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Vibrio furnissii (Formerly Aerogenic Biogroup of *Vibrio fluvialis*), a New Species Isolated from Human Feces and the Environment

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Strains formerly classified as the aerogenic (gas-producing) biogroup of *Vibrio fluvialis* were shown by DNA relatedness to be a separate species. The species was named *Vibrio furnissii* sp. nov. (type strain ATCC 35016 =CDC B3215). Three strains of *V. furnissii* were 79% or more related to the type strain of *V. furnissii* and about 50% related to the type strain of *V. fluvialis*. *V. fluvialis* strains were 40 to 64% related to the type strain of *V. furnissii*. Divergence in related sequences was only 0.0 to 1.5% among strains of *V. furnissii* and among strains of *V. fluvialis* but was 5.0 to 8.0% in interspecific reactions between *V. fluvialis* and *V. furnissii*. *V. furnissii* was aerogenic (produced gas from the fermentation of carbohydrates), whereas *V. fluvialis* was anaerogenic (did not produce gas from the fermentation of carbohydrates). Another test of some help in differentiating the two species was fermentation of L-rhamnose (57% positive for *V. furnissii* and negative for *V. fluvialis*). In addition to the reactions above, *V. furnissii* is distinguished from other salt-requiring vibrios on the basis of its positive reactions in tests for Møller L-arginine, L-arabinose, maltose, and D-mannitol and its negative reactions for Møller L-lysine and L-ornithine, lactose, and Voges-Proskauer. *V. furnissii* has been isolated from patients with acute gastroenteritis in at least two outbreaks of food poisoning; its role as a cause of diarrhea needs further study.

Strains of the organism now classified as *Vibrio fluvialis* were first described by Furniss et al. in 1977 (8). Their organisms, designated group F, were isolated in 1975 from a patient with diarrhea in Bahrain, from patients with diarrhea in Bangladesh, and from shellfish and estuarine waters in England. Group F required salt and had a number of properties compatible with or midway between those of vibrios and aeromonads. In a numerical taxonomic study, Lee et al. showed that six group F strains were a distinct phenon that probably represented a new species (13) and that the group contained two subgroups on the basis of gas production during fermentation of glucose (14).

Huq et al. (12; I. Huq, B. R. Davis, R. E. Weaver, D. G. Hollis, W. T. Martin, and D. J. Brenner, 13th Joint Conf. on Cholera, Atlanta, Ga. p.82, 1977) studied a large number of strains associated with an outbreak of diarrhea in Bangladesh as well as strains isolated from patients with diarrhea in Indonesia, strains from sewage in Brazil, and U.S. strains that had been called group EF-6 in the Special Bacterial Reference Activity at the Centers for Disease Control, Atlanta, Ga. By both phenotypic tests and DNA relatedness, they found that the organism was closer to the genus *Vibrio* than to the genus *Aeromonas*. All of their strains produced no gas from the fermentation of glucose (were anaerogenic) and formed a single DNA relatedness group (A. G. Steigerwalt and D. J. Brenner, unpublished data). Thus, the EF-6 group appeared to be identical to group F.

Group F strains isolated from several parts of the world were compared phenotypically and genetically by Seidler et al. (16). They confirmed and extended the observation that group F was

more closely related to *Vibrio* than to *Aeromonas*. They further showed that the aerogenic group F strains were in a different DNA relatedness group from the anaerogenic strains, and they recommended that the two biogroups be considered as two separate species within the genus *Vibrio*.

Lee et al. (15) proposed the name *V. fluvialis*, which included both aerogenic and anaerogenic strains of group F and the synonymous group EF-6. An anaerogenic strain was chosen as the type strain of *V. fluvialis*. These authors noted that both aerogenic and anaerogenic strains of *V. fluvialis* were found in the environment but that only anaerogenic strains had been isolated from humans with diarrhea (15).

We now confirm the evidence that the aerogenic strains of group F are a species separate from *V. fluvialis*. In this paper, we name the new species *Vibrio furnissii*. Information on the isolation of *V. furnissii* from humans with acute gastroenteritis is given, and its possible causative role is discussed.

MATERIALS AND METHODS

Bacterial strains. The 17 *V. fluvialis* strains and 4 *V. furnissii* strains used in DNA relatedness studies and their sources are listed in Table 1. Also listed are the type and reference strains of other *Vibrionaceae* used in the DNA relatedness studies. Biochemical tests were done on a total of 24 *V. fluvialis* and 7 *V. furnissii* strains. The three *V. furnissii* strains not listed in Table 1 are 1273-78, isolated from a human diarrheal stool specimen in Indonesia and sent by S. W. Joseph; 9550-78(VL2386), isolated from river water in England and sent by J. V. Lee; and 292-80, isolated from the stool of a healthy woman in Lima, Peru, and sent by J. M. Guevara. The growth and maintenance of strains have been described (10).

