Budvicia aquatica gen. nov., sp. nov.: a Hydrogen Sulfide-Producing Member of the *Enterobacteriaceae*

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Deoxyribonucleic acid relatedness studies (S1 nuclease method) showed that 60 strains proposed as *Budvicia aquatica* form a homogeneous deoxyribonucleic acid hybridization group. Three strains labeled *Budvicia*-like were 9 to 22% related to *B. aquatica*. A total of 74 strains representing known species and genera in the *Enterobacteriaceae* were 0 to 8% related to *B. aquatica*. These findings support designation of *Budvicia aquatica* as a new genus and new species. This new species in the family *Enterobacteriaceae* is composed of strains which produce H₂S; hydrolyze urea and *o*-nitrophenyl- β -D-galactopyranoside; do not produce acid from trehalose, D-mannose, glycerol, sucrose, maltose, and D-melibiose; do not decarboxylate lysine, ornithine, or arginine; do not produce phenylalanine deaminase; and have complex growth factor requirements. The guanine-plus-cytosine content of the deoxyribonucleic acid is 46 mol%. The type strain is strain 20186HG01 (= ATCC 25567).

A new, hydrogen sulfide-producing member of the *Enterobacteriaceae* was recently described and named *Budvicia aquatica* by Aldová et al. (2). However, this name was not validated prior to the publication of the present article. The 28 strains studied by Aldová et al. (2) were isolated from freshwater in Czechoslovakia. At the same time, one of us (C.R.) was independently studying a new bacterial group that was composed of six strains isolated from stream water in Sweden and one strain isolated from a shrew in Spain and provisionally named "atypical *Citrobacter*" (unpublished data). After an exchange of biochemical data and strains between the Institute of Hygiene and Epidemiology (IHE) and the Institut Pasteur (IP), it became obvious that both institutes were working on the same group of microorganisms.

The purpose of the present study was to delineate *Budvicia aquatica* sp. nov. by using deoxyribonucleic acid (DNA)-DNA hybridization and to describe formally the new genus and new species.

MATERIALS AND METHODS

Bacterial strains. The *B. aquatica* strains used in this study are listed in Table 1. Three biochemically atypical *Budvicia*-like strains isolated from water in Czechoslovakia and the reference strains used in the DNA relatedness study are listed in Table 2. The reference strains used have been described previously (3, 5, 11).

DNA-DNA hybridization. We used previously described procedures to extract, purify, and shear unlabeled DNAs (4). The exact procedures used for in vitro labeling of DNA with tritium-labeled nucleotides and for hybridization experiments (S1-Trichloroacetic acid method) have been described previously (13). The temperature at which 50% of the reassociated DNA became hydrolyzable by S1 nuclease was determined by the method of Crosa et al. (7). The divergence value was calculated from the difference in such temperatures between the heterologous DNA reaction and the homologous DNA reaction.

G+C content determination. The melting temperatures of 50- μ g/ml DNA solutions in 0.1 $\times$ SSC buffer (1 $\times$ SSC is 0.15

M NaCl plus 0.015 M trisodium citrate) were measured with a Gilford spectrophotometer. The guanine-plus-cytosine (G+C) contents of DNAs were determined from melting temperatures by the equation of Owen et al. (18). The DNA of *Escherichia coli* K-12 was used as a standard (G+C content, 51.2 mol%). In all cases, the DNAs to be tested and *Escherichia coli* K-12 DNA were dialyzed against the same buffer solution in the same flask to avoid any difference in salt concentration.

Biochemical tests. All tests done at the IP were incubated at 30°C unless otherwise stated. Tests done at the IHE were incubated at 37°C unless otherwise stated. The biochemical reactions of 27 strains studied at the IHE have been published previously (2), and the data were extended to a total of 53 strains (strains 20186HG01^T [T = type strain] to 23879 in Table 1) by using the same methods at the IHE. The biochemical reactions of eight strains (including strain 20186HG01^T) were checked at the IP to evaluate between-laboratory variation and to add a few tests that are not currently done elsewhere. The tests done at the IP included production of gas and H₂S in glucose-lactose-iron agar (Institut Pasteur Production, Marne-la-Coquette, France), urea hydrolysis and indole production from tryptophan in urea-indole medium (Institut Pasteur Production), film method for gelatin hydrolysis (17), the β -glucuronidase test (15), the γ -glutamyltransferase test (10), growth in tryptic soy broth (Institut Pasteur Production) at different temperatures, growth in peptone-water (Bacto-Peptone [Difco Laboratories, Detroit, Mich.], 10 g; distilled water, 1 liter; pH 7) containing 0, 2, 6, or 8% (wt/vol) NaCl, and the Voges-Proskauer test using the modification of Richard (20). All other tests performed at the IP were done by using procedures described elsewhere (12).

