Characterization, Taxonomy, and Emended Description of *Vibrio metschnikovii*

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A numerical taxonomic study was carried out to establish: (i) the relationship of freshly isolated, oxidase-negative, vibrio-like organisms to strains listed in certain culture collections as *Vibrio metschnikovii* and *V. proteus*; (ii) the relationship of all these oxidase-negative organisms to the genus *Vibrio* and related organisms. Eighty-three strains were tested for their abilities to grow on 49 compounds as the sole organic source of carbon, their fermentation of carbohydrates, and their production of extracellular enzymes; various physiological tests were also carried out. In all, 130 characters were determined for each strain. The collection of strains were of *Vibrio* and *Aeromonas* species, certain other possibly related organisms, and 40 oxidase-negative organisms from a wide range of habitats. It was concluded that all of the oxidase-negative, vibrio-like strains except one belong to the species *Vibrio metschnikovii* Gamaleia 1888 (synonyms *V. proteus* Buchner 1885; *V. cholerae* biotype *proteus* Shewan and Veron 1974). The lectotype is NCTC 8443.

Certain culture collections contain strains listed as *Vibrio proteus* (Buchner) and *V. metschnikovii* (Gamaleia). *V. proteus* was originally isolated in 1884 from feces of patients suffering from cholera, and *V. metschnikovii* was isolated from a fowl that had died of a choleraic disease. It has been suggested that these two species be considered synonyms of *V. cholerae* biotype *proteus* (23). These organisms are unusual for vibrios since they are negative in the Kovács oxidase test (14).

In recent years we have frequently isolated, both from the marine environment and from freshwater, organisms that are vibrio-like and grow on thiosulfate-citrate-bile-salt-sucrose agar (TCBS) but are oxidase negative. We have also received many similar strains for identification from workers throughout the world. These strains are widely distributed both geographically and in their habitat.

The Subcommittee on the Taxonomy of Vibrios of the International Committee on Systematic Bacteriology has pointed out that very little is known about the relationship of these oxidase-negative organisms to the oxidase-positive *Vibrio* species and that their classification has not been studied in detail (12). To our knowledge no previous taxonomic study has included freshly isolated oxidase-negative strains similar to those described here. We have, therefore, examined the relationships between the wild strains and the culture collection strains. We have also studied the relationships between this oxidase-negative group, the genus *Vibrio*, and

other possibly related organisms. We selected our wild oxidase-negative strains to be as representative as possible of the isolates available to us at the time.

MATERIALS AND METHODS

Bacterial strains. The cultures used are listed in Table 1. Four strains, NCTC 8443, ATCC 7708, NCTC 8563, and NCTC 8564, were tested in duplicate since we had received separate cultures of them direct from the appropriate culture collection and via R. Hugh. These duplicate cultures did not behave identically.

The strains that required sodium for growth were maintained on slopes of marine agar (MA; Difco) at 4°C, in the dark under sterile liquid paraffin. Other cultures were maintained on slopes of heart infusion agar (Difco) under sterile liquid paraffin, in the dark at room temperature. Unless otherwise stated, strains were incubated at 30°C for all tests. *V. fischeri* 62 and *Photobacterium phosphoreum* 82 grow poorly at 30°C and were therefore tested at 20°C.

Motility and morphology. Motility was determined by the hanging drop method from 1% tryptone water (Oxoid) containing 2% (wt/vol) NaCl (T_1N_2). Flagella were stained by the Rhodes method (19) as modified by Gauthier et al. (9). A few strains were also examined by electron microscopy.

Salt tolerance. All strains were tested for their ability to grow in 1% tryptone water containing 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 10% (wt/vol) NaCl. Tubes containing 3 ml of medium were inoculated with 10 μ l of an early-log-phase T_1N_2 broth culture containing approximately 10^3 to 10^4 colony-forming units per ml. Growth was determined visually after 48 h of incubation.

Temperature range. Strains were tested for their ability to grow at different temperatures in T_1N_2 . The

