix N_2 differed in a number of traits from strains 1 and 2. These nitrogenase-negative strains were also indole and methyl red

TABLE 2. DNA-DNA hybridization relationships of *V. diazotrophicus* strain 1 to other strains of *V. diazotrophicus* and to strains of *Vibrio* and *Oceanospirillum*

Strain or species	Relatedness to *V. diazotrophicus* strain 1 (%)[a]	
3	100	(1)[b]
4	94.7	(2)
5	87.7	(2)
8	100	(3)
9	62	(3)
13	100	(3)
14	12.7	(3)
15	4	(1)
16	4.8	(3)
17	11.0	(3)
18	10.3	(3)
19	4.6	(3)
20	5.7	(3)
21	33.3	(3)
Vibrio alginolyticus	5	(1)
Vibrio anguillarum	0.7	(1)
Vibrio cholerae	8.8	(1)
Vibrio fluvialis	4	(3)
Vibrio metschnikovii	4	(1)
Vibrio nereis	6.0	(3)
Vibrio parahaemolyticus	12.7	(3)
Oceanospirillum beijerinckii	2.0	(3)
Oceanospirillum linum	1.3	(1)
Oceanospirillum maris subsp. *maris*	1.5	(3)

[a] Arithmetic means of percentages of relatedness, as determined by DNA-DNA hybridization.

[b] The numbers in parentheses are numbers of determinations.

negative and possessed the ability to hydrolyze Tween 80. In addition, two of the strains produced gas from glucose. The nitrogenase-negative strains examined in this study demonstrated only 5 to 11% homology to strain 1 (Table 2). Therefore, these strains were not considered to be taxonomically related to strain 1.

We also concluded that strain 21 was not related to strain 1; strain 21 showed only 33.3% homology to strain 1. Strain 21 was included in the study because it was isolated from an estuarine environment, demonstrated an ability to fix N_2, and had a G+C content of 46 mol%. This strain is phenotypically similar to other strains included in this study (C. R. McClung, personal communication).

Of the four strains in phenon 4 included in the hybridization study, three showed 88 to 100% homology to strain 1 (Table 2). However, strain 9 was only 62% homologous to strain 1 (Table 2) and differed in a number of traits from strains 1 and 2; e.g., it showed no growth at 42°C, produced acid from mannose, hydrolyzed urea, and grew on L-serine. Although 62% homology can be considered a low homology value for strains included within a single species, for the present we conclude that strain 9 belongs to the same species as strain 1.

Strain 1 showed very little homology to representatives of other species of *Vibrio* or to selected *Oceanospirillum* spp. (Table 2). *Oceanospirillum* strains were included in the DNA-DNA hybridization studies even though the metabolism of these strains is strictly respiratory because their DNAs have approximately the same G+C contents as the DNAs of the N_2-fixing vibrios. Like the N_2-fixing vibrios, they are oxidase-positive and require salt for growth. In addition, members of the *Spirillaceae* have been shown to fix N_2 (14, 20, 21), whereas before the work of Guerinot and Patriquin (6, 7) the ability to fix N_2 had not been reported for any member of the *Vibrionaceae*.

Strain 1 was selected as the type strain of a new species, for which the name *Vibrio diazotrophicus* is proposed. Strains 1 through 13 are considered to belong to *V. diazotrophicus*. The phenotypic traits of *V. diazotrophicus* are listed in Tables 3 through 5. The DNA base compositions of selected strains of *V. diazotrophicus* and of other strains used in this study are given in Table 6. Traits which distinguish *V. diazotrophicus* from other arginine dihydrolase-positive *Vibrio* species are given in Table 7.