Growth factor requirements. Attempts to determine growth factor requirements were done by using M70 minimal medium without agar (21) dispensed into tubes (5 ml/tube) adaptable to the Klett-Summerson colorimeter (Arthur H. Thomas Co., Philadelphia, Pa.) and supplemented with 0.2% (wt/vol) glucose and various combinations of growth factors in the concentrations recommended by Clowes and Hayes (6). The tubes were inoculated with about 150 μ l of a bacterial suspension (80 Klett units) in distilled water.

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TABLE 1. Reassociation of DNA from *B. aquatica* 23227HG26 with DNAs from other *B. aquatica* strains

Source of unlabeled DNA	Source of strain ^a	% Relative binding at 60°C
20186HG01 ^T	Well water, Bílá (FM)	99
21297HG02	Water, České Budějovice	96
21800HG03	Water, Česlý Krumlov	99
21801HG04	Water, Pelhřimov	93
21845HG05	Well water, Bohumilice (PR)	92
21927HG06	Well water, Dobšice (CB)	77
21929	Well water (PR)	91
21930HG07	Well water, Bohumilice (PR)	93
21998HG08	Water (PE)	90
21999HG09	Water (PE)	94
22176HG10	Water (CB)	94
22414HG11	Well water, Šebanov (CK)	75
22415HG12	Well water, Řehonice (PR)	71 (0.7) ^b
22416HG13	Water conduit, Slavče u Trhových Svinů (CB)	80
22529HG14	Water conduit, Svatý Jan n/Malší (CB)	91
22530HG15	Well water, Přísečná (CK)	85
23012HG17	Water conduit, Lipí (CB)	84
23013HG18	Water conduit, Míčovice (PR)	87
23027HG19	Water conduit, Přídolí (CK)	92
23149HG20	Well water, Lčovice (PR)	81
23166HG21	Well water, Horní Ždár (JH)	78
23167HG22	Water conduit, Stachy (PR)	79
23168HG23	Water (PR)	96
23169HG24	Brook water, Dolní Dvořiště (CK)	92
23170HG25	Malše River, Pořešín (CK)	84
23227HG26	Swimming pool, Lipiny (PB)	100 (0.0)
23236HG27	Public well, Rodvínov (JH)	97
23237HG28	Public well, Jindřichův Hradec district	92
23269	Tributary of a swimming pool, Příbram	97
23270	Tributary of a swimming pool, Zálány (PB)	83
23271	Tributary of a swimming pool, Luhy (PB)	87
23272	Well water, Bukovany (PB)	86
23273	Well water, Bukovany (PB)	76 (1.1)
23274	Public well, Orlov (PB)	81
23275	Well water, Skuhrov (PB)	91
23302	Water conduit, Svatý Jan n/Malší (CB)	77 (1.6)
23303	Water conduit, Nový Svět (PR)	92
23367	Well water, Čenkov (PB)	89
23436	Well water, Přílepy (PI)	82
23471	Stěžocký Brook, Radětice (PB)	96
23506	Well water, Boubská (PR)	86
23516	Besina Brook, Besina (PB)	87
23640	Water conduit, Želnavá (PR)	91
23641	Water conduit, Stachy (PR)	94
23674	Well water, Milín (PB)	79
23677	Water conduit, Libněč (CB)	82
23678	Public well, Žárová (PR)	88
23681	Skalice river, Mirovice (PI)	91
23706	Well water, Plav (CB)	93
23777	Water conduit, Rožmberk n/Vltavou (CK)	92
23795	Pool water, Libice (PB)	89
23878	Well water, Klínek (PW)	92
23879	Swimming pool, Březnice (PB)	97
3-82	Stream water, Gruvsjön, Sweden ^c	77 (2.0)
5-82	Stream water, Gruvsjön, Sweden	81
6-82	Stream water, Gruvsjön, Sweden	87
7-82	Stream water, Gruvsjön, Sweden	79
8-82	Stream water, Gruvsjön, Sweden	76 (1.0)
9-82	Stream water, Gruvsjön, Sweden	89
5-81	Digestive tract of <i>Sorex araneus</i> , Spain ^d	86 (2.0)