TABLE 1. *Strains used*

Computer no.	Laboratory no.	Received as:		Source ^a	Habitat
		Name	Strain		
Oxidase-negative vibrios					
1	MV 409	<i>V. metschnikovii</i>	NCTC 8443	(1)	Fowl
2	MV 501	<i>V. proteus</i>	P 6915	(2)	
3	MV 2066	Original isolate			Lobster
4	MV 2067	Original isolate			Lobster
5	MV 2068	<i>V. proteus</i>	NCTC 8563	(1)	
6	MV 2069	<i>V. proteus</i>	NCTC 8564	(1)	
7	MV 2196	<i>V. metschnikovii</i>	ATCC 7708	(3)	
8	MV 2229	<i>Vibrio</i> sp.		(4)	Sewage
9	MV 2318	Original isolate			Cockles
10	MV 2338	<i>Vibrio</i> sp.		(5)	River water
11	MV 2339	<i>Vibrio</i> sp.		(5)	River water
12	MV 2345	<i>Vibrio</i> sp.		(5)	River water
13	MV 2346	<i>Vibrio</i> sp.		(5)	River water
14	MV 2360	<i>Vibrio</i> sp.		(4)	Sewage
15	MV 2361	<i>Vibrio</i> sp.		(4)	Sewage
16	MV 2362	<i>Vibrio</i> sp.		(4)	Sewage
17	MV 2365	<i>Vibrio</i> sp.		(4)	Sewage
18	MV 2366	<i>Vibrio</i> sp.		(4)	Sewage
19	MV 2375	<i>Vibrio</i> sp.		(4)	Sewage
20	MV 2376	<i>Vibrio</i> sp.		(4)	Sewage
21	MV 2377	<i>Vibrio</i> sp.		(4)	Sewage
22	MV 2383	<i>Vibrio</i> sp.		(5)	River water
23	MV 2401	<i>V. metschnikovii</i>	ATCC 7708	(6)	
24	MV 2402	<i>V. metschnikovii</i>	NCTC 8443	(6)	Fowl
25	MV 2403	<i>V. metschnikovii</i>		(6)	
26	MV 2404	<i>Vibrio</i> sp. (cholera gp.)	ATCC 11554	(6)	
27	MV 2406	<i>V. proteus</i>	NCTC 8563	(6)	
28	MV 2407	<i>V. proteus</i>	NCTC 8564	(6)	
29	MV 2409	<i>V. tyrogenes</i>		(6)	
30	MV 2423	<i>Vibrio</i> sp.		(5)	River water
31	MV 2468	Original isolate			Cockles
32	MV 2469	Original isolate			Cockles
33	MV 2476	<i>Vibrio</i> sp.		(5)	River water
34	MV 2477	<i>Vibrio</i> sp.		(5)	River water
35	MV 2478	<i>Vibrio</i> sp.		(5)	River water
36	MV 2480	<i>Vibrio</i> sp.		(5)	River water
37	MV 2481	<i>Vibrio</i> sp.		(5)	River water
38	MV 2484	Original isolate			Cockles
39	MV 2488	Original isolate			Prawns
40	MV 400	<i>V. cholerae</i> classical		(7)	Feces, human
41	MV 1208	<i>V. cholerae</i> classical	NCTC 7254	(1)	Feces, human
42	MV 1330	<i>V. cholerae</i> eltor	NCTC 10954	(1)	Feces, human
43	MV 1214	<i>V. cholerae</i> (NCV)		(8)	Feces, human
44	MV 1401	<i>V. cholerae</i> (NCV)			Feces, human
45	MV 1479	<i>V. cholerae</i> (NCV)			Prawns
46	MV 463	<i>V. alginolyticus</i>		(9)	Seawater
47	MV 1251	<i>V. alginolyticus</i>			Oysters
48	MV 2204	<i>V. alginolyticus</i>	ATCC 17749	(3)	Spoiled horse mackerel
49	MV 515	<i>V. parahaemolyticus</i>			Feces, human
50	MV 1225	<i>V. parahaemolyticus</i>			Feces, human
51	MV 1424	<i>V. parahaemolyticus</i>	ATCC 17802	(3)	Feces, human
52	MV 1490	<i>V. anguillarum</i>	NCMB 6	(10)	Diseased cod
53	MV 2045	<i>V. anguillarum</i>			Oyster
54	MV 2176	<i>V. anguillarum</i>			Clam
55	AM 2206	<i>V. anguillarum</i>			Clam
56	MV 2600	<i>V. anguillarum</i>		(5)	Mussel
62	MV 1488	<i>V. fischeri</i>	NCMB 1143	(10)	Pacific cod
63	MV 1493	<i>V. fischeri</i>			Prawns

TABLE 1—(Continued)

Computer no.	Laboratory no.	Received as:		Source ^a	Habitat
		Name	Strain		
<i>Aeromonas</i> species					
64	MV 1121	<i>A. hydrophila</i>	NCTC 8049	(1)	Milk
65	MV 1122	<i>A. hydrophila</i>	NCTC 7810	(1)	Frog "red leg"
66	MV 2071	Original isolate			Feces, human
67	MV 2169	Original isolate			Feces, human
68	MV 2415			(5)	River water
69	MV 1133	Original isolate			Feces, human
70	MV 1140	Original isolate			Prawns
71	MV 1931	Original isolate			River water
72	MV 1933	Original isolate			River water
73	MV 2224			(4)	Sewage
74	MV 2374			(4)	Sewage
<i>Beneckeia</i> species and others					
57	MV 2386			(5)	River water
58	MV 2387			(5)	River water
59	MV 2389			(5)	River water
60	MV 2391			(5)	River water
61	MV 2418			(5)	River water
75	MV 2189	<i>B. campbellii</i>	ATCC 25920	(3)	Seawater
76	MV 2190	<i>B. natriegens</i>	ATCC 14048	(3)	Salt marsh mud
77	MV 2191	<i>B. neptuna</i>	ATCC 25919	(3)	Seawater
78	MV 2192	<i>B. nereida</i>	ATCC 25917	(3)	Seawater
79	MV 2193	<i>B. nigrapulchrituda</i>	ATCC 27043	(3)	Seawater
80	MV 2194	<i>B. pelagia</i>	ATCC 25916	(3)	Seawater
81	MV 2195	Group B2 of Bau-mann et al. (1)	ATCC 25915	(3)	Seawater
82		<i>Photobacterium phosphoreum</i>	NCMB 844	(10)	
83		<i>Escherichia coli</i>	NCTC 10418	(1)	

