Emended Description of *Buttiauxella agrestis* with Recognition of Six New Species of *Buttiauxella* and Two New Species of *Kluyvera*: *Buttiauxella ferragutiae* sp. nov., *Buttiauxella gaviniae* sp. nov., *Buttiauxella brennerae* sp. nov., *Buttiauxella izardii* sp. nov., *Buttiauxella noackiae* sp. nov., *Buttiauxella warmboldiae* sp. nov., *Kluyvera cochleae* sp. nov., and *Kluyvera georgiana* sp. nov.

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A total of 219 strains belonging to the genera *Buttiauxella* and *Kluyvera* were studied; 171 of these strains were isolated from mollusks, mainly snails and slugs, obtained from around the world. On the basis of DNA-DNA hybridization data, the strains were grouped into 11 genomospecies. A total of 44 phenotypic characters were used to differentiate the genera *Buttiauxella* and *Kluyvera* at the genus level and to identify genomospecies. There were significantly higher phenotypic probability distances between the genomospecies in the genus *Buttiauxella* and the genomospecies in the genus *Kluyvera* than between the genomospecies in the same genus. Therefore, the existence of *Buttiauxella* and *Kluyvera* as different genera was confirmed. The existence of new species necessitated broadening the definitions of both genera. In two cases, two *Buttiauxella* species could not be quantitatively differentiated biochemically, and several other pairs of species could be separated only by the results of one biochemical test. Nonetheless, combinations of several characteristics were used to differentiate all of the species with levels of certainty ranging from log 10.79 to log 57.77 (calculated as probability distances). The following new species are proposed: *Buttiauxella ferragutiae* (type strain, ATCC 51602 [DSM 9390]), *Buttiauxella gaviniae* (type strain, ATCC 51604 [DSM 9393]), *Buttiauxella brennerae* (type strain, ATCC 51605 [DSM 9396]), *Buttiauxella izardii* (type strain, ATCC 51606 [DSM 9397]), *Buttiauxella noackiae* (type strain, ATCC 51607 [DSM 9401]), *Buttiauxella warmboldiae* (type strain, ATCC 51608 [DSM 9404]), *Kluyvera cochleae* (type strain, ATCC 51609 [DSM 9406]), and *Kluyvera georgiana* (type strain, ATCC 51603 [DSM 9409]).

In 1976, a taxon designated group F was described by Gavini et al. (15). The strains belonging to group F were originally isolated from humans, soil, and water in the course of a study on the genus *Citrobacter*, and they were lactose positive, as are members of the genus *Citrobacter*. However, these organisms differed from *Citrobacter* strains in several biochemical characteristics, as well as in their DNA-DNA-hybridization characteristics and guanine-plus-cytosine (G+C) ratios (13, 14). Hence, the 17 strains were considered members of a new genus in the family *Enterobacteriaceae*. This genus was named *Buttiauxella* and contained a single species *Buttiauxella agrestis*. Four strains subsequently studied by Ferragut et al. were placed in the genus *Buttiauxella* but were not *B. agrestis* strains (13). At the Centers for Disease Control and Prevention, three of these strains were placed in a group that was given the vernacular name enteric group 63, and the fourth was placed in enteric group 64 (10).

Also in the Centers for Disease Control and Prevention

culture collection were more than 100 strains that were placed in a group designated enteric group 8. In 1981, Farmer et al. redefined the genus *Kluyvera* to include enteric group 8 along the named species *Kluyvera ascorbata* and *Kluyvera cryocrescens* and a third unnamed species (11). The genera *Buttiauxella* and *Kluyvera* are generally similar phenotypically, although they can be differentiated. The members of these genera have different G+C contents and exhibit low levels of DNA relatedness (14).

The frequent and profuse occurrence of *Buttiauxella* and *Kluyvera* strains in snails and slugs is an intriguing finding. Numerous strains belonging to these previously rarely seen genera have been isolated. However, the phenotypic characteristics that separate these genera did not allow clear assignment of these isolates at the genus level. The biochemical reactions of the previously described species and the new isolates suggested that most of the new isolates belong to new species. In this paper we describe the results of a taxonomic study of *Buttiauxella* and *Kluyvera* strains.

MATERIALS AND METHODS

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Bacterial strains. The *Buttiauxella* and *Kluyvera* strains used in this study are listed in Table 1. The field strains were isolated from snails and slugs and samples

TABLE 1. Strains investigated in this study

Group	Serial no.	Strain	Laboratory(ies) ^a	Origin	Other designation(s) ^b
DNA group 1 (<i>B. agrestis</i>)	001 ^c	CDC 1176-81 ^T (= CUETM 77-167 ^T)	1, 4	Unpolluted soil, France ^d	ATCC 33320 ^T
	002	CUETM 77-157	4	Drinking water, France ^d	
	003	CUETM 78-3	4	Unpolluted soil, France ^d	
	004	CUETM 78-5	4	Unpolluted soil, France ^d	
	005	CUETM 78-8	4	Drinking water, France ^d	
	006	CUETM 78-18	4	Unpolluted soil, France ^d	
	007	CUETM 78-19	4	Drinking water, France ^d	
	008	CUETM 78-27	4	Drinking water, France ^d	
	009	CUETM 78-29	4	Drinking water, France ^d	ACTT 33994
	010	CUETM 78-34	4	Unpolluted soil, France ^d	ATCC 33995
	011	CUETM 78-44	4	Drinking water, France ^d	
	012 ^c	S3/3-162	3	Snail, Braunschweig, Germany	DSM 9388
	013 ^c	S3/3-203	3	Snail, Braunschweig, Germany	
	014	S3/4-204	3	Snail, Braunschweig, Germany	
	015	S3/4-556	3	Snail, Braunschweig, Germany	
	016	S3/4-775	3	Snail, Braunschweig, Germany	
	017 ^e	S3/4-776	3	Snail, Braunschweig, Germany	
	018 ^e	S3/4-779	3	Snail, Braunschweig, Germany	
	019	S3/4-871	3	Snail, Braunschweig, Germany	
	020	S3/4-1098	3	Slug, Braunschweig, Germany	
	021 ^c	S3/5-201	3	Snail, Braunschweig, Germany	
	022 ^e	S3/5-757	3	Snail, Braunschweig, Germany	
	023 ^c	S3/6-333	3	Slug, Braunschweig, Germany	DSM 9389
	024 ^c	S3/7-896	3	Slug, Braunschweig, Germany	
	025 ^c	S3/7-897	3	Snail, Braunschweig, Germany	
	026 ^c	S3/8-910	3	Slug, Salisbury, Great Britain	
	027	S3/9-488	3	Slug, Braunschweig, Germany	
	028	S3/9-602	3	Snail, Braunschweig, Germany	
	029 ^c	S3/9-878	3	Snail, Braunschweig, Germany	
	030 ^c	S3/9-887	3	Slug, Braunschweig, Germany	
	031 ^c	S3/10-769	3	Snail, Braunschweig, Germany	
	032 ^c	S3/11-533	3	Slug, Braunschweig, Germany	
	033 ^c	S3/11-794	3	Snail, Braunschweig, Germany	
	034	S3/11-832	3	Snail, Braunschweig, Germany	
	035	S3/11-859	3	Snail, Braunschweig, Germany	
	036	S3/14-770	3	Snail, Braunschweig, Germany	
	037 ^c	S3/14-1071	3	Slug, Braunschweig, Germany	
	038	S3/14-1073	3	Slug, Braunschweig, Germany	
	039	S3/14-1080	3	Slug, Braunschweig, Germany	
	040	S3/14-1081	3	Slug, Braunschweig, Germany	
	041	S3/14-1082	3	Slug, Braunschweig, Germany	
	042 ^c	S3/14-1085	3	Slug, Braunschweig, Germany	
	043	S3/14-1094	3	Slug, Braunschweig, Germany	
	044	S3/14-1096	3	Slug, Braunschweig, Germany	
	045	S3/14-1099	3	Slug, Braunschweig, Germany	
	046	S3/14-1101	3	Slug, Braunschweig, Germany	
	047	S3/14-1127	3	Slug, Braunschweig, Germany	
	048	S3/14-1129	3	Slug, Braunschweig, Germany	
	049	S3/14-1132	3	Slug, Braunschweig, Germany	
	050	S3/14-1146	3	Slug, Braunschweig, Germany	
	051	S3/14-1149	3	Slug, Braunschweig, Germany	
	052	S3/14-1166	3	Slug, Braunschweig, Germany	
	053 ^c	S3/13-692	3	Slug, Braunschweig, Germany	
DNA group 2 (enteric group 63, <i>B. ferruginae</i>)	054 ^c	CDC 1180-81 ^T (= CUETM 78-31 ^T)	1, 4	Unpolluted soil, France (13)	ATCC 51602 ^T , DSM 9390 ^T
	055	CUETM 78-50	4	Drinking water, France (13)	DSM 9391
	056	CUETM 78-35	4	Unpolluted soil, France (13)	
	057	CUETM "H. alvei 125"	4	Unknown, France	
DNA group 3 (enteric group 64, <i>B. gaviniae</i>)	058 ^c	CDC 1175-81 (= CUETM 77-159)	1, 4	Unpolluted soil, France (13)	DSM 9392
	059	S1/1-895	3	Slug, Braunschweig, Germany	
	060	S1/1-978	3	Snail, Braunschweig, Germany	
	061	S1/1-981	3	Snail, Braunschweig, Germany	
	062 ^c	S1/1-984 ^T	3	Snail, Braunschweig, Germany	ATCC 51604, DSM 9393
	063 ^c	S1/1-986	3	Snail, Braunschweig, Germany	
	064	S1/1-987	3	Snail, Braunschweig, Germany	
	065	S1/1-988	3	Snail, Braunschweig, Germany	
	066	S1/1-993	3	Snail, Braunschweig, Germany	