^a Strains were isolated in Czechoslovakia unless otherwise stated. The Czechoslovakian districts, indicated by letters in parentheses, are as follows: CB, České Budějovice; CK, Český Krumlov; FM, Frýdek-Místek; JH,

Assimilation tests. Carbon source utilization tests were done at the IP by using specially manufactured API strips (API System, La Balme les Grottes, France) which contained pure carbon sources and were similar to commercial API CH, API AO, and API AA galleries (9), except that the total number of tests were limited to 99 carbon sources (these compounds are listed below). The minimal medium without growth factors (supplied by API System) was supplemented with the following 31 growth factors (final concentration in micrograms per milliliter): biotin and thiamine, 0.002; *para*-aminobenzoic acid, folic acid, nicotinamide, nicotinic acid, pantothenic acid, and pyridoxine, 0.08; riboflavin, 0.4; inositol, 0.8; sodium thiosulfate, 8; and L-alanine, L-arginine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, histidine, isoleucine, L-leucine, L-lysine, methionine, ornithine, L-phenylalanine, proline, serine, threonine, L-tryptophan, L-tyrosine, and L-valine, 17. A total of 16 strains (strains 20186HG01^T, 21999HG09, 22529HG14, 23013HG13, 23027HG19, 23168HG23, 23227HG26, 23275, 23471, 23677, 23678, 23706, 23795, 23878, 3-82, and 5-82) were tested. A 2-ml portion of a calibrated (80-Klett unit) bacterial suspension in sterile distilled water was added to 60 ml of growth factor-supplemented minimal medium. The mixture was distributed into API cupules. The strips were incubated at 30°C and examined for growth daily for 6 days.

Production of enterobacterial common antigen. Production of enterobacterial common antigen by strain 23227HG26 was studied by using a previously described hemagglutination test (16).

RESULTS

DNA-DNA hybridization. The DNA relatedness results obtained with labeled reference DNA from strain 23227HG26 and unlabeled DNAs from 60 strains of *B. aquatica* are shown in Table 1. Although the type strain was not used for the labeled DNA preparation, hybridization showed the type strain and strain 23227HG26 to be related at the 99% level. The DNA relatedness results obtained with the same labeled DNA and DNAs from 74 other representative strains of the family *Enterobacteriaceae* are shown in Table 2. The S1 nuclease-resistant core (in the incubated control tubes containing only denatured labeled DNA) was calculated to be $6 \pm 1\%$ (mean $\pm$ standard deviation). At 60°C, the *B. aquatica* strains were $87.2 \pm 7.6\%$ related to strain 23227HG26.

Atypical *Budvidia*-like strains 20125HG, 23635, and 23679 were 9 to 22% related to strain 23227HG26.

All other species tested were 0 to 8% related to strain 23227HG26. The divergence values between *B. aquatica* strains and strain 23227HG26 were less than 2°C.

DNA base composition. The average G+C content of the DNAs of seven strains were 46 ± 1 mol%.

Phenotypic characterization. Characteristics that were common to 95 to 100% of the strains are given below in the species description. The enterobacterial common antigen was detected in strain 23227HG26. In semisolid nutrient agar, 91% of the strains showed motility in 2 days at 22°C (44% of the strains showed motility at 37°C), and 5% of the

Jidřichův Hradec; PB, Příbram; PE, Pelhřimov; PI, Písek; RP, Prachatic; PW, Prague-West.

^b The values in parentheses are thermal stabilities of heteroduplexes (expressed in degrees Celsius).

^c Strains from Sweden were received from Alasdair H. Neilson.

^d Received from Guillermo Prats Pastor.