Phenotypically, *V. diazotrophicus* comprises a very well-defined cluster of strains. Like many other species of *Vibrio*, if this species is grown in liquid media, the cells have a single, long-wavelength flagellum which is sometimes sheathed (Fig. 1). On solid media, the cells may also possess lateral, unsheathed flagella having a shorter wavelength (Fig. 2). Characteristically, strains of *V. diazotrophicus* produce only a limited range of extracellular enzymes and are not able to hydrolyze casein, chitin, DNA, gelatin, lecithin, and Tween 80. Lack of extracellular enzymes apparently is very unusual in the genus *Vibrio* (1), as is the ability to ferment or grow on D-xylose as the sole source of carbon (11). However, until more species of *Vibrio* have been described, such a generalization may be premature. Strains of *V. diazotrophicus* yield reactions similar to those of *Vibrio anguillarum* in the tests routinely used for identification of members of the *Vibrionaceae*. Nevertheless, *V. diazotrophicus* strains can be distinguished easily by a few tests (Table 7).

In a study of *Beneckea* Reichelt et al. (18) reported that two closely related strains (strains

TABLE 3. Biochemical characteristics of *V. diazotrophicus* for which all strains are positive

Fermentative in oxidation/fermentation medium
Motility
Catalase production
Oxidase production
Indole production
Methyl red reaction
Growth in 3% NaCl
Growth at:
20°C
37°C
Arginine dihydrolase
Nitrate reduction
o-Nitrophenyl-β-D-galactopyranoside reaction
Susceptibility to 0/129 (150 μg/ml)
Amylase
Nitrogenase
Phosphatase
Acid production from:
L-Arabinose
Arbutin
Cellobiose
Glucose
Mannitol
Salicin
Sucrose
Trehalose
D-Xylose
Growth on:
Acetate
D-Alanine
L-Alanine
L-Arginine
Cellobiose
Citrate
Glutamate
L-Proline
Pyruvate
Salicin
Succinate
D-Xylose

TABLE 4. Biochemical characteristics of *V. diazotrophicus* for which all strains are negative

Gram reaction
Gas from glucose
Growth in:
0% NaCl
8% NaCl
Lysine decarboxylase
Ornithine decarboxylase
Voges-Proskauer reaction
H_2S production
Swarming on solid media
Hemolysis
Luminescence
Acid production from:
Inositol
Sorbitol
Albumin hydrolysis
Casein hydrolysis
Chitinase
Chondroitinase
Deoxyribonuclease
Elastin hydrolysis
Gelatin hydrolysis
Lecithinase
Tween 80 hydrolysis
Growth on:
Formate
Glycine
Melizitose
Proprionate
Sorbitol
Ethanol
1-Propanol

84 and 85) had little relationship to other strains of *Beneckea*; these strains are now regarded as members of *Vibrio* (1) and are similar to *V. diazotrophicus* in that they are arginine dihydrolase positive, lack the extracellular enzymes gelatinase and lipase, and have similar G+C contents. However, in a numerical taxonomy study in which 148 characteristics were used (West, Ph.D. thesis), these strains did not cluster with strains of phenon 4. Therefore, it is unlikely that they are related to *V. diazotrophicus*.

TABLE 6. DNA base compositions of selected strains of *V. diazotrophicus* and other strains used in this study

Strain	G+C content of DNA (mol%)
V. diazotrophicus strains	
1	45.9
2	46.6
4	46.1
5	46.1
7	46.1
8	47.2
9	46.8
11	46.5
Other strains	
14	48.4
15	47.9
16	46.3
20	47.0
21	46.0

West (Ph.D. thesis) tentatively suggested the name "*Vibrio fossarum*" (*fossarum*, meaning of ditches, referring to the original site of isolation) for strains 5 through 13 and stated that additional data, including DNA-DNA hybridization data and G+C values, would be required to confirm the taxonomic status of these strains. As this name has not been validly published, the name *V. diazotrophicus* is here proposed for this organism.