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TABLE 1—Continued

Group	Serial no.	Strain	Laboratory(ies) ^a	Origin	Other designation(s) ^b
	067	S1/1-996	3	Snail, Braunschweig, Germany	
	068	S1/1-998	3	Snail, Braunschweig, Germany	
	069	S1/1-999	3	Snail, Braunschweig, Germany	
	070	S1/1-1000	3	Snail, Braunschweig, Germany	
	071	S1/1-1003	3	Snail, Braunschweig, Germany	
	072	S1/1-1007	3	Snail, Braunschweig, Germany	
	073	S1/1-1032	3	Snail, Braunschweig, Germany	
	074	S1/1-1033	3	Snail, Braunschweig, Germany	
	075	S1/1-1039	3	Snail, Braunschweig, Germany	
	076	S1/1-1041	3	Snail, Braunschweig, Germany	
	077	S1/1-1049	3	Snail, Braunschweig, Germany	
	078	S1/1-1050	3	Snail, Braunschweig, Germany	
	079	S1/1-1051	3	Snail, Braunschweig, Germany	
	080	S1/1-1116	3	Slug, Braunschweig, Germany	
	081	S1/1-1118	3	Slug, Braunschweig, Germany	
	082	S1/1-1161	3	Slug, Braunschweig, Germany	
	083	S1/1-1162	3	Slug, Braunschweig, Germany	
	084	S1/2-992	3	Snail, Braunschweig, Germany	
	085 ^c	S1/2-994	3	Snail, Braunschweig, Germany	
	086	S1/2-1020	3	Snail, Braunschweig, Germany	
	087 ^c	S1/2-1111	3	Slug, Braunschweig, Germany	
	088 ^c	S1/3-1052	3	Snail, Braunschweig, Germany	
	089 ^c	S1/3-1200	3	Snail, Braunschweig, Germany	
	090 ^c	S1/4-695	3	Slug, Braunschweig, Germany	
	091	S1/5-778	3	Snail, Braunschweig, Germany	
	092	S1/5-791	3	Snail, Braunschweig, Germany	
	093 ^c	S1/5-857	3	Snail, Braunschweig, Germany	
	094 ^c	S1/5-860	3	Snail, Braunschweig, Germany	
	095 ^c	S1/7-674	3	Snail, Braunschweig, Germany	
	096 ^c	S1/7-1143	3	Slug, Braunschweig, Germany	
	097	S1/9-505	3	Slug, Braunschweig, Germany	
	098	S1/9-512	3	Slug, Braunschweig, Germany	
	099 ^c	S1/9-514	3	Slug, Braunschweig, Germany	
	100 ^c	S1/9-518	3	Slug, Braunschweig, Germany	
	101	S1/9-1177	3	Slug, Braunschweig, Germany	
	102	S1/10-549	3	Snail, Braunschweig, Germany	
	103 ^c	S1/10-801	3	Snail, Braunschweig, Germany	
	104 ^c	S1/10-853	3	Snail, Braunschweig, Germany	
	105 ^c	S1/10-863	3	Snail, Braunschweig, Germany	
	106	S1/10-1044	3	Snail, Braunschweig, Germany	
	107	S1/10-1179	3	Slug, Braunschweig, Germany	
	108 ^c	S1/11-577	3	Snail, Braunschweig, Germany	
	109	S1/11-630	3	Snail, Braunschweig, Germany	
	110 ^c	S1/11-707	3	Slug, Braunschweig, Germany	
	111	S1/11-787	3	Snail, Braunschweig, Germany	
	112	S1/11-893	3	Slug, Braunschweig, Germany	
	113 ^c	S1/12-1180	3	Slug, Braunschweig, Germany	
	114 ^c	S1/14-564	3	Snail, Braunschweig, Germany	
	115 ^c	S1/14-669	3	Snail, Braunschweig, Germany	
	116	S1/14-873	3	Snail, Braunschweig, Germany	
	117	S1/14-875	3	Snail, Braunschweig, Germany	
	118	S1/15-773	3	Snail, Braunschweig, Germany	
	119 ^c	S1/15-774	3	Snail, Braunschweig, Germany	
	120	S1/15-786	3	Snail, Braunschweig, Germany	
	121 ^c	S1/15-828	3	Snail, Braunschweig, Germany	
	122	S1/15-845	3	Snail, Braunschweig, Germany	
	123 ^c	S1/16-12	3	Slug, Braunschweig, Germany	
	124 ^c	S1/17-7	3	Slug, Braunschweig, Germany	
	125	S1/17-702	3	Slug, Braunschweig, Germany	
	126	S1/17-705	3	Slug, Braunschweig, Germany	
	127	S1/17-903	3	Slug, Salisbury, Great Britain	
	128	S1/17-906	3	Slug, Salisbury, Great Britain	
	129 ^c	S1/17-911	3	Slug, Salisbury, Great Britain	DSM 9394
	130 ^c	S1/18-662	3	Snail, Braunschweig, Germany	
	131	S1/18-665	3	Snail, Braunschweig, Germany	
	132 ^c	S1/18-784	3	Snail, Braunschweig, Germany	
	133	S1/19-388	3	Slug, Braunschweig, Germany	
	134 ^c	S1/19-661	3	Snail, Braunschweig, Germany	