^a (1) NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England. (2) P. Institut Pasteur, Paris, France. (3) ATCC, American Type Culture Collection, Rockville, Md. (4) V. Muic, Institute of Public Health of SR Croatia, Zagreb, Yugoslavia. (5) J. McCoy, Public Health Laboratory, Hull Royal Infirmary, Hull, England. (6) R. Hugh, Department of Microbiology, George Washington University School of Medicine, Washington D.C. (7) Cholera Research Laboratory, Dacca, Bangladesh. (8) School of Public Health and Tropical Medicine, Sydney, Australia. (9) E. Aldova, Institute of Epidemiology and Microbiology, Prague, Czechoslovakia. (10) National Collection of Marine Bacteria, Torry Research Station, Aberdeen, Scotland.

media were inoculated as for the salt tolerance test. Growth was recorded after incubation for 14 days at 4°C, 2 days at 15°C, and 1 day at 30, 37, and 42°C.

Swarming. Marine agar was prepared according to the manufacturer's instructions, and the sterile molten agar was cooled in a water bath at 45°C for 1 h before the plates were poured. After the plates had set, they were not dried, but gutters were cut in them to divide the agar into quadrants. Each quadrant was inoculated with a 10-mm streak of one of the test organisms. Plates were examined for swarming after overnight incubation. A known positive control organism was included with each batch of strains tested.

Luminescence. Strains were inoculated onto plates of nutrient agar containing (per liter): nutrient broth dried base (Oxoid), 25 g; NaCl, 17.5 g; KCl, 1.0 g; MgCl₂ · 6H₂O, 4.0 g; Davis agar, 15.0 g. After overnight incubation at 20°C, luminescence was recorded

after the plates were observed for 5 min in the dark.

Antibiotic susceptibilities. Susceptibility to 2,4-diamino-6,7-diisopropyl-pteridine phosphate (0/129) was determined on heart infusion agar (Difco). Susceptibilities to other antibiotics were determined on Sensitest agar (Oxoid). Strains 53, 62, 78, 79, 81, and 82 would not grow on this medium and were tested on marine agar. The antibiotics and the concentrations used are shown in Table 2. For novobiocin, 0/129, and methylene blue, the susceptibilities were read against *V. cholerae* 41 (NCTC 1208) as a control. For the remaining antibiotics, *Escherichia coli* 83 (NCTC 10418) was used.

General physiological and biochemical properties. Unless otherwise stated, tests were read during an incubation period of 5 days.

The mode of glucose metabolism was examined by the method of Hugh and Leifson (10), but the NaCl

concentration of the medium was increased to 3% (wt/vol).

Strains were tested for: growth after 24 h on TCBS, cystine-lactose electrolyte-deficient medium, and MacConkey agar (all Oxoid); hemolysis of sheep erythrocytes, as determined by the method of Sakazaki et al. (21); agglutination of chicken erythrocytes by the method of Finkelstein and Mukerjee (7); chitinase production on MA overlaid with MA containing 1% (wt/vol) colloidal chitin; alginase on MA overlaid with MA containing 2% (wt/vol) sodium alginate; lecithinase on Oxoid blood agar base CM55 containing 1% (wt/vol) NaCl and 10% (wt/vol) egg yolk emulsion (Oxoid); arginine dihydrolase by the method of Thornley (25); and the reduction of trimethylamine oxide to trimethylamine by the method of Laycock and Regier (15), except that 20% (wt/vol) KOH was used instead of K_2CO_3 (18).

The following tests were performed as described by Cowan (5). When alternatives are listed, the one chosen is shown in parentheses. The sodium chloride concentration in all the media was raised to 1% (wt/vol). The tests were: indole production after 24 h (method 2); Kovács (14) oxidase; catalase (method 1); reduction of nitrate to nitrite, but using reagents A and B of Crosby (6); acid and gas production from carbohydrates in peptone water containing Andrade indicator; *o*-nitrophenyl- β -D-galactopyranoside; Voges-Proskauer after 1 day (method 1); methyl red after 2 days of incubation; gluconate oxidation (method 2); decarboxylation of arginine, lysine, and ornithine (method 1), using Difco decarboxylase base; urease (method 1); hydrogen sulfide production (method 1); deoxyribonuclease on Difco deoxyribonuclease test agar; gelatinase (method 3); Tween 80 hydrolysis (method 1); phosphatase (method 1), substituting MA for nutrient agar; citrate utilization (method 2); malonate utilization (method 1); phenylalanine deamination (method 1); starch hydrolysis (method 1), substituting MA for nutrient agar.