Because the N_2-fixing isolates are facultatively anaerobic, oxidase-positive, rigid, gram-negative, motile rods having a single polar flagellum, we concluded that they belong to the family *Vibrionaceae*. They are placed in the genus *Vibrio* on the basis of their susceptibility to the vibriostatic agent 0/129 (150 μg/ml), their DNA base composition (45 to 47 mol% G+C), their anaerogenic fermentation of glucose, their production of a sheathed polar flagellum, and their requirement for NaCl. Inclusion of N_2-fixing strains in the genus *Vibrio* requires a redefinition of the genus since the current definition of

TABLE 5. Biochemical characteristics of *V. diazotrophicus* for which strain differences were observed

Characteristic	No. of strains positive[a]	Reaction of type strain[b]	Strain giving less frequent result
Growth at 4°C	12	+	13
Growth at 42°C	9	+	3, 9, 10, 11
Acid from mannose	2	−	9, 10
Acid from rhamnose	6	+	1, 2, 4, 5, 6, 7
Sulfatase	3	−	5, 7, 11
Urease	1	−	9
Growth on L-serine	3	−	9, 10, 11
Growth on threonine	6	−	4, 5, 6, 7, 8, 11, 12

[a] In each case 13 strains were tested.
[b] Strain 1 (= ATCC 33466)

TABLE 7. Characteristics useful in distinguishing strains of *V. diazotrophicus* from phenotypically similar bacteria[a]

Character	Reaction of:[b]						
	V. diazotrophicus	*V. anguillarum*	*V. nereis*	*V. metschnikovii*	*V. fluvialis*	*Vibrio proteolyticus*	*Vibrio splendidus* I
Luminescence	−	−	−	−	−	−	+
Gas from glucose	−	−	−	−	v	−	−
Oxidase	+	+	+	−	+	+	+
Nitrate reduction	+	+	+	−	+	+	+
Indole	+	v	+	v	−	+	+[c]
Methyl red	+	−	+	+	+	+	−[c]
Nitrogenase	+	−	−	−	−	−	−
Voges-Proskauer	−	+	−	+	−	+	−
Acid from:							
L-Arabinose	+	v	−	−	+	−	−
Cellobiose	+	+	−	−	v	+	+
Salicin	+	−	−	−	v	−	−[c]
D-Xylose	+	−	−	−	−	−	−
Casein hydrolysis	−	+	−	+	+	+	v[c]
Chitinase	−	+	+	+	+	+	+
Deoxyribonuclease	−	+	+	+	+	+	+
Gelatinase	−	+	−	+	+	+	+
Lecithinase	−	v	−	+	+	+	+[c]
Tween 80 hydrolysis	−	+	−	+	+	+	+

[a] Data from this study and references 5, 10, 11, 18 and 19, except as noted below.
[b] −, Negative; +, positive; v, variable between strains.
[c] Data from West (Ph.D. thesis).