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TABLE 1—Continued

Group	Serial no.	Strain	Laboratory(ies) ^a	Origin	Other designation(s) ^b
	135	S1/19-664	3	Snail, Braunschweig, Germany	
	136 ^c	S1/19-760	3	Snail, Braunschweig, Germany	
	137	S1/19-800	3	Snail, Braunschweig, Germany	
	138	S1/19-861	3	Snail, Braunschweig, Germany	
	139 ^c	EG59-like strain K 2258	3	Snail, Kaernten, Austria	
	140 ^c	EG59-like strain K 2286	3	Snail, Kaernten, Austria	
	141 ^c	EG59-like strain K 2340	3	Snail, Kaernten, Austria	
	142 ^c	EG59-like strain SA 1191	3	Snail, Cape Town, South Africa	
	143 ^c	EG59-like strain Z 2132	3	Snail, Zürich, Switzerland	DSM 9395
DNA group 4 (<i>B. brennerae</i>)	144 ^c	S1/6-360	3	Slug, Braunschweig, Germany	
	145 ^c	S1/6-571 ^T	3	Snail, Braunschweig, Germany	ATCC 51605 ^T DSM 9396 ^T
	146 ^c	S1/6-706	3	Slug, Braunschweig, Germany	DSM 9413
	147 ^c	S1/8-1022	3	Snail, Braunschweig, Germany	
	148 ^c	S1/8-783	3	Snail, Braunschweig, Germany	
	149 ^c	S3/8-879	3	Snail, Braunschweig, Germany	
	150 ^c	S3/12-695	3	Snail, Braunschweig, Germany	
DNA group 5 (<i>B. izardii</i>)	151 ^c	S3/2-161 ^T	3	Snail, Braunschweig, Germany	ATCC 51606 ^T , DSM 9397 ^T
	152 ^c	S3/2-199	3	Snail, Braunschweig, Germany	DSM 9398
	153	S3/2-525	3	Slug, Braunschweig, Germany	
	154	S3/2-589	3	Snail, Braunschweig, Germany	
	155	S3/2-917	3	Earthworm, Salisbury, Great Britain	
	156	S3/2-1113	3	Slug, Braunschweig, Germany	
	157	S3/2-1178	3	Slug, Braunschweig, Germany	
	158 ^c	S3/10-487	3	Slug, Braunschweig, Germany	
	159 ^c	S3/13-626	3	Snail, Braunschweig, Germany	
	160 ^c	EG59-like strain NSW 150	3	Snail, Sydney, Australia	
	161 ^c	EG59-like strain NSW 155	3	Snail, Sydney, Australia	DSM 9399
	162 ^c	EG59-like strain RA 144	3	Snail, Rabat, Morocco	DSM 9400
	163 ^c	EG59-like strain RA 151	3	Snail, Rabat, Morocco	
DNA group 6 (enteric group 59, <i>B. noackiae</i>)	164 ^c	EG59-like strain BR 489	3	Snail, Sao Paulo, Brazil	
	165 ^c	EG59-like strain BR 539	3	Snail, Sao Paulo, Brazil	
	166 ^c	EG59-like strain BR 588	3	Snail, Sao Paulo, Brazil	
	167 ^c	EG59-like strain BR 607	3	Snail, Sao Paulo, Brazil	
	168 ^c	EG59-like strain BR 626	3	Snail, Sao Paulo, Brazil	
	169 ^c	EG59-like strain BR 637	3	Snail, Sao Paulo, Brazil	
	170 ^c	EG59-like strain NSW11 ^T	3	Snail, Sydney, Australia	ATCC 51607 ^T , DSM 9401 ^T
	171 ^c	EG59-like strain NSW87	3	Snail, Sydney, Australia	
	172 ^c	EG59-like strain NSW 120	3	Snail, Sydney, Australia	
	173 ^c	EG59-like strain NSW 245	3	Snail, Sydney, Australia	
	174 ^c	EG59-like strain NSW 297	3	Snail, Sydney, Australia	
	175 ^c	EG59-like strain NSW 336	3	Snail, Sydney, Australia	
	176 ^c	EG59-like strain NSW 402	3	Snail, Sydney, Australia	
	177 ^c	EG59-like strain RA 116	3	Snail, Rabat, Morocco	DSM 9402
	178 ^c	EG59-like strain SA 1313	3	Snail, Cape Town, South Africa	
	179 ^c	CDC 1650-80 (= EG59)	1	Human clinical specimen ^d	DSM 9403
DNA group 7 (<i>B. warmboldiae</i>)	180 ^c	EG59-like strain NSW 184	3	Snail, Sydney, Australia	
	181 ^c	EG59-like strain NSW 253	3	Snail, Sydney, Australia	
	182 ^c	EG59-like strain NSW 326	3	Snail, Sydney, Australia	ATCC 51608 ^T , DSM 9404 ^T
	183 ^c	EG59-like strain NSW 393	3	Snail, Sydney, Australia	
	184 ^c	EG59-like strain SA 420	3	Snail, Cape Town, South Africa	DSM 9405
DNA group 8 (<i>K. cochleae</i>)	185 ^c	S3/1-49 ^T	3	Snail, Braunschweig, Germany	ATCC 51609 ^T , DSM 9406 ^T
	186 ^c	S3/1-913	3	Slug, Salisbury, Great Britain	DSM 9407
	187 ^c	S3/6-835	3	Snail, Braunschweig, Germany	
DNA group 9 (enteric group 36/37, <i>Kluyvera</i> species 3, <i>K. georgiana</i>)	188	CDC/CUETM 4246-74	1, 4	Human clinical specimen ^e	DSM 9408
	189 ^c	CDC 2891-76 ^T	1	Human sputum ^e	ATCC 51603 ^T , DSM 9409 ^T
	190	CDC/CUETM 2065-76	1, 4	Human clinical specimen ^e	
	191	CDC/CUETM 2774-70	1, 4	Human clinical specimen ^e	
	192	CDC/CUETM 3108-76	1, 4	Human clinical specimen ^e	
DNA group 10 (<i>K. ascorbata</i>)	193 ^c	CDC/CUETM 648/74 ^T	1, 4	Human sputum, North Carolina ^e	ATCC 33433 ^T
	194	CDC 4450-74	1	Unknown ^e	
	195	CDC 4417-74	1	Unknown ^e	
	196	CDC/CUETM 762-75	1, 4	Unknown ^e	
	197	CDC 3858-69	1	Unknown ^e	

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TABLE 1—Continued

Group	Serial no.	Strain	Laboratory(ies) ^a	Origin	Other designation(s) ^b
DNA group 11 (<i>K. cryocrescens</i>)	198	CDC 1220-73	1	Unknown ^c	
	199	CDC 972-78	1	Unknown ^c	
	200	CDC 2070-78	1	Unknown ^c	
	201	CUETM 1-79	4	Unknown	
	202	CDC 2064-78	1	Unknown ^c	
	203	CDC 2589-70	1	Unknown ^c	
	204	CDC 2567-61	1	Sewage	ATCC 14236
	205	CDC 2221-78	1	Human (?), New York ^c	ATCC 33434
	206	CUETM 9-79	4	Unknown	
	207	CUETM 20-74	4	Unknown	
	208	CUETM 14-79	4	Unknown	
	209	CUETM 3-79	4	Unknown	
	210	CDC/CUETM 1734-74	1, 4	Unknown ^c	
	211	CDC/CUETM 1396-73	1, 4	Unknown ^c	
	212	CUETM 2-79	4	Unknown	
	213	CDC 410-78	1	Unknown ^c	
	214 ^e	CDC 2065-78 ^T	1	Kitchen food, Persian Gulf ^f	ATCC 33435 ^T
	215	CDC 2568-61	1	Unknown	ATCC 14237
	216	CDC 2569-61	1	Unknown	ATCC 14238
	217	BU 005 12810484	2	Unknown	DSM 9410
	218	BU 009 33728683	2	Unknown	DSM 9411
	219	BU 010 14812T	2	Unknown	DSM 9412

^a 1, Centers for Disease Control and Prevention, Atlanta, Ga., 2, Fachgebiet Hygiene, Technische Universität, Berlin, Germany; 3, Staatliches Medizinaluntersuchungsamt, Braunschweig, Germany; 4, Institut Pasteur, Paris, France.

^b ATCC, American Type Culture Collection; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen.

^c P-labeled strain.

^d See reference 13.

^e Strain used in DNA-DNA hybridization experiments.

^f See reference 10.

^g See reference 11.

were obtained from around the world. The strains were identified in two steps. The first step involved screening tests to identify organisms that were members of the family *Enterobacteriaceae* (rod-shaped, gram-negative, oxidase-negative, catalase-positive organisms that fermented D-glucose, reduced nitrate, and exhibited unusual L-arginine dihydrolase, L-lysine decarboxylase, and L-ornithine decarboxylase activity patterns [i.e., strains that were L-arginine dihydrolase negative, L-lysine decarboxylase negative, and L-ornithine decarboxylase positive, strains that were L-arginine decarboxylase positive, L-lysine decarboxylase negative, and L-ornithine decarboxylase positive, and strains that were L-arginine decarboxylase positive, L-lysine decarboxylase negative, and L-ornithine decarboxylase positive]). After this step additional tests (Table 2) were performed.

DNA relatedness. DNA-DNA hybridization experiments were performed with 95 strains. To obtain DNA, cells were grown in brain heart infusion broth at 36 ± 1°C with shaking until they reached the late logarithmic phase. The methods used to extract and purify DNA and the batch hydroxyapatite method used for DNA-DNA hybridization have been described previously (6). An incubation temperature of 60°C was used for optimal DNA reassociation, and an incubation temperature of 75°C was used for stringent DNA reassociation. Labeled DNAs from strains 001, 012, 054, 058, 062, 143, 145, 149, 151, 161, 170, 179, 182, and 185 (serial numbers [Table 1]) were prepared enzymatically in vitro with [³²P]dCTP by using a nick translation reagent kit (Gibco BRL, Gaithersburg, Md.) as directed by the manufacturer. Levels of relatedness were expressed as relative binding ratios, and the level of divergence of related sequences was determined by calculating the decrease in thermal stability in a heteroduplex (reassociated labeled and unlabeled DNAs from two different strains) compared with the thermal stability in a homoduplex (reassociated labeled and unlabeled DNAs from the same strain). Levels of divergence were calculated to the nearest 0.5% on the assumption that each 1°C decrease in heteroduplex DNA stability was due to approximately 1% unpaired (diverged) bases within the related DNA (3, 4, 11, 16). A species was defined as a group of strains that exhibited levels of DNA relatedness at the optimal reassociation temperature of 70% or more and whose related sequences exhibited 5% or less divergence (19).