TABLE 2. Reassociation of DNA from *B. aquatica* 23227HG26 with DNAs from other members of the *Enterobacteriaceae*

Source of unlabeled DNA ^a	% Relative binding at 60°C
<i>Budvicia</i> -like strain 20125HG	9
<i>Budvicia</i> -like strain 23635	22
<i>Budvicia</i> -like strain 23679	16
<i>Cedecea davisae</i> 005 ^T (= CDC 3278-77 ^T)	1
<i>Cedecea</i> sp. strain 001 (= CDC 4853-73)	2
<i>Citrobacter amalonaticus</i> 25406	0
<i>Citrobacter diversus</i> 1066-71	1
<i>Citrobacter freundii</i> 460-61	3
<i>Edwardsiella tarda</i> NCTC 10396 ^T	3
<i>Edwardsiella tarda</i> (atypical) strain 389-67	3
<i>Edwardsiella hoshinae</i> 2-78 ^T	1
<i>Edwardsiella ictaluri</i> 1976-78 ^T	1
<i>Enterobacter aerogenes</i> Al ^T (= ATCC 13048 ^T)	1
<i>Enterobacter agglomerans</i> E20 ^T (= NCTC 9381 ^T)	3
<i>Enterobacter agglomerans</i> II 3123-70	0
<i>Enterobacter agglomerans</i> III 1429-71	3
<i>Enterobacter agglomerans</i> IV 1741-71	2
<i>Enterobacter agglomerans</i> V 3482-71	4
<i>Enterobacter agglomerans</i> VI 6070-69	1
<i>Enterobacter agglomerans</i> VII 6003-71	2
<i>Enterobacter agglomerans</i> VIII 5422-69	0
<i>Enterobacter agglomerans</i> IX 4388-71	0
<i>Enterobacter agglomerans</i> X 1600-71	5
<i>Enterobacter agglomerans</i> XI 5378-71	0
<i>Enterobacter agglomerans</i> XII 219-71	4
<i>Enterobacter agglomerans</i> XIII 1645-71	1
<i>Enterobacter amnigenus</i> 7879	3
<i>Enterobacter cloacae</i> 1347-71	0
<i>Enterobacter gergoviae</i> 2-78	2
<i>Enterobacter intermedium</i> 77-127 (= ATCC 33422)	1
<i>Enterobacter sakazakii</i> 4562-70 (= ATCC 29544)	1
<i>Erwinia amylovora</i> EA178	3
<i>Erwinia carnegieana</i> EC186	2
<i>Erwinia carotovora</i> 495	2
<i>Erwinia chrysanthemi</i> SR32	3
<i>Erwinia cypripedii</i> EC155	4
<i>Erwinia mallotivora</i> 2851 ^T	1
<i>Erwinia nigrifluens</i> EN104	2
<i>Erwinia rhapontici</i> ER106	2
<i>Erwinia rubrifaciens</i> ER105	2
<i>Erwinia salicis</i> ES102	1
<i>Escherichia blattae</i> 9005-74 ^T (= ATCC 29907 ^T)	1
<i>Escherichia coli</i> K-12	1
<i>Ewingella americana</i> 1468-78 ^T (= ATCC 33852 ^T)	4
<i>Hafnia alvei</i> 329-73	2
<i>Klebsiella oxytoca</i> 13182 ^T	0
<i>Klebsiella pneumoniae</i> 2	0
<i>Klebsiella</i> sp. strain 4241-72	1
<i>Kluyvera ascorbata</i> 1-79 (= ATCC 14236)	1
<i>Kluyvera cryocrescens</i> 2-79 (= ATCC 14239)	2
<i>Morganella morganii</i> 25830 ^T	4
<i>Obesumbacterium proteus</i> 4302-74	0
<i>Proteus mirabilis</i> PR14	2
<i>Proteus myxofaciens</i> 19692 ^T	0
<i>Proteus rettgeri</i> 1163 (= ATCC 29944)	0
<i>Proteus vulgaris</i> PR1	0
<i>Providencia stuartii</i> 2896-68 ^T (= ATCC 29914 ^T)	1
<i>Rahnella aquatilis</i> 77-115 ^T (= ATCC 33071 ^T)	2
<i>Salmonella typhimurium</i> LT2	1
<i>Serratia grimesii</i> 3692	3
<i>Serratia grimesii</i> 503 ^T (= ATCC 14460 ^T)	3
<i>Serratia marcescens</i> 504 ^T (= ATCC 13880 ^T)	3
<i>Serratia odorifera</i> 1073 ^T (= ATCC 33077 ^T)	5
<i>Shigella boydii</i> 1610-55	1
<i>Tatumella ptyseos</i> H3	3
<i>Xenorhabdus luminescens</i> Hb ^T	5
<i>Xenorhabdus luminescens</i> NC-19	8