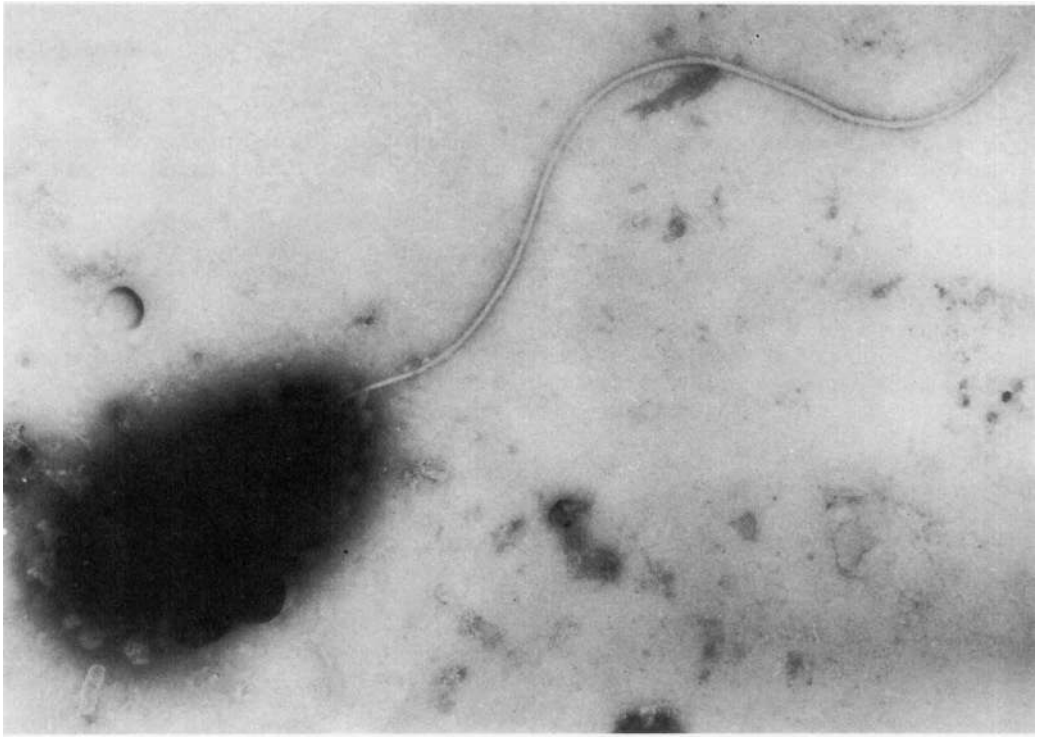

FIG. 1. *V. diazotrophicus* strain 8 grown for 4 h in a broth culture, showing a sheathed, long-wavelength polar flagellum. ×21,000.