Biochemical tests. Routine biochemical tests were performed by using standardized procedures, and results were recorded after 48 h of incubation at 30 ± 1°C (8, 9, 12). Consistent results for the decarboxylase reactions were obtained by using the medium of Fay and Barry at pH 5.5 (1). Carbon source utilization tests were performed at the Institut Pasteur Paris by using Biotype strips (bio-Mérieux, La Balme les Grottes, France) that contained 99 pure carbon sources. The strips were inoculated by using Biotype medium 1 as recommended by the manufacturer. The strips were incubated at 30°C for 4 days, and growth was

assessed visually daily. In the esculin, hydroxyquinoline, β-glucuronide, tryptophan, and histidine tests development of a color (black, orange to brown, and dark red, respectively) was considered a positive reaction (5). At the Technische Universität Berlin carbon source utilization tests were performed in microtiter plates (flat-bottom wells) by using filter-sterilized M70 minimal medium (18) supplemented with 0.002% (wt/vol) yeast extract (Oxoid) and 0.002% (wt/vol) bio-Lactysat (bio-Mérieux). Each filter-sterilized carbon source was added at a final concentration of 0.2% (wt/vol). For aromatic compounds, a concentration of 0.05% (wt/vol) was used. Qualitative enzyme tests were performed in filter-sterilized media (pH 7.2) containing 0.05 M Tris HCl buffer, 0.05% yeast extract (Oxoid), and 0.05% bio-Lactysat (bio-Mérieux). Filter-sterilized solutions of chromogenic substrates (*p*-nitrophenyl-linked compounds) were added at a final concentration of 2 mM. For *p*-nitroanilides, a concentration of 1 mM was used. The preparations were incubated at 30°C, and growth was assessed visually daily for 4 days by comparing the cultures with a control that did not contain substrate.

Calculation of phenotypic probability distances. The probability distance (pd) for each pair of species was calculated in two steps by the using average percentages (*v*₁ and *v*₂) obtained from discriminating tests (*t*₁ to *t*_n). First, the larger of the two average percentages for two taxa was divided by the smaller, giving a value of >1 (*v*₂/*v*₁). Because this calculation failed for values of 0 and 100%, these values were replaced by 0.001 and 99.99%, respectively, without any practical disadvantage. Thereafter, *n* quotients of *n* tests were multiplied (pd = *v*₂/*v*₁₁ × *v*₂/*v*₁₂ × ... × *v*₂/*v*₁_n). Low values represented close probability distances and high levels of phenotypic relatedness and vice versa (Tables 3 through 5).

RESULTS AND DISCUSSION

Habitat. The few previously described *Buttiauxella* strains were isolated from unpolluted soil and drinking water, surface water, sewage, soil, and fecal samples, and to our knowledge no *Buttiauxella* strains have been isolated from primary sterile clinical specimens. *Kluyvera* strains have been isolated more frequently than *Buttiauxella* strains but often appear to be biochemically different from members of the previously described species, *K. ascorbata* and *K. cryocrescens* (2, 7, 17). *Kluyvera* strains have been isolated from both soil and clinical

TABLE 2. Biochemical reactions that differentiate DNA groups 1 through 11

Test	Method(s) used ^a	DNA group 1 (<i>B. agrestis</i>)		DNA group 2 (<i>B. fermentalis</i>)		DNA group 3 (<i>B. garviniae</i>)		DNA group 4 (<i>B. bremeriae</i>)		DNA group 5 (<i>B. izantii</i>)		DNA group 6 (<i>B. moockiae</i>)		DNA group 7 (<i>B. wamboldiae</i>)		DNA group 8 (<i>K. cochlear</i>)		DNA group 9 (<i>K. georgiana</i>)		DNA group 10 (<i>K. ascorbata</i>)		DNA group 11 (<i>K. chrysocens</i>)	
		Reaction of strain CDC 1176-81 ^T	% of positive strains (n = 53)	Reaction of strain CDC 1180-81 ^T	% of positive strains (n = 4)	Reaction of strain S1/1-984 ^T	% of positive strains (n = 86)	Reaction of strain S1/6-571 ^T	% of positive strains (n = 7)	Reaction of strain S3/2-161 ^T	% of positive strains (n = 13)	Reaction of strain NSW 11 ^T	% of positive strains (n = 15)	Reaction of strain NSW 326 ^T	% of positive strains (n = 5)	Reaction of strain S3/1-49 ^T	% of positive strains (n = 3)	Reaction of strain CDC 2891-76 ^T	% of positive strains (n = 5)	Reaction of strain CDC 3858-69 ^T	% of positive strains (n = 14)	Reaction of strain CDC 2065-78 ^T	% of positive strains (n = 14)
Acetate	Ut. (FGH)	+	78	+	100	+	80	+	40	+	100	+	100	+	100	+	100	+	100	+	100	+	100
N-Acetyl-D-galactosamine ^b	Ut. (FGH)	+	94	+	100	+	70	+	100	+	100	+	93	+	100	+	0	+	60	+	67	+	100
N-Acetyl-L-glutamate ^b	Ut. (FGH)	+	94	+	100	+	90	+	100	+	100	+	100	+	100	+	0	+	0	+	0	+	0
N-Acetyl-L-glutamine	Ut. (FGH)	-	6	-	0	-	20	-	0	-	8	-	80	-	0	-	0	-	20	+	67	-	0
N-Acetyl-glycine	Ut. (FGH)	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	+	0	-	0
Adonitol	Acid. (SM), Ut. (FGH, IP)	+	0	-	0	+	65	+	60	-	8	-	0	-	0	-	0	-	0	-	0	-	0
D-Arabinose ^b	Ut. (FGH)	+	69	-	0	+	45	+	0	+	100	+	73	+	100	-	0	-	20	-	33	+	67
D-Arabinol	Acid. (SM), Ut. (FGH, IP)	-	0	-	0	+	65	+	60	-	8	-	0	-	0	-	0	-	0	-	0	-	0
L-Arginine dihydrolase ^b	Alcal. (FGH, SM)	-	0	-	0	+	100	+	70	-	15	+	100	+	100	-	0	-	20	+	0	-	0
L-Arginine	Ut. (FGH)	-	0	-	0	-	0	-	0	-	0	-	20	-	0	-	0	-	0	+	67	-	0
Citrate utilization (Simmons) ^b	Ut. (FGH, SM, IP)	+	50	-	0	-	36	+	60	+	100	+	100	-	0	+	100	+	80	+	96	-	80
m-Coumarate (API)	Ut. (IP)	+	0	-	0	-	0	-	0	-	ND ^c	-	ND	-	ND	+	0	+	100	+	73	+	100
Dulcitol ^b	Acid. (FGH, SM), Ut. (FGH, IP)	-	10	-	0	-	20	-	0	-	0	-	0	-	0	-	0	+	40	+	45	-	0
1-Fucose ^b	Acid. (SM), Ut. (FGH, IP)	+	80	-	0	-	60	-	0	+	85	-	7	+	100	-	0	-	20	-	10	+	44
Gentisate (API) ^b	Ut. (IP)	-	0	-	0	-	1	-	0	-	0	-	ND	-	ND	-	0	+	60	+	100	-	0
Glycerol ^b	Acid. (SM)	+	60	-	0	-	0	-	0	-	0	-	0	-	0	+	0	+	25	-	40	-	5
3-Hydroxybenzoate ^b	Ut. (FGH, IP)	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	+	100	-	80	-	100
3-Hydroxyphenylacetate ^b	Ut. (FGH)	-	0	+	75	-	0	-	0	-	0	-	0	-	0	-	0	+	60	+	100	+	100
Indole production ^b	Kovacs (SM)	-	0	-	0	-	30	-	0	-	0	-	40	-	0	-	0	+	100	+	92	+	90
Indole production ^b	Acid. (FGH, SM)	-	0	-	0	-	0	-	0	-	0	-	+	-	100	-	0	+	0	-	0	-	0
Indole production ^b	Growth (SM)	+	90	+	100	+	82	+	100	+	100	+	100	+	0	+	100	+	100	+	92	+	86
KCN ^b	Ut. (IP)	+	10	-	0	+	97	+	100	+	50	+	93	-	0	+	100	+	80	+	98	+	100
5-Ketogluconate ^b	Acid. (SM)	+	100	-	0	-	1	-	100	+	100	+	0	-	0	+	67	+	80	+	98	+	100
Lactose ^b	Acid. (FGH), Ut. (IP)	+	10	-	0	-	0	-	0	-	0	-	0	-	0	-	0	+	60	+	95	-	50
Lactulose ^b	Alcal. (FGH, SM)	-	0	-	100	-	0	-	0	-	0	-	0	-	0	-	0	+	23	+	97	-	23
L-Lysine decarboxylase ^b	Ut. (FGH, SM)	+	96	+	0	-	100	+	100	+	100	+	93	+	100	+	67	+	60	+	96	+	86
Malonate utilization ^b	Acid. (FGH), Ut. (IP)	+	75	+	100	+	88	+	90	+	30	+	87	+	0	+	100	+	100	+	99	+	100
Maltitol ^b	Acid. (SM)	+	55	+	75	+	1	+	40	+	50	+	0	-	0	+	100	+	100	+	100	+	100
α-Methyl-D-glucopyranoside ^b	Acid. (SM), Ut. (IP)	+	95	-	25	-	2	-	40	-	0	-	87	-	0	+	100	+	100	+	5	-	0
3-Methyl-D-glucopyranoside ^b	Ut. (FGH, IP)	+	95	+	75	+	95	+	100	+	85	+	100	+	0	-	0	+	100	+	100	+	100
Methyl red	Acid. (SM)	+	100	+	100	+	100	+	100	+	100	+	100	+	0	-	0	+	100	+	100	+	100
Mucate ^b	Acid. (SM)	+	71	+	25	+	55	+	100	+	30	+	7	-	0	+	100	+	100	+	90	+	81
L-Ornithine decarboxylase ^b	Alcal. (FGH, SM)	+	98	+	100	+	0	+	100	+	100	+	100	+	0	+	100	+	100	+	100	+	100
Palmitose ^b	Acid. (SM), Ut. (IP)	+	60	+	50	+	20	+	0	-	70	+	30	+	0	+	100	+	60	+	100	+	100
Phenylacetate ^b	Ut. (FGH, IP)	-	0	-	0	-	0	-	0	-	0	-	33	-	0	-	0	-	0	-	0	-	0
1-Phenylalanine decarboxinase	FeCl ₃ test (SM)	+	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	20	-	30	-	40
3-Phenylpropionate ^b	Ut. (FGH, IP)	+	80	-	75	+	98	+	100	+	100	+	80	+	100	+	0	+	100	+	50	+	98
Raffinose ^b	Acid. (SM), Ut. (IP)	+	28	-	25	+	2	+	90	+	0	+	0	+	0	+	67	+	100	+	98	+	100
D-Sorbitol ^b	Acid. (SM), Ut. (IP)	+	60	+	100	-	25	+	0	-	0	-	0	-	0	+	33	+	100	+	40	+	45
Sucrose ^b	Acid. (SM)	-	2	-	0	-	14	-	0	-	0	-	0	-	0	+	81	+	100	+	98	+	0
D-Tagatose ^b	Acid. (FGH, SM), Ut. (FGH, IP)	-	10	-	0	-	20	-	0	-	0	-	0	-	0	+	100	-	40	-	45	-	0
Tartrate (Jordan's) ^b	Acid. (SM)	+	60	-	0	+	30	-	0	-	20	+	90	-	0	+	0	-	0	-	0	-	0
Voges-Proskauer	Acetoin (SM)	-	0	-	0	-	0	-	15	-	0	-	0	-	0	+	100	-	0	-	0	-	0