Media and biochemical and other tests. Media used in carbohydrate fermentation and several other standard tests contained 0.5% NaCl. Media that did not contain NaCl were supplemented with 1% NaCl. These were methyl red, Voges-Proskauer, Møller L-lysine, L-arginine, and L-ornithine, gelatin, esculin broth, nitrate, and nutrient agar. Peptone water and heart infusion broth used to test for indole production contained 0.5% NaCl and were supplemented with 0.5% NaCl to bring the final concentration to 1%. All tests in the standard biochemical test set used for *Vibrionaceae* in the Enteric Reference Laboratory (formerly the Enteric Section) of the Enteric Bacteriology Section at the Centers for Disease Control were done on all strains. The media and test procedures used have been described (7, 9–11). Antibiograms were done by the disk method of Bauer et al. as previously described (10).

DNA methods. Guanine-plus-cytosine determinations, the preparation and labeling of DNA, and DNA relatedness experiments assayed on hydroxyapatite with $^{32}PO_4$ were done as described previously (1, 10). Relatedness was expressed as the relative binding ratio (RBR) and as divergence (D) (see Tables 2 and 3). RBR is the amount of double-stranded DNA formed between labeled and unlabeled DNAs from different strains divided by the amount of double-stranded DNA formed between labeled and unlabeled DNA from the same strain. RBR was expressed as a percentage. D is the amount of divergence, or unpaired bases, in DNA sequences held in common between two bacteria. D was calculated on the assumption that each decrease of 1°C in the thermal stability of a heterologous DNA duplex, compared with that of the homologous DNA duplex, is caused by approximately 1% of unpaired bases. D was calculated to the nearest 0.5%. The amount of reassociation in homologous (labeled and unlabeled DNA from the same strain) *V. furnissii* B3215 reactions was 55 to 65%. For *V. fluvialis* 9555-78, the homologous reaction values were 50 to 75%. These observed homologous reassociation values were arbitrarily deemed as 100%. Control reactions in which labeled DNA was incubated in the absence of unlabeled DNA were 0.5 to 4.0%. These label-only control values were subtracted from all reactions before the RBR was calculated.

RESULTS

DNA relatedness. DNAs from the type strains of *V. furnissii* (B3215) and *V. fluvialis* (9555-78) were labeled with $^{32}PO_4$ and reacted with a series of unlabeled DNAs from representative *V. furnissii* and *V. fluvialis* strains (Table 2). *V. furnissii* strains were 79 to 92% related to the type strain of *V. furnissii* at the optimal reassociation temperature of 60°C. The related sequences contained only 1.0 to 1.5% D. Relatedness remained high (77 to 82%) in reactions at 75°C (a stringent incubation temperature at which only nearly identical DNA sequences can reassociate). *V. fluvialis* strains were 40 to 64% related to *V. furnissii*, with D of 6.0 to 8.0% and relatedness of 29 to 51% in reactions at 75°C. *V. fluvialis* strains were 61 to 83% related to the *V. fluvialis* type strain. D values were 0.0 to 1.5%, and relatedness was 63 to 93% in reactions of 75°C. Two *V. furnissii* strains were 51 and 52% related to *V. fluvialis* with 5.0 and 7.0% D and with 38 and 43% relatedness in reactions at 75°C. *V. furnissii* was ≤21% related to representative strains of most species in *Vibrionaceae* (Table 3) and was only 3 to 10% related to DNAs from 32 representative species of *Enterobacteriaceae* (date not shown; see reference 1 for list of strains and methods for growing cells).

The guanine-plus-cytosine content of *V. furnissii* B3215 was 50.4 mol% (mean of 49.9, 50.4, 50.9).

Biochemical reactions. Biochemical reactions for 7 *V. furnissii* and 24 *V. fluvialis* strains and those of the type strains for each species are shown in Table 4. Both species required salt for growth. They were usually indole negative (14% positive for *V. furnissii* and 4% positive for *V. fluvialis* in heart infusion broth containing 1% NaCl [Table 2]) and 0% positive for indole production in peptone water containing 1% NaCl (data not shown). Strains from both spe-