TABLE 2—Continued

Source of unlabeled DNA ^a	% Relative binding at 60°C
<i>Xenorhabdus</i> sp. strain 286	8
<i>Xenorhabdus nematophilus</i> CCEB 786 ^T	5
<i>Yersinia enterocolitica</i> 614	1
<i>Yersinia frederiksenii</i> CIP 8029	5
<i>Yersinia intermedia</i> CIP 8028	6
<i>Yersinia kristensenii</i> CIP 8030	5
<i>Yersinia pseudotuberculosis</i> P105	2
<i>Yersinia ruckeri</i> 4535-69	1

^a ATCC, American Type Culture Collection, Rockville, Md.; CCEB, Culture Collection of Entomopathogenic Bacteria, Prague, Czechoslovakia; CDC, Centers for Disease Control, Atlanta, Ga.; NCTC, National Collection of Type Cultures, London, England.

strains showed motility in 3 to 20 days at 22°C (11% of the strains showed motility in 3 to 20 days at 37°C). The methyl red test yielded variable results. At the IHE, 22 and 31% of the strains gave positive methyl red tests at 37 and 22°C, respectively. At the IP, 50% of the strains gave positive methyl red tests at 30°C. No hydrolysis of gelatin was detected at the IHE (nutrient gelatin method), whereas seven strains tested at the IP hydrolyzed gelatin as determined by the film method in 1 to 5 days. Gas was not produced from glucose in triple-sugar-iron agar (IHE) or glucose-lactose-iron agar (IP), but weak production of gas was detected in Durham tubes with peptone-water and glucose with some strains (five of eight tested at the IP). Production of acid from lactose was also variable. At the IHE, 9% of the strains produced acid from lactose in 2 days, and 67% produced acid in 3 to 20 days. At the IP (eight strains checked), five strains produced acid from lactose in 2 days, and seven strains produced acid in 3 to 6 days. Acid was produced from rhamnose by 79% of the strains in 2 days (IHE) and 18% of the strains in 3 to 20 days. The eight strains tested at the IP produced acid from rhamnose. *o*-Nitrophenyl-β-D-galactopyranoside was hydrolyzed by 98% of the strains tested at the IHE and by all eight strains tested at the IP.

The growth factor requirements were found to be complex. An absolute requirement for nicotinic acid was observed. In tubes, moderate growth (23 Klett units after 2 days) was obtained when M70 minimal medium containing glucose was supplemented with nicotinic acid (0.08 µg/ml) and L-cysteine, threonine, aspartic acid, and glycine (17 µg/ml each). No single vitamin or amino acid added to the growth factor mixture described above improved growth significantly. However, good growth (52 Klett units after 2 days) was obtained when M70 minimal medium containing glucose was supplemented with the 31 growth factors listed above (factors used in assimilation tests).

The 16 strains submitted to assimilation tests utilized 29 substrates and failed to utilize 54 substrates (see below). Variable results were obtained with the following 16 substrates (the numbers of strains positive are given in parentheses): citrate (15), L-proline (15), lactose (12), D-fructose (11), L-tyrosine (10), glycine (8), D-melibiose (2), betaine (2), *trans*-aconitate (2), adonitol (1), D-malate (1), xylitol (1), D-lyxose (1), L-tartrate (1), acetate (1), and propionate (1).

DISCUSSION

The strains of *B. aquatica* have properties (2) fitting the description of the family *Enterobacteriaceae* (8). Production

of the enterobacterial common antigen is a confirmatory test of family assignment (16, 19).

The G+C content of *B. aquatica* DNA is on the low end of the range reported for the *Enterobacteriaceae* (38 to 60 mol%). This G+C content was reported earlier as 51 to 54 mol% (2). However, no control DNA was included in these tests (E.A.). In all of the G+C determinations described in the present paper, the *B. aquatica* DNA melting curves started and ended at a lower temperature than the *Escherichia coli* melting curves did, and the calculated thermal denaturation values for *B. aquatica* (all experiments and all DNAs tested) were lower than the *Escherichia coli* K-12 thermal denaturation values.

The DNA relatedness experiments showed that the 60 strains of *B. aquatica* form a single DNA hybridization group clearly distinct from 74 known DNA relatedness groups in the family *Enterobacteriaceae*. Thus, *B. aquatica* is a good genomic species. Apart from *Budvicia*-like strains 2012SHG, 23635, and 23679, the genus most closely related to *B. aquatica* is *Xenorhabdus*. The 8% homology (S1 method) between *B. aquatica* and *Xenorhabdus* spp. would probably correspond to about 28% homology if the hydroxyapatite method were used (13). However, there is too little phenotypic relatedness, if any, between *Budvicia* (2) and *Xenorhabdus* (1, 14) to think of transferring *Budvicia* to the genus *Xenorhabdus*. Thus, the results of DNA relatedness studies support the designation of a new genus, *Budvicia*. In addition, this genomic group possesses biochemical characteristics which allow unambiguous distinction from other genera and species in the *Enterobacteriaceae* (2).