^a Ut., utilization test; Acid., acidification test; Alcal., alkalization test. The information in parentheses indicates at which institutions the tests were performed, as follows: FGH, Fachgebiet Hygiene, Technische Universität, Berlin, Germany; SM, Staatliches Medizinallaboratorium, Braunschweig, Germany; IP, Institut Pasteur, Paris, France.

^b Test useful in differentiating DNA groups 1 through 11, which correspond to *Buttiauxella* and *Kluyvera* genomespecies.

^c ND, test not done.

TABLE 3. Logarithms of probability distances and relative binding ratios

DNA group	Logarithm of probability distances or relative binding ratio at 60°C ^a									
	DNA group 1	DNA group 2	DNA group 3	DNA group 4	DNA group 5	DNA group 6	DNA group 7	DNA group 8	DNA group 9	DNA group 10
1		50.60	55.64	53.33	62.67	58.00	56.00			
2	23.92		50.2	49.83	48.33	55.00	49.00			
3	22.28	34.50		60.16	52.40	70.50	48.71			
4	23.42	24.65	23.05		52.56	55.00	49.25			
5	15.46	26.94	19.44	16.21		50.29	48.00			
6	24.77	31.60	16.77	24.31	16.93		47.92			
7	35.99	33.59	30.89	33.61	26.60	27.86				
8	26.65	33.47	40.15	31.43	36.52	43.76	50.90			
9	30.57	35.89	39.04	35.43	36.22	40.66	50.19	28.28		
10	28.82	36.41	39.83	41.42	39.95	40.01	57.77	29.73	10.79	
11	23.16	26.39	35.26	30.47	28.97	34.86	43.35	26.32	13.77	14.88

^a The values on the lower left are logarithms of phenotypic probability distances; these values were calculated by using all of the data in Table 2 except the *m*-coumarate and gentisate data. The values on the upper right are relative binding ratios at 60°C (expressed as percentages); these values were obtained from Table 6.

sources, although soil, water, and humans do not seem to be the normal habitats of *Kluyvera* species (14, 15).

Our experience indicates that strains of both genera occur frequently and abundantly in the intestines of snails, slugs, and other mollusks. The apparent diversity of the different *Buttiauxella* and *Kluyvera* species isolated from mollusks is an additional reason to consider these animals to be the ecologic niche and natural source of these bacteria.

DNA relatedness. The results of our DNA-DNA relatedness experiments revealed that there were 11 DNA hybridization groups or genomospecies (Table 6). Eighteen snail and slug isolates and the type strain of *B. agrestis* were in hybridization group 1. None of the mollusk isolates were in hybridization group 2, which corresponds to the unnamed *Buttiauxella* species called enteric group 63; this was due to the screening tests used during isolation. The strains isolated were distinguished on the basis of a negative lysine decarboxylase test. Since enteric group 63 strains are positive for this reaction, if they were present, they would have been eliminated by the screening procedure used. Hybridization group 3, represented by enteric group 64 containing one previously described strain, contained 35 snail and slug strains. Hybridization groups 4, 5, and 7 each represented previously unrecognized species containing seven, nine, and five strains, respectively. Hybridization

group 6, represented by the unnamed species enteric group 59, contained 15 snail and slug strains.

None of the *Kluyvera*-like strains hybridized at the species level to members of hybridization group 10 (*K. ascorbata*), hybridization group 11 (*K. cryocrescens*), or hybridization group 9 (unnamed *Kluyvera* species 3 [11, 14]). Three *Kluyvera*-like strains isolated from snails and slugs belonged to hybridization group 8. Essentially without exception, the levels of relatedness within each hybridization group were more than 70% at both the optimal temperature (60°C) and the stringent temperature (75°C) used for DNA reassociation (Table 6). Similarly, essentially without exception, the levels of divergence within related sequences were 5% or less. These levels of relatedness correspond to the levels recommended for strains belonging to a single species (19).

The levels of relatedness obtained for members of hybridization groups 1 through 7 were generally between 45 and 65%, a good indication that all of these groups belong to a single genus, the genus *Buttiauxella*. The hybridization groups that exhibited the highest levels of relatedness were hybridization groups 1 and 5 and hybridization groups 3 and 6. The members of hybridization group 8 were 44 to 48% related to the type strains of the two previously described and one previously unnamed *Kluyvera* species, but only 13 to 25% related to the

TABLE 4. Results obtained when DNA groups were compared

Comparison	No. of tests	Phenotypic probability distance (mean $\pm$ SD)	DNA-DNA relatedness value (mean $\pm$ SD)
Within the genus <i>Buttiauxella</i>			
DNA group 1 vs DNA groups 2 through 7	21	25.37 $\pm$ 6.31	53.49 $\pm$ 5.69
DNA group 2 vs DNA groups 1 and 3 through 7	6	24.31 $\pm$ 6.63	56.04 $\pm$ 4.12
DNA group 3 vs DNA groups 1, 2, and 4 through 7	6	29.20 $\pm$ 4.62	50.49 $\pm$ 2.36
DNA group 4 vs DNA groups 1, 2, and 4 through 7	6	24.49 $\pm$ 6.83	56.27 $\pm$ 8.09
DNA group 5 vs DNA groups 1 through 3 and 5 through 7	6	24.21 $\pm$ 5.56	53.36 $\pm$ 3.97
DNA group 6 vs DNA groups 1 through 4, and 7	6	20.26 $\pm$ 5.22	52.38 $\pm$ 5.40
DNA group 7 vs DNA groups 1 through 5 and 7	6	23.71 $\pm$ 5.92	56.12 $\pm$ 7.93
DNA group 7 vs DNA groups 1 through 6	6	31.42 $\pm$ 3.65	49.81 $\pm$ 3.08
<i>Buttiauxella</i> DNA groups vs <i>Kluyvera</i> DNA groups	28	37.06 $\pm$ 7.79	
Within the genus <i>Kluyvera</i>			
DNA group 8 vs DNA groups 9 through 11	6	20.63 $\pm$ 8.37	
DNA group 9 vs DNA groups 8, 10, and 11	3	26.55 $\pm$ 2.55	
DNA group 10 vs DNA groups 8, 9, and 11	3	17.61 $\pm$ 9.36	
DNA group 11 vs DNA groups 8 through 10	3	18.47 $\pm$ 9.97	
	3	18.32 $\pm$ 6.95	