TABLE 1. Strains of *Vibrionaceae* used in DNA relatedness studies

Strain[a]	Source	Sender[b]
Aeromonas hydrophila 9079-79 (ATCC 9766)[c]	Milk	ATCC
Aeromonas punctata 9179-79 (NCMB 74)		NCMB
Aeromonas salmonicida subsp. *achromogenes* 9180-79 (NCMB 1110)		NCMB
Aeromonas sobria 9538-76 (CIP 208)[c]		M. Popoff, IP
Photobacterium angustum 9093-79 (ATCC 25915)[c]	Seawater	ATCC
Photobacterium leiognathi 9094-79 (ATCC 25520)	Teleostean fish, light organ	ATCC
Plesiomonas shigelloides 9091-79 (ATCC 14029)[c]		ATCC
Vibrio alginolyticus 9065-79 (ATCC 17749)[c]	Mackeral, spoiled	ATCC
Vibrio anguillarum 9063-79 (ATCC 19264)[c]	Cod, ulcerous lesion	ATCC
Vibrio campbellii 9099-79 (ATCC 25920)[c]	Seawater	ATCC
Vibrio cholerae 9060-79 (ATCC 14035)[c]		ATCC
Vibrio damsela 2588-80 (ATCC 33539)[c]	Damselfish	M. Love, Occidental College
Vibrio fischeri 9064-79 (ATCC 7744)[c]		ATCC
Vibrio fluvialis D2326	Human, stool, Guam	R. E. Weaver, CDC
Vibrio fluvialis 519-77	Human, diarrheal stool	M. I. Huq, ICDDR
Vibrio fluvialis 715-77	Human, diarrheal stool	M. I. Huq, ICDDR
Vibrio fluvialis 716-77	Human, diarrheal stool	M. I. Huq, ICDDR
Vibrio fluvialis 807-77	Human, diarrheal stool	M. Q. Huq, ICDDR
Vibrio fluvialis 1197-78	Sewage, Brazil	G. Pessoa, Saõ Paulo, Brazil
Vibrio fluvialis 1198-78	Sewage, Brazil	G. Pessoa, Saõ Paulo, Brazil
Vibrio fluvialis 1199-78	Sewage, Brazil	G. Pessoa, Saõ Paulo, Brazil
Vibrio fluvialis 1277-78	Human, diarrheal stool, Indonesia	S. W. Joseph, NMRI
Vibrio fluvialis 1278-78	Human, diarrheal stool, Indonesia	S. W. Joseph, NMRI
Vibrio fluvialis 1279-78	Human, diarrheal stool, Indonesia	S. W. Joseph, NMRI
Vibrio fluvialis 1280-78	Human, diarrheal stool, Indonesia	S. W. Joseph, NMRI
Vibrio fluvialis 1283-78	Human, diarrheal stool, Indonesia	S. W. Joseph, NMRI
Vibrio fluvialis 1284-78	Human, diarrheal stool, Indonesia	S. W. Joseph, NMRI
Vibrio fluvialis 1285-78	Human, diarrheal stool, Indonesia	S. W. Joseph, NMRI
Vibrio fluvialis 9554-78 (VL 2926)	Human, feces, England	J. V. Lee, PHLS
Vibrio fluvialis 9555-78 (VL 5125, NCTC 11327)[c]	Human feces, England	J. V. Lee, PHLS
Vibrio furnissii B3215 (ATCC 35016)[c]	Human, feces	R. E. Weaver, CDC
Vibrio furnissii 9551-78 (VL 2391)	River water, England	J. V. Lee, PHLS
Vibrio furnissii 9552-78 (VL 5909)	Pig, feces, England	J. V. Lee, PHLS
Vibrio furnissii 9553-78 (VL 5975)	Rabbit, feces, England	J. V. Lee, PHLS
Vibrio gazogenes 2820-79 (ATCC 29988)[c]	Marine mud	ATCC
Vibrio harveyi 9098-79 (ATCC 14126)[c]	Dead luminescing amphipod	ATCC
Vibrio hollisae 75-80 (ATCC 33564)[c]	Human, stool	Maryland, SHD
Vibrio metschnikovii 9528-79 (ATCC 7708)[c]		ATCC
Vibrio mimicus 1721-77 (ATCC 33653)[c]	Human, ear infection	North Carolina, SHD
Vibrio natriegens 9101-79 (ATCC 14048)[c]	Mud, salt marsh	ATCC
Vibrio nereis 9103-79 (ATCC 25917)[c]	Seawater	ATCC
Vibrio nigrapulchritudo 9104-79 (ATCC 27043)[c]	Seawater	ATCC
Vibrio parahaemolyticus 9062-79 (ATCC 17802)[c]	Shirasu food poisoning	ATCC
Vibrio pelagius 9105-79 (ATCC 25916)[c]	Seawater	ATCC
Vibrio proteolyticus 9082-79 (ATCC 15338)[c]		ATCC
Vibrio splendidus 9106-79 (ATCC 25914)	Seawater	ATCC
Vibrio vulnificus 9107-79 (ATCC 27562)[c]	Human, blood	ATCC

[a] The first strain number is the Centers for Disease Control strain number.