Utilization of carbon compound. Strains were screened for their ability to utilize 49 different compounds as the sole organic source of carbon, using the basal medium of Baumann et al. (1). All of the strains studied were capable of growth on basal medium containing 0.1% (wt/vol) vitamin-free Casamino Acids (Difco) and were therefore considered to have no vitamin requirements. If sufficiently soluble, the substrates were prepared as concentrated solutions, which were sterilized by passage through a 0.2- μ m-pore membrane filter (Sartorius) and added to the medium immediately before plates were poured. Otherwise, compounds were dissolved in the medium and autoclaved at 115°C for 10 min. In all cases the final concentration of the substrate in the medium was 0.1% (wt/vol). Plates of medium were inoculated by means of a mechanical multipoint inoculator. Strains were grown in T_1N_2 , and the inoculum was prepared by diluting the culture with fresh T_1N_2 such that the final inoculum on the plate contained approximately 10^4 organisms in a spot of 2- to 3-mm diameter. Up to 24 strains could be inoculated onto one 90-mm-diameter petri dish. Strains that swarmed were isolated from other strains by gutters in the agar, and only six were then tested on one plate. Growth was

recorded for 7 days. A positive (basal medium plus 0.1% vitamin-free Casamino Acids) control and a negative (basal medium only) control were put up for every strain.

Base composition of DNA. The deoxyribonucleic acid (DNA) base compositions of selected strains were determined by thermal denaturation (17) after extraction and purification by the method of Marmur (16).

Numerical analysis of results. Results from all the tests shown in Table 2, except mode of flagellation, were used in a computer-assisted taxonomic analysis. The results were regarded as two-state characters with the exception of temperature range and salt tolerance. Temperature range was coded as two multistate quantitative characters, i.e., the minimum and maximum temperatures at which the strains grew. Growth in the absence of sodium chloride was considered as a single two-state character, and growth at the remaining salt concentrations was coded as two multistate quantitative characters, i.e., the minimum and maximum salt concentrations in which the strains grew. If a strain grew in the absence of sodium chloride, the character "salt minimum" was coded as "no comparison," as was the character "production of gas from glycerol" if the strain did not produce acid from glycerol, and as was the character "acid from lactose" if the strain was negative in the ONPG test. Similarities were calculated by using the general similarity coefficient of Gower (negative matches were counted for all two-state characters), and dendrograms were constructed by single-linkage and weighted pair-group centroid (median) clustering methods (24).

(The full list of code characters and strain data have been deposited with the World Data Center for Microorganisms, Department of Microbiology, University of Queensland, St. Lucia Brisbane 4067, Australia, from which it can be obtained.)

RESULTS AND DISCUSSION

The characteristics of the strains are shown in Table 2. The two methods of cluster analysis, with few exceptions, gave identical groupings (Fig. 1 and 2). We shall therefore confine our discussion to the dendrogram constructed from the single-linkage cluster analysis (Fig. 1). The clustering is very much as we would have expected from our experience in identifying members of the *Vibrionaceae* and from the work of others (1, 4, 20). Seven major phenons, described below, were formed in the analysis.

V. metschnikovii. The naming of the phenon *V. metschnikovii* and its taxonomic position are discussed below. It is formed at the 89% level of similarity (S), contains only oxidase-negative, vibrio-like organisms, and includes all such organisms studied with one exception. Within this phenon there are two subphenons. The smaller one formed at 92% S contains all of the *V. metschnikovii* strains, no. 29 received as *V. tyrogenes* and no. 26 received as "*Vibrio* sp. (cholera gp.)." The second subphenon formed at 91% S includes all of the wild strains