TABLE 5. Probability distances (based on phenotypic characteristics) that differentiate the genera *Buttiauxella* and *Kluyvera* from other genera belonging to the *Enterobacteriaceae*

Genus	Logarithm of probability distances to the following genera ^a :	
<i>Buttiauxella</i>	0	16.61
<i>Kluyvera</i>	16.61	0
<i>Budvicia</i>	40.99	22.27
<i>Cedecea</i>	22.27	16.57
<i>Citrobacter</i>	16.57	40.67
<i>Edwardsiella</i>	40.67	37.19
<i>Enterobacter</i>	37.19	15.33
<i>Escherichia and Shigella</i>	15.33	17.20
<i>Ewingella</i>	17.20	33.99
<i>Hafnia</i>	33.99	29.27
<i>Klebsiella</i>	29.27	17.82
<i>Leclercia</i>	17.82	22.91
<i>Leminorella</i>	22.91	50.58
<i>Moellerella</i>	50.58	39.60
<i>Morganella</i>	39.60	47.68
<i>Obesumbacterium</i>	47.68	45.55
<i>Pantoea</i>	45.55	21.39
<i>Pragia</i>	21.39	50.22
<i>Proteus</i>	50.22	50.47
<i>Providencia</i>	50.47	37.87
<i>Rahnella</i>	37.87	24.44
<i>Salmonella</i>	24.44	22.54
<i>Serratia</i>	22.54	22.57
<i>Tatumella</i>	22.57	49.83
<i>Trabulsilla</i>	49.83	20.25
<i>Xenorhabdus</i>	20.25	54.96
<i>Yersinia</i>	54.96	27.43
<i>Yokenella</i>	27.43	25.09

^a The values are not identical to the values in Tables 3 and 4 because we used more characteristics and different characteristics in this analysis.

members of the seven *Buttiauxella* hybridization groups, which is consistent with placement of hybridization group 8 in the genus *Kluyvera*. The levels of relatedness between members of the *Buttiauxella* and *Kluyvera* hybridization groups and more than 50 other species belonging to the *Enterobacteriaceae* were generally between 15 and 30% (data not shown).

Certain strains included in the hybridization groups exhibited higher levels of divergence (more than 5%) or lower levels of relatedness (less than 70% in 60°C reactions or less than 60% in 75°C reactions) than the levels used in the definition of a genetic species (19). In almost all cases, these borderline strains deviated in only one of the three parameters used to determine species level relatedness, and their levels of relatedness with a second reference strain were fully within the species definition. For example, EG59-like strain NSW 155 in hybridization group 4 was 66% related to the reference strain at 60°C but exhibited only 0.5% divergence and the level of relatedness at 75°C was 66%. Similarly, strain S1/3-1052 in hybridization group 3 exhibited 5.5% divergence with one reference strain, but 5.0% divergence with the second reference strain, and, in both cases the levels of relatedness at both temperatures were well within the limits used to define species. In every case, all of the strains belonging to a relatedness group exhibited similar biochemical reactions. Therefore, we decided that they should be included in the relatedness group, at least for the present.

Phenotypic and chemotaxonomic characterization. All of the strains studied were gram-negative, oxidase-negative, catalase-positive, D-glucose-fermenting, nitrate-reducing, rod-shaped organisms belonging to the family *Enterobacteriaceae*. They grew on MacConkey agar and on Endo agar at 30°C. With few exceptions, all of the strains were positive in standard tests for motility, acid and gas production from D-glucose, fermentation of D-mannitol, salicin, L-arabinose, L-rhamnose, maltose, D-xylose, trehalose, cellobiose, D-mannose, galactose, gentiobiose, D-ribose, and arbutin, esculin hydrolysis, and *o*-nitrophenyl-β-D-galactopyranoside (β-galactosidase) test. They were negative for production of hydrogen sulfide on triple sugar iron, Christensen urease activity, gelatin liquefaction, lipase activity (Tween 80 and corn oil), DNase activity, and production of a yellow pigment or some other pigment and did not ferment 2-deoxy-D-glucose, 2-deoxy-D-ribose, erythritol, α-D-fucose, D-lyxose, and xylitol.

Almost all of the strains utilized the following compounds as sole carbon and energy sources: *N*-acetyl-D-glucosamine, D-alanine, L-alanine, L-arabinose, arbutin, DL-asparagine, D-cellobiose, D-fructose, D-galactose, D-galacturonate, gentiobiose, D-gluconate, D-glucosamine, D-glucuronate, L-glutamine, L-glutamate, DL-glycerate, glycerol, 4-hydroxybenzoate, 2-ketoglucuronate, D-lactate, DL-lactate, maltose, maltotriose, mannitol, D-mannose, methyl-α-galactoside, methyl-β-galactoside, methyl-β-D-glucoside, mucate, oxaloacetate, palatinose, protocatechuate, pyruvate, quinate, L-rhamnose, D-ribose, D-saccharate, salicin, L-serine, starch, D-trehalose, and D-xylose.

Almost all of the strains were not able to utilize the following compounds as sole carbon and energy sources: acetamide, acetamidocaprinate, *N*-acetyl-DL-methionine, *N*-acetyl-L-proline, *cis*-aconitate, *trans*-aconitate, adipate, β-alanine, allantoin, al-trose, DL-2-aminoadipate, 2-aminobenzoate, 3-aminobenzoate, 4-aminobenzoate, DL-2-aminobutyrate, DL-3-aminobutyrate, 4-aminobutyrate, DL-2-amino-isobutyrate, DL-3-amino-isobutyrate, 5-aminovalerate, aminoxyacetate, amygdalin, anthranilate, 2-hydroxy-phenylacetate, L-arabitol, arabonate, D-arginine, D-asparagine, azelate, benzoate, betaine, 1-butanol, 2-butanol, *n*-butyrate, isobutyrate, cadaverine, caprate, caprylate, carnitine, carnosine, L-citrulline, citraconate, L-cysteinate, dextran,

TABLE 6. Identification of *Buttiauxella* and *Kluyvera* strains by DNA-DNA hybridization

Source of unlabeled DNA		Source of labeled DNA																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																									
		DNA group 1 strains				DNA group 2 strain CDC 1180-81 ^T (serial no. 054)				DNA group 3 strains				DNA group 4 strains				DNA group 5 strains				DNA group 6 strains				DNA group 7 strain NSW 326 ^T (serial no. 182)				DNA group 8 strain S31-49 ^T (serial no. 185)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
		CDC 1176-81 ^T (serial no. 001)		S3/2-162 (serial no. 012)		D ^b		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR		RBR	

DL-2,4-diaminobutyrate, diaminopimelate, 2,3-diaminopropionate, dimethylglycine, *m*-erythritol, ethanol, ethanolamine, ethylamine, D-fucose, D-glutamate, glutarate, glycinamide, glycogen, glycolate, glycyrrhizinate, 1-hexanol, 1,6-hexandiol, hexylamine, hippurate, histamine, D-histidine, L-histidine, L-homoserine, DL-3-hydroxybutyrate, 4-hydroxybutyrate, DL-2-hydroxycaproate, DL-2-hydroxy-isobutyrate, L-2-hydroxyisocaproate, 2-hydroxy-isovalerate, DL- γ -hydroxylysine, 4-hydroxyphenylglycine, DL-hydroxyproline, 2-hydroxyvalerate, HQ- β -glucuronide, heptanoate, indole-3-acetate, isophthalate, itaconate, 2-ketoglutarate, 2-ketoisocaproate, D-leucine, L-leucine, levulinate, D-lysine, D-malate, maleate, D-mandelate, L-mandelate, D-mannoheptulose, D-melezitose, mesaconate, mesoxalate, D-methionine, L-methionine, DL-methioninesulfone, methyl- α -D-glucoside, α -methyl-D-mannoside, β -methyl-D-xyloside, L-norleucine, D-norvaline, L-norvaline, 1,8-octandiol, D-ornithine, phenoxyacetate, D-phenylalanine, phenylglycine, phenyllactate, phosphoenolpyruvate, phthalate, pimelate, poly-D-galactomannan, polygalacturonate, D-proline, propionate, protocatechuate, putrescine, salicylamide, salicylate, sarcosine, sorbate, L-sorbose, spermine, suberate, D-tartrate, *meso*-tartrate, tartronate, taurine, thiamine, tricarballoylate, trigonelline, tropate, tryptamine, D-tryptophan, L-tryptophan, tyramine, L-tyrosine, ureidosuccinate, *n*-valerate, isovalerate, L-valine, xyloitol, and L-xylose.