[b] Abbreviations: ATCC, American Type Culture Collection, Rockville, Md.; NCMB, National Collection of Marine Bacteria, Aberdeen, Scotland; CIP, Collection of the Institut Pasteur, Paris, France; IP, Institut Pasteur, Paris, France; CDC, Centers for Disease Control, Atlanta, Ga.; ICDDR, International Centre for Diarrhoeal Disease Research, Dacca, Bangladesh; NMRI, Naval Medical Research Institute, Bethesda, Md.; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England; PHLS, Public Health Laboratory Service, Maidstone, England; VL, Vibrio Laboratories, PHLS, Maidstone, England; SHD, State Health Department.

[c] Type strain.

TABLE 2. DNA relatedness between *V. furnissii* and *V. fluvialis*

Source of unlabeled DNA	Data (%) for labeled DNA from[a]:					
	V. furnissii B3215			*V. fluvialis* 9555-78		
	RBR, 60°C	D	RBR, 75°C	RBR, 60°C	D	RBR, 75°C
V. furnissii strains						
B3215	100	0.0	100			
9552-78	92	1.0	82	51	5.0	38
9551-78	91	1.5	89	52	7.0	43
9553-78	79	1.5	77			
V. fluvialis strains						
9555-78	42	6.0	31	100	0.0	100
807-77	58			83	0.0	85
715-77	64	8.0	39	81	1.0	77
1198-78	60			76	1.0	93
519-77	54			75	1.0	83
D2326	62	6.5	51	75	1.0	82
1199-78				73	1.0	74
1280-78	51			69	0.5	73
1197-78	53			67	1.5	71
1278-78	48			66	1.5	71
1284-78	43			62	0.5	64
1285-78	44	6.5	35	61	1.0	65
1279-78	43					66
1283-78	48					63
1277-78	40					
9554-78	42	7.0	29			
716-77	58					

[a] See text for definitions of RBR and D. Blank space indicates not done.

cies were gram-negative, oxidase-positive, motile, straight to slightly curved rods. They were positive in tests for methyl red and Simmons citrate. Their reactions in Møller decarboxylases were L-arginine positive, L-lysine negative, and L-ornithine negative. *V. furnissii* produced gas from D-glucose; *V. fluvialis* did not. Other tests of help in distinguishing these two species were acid from D-arabitol, glycerol, and L-rhamnose and growth in KCN (Table 5). Positive reactions for growth in KCN, Møller L-arginine dihydrolase, gas production from D-glucose, acid from D-arabitol, L-arabinose, maltose, and D-mannitol, and negative reactions for indole, Voges-Proskauer, and Møller lysine and ornithine decarboxylases were helpful in differentiating *V. furnissii* from other *Vibrio* species (Table 5).

Antibiotic susceptibility tests. The results of disk susceptibility tests for *V. furnissii* and *V. fluvialis* are given in Table 6. Both species had similar susceptibility patterns for all antibiotics.

DISCUSSION

Lee et al. (15) described two biogroups within *V. fluvialis*. Their group I, represented by the type strain, was composed of strains that were all anaerogenic and most of which hydrolyzed esculin (72%), grew on citrulline (97%), glucuronate (94%), and usually cellobiose (63%) but usually not on putrescine (31%) and never on delta-amino-valerate. Their group II contained strains that were aerogenic (89%), did not hydrolyze esculin (0%), did not grow on citrulline (4%), glucuronate (7%), or cellobiose (4%), but did grow on putrescine (100%) and usually grew on delta-amino-valerate (63%).

Seidler et al. (16) showed that DNAs from five aerogenic "*V. fluvialis*" strains were 86% or more interrelated in reassociation reactions done at a stringent (75°C) incubation temperature. At the same stringent condition, 14 anaerogenic *V. fluvialis* strains were 85 to 100% interrelated. Relatedness between members of these two groups was 29 to 57%.

TABLE 3. DNA relatedness of *V. furnissii* to *Vibrionaceae*

Source of unlabeled DNA	Data (%) for ^{32}P-labeled DNA from *V. furnissii* B3215[a]	
	RBR, 60°C	D
Vibrio furnissii B3215	100	0.0
Vibrio cholerae 9060-79	21	16.0
Vibrio proteolyticus 9082-79	21	
Vibrio mimicus 1721-77	20	
Vibrio natriegens 9101-79	17	
Vibrio nereis 9103-79	17	
Vibrio metschnikovii 9528-79	16	
Vibrio parahaemolyticus 9062-79	16	15.5
Vibrio vulnificus 9107-79	16	
Vibrio anguillarum 9063-79	15	
Vibrio alginolyticus 9065-79	15	
Vibrio campbellii 9099-79	15	
Vibrio pelagius 9105-79	15	
Vibrio harveyi 9098-79	13	
Vibrio nigrapulchritudo 9104-79	12	
Vibrio splendidus 9106-79	12	
Vibrio gazogenes 2820-79	11	
Vibrio hollisae 75-80	10	
Photobacterium leiognathi 9094-79	9	
Vibrio damsela 2588-80	8	
Photobacterium angustum 9093-79	8	
Vibrio fischeri 9064-79	8	
Aeromonas hydrophila 9079-79	8	
Plesiomonas shigelloides 9091-79	8	
Aeromonas salmonicida 9180-79	5	
Aeromonas punctata 9179-79	5	
Aeromonas sobria 9538-76	5	
Alteromonas haloplanktis 9111-79	4	
Alteromonas espejiana 9112-79	4	
Alteromonas communis 9108-79	3	
Alteromonas macleodii 9109-79	3	
Alteromonas vaga 9110-79	3	
Alteromonas undina 9113-79	3	