TABLE 2. Characteristics of *V. metschnikovii* and the other phenons^a

Determination	All strains of <i>V. metschnikovii</i>	<i>V. metschnikovii</i> lectotype NCTC 8443	<i>V. metschnikovii</i> NCTC 11170	<i>V. cholerae</i>	<i>V. anguillarum</i>	Group D	Marine vibrios	Group F	<i>A. hydrophila</i>	<i>B. pelagia</i> ATCC 25916	<i>V. fischeri</i> NCMB 1143	<i>P. phosphoreum</i> NCMB 844	<i>B. natrigens</i> ATCC 14048	<i>B. nereida</i> ATCC 25917	Group B2 ATCC 25915	<i>E. coli</i> NCTC 10418
No. of strains	39	1	1	6	5	6	10	6	4	1	1	1	1	1	1	1
Motility	+	+	+	+	+	✓	+	+	+	+	+	+	+	+	+	+
Growth on:																
TCBS	+-	-	+	+	+	+-	+	+	✓	+	-	-	+	-	-	-
CLED	+	+	+	+	-	+	-	-	+	+	-	-	-	-	-	-
MacConkey	+-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	+
Swarming	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Luminescence	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Growth in % NaCl:																
0	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	+
0.5	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	+
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	✓	+	+	+	+	+	+	+
5	+	+	+	✓	+	+	+	+	-	+	-	+	+	+	+	+
6	+	+	+	✓	+	+	+	+	-	+	-	+	+	+	+	+
7	+	+	+	+	+	-	+	+	-	+	-	+	+	+	-	+
8	✓	-	+	-	+	-	✓	+	-	-	-	-	+	+	-	-
10	-	-	-	-	-	-	✓	+	-	-	-	-	-	+	-	-
Growth at:																
15°C	+-	+	+	+-	+	+-	✓	+	✓	-	+	+	+	+	-	-
30°C	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+
37°C	+	+	+	+	+	+	+-	+	+	-	-	-	+	+	-	+
42°C	✓	-	+	+	-	✓	✓	✓	✓	-	-	-	+	+	-	+
Hemolysis	+	+	+	✓	+	✓	+	+	✓	-	NC	NC	-	+	+	-
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+
Oxidase	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-
NO ₃	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
TMAO	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
MR	+-	-	+	+	✓	+-	+	+	✓	+	-	+	+	+	+	+
VP	+	+	+	✓	+-	+	+-	+	+	-	-	-	-	-	+	+
Indole	✓	+	-	+	+-	+	+	+	+	-	-	-	-	-	+	+
ONPG	+-	+	+	+	+-	+	✓	+	+	+	+	-	-	NC	-	+
Gas from:																
Glucose	-	-	-	-	-	✓	-	+-	+	-	-	+	-	-	-	+
Glycerol	-	-	-	-	-	-	-	+-	+	-	-	-	-	-	-	-
Acid from:																
Arabinose	-	-	-	-	-	✓	✓	+	✓	-	-	-	-	-	-	+
Cellobiose	-	-	-	-	+-	+-	+	+-	-	-	+	-	+	-	-	-
Dulcitol	-	-	-	-	-	-	-	+-	-	-	-	-	-	-	-	+
Glycerol	+	+	+	-	-	✓	✓	+	+	-	-	-	-	-	-	-
Inositol	✓	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lactose	✓	+	+	-	-	✓	-	-	✓	-	-	-	-	-	-	+
10% Lactose	+-	+	+	✓	-	✓	-	-	✓	-	-	-	-	-	+	+
Maltose	+	+	+	+	+-	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+
Mannose	+-	+	+	+-	+-	✓	+	+-	+	-	-	+	-	-	+	+
Raffinose	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
Rhamnose	-	-	-	-	-	-	-	+-	-	-	-	-	+	-	-	+
Salicin	-	-	-	-	-	+-	-	-	-	-	-	-	+	-	-	-
Sorbitol	+-	-	-	-	✓	-	-	-	-	-	-	-	-	-	-	+
Starch	+	+	+	+-	+	+	✓	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+-	+	+-	+	+	+	+	+	-	+	+	+	+
Trehalose	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Gluconate oxidation	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-

TABLE 2—(Continued)

Determination	All strains of <i>V. metschnikovii</i>	<i>V. metschnikovii</i> lectotype NCTC 8443	<i>V. metschnikovii</i> NCTC 11170	<i>V. cholerae</i>	<i>V. anguillarum</i>	Group D	Marine vibrios	Group F	<i>A. hydrophila</i>	<i>B. pelagia</i> ATCC 25916	<i>V. fischeri</i> NCMB 1143	<i>P. phosphoreum</i> NCMB 844	<i>B. natrigens</i> ATCC 14048	<i>B. nereida</i> ATCC 25917	Group B2 ATCC 25915	<i>E. coli</i> NCTC 10418
Thornley's arginine	+-	+	+	-	+	+	-	+	+	-	-	+	-	+	-	-
Moeller's																
Arginine	+-	+	+	-	+-	+	-	+	+	-	-	+	-	+	+	-
Lysine	+-	-	+	+	+-	+-	+	+	+	-	+	+	-	-	-	+
Ornithine	-	-	-	+	+	-	+	+	+	-	-	+	-	-	-	+
Amylase	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+
Alginase	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Chitinase	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-
Deoxyribonuclease	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Gelatinase	+	+	+	+	+	+	+	+	+	+	+	NC	+	+	+	-
Lecithinase	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-
Tween 80	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-
Phosphatase	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
Urease	-	-	-	-	-	+	+	-	-	-	NC	-	-	-	-	-
Malonate	-	-	-	-	+	-	-	+	+	+	NC	-	-	-	-	-
PPA	-	-	-	-	+	-	-	+	+	+	+	-	-	-	-	-
Simmons' citrate	+	-	+	+	-	+	-	+	+	+	+	-	-	-	-	-
CCA	+	-	-	+	+	+	-	-	+	-	+	-	-	-	+	+
Susceptibility to:																
Ampicillin, 10 µg	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+
Carbenicillin, 100 µg	+-	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+
Cephaloridine, 15 µg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Kanamycin, 30 µg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Methylene blue, 100 µg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Novobiocin, 5 µg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Polymyxin B, 50 IU	+	+	+	+	+	+	+	+	+	+	NC	+	+	+	+	+
Streptomycin, 25 µg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sulfafurazole, 100 µg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tetracycline, 10 µg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trimethoprim, 1.25 µg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0/129, 100 µg	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-
Growth on:																
Alanine	+	+	+	-	+	-	+	+	-	-	-	-	+	+	-	+
Arginine	+-	+	-	-	-	-	+	+	-	+	-	-	+	+	-	-
Asparagine	+	-	+	+	+	+	+	+	+	+	-	-	+	+	+	-
Aspartate	-	-	-	+	+	-	+	+	-	+	-	-	-	+	+	-
Glutamate	+-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Glutamine	+-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Glycine	-	-	-	-	-	-	+	+	+	+	-	-	-	+	+	-
Histidine	+-	+	-	+	NC	+	+	NC	NC	NC	-	-	NC	NC	NC	-
Leucine	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-
Lysine	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-
Proline	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+
Putrescine	-	-	-	-	+	-	+	+	-	+	-	-	+	+	-	+
Serine	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+
Threonine	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+	-
Arabinose	-	-	-	-	-	+	+	+	+	-	-	-	+	+	-	+
Cellobiose	-	-	-	-	+	+	+	+	-	-	-	-	+	-	-	-
Galactose	-	-	-	-	+	+	+	+	-	-	-	-	+	-	-	-
Gluconate	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+
Glucuronate	-	-	-	+	-	-	+	-	-	+	-	+	+	+	+	-
Glycerol	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Inositol	+	+	-	-	-	-	-	-	+	+	-	-	-	+	+	-
Lactose	+	-	-	-	+	+	-	-	+	+	-	-	-	+	+	-
Salicin	-	-	-	-	-	+	+	-	-	+	-	-	+	+	-	-