The strains varied in their ability to utilize the following compounds as sole sources of carbon and energy, and these characteristics could not be used to differentiate hybridization groups: D-alanine, L-asparagine, L-aspartate, benzoate, fumarate, D-glucarate, D-glucosaminates, glycerate, glycerophosphate, glycine, glyoxylate, 4-hydroxybenzoate, 4-hydroxyphenylacetate, inulin, DL-isocitrate, DL-isoleucine, 2-ketoglutarate, L-lactate, D-lyxose, L-lyxose, L-malate, L-mannose, phenylpyruvate, D-serine, spermidine, succinate, L-tartrate, L-threonine, and D-turanose.

The results of 44 biochemical tests that were used to differentiate the 11 DNA hybridization groups belonging to the genera *Buttiauxella* and *Kluyvera* in our numerical taxonomic study are shown in Table 2. The most useful tests for differentiating the 11 genomospecies with binary phenetic data are indicated. Hybridization groups 1 and 5 could not be distinguished on the basis of the results of any single test. These groups had to be distinguished on the basis of their overall biochemical profiles. Hybridization groups 3 and 6 could not be separated on the basis of the results of a single test, but could be differentiated on the basis of their *N*-acetyl-L-glutamine, adonitol, D-arabitol, L-fucose, mucate, and tartrate reactions. As stated above, these two pairs of hybridization groups exhibited the highest levels of interspecies DNA relatedness.

The probability distances that separated pairs of hybridization groups when the biochemical reaction results shown in Table 2 were used are shown in Table 3. The probability distances for the 11 DNA hybridization groups varied from log 10.79 to log 57.77. The lowest probability distances between hybridization groups in the same genus were log 15.46 for *Buttiauxella* hybridization groups 1 and 5 and log 10.79 for *Kluyvera* hybridization groups 9 and 10. The intergeneric probability distances ranged from log 23.16 to log 57.77. Table 4 shows that the mean value for 21 phenotypic probability distances in the genus *Buttiauxella* was log 25.37 (standard deviation, log 6.31). The corresponding value for 21 DNA-DNA relatedness pairs (based on the relative binding ratios at 60°C shown in Table 6) was 53.49% (standard deviation, 5.69%). The phenotypic and genomotypic values for DNA groups 7 and 2 correlate well; these values are the farthest from the

mean values. The most typical DNA group could not be determined with certainty. *Buttiauxella* DNA group 5 exhibited the closest phenotypic probabilistic distance with other *Buttiauxella* DNA groups; however, DNA groups 3, 6, and 1 were the closest groups on the basis of DNA relatedness data.

The mean value for six total phenotypic probability distances in the genus *Kluyvera* was log 20.63, and the standard deviation was log 8.37. However, the mean value for 28 total phenotypic probability distances between the genera *Buttiauxella* and *Kluyvera* was log 37.06 (standard deviation, log 7.79). These high phenotypic probability distances indicate that there are significant differences between the genus *Buttiauxella* and the genus *Kluyvera*. Although these two genera have many similar phenotypic characteristics, the phenotypic data correlate with the DNA hybridization data and indicate that they are separate genera.

Previously reported conventional test results for *Buttiauxella* and *Kluyvera* strains are compared with the results obtained for other genera belonging to *Enterobacteriaceae* in Table 7 (11, 12, 13). Although some biochemical characteristics can be used to differentiate various *Buttiauxella* and *Kluyvera* species (*N*-acetyl-L-glutamate, *m*-coumarate, glycerol, 3-hydroxyphenylacetate, indole production, lactulose, lysine decarboxylase activity, melibiose, α -methyl-D-glucopyranoside, raffinose, sucrose) (Table 2), no single biochemical test result differentiates these genera with 90 to 100% certainty. Nine test results (indole production, citrate utilization, L-arginine dihydrolase activity, ornithine decarboxylase activity, and fermentation of lactose, sucrose, raffinose, α -methyl-D-glucopyranoside, and melibiose) do give differences of 40 to 50% between *Buttiauxella* and *Kluyvera* species. The phenotypic probability distances between *Buttiauxella* and *Kluyvera* species and other genera that belong to the *Enterobacteriaceae* are shown in Table 5. These data are not identical to the data in Table 3 because they were calculated by using 47 conventional tests (12). The lowest probability distance (log 12.46) was the distance between the genus *Buttiauxella* and the genus *Enterobacter*. The probability distance between the genus *Kluyvera* and the genus *Enterobacter* was log 15.33. It will be interesting to determine whether the probability distances correlate with relatedness trees generated from rRNA sequence similarity data.

On the basis of the biochemical and DNA hybridization data, we concluded that the genus *Buttiauxella* contains the following seven species: *B. agrestis*, species corresponding to previously described enteric groups 59, 63, and 64, and three previously undescribed species. The genus *Kluyvera* contains four species, *K. ascorbata*, *K. cryocrescens*, a species corresponding to *Kluyvera* unnamed species 3 (11), and a previously undescribed species. Taxonomic proposals to name and define the new species and to amend the description of *B. agrestis* are given below.

Emended description of *Buttiauxella agrestis* Ferragut, Izard, Gavini, Lefebvre, and Leclerc 1981. *B. agrestis* was called DNA hybridization group 1 in this study. The original description (13), which was based on the 17 strains originally described by Gavini et al. (15), stated that 60% of *B. agrestis* strains were malonate positive; we found (Table 2) that 96% of the strains were malonate positive. The biochemical tests that are useful for differentiating *B. agrestis* from other *Buttiauxella* species are the arginine dihydrolase, fucose, glycerol, lactose, melibiose, ornithine decarboxylase, palatinose, and D-sorbitol tests. This species has been isolated from mollusks, as well as water, soil, and humans.

Description of *Buttiauxella ferragutiae* sp. nov. *Buttiauxella ferragutiae* (fer'ra.gut.i.ae. M. L. fem. gen. n. *ferragutiae*, of Ferragut, in honor of Carmen Ferragut, a French microbiologist,

TABLE 7. Mean percentages of positive strains in biochemical reactions for genera belonging to the *Enterobacteriaceae*^a