[a] See text for definitions of RBR and D. Blank space indicates not done.

TABLE 4. Biochemical reactions of 7 *V. furnissii* strains and the type strain, and of 24 *V. fluvialis* strains and the type strain

Test[a]	*V. furnissii*				*V. fluvialis*			
	Cumulative % positive at day:			Reaction for type strain B3215 (ATCC 35016)[b]	Cumulative % positive at day:			Reaction for type strain 9555-78 (NCTC 11327)
	1	2	7		1	2	7	
Indole (1% NaCl)[c]	[d]	14		−		4		−
Methyl red (1% NaCl)		100		+		100		+
Voges-Proskauer (1% NaCl)		0		−		0		−
Citrate (Simmons)	100	100	100	+	88	100	100	+
H_2S on TSI	0	0	0	−	0	0	0	−
H_2S on PIA	0	0	0	−	0	0	0	−
Urea	0	0	0	−	0	0	0	−
Phenylalanine	0			−	0			−
L-Lysine (Møller) (1% NaCl)	0	0	0	−	0	0	0	−
L-Arginine (Møller) (1% NaCl)	100	100	100	+	92	100	100	+
L-Ornithine (Møller) (1% NaCl)	0	0	0	−	0	0	0	−
Motility	100	100	100	+	71	75	100	+[2]
Gelatin (22°C) (1% NaCl)	29	86	100	+	17	79	100	+[2]
Growth in KCN	29	100	100	+	46	71	71	−
Malonate	0	14	14	−	0	0	4	−
D-Glucose								
Acid	100	100	100	+	100	100	100	+
Gas	100	100	100	+	0	0	0	−
Acid from:								
Adonitol	0	0	0	−	0	0	0	−
L-Arabinose	100	100	100	+	88	100	100	+
D-Arabitol	100	100	100	+	67	67	67	+
Cellobiose	14	14	14	−	33	38	38	+
Dulcitol	0	0	0	−	0	0	0	−
Erythritol	0	0	0	−	0	0	0	−
D-Galactose	100	100	100	+	96	100	100	+
Glycerol	14	57	100	+[3–7w]	0	8	33	−
i-Inositol	0	0	0	−	0	0	0	−
Lactose	0	0	14	−	0	0	4	−
Maltose	100	100	100	+	100	100	100	+
D-Mannitol	100	100	100	+	100	100	100	+
D-Mannose	100	100	100	+	100	100	100	+
Melibiose	0	0	0	−	4	4	4	−
α-CH_3-glucoside	0	0	0	−	0	0	0	−
Raffinose	0	0	0	−	0	0	0	−
L-Rhamnose	0	57	57	+[2]	0	0	4	−
Salicin	0	0	0	−	0	0	0	−
D-Sorbitol	0	0	0	−	0	0	0	−
Sucrose	100	100	100	+	100	100	100	+
Trehalose	100	100	100	+	100	100	100	+
D-Xylose	0	0	0	−	0	0	0	−
Esculin (1% NaCl)	0	0	0	−	0	12	25	−

TABLE 4—*Continued*

Test[a]	*V. furnissii*				*V. fluvialis*			
	Cumulative % positive at day:			Reaction for type strain B3215 (ATCC 35016)[b]	Cumulative % positive at day:			Reaction for type strain 9555-78 (NCTC 11327)
	1	2	7		1	2	7	
Mucate	0	0	0	−	0	0	0	−
Tartrate (Jordan)	0	14	14	$+^{2w}$	12	42	42	−
Acetate	14	71	71	−	29	75	88	+
Lipase (corn oil)	71	86	86	$+^{2w}$	92	92	92	+
DNase								
25°C	100	100	100	+	96	100	100	+
36°C	67	83	100[e]	$+^{3-7w}$	96	96	96	+
$NO_3^- \rightarrow NO_2^-$ (1% NaCl)	100			+	100			+
Oxidase (Kovacs) (1% NaCl)	100			+	100			+
ONPG	29	43	57	−	25	42	54	$+^{2}$
Citrate (Christensen)	57	71	86	$+^{2}$	71	88	100	+
Tyrosine clearing	14	43	43	−	42	79	88	$+^{2}$
String test	100			+	100			+
Growth in nutrient broth plus NaCl:								
0%	0	0	0	−	0	0	0	−
1%	100	100	100	+	100	100	100	+
6%	100	100	100	+	96	96	96	+
8%	57	71	71	+	67	79	79	−
10%	0	0	14	$+^{3-7w}$	0	25	38	−
12%	0	0	0	−	0	0	0	−
Vibriostatic compound 0/129, sensitivity	0			−	25			−