TABLE 2—(Continued)

Determination	All strains of <i>V. metschnikovii</i>	<i>V. metschnikovii</i> lectotype NCTC 8443	<i>V. metschnikovii</i> NCTC 11170	<i>V. cholerae</i>	<i>V. anguillarum</i>	Group D	Marine vibrios	Group F	<i>A. hydrophila</i>	<i>B. pelagia</i> ATCC 25916	<i>V. fischeri</i> NCMB 1143	<i>P. phosphoreum</i> NCMB 844	<i>B. natrigens</i> ATCC 14048	<i>B. neredia</i> ATCC 25917	Group B2 ATCC 25915	<i>E. coli</i> NCTC 10418
Sorbitol	— ⁺	—	—	—	V	—	—	—	—	+	—	—	—	—	+	—
Sucrose	+	+	+	+	+	+	V	+	—	+	—	—	+	+	+	—
Xylose	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—
Acetate	+	—	—	+	+	+	+	+	—	+	—	—	+	+	+	—
Butyrate	—	—	—	—	—	—	V	—	—	—	—	—	+	+	+	—
Decanoate	+	—	—	+	—	V	+	+	—	—	—	—	+	+	—	—
Formate	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
p-OH benzoate	—	—	—	—	—	—	V	V	—	—	—	—	+	+	—	—
3-OH butyrate	—	—	—	—	—	—	—	V	—	+	—	—	+	+	—	—
α-Ketoglutarate	—	—	—	+	V	—	+	+	V	+	—	—	+	+	+	+
Pyruvate	+	+	+	V	—	—	—	V	—	—	—	—	—	+	—	—
Succinate	+	—	+	+	+	+	V	+	—	+	—	—	+	+	+	—
Ethanol	—	—	—	—	—	—	V	+	—	—	—	—	—	+	—	—
Propanol	—	—	—	—	—	—	—	V	—	—	—	—	—	+	—	—
Flagella	m	m	m	m	m	m	m	m	m	m	m	m	m	m	m	p

^a All strains were positive for: glucose fermentation; growth in 1% NaCl; susceptibility to nalidixic acid, septrin, nitrofurantoin, chloramphenicol, and gentamicin. All strains were negative for: Gram reaction: adonitol (peptone-water-sugar); H₂S; growth at 4°C; growth on cysteine, isoleucine, methionine, phenylalanine, tryptophan, tyrosine, valine, DL-2-hydroxybutyrate, tartrate, butanol, and erythrose. Symbols/abbreviations: +, reaction positive for at least 90% of strains; —, reaction negative for at least 90% of strains; V, variable reaction for group; ++ or —+, first reaction present in 80 to 89% of the strains; m, monotrichous polar flagella; p, peritrichous flagella; CLED, cystine-lactose electrolyte-deficient medium; TMAO, trimethylamine oxide; MR, methyl red; VP, Voges-Proskauer; ONPG, o-nitrophenyl-β-D-galactopyranoside; PPA, phenyl pyruvic acid test; CCA, chick cell agglutination.

^b Included in total for phenon.

and the *V. proteus* strains, the latter forming a single cluster at 94% S.

Number 81 (ATCC 25915), which represents group B2 of Baumann et al. (1), was the only oxidase-negative, vibrio-like strain in our study that did not cluster in the *V. metschnikovii* phenon. It is clearly not closely related to any of the organisms in the study since it is the last but one strain to cluster.

V. cholerae. The phenon *V. cholerae* contains all the strains of the classical and *eltor* biotypes of *V. cholerae*, including the type strain, and all the so-called noncholera vibrios and no others. It is now recognized that the noncholera vibrios, also called non-agglutinable vibrios by some workers, should be considered as different serotypes of the species *V. cholerae* (13). This phenon therefore corresponds to *V. cholerae*.

V. anguillarum. The phenon *V. anguillarum*, formed at 84% S, consists of the four wild strains we had previously identified as *V. anguillarum* and no. 52 (NCMB 6), one of the strains recommended as a working type by Shewan and Véron (23). Numbers 54 and 53, which were the last to join the *V. anguillarum* phenon by single linkage, are separated from it by me-

dian sorting (Fig. 1 and 2). This suggests that the phenon may be slightly heterogenous, but it corresponds to *V. anguillarum* as described by Shewan and Véron (23).