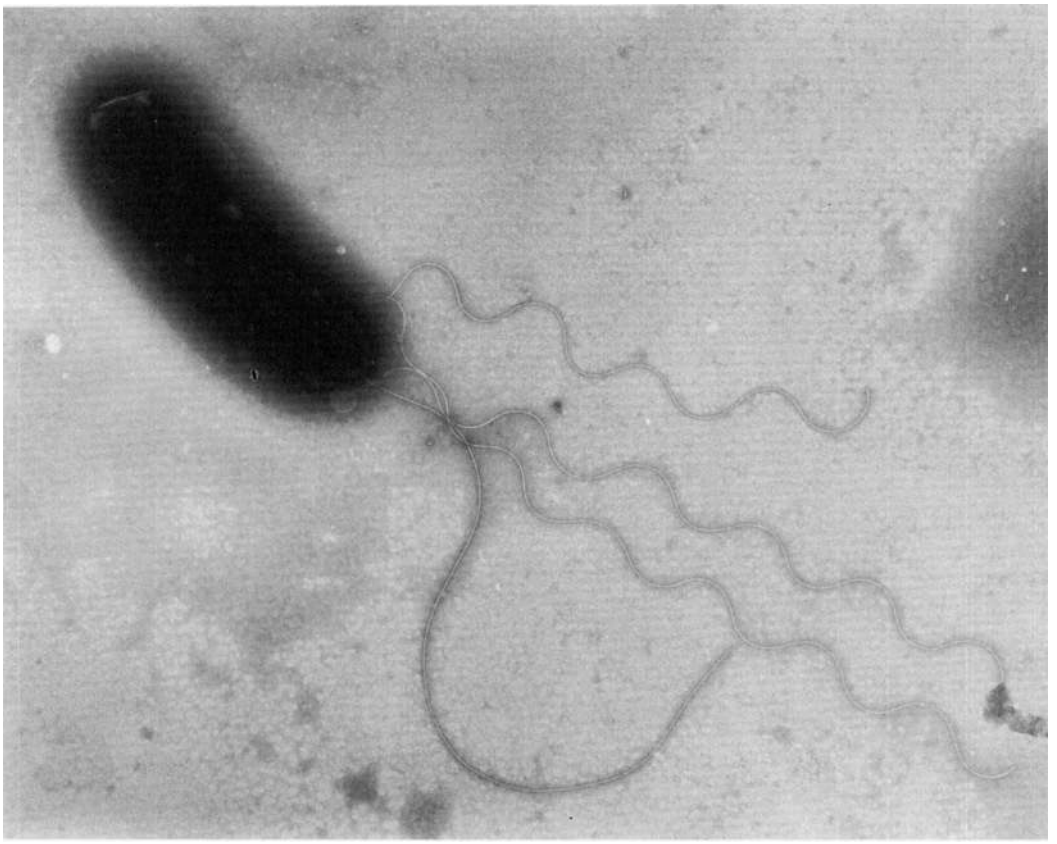

FIG. 2. *V. diazotrophicus* strain 5 grown for 8 h on marine agar, showing a polar flagellum and three short-wavelength lateral flagella. ×14,000.

Vibrio states that no species fixes molecular nitrogen (1).

It is not surprising that vibrios that are capable of fixing N_2 exist. Indeed, the ability to fix N_2 is widespread among bacterial genera, but it is often found in only one or two species or strains within a genus (2, 17). The ability of vibrios to fix N_2 may prove to be important in certain marine habitats limited by available nitrogen since organisms capable of nitrogen fixation have the potential for significantly affecting the nitrogen budget of their surroundings. The role of the N_2-fixing vibrios in the nitrogen cycle of estuarine and marine environments remains to be elucidated (9).

***Vibrio diazotrophicus* sp. nov.** (di.a.zo.tro'.phi. cus. Gr. prefix *di* two, double; NL.n. *azotum* nitrogen; Gr. n. *trophos* one that feeds; L. suffix *icus* relating to; ML. adj. *diazotrophicus* one that feeds on dinitrogen). Gram-negative, short rods. 0.5 by 1.5 to 2.0 μm; axis straight or curved. Cells occur singly or are sometimes united into S-shapes or spirals. No resting stages have been observed. Cells are motile by means of a single polar flagellum, which may be sheathed when the cells are grown in liquid media. On solid media, lateral, unsheathed, short-wavelength flagella may be produced. Colonies on marine agar (Difco) are flat, circular, and off-white. Strains fail to grow in the absence of sodium chloride. Facultatively anaerobic. Acid, but not gas, produced from glucose. Reduces nitrates to nitrites. Oxidase, catalase, and nitrogenase are produced. Gelatinase, deoxyribonuclease, and chitinase are not produced. Voges-Proskauer negative. The G+C content of the DNA ranges from 45.9 to 47.2 mol%.

Habitat: distributed throughout marine and estuarine environments, occurring in seawater, sediments, and the gastrointestinal tracts of marine animals.

Type strain: ATCC 33466. The description of the type strain is the same as that given above for the species. The base composition of its DNA is 45.9 mol% G+C, as determined by the buoyant density method.

ACKNOWLEDGMENTS

We thank L. A. McNicol for invaluable assistance and advice with the DNA procedures and C. R. McClung for

encouragement during the study and for a critical reading of the manuscript. We are also grateful to A. A. Porter for assistance with the electron microscopy, to L. R. Hill for allowing us to use the facilities of the National Collection of Type Cultures to determine the DNA base compositions, and to R. J. Owen for advice and assistance in these determinations.

Support was provided by grant DEB-77-14646 from the National Science Foundation.

REPRINT REQUESTS

Address reprint requests to: Dr. R. R. Colwell, Department of Microbiology, University of Maryland, College Park, MD 20742.