[a] Abbreviations: TSI, Triple sugar iron agar; PIA, peptone iron agar; ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

[b] Symbols: +, Positive at 24 h or at time of test; −, negative at 7 days or at time of test; $+^{2}$, positive at 48 h; $+^{3-7}$, positive between 3 and 7 days; ^{w}, weakly positive.

[c] (1% NaCl) indicates that NaCl was added to each of these media to reach a final concentration of 1%.

[d] Blank space indicates not done.

[e] Six strains tested.

In this study (Table 2), we used 4 aerogenic strains (strain 9555-78 [VL 2391] was used in both studies) and 17 anaerogenic strains to confirm that the aerogenic strains are a separate species from *V. fluvialis*. Relatedness among the aerogenic strains was 77% or more at both optimal and stringent incubation temperatures. Relatedness among the anaerogenic strains was 61 to 85% in reactions at the optimal reassociation temperature and 63 to 93% at the stringent reassociation termperature. D in related sequences was 0.0 to 1.5%. Relatedness of 70% or more with less than 6% D in related sequences in optimal reassociation reactions and 55% or more relatedness at a stringent reassociation temperature is usually considered species-level relatedness. Although relatedness fell to below 70% in some reactions at 60°C, the fact that relatedness did not decrease in reactions at 75°C and that D was very low in the related sequences makes us confident that all of the anaerogenic strains are a single species. The lower relatedness of some strains to labeled DNA from strain 9555-78 would be explained if 9555-78 has a genome size some 25 to 33% larger than that of these strains. There is precedence for this type of difference in genome size of strains of the same species, but this possibility was not tested experimentally.

TABLE 5. Reactions useful in distinguishing *V. furnissii* from other halophilic *Vibrio* species isolated from human clinical specimens

Test	% Positive in 48 h[a]							
	V. furnissii	*V. fluvialis*	*V. alginolyticus*	*V. damsela*	*V. hollisae*	*V. metschnikovii*	*V. parahaemolyticus*	*V. vulnificus*
Growth in KCN	100	71	19	6	0	0	12	0
Gas from D-glucose	100	0	0	0	100	0	0	2
Acid from:								
D-Arabitol	100	67	0	0	0	0	0	0
Glycerol	57 (100)	8 (33)	72	0	0	100	46	0
L-Rhamnose	57	0	0	0	0	0	7	0
L-Arabinose	100	100	3	0	94	0	80	0
Lactose	0	0	0	0	0	59	0	95
Maltose	100	100	100	100	0	100	99	100
D-Mannitol	100	100	100	0	0	100	100	41
Indole (1% NaCl)[b]	14	4	50	0	100	24	88	92
Voges-Proskauer (1% NaCl)	0	0	93	100	0	100	0	0
L-Lysine (Møller) (1% NaCl)	0	0	100	56	0	24	100	98
L-Arginine (Møller) (1% NaCl)	100	100	0	100	0	53	0	0
L-Ornithine (Møller) (1% NaCl)	0	0	65	0	0	0	82	57
Oxidase (1% NaCl)	100	100	100	94	100	0	100	100

[a] Numbers in parentheses show percent positive at 3 to 7 days.
[b] (1% NaCl), See Table 4, footnote *c*.

Relatedness between the anaerogenic and the aerogenic strains was 40 to 64% with 5.0 to 8.0% D in related sequences. At the stringent incubation temperature, relatedness between these groups was 29 to 51%. This level of relatedness indicates two separate species.

Since the type strain of *V. fluvialis*, NCTC 11327 (CDC 9555-78), is anaerogenic, the anaerogenic strains remain as *V. fluvialis*. The new species name, *V. furnissii*, was therefore coined for the anaerogenic strains.

Three of Lee's group II strains did not produce gas (15). These were classified in group II on the bases of negative reactions for esculin hydrolysis, growth on cellobiose, glucuronate, and citrulline, and a positive reaction for growth on putrescine. The substrate utilization tests were not done in our study. Therefore, we could not identify such strains phenotypically. It remains to be seen whether these "anaerogenic group II" strains are *V. furnissii*, *V. fluvialis*, or a third species.