Group D. The phenon group D contains six strains that we would identify as *Aeromonas*, but no type strains are included.

Marine vibrios. The rather heterogenous group marine vibrios contains the type strains and all the other strains of *V. alginolyticus* and *V. parahaemolyticus*, along with the type strains of *Beneckea neptuna* (no. 77), *B. campbellii* (no. 75), and *B. nigrapulchrituda* (no. 79). The remaining strain in this group is no. 63. It is luminescent and closely matches the description of *V. fischeri* (23), except that it grows at 37°C and possesses ornithine decarboxylase. The particular strain of *V. fischeri* used in the study (no. 62, NCMB 1143) was rather unreactive and differs from the published description in being negative for methyl red, gelatinase production, and acid production from mannose. Number 62 did not fall into any distinct phenon in our study.

The marine vibrio phenon has no clear sub-cluster within it corresponding to the species *V. parahaemolyticus* or *V. alginolyticus*. Probably, with a much larger number of strains, subclus-

FIG. 1. Dendrogram constructed by single-linkage cluster analysis. (a) Culture collection strains of *V. proteus*; (b) culture collection strains of *V. metschnikovii*.

ters would have been formed within this phenon corresponding to the species represented in it. Nevertheless over the past 2 years, in attempting to identify many hundreds of vibrio isolates from the marine environment, we have found very many strains with characteristics between *V. alginolyticus*, *V. parahaemolyticus*, *B. neptuna*, *B. campbellii*, and *V. fischeri*. This marine

vibrio phenon is therefore much as we would have expected.

Group F. Group F is a very interesting group, which we had not suspected existed until it was revealed by this study and another one running concurrently in our laboratory on the antibiotic susceptibilities of vibrios. It includes six strains of which five produce gas from glucose, and all

FIG. 2. Dendrogram constructed by weighted pair-group centroid (median) cluster analysis. (a) Culture collection strains of *V. proteus*; (b) culture collection strains of *V. metschnikovii*.

six had previously been identified as *Aeromonas* spp. However, unlike other aeromonads, they are unable to grow in the absence of NaCl, they can grow on a much wider range of compounds as the sole organic source of carbon, and they show some degree of susceptibility to 0/129. In this study they are recorded as having variable susceptibility to 0/129 when tested with disks containing 100 μ g of the drug. If tested with disks containing 150 μ g, all of these strains are susceptible, whereas *Aeromonas* strains remain resistant.

We now realize that this group is extremely widely distributed in the marine and estuarine environment around Britain. We have also received similar strains from patients with diarrhea whose sources of infection were in Bangladesh, Bahrain, and Jordan. Two of the strains from this study and one from a patient whose illness was contracted in Bahrain have had their DNA base composition examined. The guanine-plus-cytosine (G+C) content of the DNA of these strains was in the range 51.5 to 52.2 mol% (Table 3). This is too low for the genus *Aeromonas* (57 to 63 mol%) and perhaps too high for the genus *Vibrio*, which has 40 to 50 mol% G+C (22, 23). It seems probable that they constitute a new species if not a new genus. However, at the moment, we do not feel justified in proposing a new species on the information so far available.

***Aeromonas hydrophila*.** The four strains of the *Aeromonas hydrophila* phenon include the neotype *A. hydrophila* subsp. *hydrophila* no. 64

TABLE 3. DNA base composition of selected *V. metschnikovii* and group F strains

Strain	Mol% G+C
<i>V. metschnikovii</i>	
1 Lectotype NCTC 8443	45.4
3	45.6
4	44.9
5 NCTC 8564	45.4
11 NCTC 11171	45.9
18 NCTC 11172	45.4
19	46.9
31	46.9
38 NCTC 11170	44.2
Group F ^a	
57	52.0
60	52.2

^a The mole percent G+C of a similar strain isolated from a patient with diarrhea was 51.5.

(NCTC 8049), and all the strains conform to the description of Schubert (22).

Taxonomy and nomenclature of *V. metschnikovii*. The formation of separate clusters corresponding to *V. metschnikovii*, *V. proteus*, and the wild strains could be interpreted as an indication of separate strains or biotypes. However, two of the *V. proteus* strains and two of the *V. metschnikovii* strains are supposedly the same organism received from two sources, in each case one from the American Type Culture Collection or the National Collection of Type Cultures (NCTC) and the other via R. Hugh. None of these strains was as highly related to one another as would have been expected. Furthermore, all of the strains falling in the clusters corresponding to *V. metschnikovii* and *V. proteus* are culture collection strains that, having been subcultured over many years, may have changed. We believe this has happened with certain old NCTC strains of *V. cholerae*, which differ from strains isolated more recently in being less versatile, growing less rapidly, and being unable to grow on TCBS. It is possible that *V. proteus* and *V. metschnikovii* strains may have "changed" in a similar way. We have not yet discovered any wild isolate that matches the culture collection strains exactly. In the case of *V. metschnikovii*, this may be because they are unable to grow on TCBS, but we do have a few fresh isolates, such as strain 20, that are unable to grow on TCBS and otherwise match the other wild strains. We therefore do not believe we are justified in dividing the oxidase-negative group into separate species or biotypes at the moment. We think they should be included in a single species.