A practical reason to recognize *B. aquatica* as an environmental bacterial species is that it might be misidentified as a *Yersinia* (strains from Sweden were received as H₂S-positive *Yersinia*), *Citrobacter*, or possibly *Salmonella* or *Edwardsiella* species.

To recognize the priority of three authors in the delineation of the new genus and species, we propose the following citations of names: *Budvicia* Aldová, Hausner, and Gabrhelová in Bouvet, Grimont, Richard, Aldová, Hausner, and Gabrhelová 1985; and *Budvicia aquatica* Aldová, Hausner, and Gabrhelová in Bouvet, Grimont, Richard, Aldová, Hausner, and Gabrhelová 1985.

Description of *Budvicia* gen. nov. *Budvicia* (Bud. vi' ci. a. L. fem. n. *Budvicia* derived from Budvicium, the Latin name of the city České Budějovice, where the bacterium was first isolated) cells are gram-negative, nonsporeforming, non-encapsulated, straight rods with peritrichous flagella (when grown at 22°C). Members of the genus conform to the definition of the family *Enterobacteriaceae* (8). Facultatively anaerobic; glucose fermenters; nitrate reducers. Oxidase test negative. Small colonies are produced on nutrient agar. Growth occurs between 4 and 37°C, but not at 42°C. No growth occurs in KCN medium. H₂S produced; urea hydrolyzed. Indole not produced. Tryptophan and phenylalanine not deaminated. Lysine, arginine, and ornithine not decarboxylated. Voges-Proskauer test negative. Acid not produced from maltose, D-mannose, glycerol, and trehalose. Esculin not hydrolyzed. Glucuronate test negative. *o*-Nitrophenyl-β-D-galactopyranoside hydrolyzed. Extracellular enzymes (lipase, Tween esterase, amylase, deoxyribonuclease, gelatinase [tube method]) not produced (in 6 days) or weakly produced (gelatinase [film method]). No growth on Simmons citrate or Trabulsi acetate. Growth factor requirements are complex. The enterobacterial common antigen is produced. The G+C content of the DNA is about 46 mol%. The type species is *Budvicia aquatica*.

Description of *Budvicia aquatica* sp. nov. *Budvicia aquatica* (a. qua' ti. ca. L. fem. adj. *aquatica* living in water) cells are gram-negative, nonsporeforming, non-encapsulated, straight rods with peritrichous flagella (when grown at 22°C). Nonmotile strains occur. The species conforms to the definition of the *Enterobacteriaceae* (8).

All strains grow on nutrient agar, producing small colonies about 0.1 mm in diameter after 24 or 48 h at 37°C or about 0.5 mm in diameter after 24 h and 1.5 mm in diameter after 48 h at 30°C. The colonies are translucent and have smooth, entire edges. Facultatively anaerobic. All strains grow in 1 day at 4, 10, 30 or 37°C in tryptic soy broth and at 30°C in peptone-water containing 0 to 4% NaCl. Growth in peptone-water containing 6% NaCl occurs in 2 days. No growth at 42°C (in either tryptic soy broth or nutrient broth). Growth occurs on MacConkey agar.