All strains of *V. furnissii* and 75% of *V. fluvialis* strains were esculin negative with our test (Table 4). Lee et al. (15) found 72% of *V. fluvialis* strains to be esculin positive. Esculin broth was used in both studies, but the formulations differed somewhat. We used the formulation of Vaughn and Levine, as cited in Edwards and Ewing (7), to which 1% NaCl was added. In this method, 3 g of esculin and 0.5 g of ferric citrate were added to a solution of 5 g of peptone, 1 g of dibasic potassium phosphate, 10 ml of Andrade indicator, and 1,000 ml of distilled water, and the solution was heated to dissolve the ferric citrate and autoclaved. Lee et al. used the method cited by Cowan (6), in which 1 g of esculin and 0.5 g of ferric citrate are dissolved in 1,000 ml of peptone water (10 g of peptone, 5 g of NaCl, and 1,000 ml of tap water, adjusted to pH 8.0 to 8.4, boiled 10 min, filtered, adjusted to pH 7.2 to 7.4, and autoclaved). These differences in the formulation of esculin broth may have been responsible for the different results obtained in the two studies. Another biochemical test difference between the two laboratories was growth in the absence of NaCl (0% in our study, positive or negative in the study of Lee).

Several differences were seen in biochemical results obtained in our study and of Seidler et al.

TABLE 6. Susceptibility of 7 *V. furnissii* strains and 19 *V. fluvialis* strains by agar diffusion

Antibiotic (disk concn)	Zone diam (mm)					
	V. furnissii			*V. fluvialis*		
	Range	Mean	SD	Range	Mean	SD
Ampicillin (10 μg)	6–12	10	2.5	6–22	10	4.0
Carbenicillin (100 μg)	15–22	19	2.1	13–29	20	3.5
Cephalothin (30 μg)	17–24	19	2.3	6–26	12	7.4
Chloramphenicol (30 μg)	25–33	28	2.7	7–29	24	7.6
Colistin (10 μg)	12–14	13	0.9	11–16	12	1.4
Gentamicin (10 μg)	18–21	20	1.3	16–25	19	2.2
Kanamycin (30 μg)	18–21	20	1.0	16–25	19	2.0
Nalidixic acid (30 μg)	19–32	24	4.2	19–34	24	3.4
Penicillin (10 U)	6	6	0.0	6	6	0.0
Polymyxin (50 U)	9–12	11	1.1	9–12	10	0.8
Streptomycin (10 μg)	16–18	17	0.8	6–19	15	3.1
Sulfadiazine (250 μg)	12–21	15	3.0	6–22	13	5.4
Tetracycline (30 μg)	16–30	22	4.4	6–27	20	4.0

(16), in which bacteria suspended in 0.85% NaCl were tested with the API 20E test system with incubation at 37°C for 48 h Analytab Products, Inc., Plainview, N.Y.). With the API 20E, strains (*V. fluvialis* and *V. furnissii*) were 100% positive for ONPG (*o*-nitrophenyl-β-D-galactopyranoside), usually positive for indole production and Voges-Proskauer, and negative for L-rhamnose. In our study, strains of both species were less than 60% positive for ONPG, *V. fluvialis* was 4% positive and *V. furnissii* 14% positive for indole, both species were 0% positive for Voges-Proskauer, and 57% of *V. furnissii* were positive for L-rhamnose (Table 4). The biochemical test differences obtained in these three studies emphasize the variability in results that can occur when different media and methods are used.

Description of *V. furnissii* sp. nov. Genetic and phenotypic tests indicate that the aerogenic strains formerly included in *V. fluvialis* represent a new species in the genus *Vibrio*. *V. furnissii* sp. nov. is the name proposed for this new species. It is treated as a modern Latin genitive noun *furnissii* (fur niss' i i) in honor of A. L. Furniss, Maidstone Public Health Laboratory, Maidstone, England, for his role in the classification of *V. fluvialis* and for his many contributions to the knowledge of the genus *Vibrio*.

V. furnissii is a gram-negative, straight to slightly curved rod that is motile by means of polar flagella. It is an NaCl-requiring, oxidase-positive, nitrate-positive organism that ferments D-glucose and other carbohydrates with the production of acid and gas, has 50 mol% guanine plus cytosine in its DNA, and is isolated from water, animal feces, and human feces. It is positive in reactions for methyl red, Simmons citrate, Møller arginine dihydrolase, KCN, DNase, growth in 1 and 6% NaCl, and fermentation of L-arabinose, D-arabitol, D-galactose, maltose, D-mannitol, D-mannose, sucrose, and trehalose. It is negative in reactions for Voges-Proskauer, H_2S, urea, phenylalanine, Møller lysine and ornithine decarboxylases, esculin, mucate, growth in 10% NaCl, and fermentation of adonitol, dulcitol, erythritol, *i*-inositol, lactose, melibiose, α-methylglucoside, raffinose, salicin, D-sorbitol, and D-xylose. Most strains are negative in tests for indole, malonate, Jordan tartrate, and fermentation of cellobiose and are positive in tests for acetate, lipase, and growth in 8% NaCl. Strains of *V. furnissii* give variable results in tests for ONPG, tyrosine clearing, and fermentation of L-rhamnose. *V. furnissii* gives a positive string test and grows in the presence of disks saturated with 0.1% of the vibriostatic compound 0/129 in acetone. A further description of *V. furnissii* is given in the tables as well as below. The type strain (holotype) of *V. furnissii* is ATCC 35016 (CDC B3215), which was isolated from feces of an adult woman with gastroenteritis in 1969.