Although these strains are all oxidase negative and unable to reduce nitrate to nitrite or trimethylamine oxide to trimethylamine, they are

otherwise similar to the other species of the genus *Vibrio*. The DNA base composition of the nine strains examined was in the range 44.2 to 46.9 mol% G+C, with a mean of 45.6 mol% G+C (Table 3). This is within the range for the genus *Vibrio* (40 to 50 mol%) and too low for the genus *Aeromonas* (57 to 63 mol%). We do not think that they are closely related to the genus *Photobacterium*, since they do not produce gas, are nonluminescent, and possess amylase and gelatinase. The two species in our study with which they have greatest similarity are *V. cholerae* and *V. anguillarum*. Thus, there is insufficient evidence to create a new genus for this oxidase-negative group of organisms; they should be retained within the genus *Vibrio*.

The *V. cholerae* phenon does not link with the oxidase-negative phenon until the 85% level of similarity. There are also several consistent differences between strains of the two phenons, notably decarboxylase reactions, salt requirement for growth, and acid production from inositol and lactose. Therefore, we cannot support the suggestion of Shewan and Véron (23) that this group be considered as a biotype of *V. cholerae*.

According to the *Index Bergeyana* (2) *V. proteus* Buchner is an illegitimate name. From the records of the NCTC (Lapage, personal communication, 1977), *V. metschnikovii* M34, NCTC 8443 (our computer number 1), is the

same as the strain listed as NCTC 262 in the first NCTC catalog of 1922. In this it says "originally isolated by Gamaleia from disease of fowls." Accordingly, we conclude that this phenon should be named *V. metschnikovii* and that the lectotype is NCTC 8443. Since this strain has been in culture collections for many years and differs from the wild strains in certain characteristics (Table 2), we suggest that our strain 38 (NCTC 11170), isolated from cockles, may be used as a working strain. All of the *V. metschnikovii* strains in our study were polarly flagellated, gram-negative, asporogenous, slightly curved rods. Electron micrographs of the lectotype and strains with computer numbers 11, 18, and 38 are shown in Fig. 3. The inclusion of these organisms in the genus *Vibrio* would require its definition to be changed accordingly. We also suggest that the minimal characters for the identification of vibrio species recommended by Hugh and Sakazaki (11) be emended as shown in Table 4.

Vibrio metschnikovii Gamaleia 1888 (synonyms *Vibrio proteus* Buchner 1885; *Vibrio cholerae* biotype *proteus* Shewan and Veron 1974). Named for E. Metschnikoff, a Russian bacteriologist.

Description of species. Short rods; axis straight or curved; sides parallel; ends rounded; occurring singly, in pairs, and occasionally in short chains of three to four organisms; pleo-

FIG. 3. Electron micrographs of *V. metschnikovii* strains. (A) Lectotype strain 1, NCTC 8443; (B) strain 38; (C) strain 11; (D) strain 18. Bars indicate 1 μ m.

FIGS. 3B-C

morphic; 0.5 by 1.5 to 2.5 μm . Motile by means of a single polar flagellum. Sodium chloride is required for growth, but no organic growth factors are required. The biochemical and physiological characteristics of the species and lectotype are shown in Table 2. Those characteristics particularly useful for the identification of the

species are summarized in Table 5.

Habitat: Very widely distributed throughout the aquatic environment, particularly in rivers, estuaries, and sewage. Sometimes isolated from animal intestines, including that of humans, but there is no evidence to suggest they cause enteritis in humans or animals. Frequently isolated

FIG. 3D

TABLE 4. Characters useful for the identification of *Vibrio* species

Character	Results
Polar flagellum	+
Gram-negative asporogenous rod	+
Indophenol (Kovács) oxidase	+ ^a
Glucose, acid under petroleum seal	+
Glucose, gas	-
D-Mannitol	+
40-50 mol% G+C	+
O/129 (150- μ g disk) susceptible	+
Extracellular deoxyribonuclease	+

^a Except *V. metschnikovii*.

from various shellfish, e.g., cockles, clams, oysters, and lobsters.

The G+C content of the DNA is in the range 42.5 to 46.9 mol% for the 9 strains examined (Table 3).

The lectotype is NCTC 8443. Since this strain has been in culture collections for many years and differs from the fresh isolates in certain characters (Table 2), we have also deposited strains MV2339, MV2366, and MV2376 in the NCTC under the numbers NCTC 11171, NCTC 11172, and NCTC 11173, respectively. We recommend strain MV2484 (NCTC 11170), originally isolated from cockles, as a working strain.

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TABLE 5. Characters useful for the identification of *V. metschnikovii*

Character	Result
Oxidase	-
Nitrate reduction	-
Arginine dihydrolase	+
Lysine decarboxylase	V ^a
Ornithine decarboxylase	-
O/129 (10 μ g disk) susceptible	+
Sucrose	+

^a Variable reaction.

Scotland, for determining the DNA base compositions, and W. Hodgkiss, also of Torry, for the electron micrographs.

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