Catalase produced. Oxidase not produced. Nitrates reduced to nitrites. No growth occurs in KCN medium. Tetrathionate reduced. H₂S produced in triple-sugar-iron agar or in glucose-lactose-iron agar. Urea hydrolyzed in urea-indole medium and Christensen medium. *o*-Nitrophenyl-β-D-galactopyranoside hydrolyzed. γ-Glutamyl-transferase test positive; β-glucuronidase test negative. Indole not produced in peptone-water or urea-indole medium. Tryptophan and phenylalanine not deaminated. Lysine, arginine, and ornithine not decarboxylated (Møller medium). Tributyrin, Tween 80, starch, and DNA not hydrolyzed within 6 days. Gelatin slowly hydrolyzed (film method, 2 to 5 days) or not hydrolyzed (tube method). Glucose fermented with little or no production of gas. Voges-Proskauer test negative (22, 30, or 37°C). Methyl red test positive or negative. Acid produced (in 2 days) in peptone-water from glucose, L-arabinose, galactose, galacturonate, xylose, and mucate. Most strains produce acid from rhamnose. Production of acid from D-mannitol is delayed (1 to 3 days in peptone-water at the IP) and may depend upon the method used (no acid was produced from D-mannitol in tests done at the IHE). Acid not produced (in 2 days) from adonitol, cellobiose, dulcitol, inositol, maltose, mannose, glycerol, raffinose, salicin, sorbitol, sorbose, sucrose, trehalose, D-melezitose, and D-melibiose. Acid produced (1 to 4 days) or not produced from lactose. β-Xylosidase test positive or negative. Esculin not hydrolyzed. Glucuronate test negative.

No growth on Simmons citrate or Trabulsi acetate. Growth factor requirements are complex; nicotinic acid is absolutely required, and a combination of amino acids (including L-cysteine, threonine, aspartic acid, and glycine) is necessary to yield appreciable growth.

The following compounds are used as sole carbon and energy sources (in a minimal medium supplemented with 31 growth factors): *N*-acetylglucosamine, L-arabinose, D-arabitol, L-aspartate, *meso*-erythritol, fumarate, galactose, galacturonate, gluconate, D-glucose, glucuronate, L-glutamate, DL-glycerate, glycerol, 2-ketogluconate, 5-ketogluconate, DL-lactate, L-malate, D-mannitol, mucate, 2-oxoglutarate, pyruvate, rhamnose, ribose, L-serine, succinate, D-tartrate, *meso*-tartrate, and D-xylose. The following compounds are not used as sole carbon and energy sources: D-alanine, L-alanine, β-alanine, 2-aminobenzoate, DL-4-aminobutyrate, DL-5-aminovalerate, L-arabitol, benzoate, butyrate, caprate, caprylate, D-cellobiose, dulcitol, esculin, ethanolamine, L-fucose, β-gentiobiose, glucosamine, glutarate, heptanoate, histamine, L-histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, DL-3-hydroxybutyrate, *myo*-inositol, itaconate, malonate, maltitol, maltose, D-mannose, D-melezitose, L-methionine, α-methyl-D-glucoside, 3-methyl-D-glucose, β-

methyl-D-glucoside, α -methyl-D-mannoside, phenylacetate, putrescine, quinate, D-raffinose, salicin, sarcosine, sorbitol, L-sorbose, sucrose, D-tagatose, trehalose, trigonelline, tryptamine, D-tryptophan, L-tryptophan, and D-turanose. The G+C content of the DNA is 46 mol%.

Isolated mostly from water (drinking or river water) and also from wild small mammals. No strain has yet been identified as involved in a human, animal, or plant disease.

The type strain is strain 20186HG01 (= ATCC 25567).

Description of the type strain. Strain 20186HG01^T has all of the characteristics given above for the species. In addition, this strain is motile and β -xylosidase positive, hydrolyzes gelatin (film method) in 3 days, produces a small amount of gas from glucose in peptone-water with a Durham tube, and produces acid from rhamnose (in 1 day), lactose (in 2 days), and D-mannitol (in 2 days). The following substrates are utilized by strain 20186HG01^T (API strips with carbon sources; minimal medium supplemented with 31 growth factors): D-fructose, lactose, D-lyxose, citrate, glycine, L-tyrosine, L-proline, and propionate. The following substrates are not utilized: adonitol, D-malate, *trans*-aconitate, betaine, L-tartrate, and acetate.