Differentiation of *V. furnissii* from *V. fluvialis* and other *Vibrio* species. *V. furnissii* produces gas from the fermentation of D-glucose and other carbohydrates; *V. fluvialis* does not. Additional tests of some use in differentiating these species are L-rhamnose and glycerol (Table 5). With the proposal of *V. furnissii*, the description of *V. fluvialis* should be restricted to only those anaerogenic strains with the characteristics given in Table 4 and given by Lee et al. for their anaerogenic biogroup I (15).

Some useful reactions to differentiate *V. furnissii* from other salt-requiring vibrios isolated from humans are listed in Table 5. Its distinctive reactions include gas from D-glucose, fermentation of D-arabitol, L-arabinose, and D-mannitol, positive Møller L-arginine dihydrolase reaction, and negative reactions for Voges-Proskauer,

Møller L-lysine and L-ornithine decarboxylases, and lactose fermentation.

Epidemiology. It was first thought that only *V. fluvialis* (anaerogenic strains) was isolated from human stools. *V. furnissii* was isolated from water and from animal stools, but not from human stools (15). One exception was a *V. furnissii* strain isolated from a human diarrheal stool in Indonesia (16). In Japan, however, most human isolates were aerogenic (*V. furnissii*, R. Sakazaki, personal communication).

While investigating the history of the *V. furnissii* type strain, we found that *V. furnissii* had been isolated during the investigation of three outbreaks of acute gastroenteritis in American tourists returning from the Orient in 1969 (2–5; R. E. Weaver and D. G. Hollis, unpublished data). In the first outbreak, in May 1969, a flight from Tokyo to Seattle made an emergency stop in Alaska because 23 of 42 elderly passengers developed gastroenteritis (2, 3). One woman died, and two other persons required hospitalization. The symptoms were diarrhea (91%), abdominal cramps (79%), nausea (65%), and vomiting (39%). There were no reports of fever, and 50% of the patients had recovered within 12 h. Food histories implied that a shrimp and crab salad or a cocktail sauce or both served on the plane was the cause of illness. The onset of illness was between 5 and 20 h. A second outbreak affected 24 of 59 people returning to Boston from Hong Kong (4, 5). Nine people were hospitalized en route in Chicago. They had diarrhea and abdominal cramps (100%), nausea (89%), and vomiting (78%). The other 15 ill travelers had similar symptoms of lesser severity. The illness was self-limiting and lasted less that 24 h. A food vehicle was not found. In the third outbreadk (4, 5). Five passengers on a flight between Tokyo and Seattle became ill and requested medical attention. Subsequent questioning revealed that at least 67 people became ill in an ll-day period with symptoms of diarrhea (77%), nausea (37%), vomiting (26%), and abdominal cramps (26%). No single meal or common vehicle was implicated.

In the first outbreak, *V. furnissii* was recovered from seven stool specimens, two of which also contained *Vibrio parahaemolyticus* (R. E. Weaver and D. G. Hollis, unpublished data). *V. parahemolyticus* (5 isolates), *Aeromonas shigelloides* (9 isolates), salmonellae (5 isolates), and noncholera vibrios (14 isolates) were recovered from fecal specimens in the second outbreak (5). Of the noncholera vibrios, one was *V. cholerae* non-O1, one was *V. fluvialis*, and at least five were *V. furnissii* (R. E. Weaver and D. G. Hollis, unpublished data). *V. parahaemolyticus* (five isolates), noncholera vibrios (eight isolates), salmonellae (six isolates), *A. shigelloides* (one isolate), and *Shigella flexneri* (one isolate) were isolated from persons in the third outbreak (4, 5). It is not known whether any of these noncholera vibrios were *V. furnissii*. The role of *V. furnissii* as a cause of diarrhea may be similar to that for *V. fluvialis*. *V. fluvialis* was first thought to be nonpathogenic. It was then associated with diarrheal outbreaks in Bangladesh and Indonesia, and a fatal case was just reported in the United States (17). Clearly, *V. furnissii* has been isolated from human cases of gastroenteritis. Future studies will be required to determine whether it is a causative agent.

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