LITERATURE CITED

1. **Baumann, P., L. Baumann, S. S. Bang, and M. J. Woolkalis.** 1980. Reevaluation of the taxonomy of *Vibrio, Beneckea* and *Photobacterium*: abolition of the genus *Beneckea*. Curr. Microbiol. **4**:127–132.
2. **Burns, R. C., and R. W. F. Hardy.** 1975. Nitrogen fixation in bacteria and higher plants. Springer-Verlag, New York.
3. **Cowan, S. T.** 1974. Cowan and Steel's manual for the identification of medical bacteria, 2nd ed. Cambridge University Press, New York.
4. **Denhardt, D. T.** 1966. A membrane-filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Commun. **23**:641–646.
5. **Furniss, A. L., J. V. Lee, and T. J. Donovan.** 1978. The vibrios. Public Health Laboratory Service Monograph Series, no. 11. Her Majesty's Stationery Office, London.
6. **Guerinot, M. L., and D. G. Patriquin.** 1981. N_2-fixing vibrios isolated from the gastrointestinal tract of sea urchins. Can. J. Microbiol. **27**:311–317.
7. **Guerinot, M. L., and D. G. Patriquin.** 1981. The association of N_2-fixing bacteria with sea urchins. Mar. Biol. **62**:197–207.
8. **Hardy, R. W. F., R. D. Holsten, E. K. Jackson, and R. C. Burns.** 1968. The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. Plant Physiol. **43**:1185–1207.
9. **Knowles, R.** 1977. The significance of asymbiotic dinitrogen fixation by bacteria, p. 34–83. *In* R. W. F. Hardy and A. H. Gibson (ed.), A treatise on dinitrogen fixation, sect. 4. Agronomy and ecology. John Wiley & Sons, Inc., New York.
10. **Lee, J. V., T. J. Donovan, and A. L. Furniss.** 1978. Characterization, taxonomy, and emended description of *Vibrio metschnikovii*. Int. J. Syst. Bacteriol. **28**:99–111.
11. **Lee, J. V., P. Shread, A. L. Furniss, and T. N. Bryant.** 1981. Taxonomy and description of *Vibrio fluvialis* sp. nov. (synonym group F vibrios, group EF6). J. Appl. Bacteriol. **50**:73–94.
12. **Marmur, J.** 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. **3**:208–218.
13. **Marmur, J., and P. Doty.** 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. **5**:109–118.
14. **McClung, C. R., and D. G. Patriquin.** 1980. Isolation of a nitrogen-fixing *Campylobacter* species from the roots of *Spartina alterniflora* Loisel. Can. J. Microbiol. **26**:881–886.
15. **Nishibuchi, M., K. Muroga, R. J. Seidler, and J. L. Fryer.** 1979. Pathogenic *Vibrio* isolated from cultured eels. IV. Deoxyribonucleic acid studies. Bull. Jpn. Soc. Sci. Fish. **45**:1469–1473.
16. **Owen, R. J., and L. R. Hill.** 1979. Estimation of base composition, base pairing and genome sizes of bacterial deoxyribonucleic acids, p. 277–296. *In* F. A. Skinner and D. W. Lovelock (ed.), Identification methods for microbiologists, 2nd ed. Academic Press, Inc., London.
17. **Postgate, J. R.** 1974. Evolution within nitrogen-fixing systems, p. 263–293. *In* M. J. Carlile and J. J. Shehel (ed.), Evolution in the microbes. Cambridge University Press, New York.
18. **Reichelt, J. L., P. Baumann, and L. Baumann.** 1976. Study of genetic relationships among marine species of the genera *Beneckea* and *Photobacterium* by means of in vitro DNA/DNA hybridization. Arch. Microbiol. **110**:101–210.
19. **Schubert, R. H. W.** 1969. *Aeromonas hydrophila* subsp. *proteolytica* comb. nov. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. **211**:409–412.
20. **Strength, W. J., B. Isani, D. M. Linn, F. D. Williams, G. E. Vandermolen, B. E. Laughon, and N. R. Kreig.** 1976. Isolation and characterization of *Aquaspirillum fasciculus* sp. nov., a rod-shaped nitrogen-fixing bacterium having unusual flagella. Int. J. Syst. Bacteriol. **26**:253–268.
21. **Tarrand, J. J., N. R. Krieg, and J. Dobereiner.** 1978. A taxonomic study of the *Spirillum lipoferum* group, with descriptions of a new genus, *Azospirillum* gen. nov., and two species, *Azospirillum lipoferum* (Beijerinck) comb. nov. and *Azospirillum brasiliense* sp. nov. Can. J. Microbiol. **24**:967–980.