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LITERATURE CITED

- Akhurst, R. J. 1983. Taxonomic study of *Xenorhabdus*, a genus of bacteria symbiotically associated with insect-pathogenic nematodes. *Int. J. Syst. Bacteriol.* **33**:38–45.
- Aldová, E., O. Hausner, M. Gabrhelová, J. Schindler, P. Petrás, and H. Braná. 1983. A hydrogen sulfide producing gram-negative rod from water. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A* **254**:95–108.
- Brenner, D. J., J. J. Farmer III, G. R. Fanning, A. G. Steigerwalt, P. Klykken, H. G. Wathen, F. W. Hickman, and W. H. Ewing. 1978. Deoxyribonucleic acid relatedness of *Proteus* and *Providencia* species. *Int. J. Syst. Bacteriol.* **28**:269–282.
- Brenner, D. J., A. C. McWhorter, J. K. Leete-Knudson, and A. G. Steigerwalt. 1982. *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. *J. Clin. Microbiol.* **15**:1133–1140.
- Brenner, D. J., A. G. Steigerwalt, P. D. Falcao, R. E. Weaver, and G. R. Fanning. 1976. Characterization of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* by deoxyribonucleic acid hybridization and by biochemical reactions. *Int. J. Syst. Bacteriol.* **26**:180–194.
- Clowes, R. C., and W. Hayes. 1968. Experiments in microbial genetics. Blackwell Scientific Publications, Oxford.
- Crosa, J. H., D. J. Brenner, and S. Falkow. 1973. Use of a single-strand-specific nuclease for analysis of bacterial and plasmid deoxyribonucleic acid homo- and heteroduplexes. *J. Bacteriol.* **115**:904–911.
- Edwards, P. R., and W. H. Ewing. 1972. Identification of *Enterobacteriaceae*, 3rd ed. Burgess Publishing Co., Minneapolis.
- Gavini, F., D. Izard, H. Leclerc, M. Desmonceaux, and J. P. Gayral. 1980. Carbon source assimilation tests: comparison between a conventional method and a microtechnic (API), in study of *Enterobacteriaceae*. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe C* **1**:182–187.
- Giammanco, G., J. Buissière, M. Toucas, G. Brault, and L. Le Minor. 1980. Intérêt taxonomique de la recherche de la γ -glutamyltransférase chez les *Enterobacteriaceae*. *Ann. Microbiol. (Paris)* **131A**:181–187.
- Grimont, P. A. D., J. J. Farmer III, F. Grimont, M. A. Asbury, D. J. Brenner, and C. Deval. 1983. *Ewingella americana* gen. nov., sp. nov., a new *Enterobacteriaceae* isolated from clinical specimens. *Ann. Microbiol. (Paris)* **134A**:39–52.
- Grimont, P. A. D., F. Grimont, H. L. C. Dulong de Rosnay, and P. H. A. Sneath. 1977. Taxonomy of the genus *Serratia*. *J. Gen. Microbiol.* **98**:39–66.
- Grimont, P. A. D., M. Y. Popoff, F. Grimont, C. Coynault, and M. Lemelin. 1980. Reproducibility and correlation study of three deoxyribonucleic acid hybridization procedures. *Curr. Microbiol.* **4**:325–330.
- Grimont, P. A. D., A. G. Steigerwalt, N. Boemare, F. W. Hickman-Brenner, C. Deval, F. Grimont, and D. J. Brenner. 1984. Deoxyribonucleic acid relatedness and phenotypic study of the genus *Xenorhabdus*. *Int. J. Syst. Bacteriol.* **34**:378–388.
- Le Minor, L., J. Buissière, G. Novel, and M. Novel. 1978. Relation entre le sérotype et l'activité β -glucuronidase chez les *Salmonella*. *Ann. Microbiol. (Paris)* **129B**:155–165.
- Le Minor, L., A.-M. Chalon, and M. Véron. 1972. Recherche sur la présence de l'antigène commun des *Enterobacteriaceae* (antigène Kunin) chez les *Yersinia*, *Levinea*, *Aeromonas* et *Vibrio*. *Ann. Inst. Pasteur Paris* **123**:761–774.
- Le Minor, L., and M. Piéchaud. 1963. Note technique. Une méthode rapide de recherche de la protéolyse de la gélatine. *Ann. Inst. Pasteur Paris* **105**:792–794.
- Owen, R. J., L. R. Hill, and S. P. Lapage. 1969. Determination of DNA base compositions from melting profiles in dilute buffers. *Biopolymers* **7**:503–516.
- Ramias, S., E. Neter, and D. J. Brenner. 1982. Production of enterobacterial common antigen as an aid to classification of newly identified species of the families *Enterobacteriaceae* and *Vibrionaceae*. *Int. J. Syst. Bacteriol.* **32**:395–398.
- Richard, C. 1972. Méthodes rapides pour l'étude des réactions de rouge de méthyle et Voges-Proskauer. *Ann. Inst. Pasteur Paris* **122**:979–986.
- Véron, M. 1975. Nutrition et taxonomie des entérobactéries. I. Méthode d'étude des auxanogrammes. *Ann. Microbiol. (Paris)* **126A**:267–274.