Test or characteristic	% of positive strains in the following genera ^b :																															
	<i>Buttiauxella</i>	<i>Kluyvera</i>	<i>Budvicia</i>	<i>Cedecea</i>	<i>Citrobacter</i>	<i>Edwardsiella</i>	<i>Enterobacter</i>	<i>Escherichia and Shigella</i>	<i>Ewingella</i>	<i>Hafnia</i>	<i>Klebsiella</i>	<i>Leclercia</i>	<i>Limnospila</i>	<i>Moellerella</i>	<i>Morganella</i>	<i>Obesumbacterium</i>	<i>Pantoea</i>	<i>Pragia</i>	<i>Proteus</i>	<i>Providencia</i>	<i>Rahnella</i>	<i>Salmonella</i>	<i>Serratia</i>	<i>Tatumella</i>	<i>Trabulsiella</i>	<i>Xenorhabdus</i>	<i>Yersinia</i>	<i>Yokenella</i>				
Indole production	10	71	0	0	53	62	1	53	84	0	31	100	0	0	99	0	22	0	25	79	0	1	11	0	13	30	25	0				
Methyl red	100	75	93	88	100	75	35	99	84	63	69	100	50	100	96	15	99	100	98	88	88	99	68	0	100	0	92	100				
Voges-Proskauer	0	25	0	56	0	0	93	0	95	78	66	0	0	0	0	0	87	0	38	0	100	0	77	5	0	0	2	0				
Citrate (Simmons)	49	89	0	99	70	41	0	9	95	5	66	0	50	80	0	0	87	89	33	60	94	59	88	2	88	23	2	92				
Hydrogen sulfide (TSI) ^c	0	0	80	0	41	25	0	0	0	0	0	0	100	0	23	0	0	89	56	0	0	87	0	0	100	0	0	0				
Urea hydrolysis	0	0	33	0	62	0	36	0	0	2	56	48	0	0	99	0	0	0	98	26	0	0	4	0	0	28	55	0				
Phenylalanine deaminase	5	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	10	22	0	0	95	0	0	90	0	0	0	5				
Lysine decarboxylase	14	55	0	0	0	0	21	52	0	100	77	0	0	0	50	100	22	0	0	0	0	53	0	0	100	0	0	0				
Arginine dihydrolase	55	4	0	76	69	0	45	8	0	72	1	0	0	0	97	100	6	0	25	0	0	82	46	0	100	0	0	8				
Ornithine decarboxylase	48	100	0	29	53	90	94	61	0	43	18	0	0	0	48	0	22	0	0	0	0	0	0	0	100	0	64	100				
Motility (36°C)	100	97	27	95	85	75	72	49	60	0	0	0	0	0	48	0	62	100	94	70	6	81	84	0	100	100	2	100				
Gelatin hydrolysis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	83	0	0	0	75	0	0	70	3	0				
Growth in KCN	82	95	0	90	83	0	90	14	5	48	95	97	0	0	95	0	28	0	99	81	0	16	61	0	100	0	0	0				
Malonate utilization	84	86	0	58	24	25	84	28	0	48	84	93	0	0	3	0	14	0	1	0	100	24	19	0	0	0	0	0				
Glucose (acid)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	85	100				
D-Glucose (gas)	100	97	53	94	97	59	98	61	0	49	75	97	17	0	91	0	11	0	82	25	98	80	29	0	100	0	8	100				
Lactose fermentation	43	85	87	23	62	32	46	23	70	3	75	93	0	100	1	0	35	0	78	23	100	10	48	0	0	0	56	0				
Sucrose fermentation	2	78	0	70	32	50	69	15	0	77	100	100	0	60	0	0	78	0	0	22	100	100	99	0	100	0	98	100				
D-Mannitol fermentation	100	99	60	100	100	50	15	23	85	100	0	19	86	42	0	0	2	0	0	0	88	45	9	0	0	0	0	0				
Dulcitol fermentation	4	41	0	0	34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
Salicin fermentation	100	100	0	100	9	7	13	93	23	80	99	100	0	100	0	0	47	78	13	11	100	5	92	55	13	0	33	8				
Adonitol fermentation	19	0	0	0	0	0	0	13	0	0	98	93	0	100	0	0	1	0	0	59	0	3	38	0	0	0	0	7	0			
<i>myo</i> -Inositol fermentation	14	0	0	0	0	0	20	0	0	0	88	0	0	0	0	0	18	0	0	46	0	0	52	0	0	0	0	7	0			
D-Sorbitol fermentation	26	38	0	40	100	0	50	25	0	0	0	0	0	0	0	0	24	0	0	1	94	74	75	0	100	0	78	0				
L-Arabinose fermentation	100	100	80	0	100	30	100	92	0	48	99	100	100	0	0	0	96	0	0	0	100	82	70	0	100	0	81	100				
Raffinose fermentation	21	100	0	42	18	0	54	32	0	1	97	66	0	100	0	0	39	0	1	3	94	83	32	0	100	0	15	25				
L-Rhamnose fermentation	100	100	100	0	100	100	100	100	16	50	99	100	0	30	0	0	50	70	74	12	94	90	95	0	100	8	73	100				
Maltose fermentation	100	100	93	80	100	0	99	71	13	49	99	100	92	0	5	15	100	0	73	5	94	86	73	9	100	0	71	100				
D-Xylose fermentation	100	100	0	100	100	25	100	92	99	83	100	100	0	0	0	85	100	0	71	20	100	86	100	93	100	0	98	100				
Trehalose fermentation	100	100	0	100	100	0	100	38	10	8	99	100	0	0	0	0	44	0	0	2	100	6	50	0	100	0	55	100				
Cellobiose fermentation	100	98	0	11	16	0	65	3	0	0	80	0	0	0	0	0	4	0	0	0	0	1	18	0	0	0	7	0				
α -Methyl-D-glucose fermentation	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	1	0	0	0	0	0				
Erythritol fermentation	0	0	0	0	89	2	62	86	18	50	4	87	100	0	0	0	42	78	13	7	100	1	90	0	13	0	0	34				
Esculin hydrolysis	100	100	0	40	44	75	70	36	0	0	99	100	0	100	0	0	67	0	0	1	100	66	60	25	0	0	20	92				
Melbiose fermentation	39	100	0	100	9	0	15	15	99	0	99	96	0	75	0	0	53	0	0	38	0	7	35	0	0	0	21	0				
D-Arabinol fermentation	19	0	27	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	100	6	51	49	0	88	57	68				
Glycerol fermentation	9	43	0	0	75	0	34	39	24	48	87	93	3	9	10	53	0	75	71	26	13	9	53	7	0	0	45	0				
Mucate fermentation	54	93	20	0	96	25	64	45	0	0	72	93	75	0	0	0	39	0	0	30	0	46	1	0	100	0	1	0				
Tartrate (Jordan's)	31	14	27	0	10	0	34	60	35	50	85	83	100	10	98	15	10	0	88	79	6	51	75	0	50	7	5	25				
Acetate utilization	85	84	0	32	59	0	53	42	10	8	49	28	0	0	0	0	39	0	13	40	6	51	62	0	0	0	14	0				
Lipase (corn oil)	0	0	0	88	0	100	0	0	0	0	0	0	0	0	0	0	31	0	79	0	0	0	62	0	0	0	7	0				
D-Nase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	55	2	0	1	86	0	0	0	0	14				
Nitrate reduced to Nitrite	100	100	100	100	98	90	0	0	97	100	97	100	0	9	91	100	66	100	96	100	100	92	97	98	100	7	94	100				
Oxidase (Kovacs)	0	0	0	0	0	75	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	61	100			
ONPG ^d	100	100	93	98	95	0	99	64	85	60	83	100	0	90	3	0	78	0	0	3	100	30	93	0	100	0	57	0	0			
Yellow pigment	0	0	0	0	0	0	7	19	0	0	0	37	0	0	0	0	49	0	0	0	0	0	0	0	0	0	0	0	0			
Mannose fermentation	100	100	0	100	25	100	99	99	100	100	100	100	0	100	97	85	99	0	0	100	100	99	100	100	100	93	100	100	100			

^a Data obtained from reference 12.^b Percentages of positive strain after 2 days of incubation at 36°C.^c TSI, triple sugar iron.^d ONPG, *o*-nitrophenyl- β -D-galactopyranoside.

for her contribution to the study of the genus *Buttiauxella* [13]) was previously called enteric group 63 (10, 12) and in this study was referred to as DNA hybridization group 2. Biochemical characteristics are shown in Table 2. Positive lysine decarboxylase and D-sorbitol tests and negative ketogluconate and malonate tests clearly differentiate this species from all other *Buttiauxella* species. Isolated from water and soil. Because of the screening procedure, any strains present in mollusks would have been discarded; therefore, it is not known whether this species occurs in mollusks.

Description of the type strain. The type strain of *B. ferruginea* is strain CDC 1180-81 (= CUETM 78-31 = ATCC 51602 = DSM 9390 = serial no. 054). This strain was isolated from unpolluted soil (13). Its colonies are circular, convex, greyish, and smooth on nutrient agar and blood agar. They are 1 to 2 mm in diameter after 1 day and 2 to 3 mm in diameter after 2 days. Good growth occurs at 30 to 36°C. Weak, slow growth occurs at 42°C. Biochemical characteristics are shown in Table 2.

Description of *Buttiauxella gaviniae* sp. nov. *Buttiauxella gaviniae* (ga.vin'i.ae. M. L. fem. gen. n. *gaviniae*, of Gavini, in honor of Françoise Gavini, a French microbiologist, for her contributions to the study of the genus *Buttiauxella* [14, 15]) was previously called enteric group 64 (10, 12) and in this study was referred to as DNA hybridization group 3. Biochemical characteristics are shown in Table 2. Positive arginine dihydrolase, 5-ketogluconate, and palatinose reactions and negative ornithine decarboxylase and raffinose reactions are useful in differentiating *B. gaviniae* from other *Buttiauxella* species. A combination of reaction results is necessary to differentiate this species from *Buttiauxella noackiae*. All but one strain were isolated from mollusks.

Description of the type strain. *B. gaviniae* type strain S1/1-984 (= ATCC 51604 = DSM 9393 = serial no. 062) was isolated from a snail sampled in Braunschweig, Germany. Its colonies are circular, convex, greyish, and smooth on nutrient agar and blood agar. They are 1 to 2 mm in diameter after 1 day and 2 to 3 mm in diameter after 2 days at 30 to 36°C. Grows poorly at 42°C. The type strain has all of the characteristics given for the genus in Table 7 and for the species in Table 2.

Description of *Buttiauxella brennerae* sp. nov. *Buttiauxella brennerae* (bren'ner.ae. M. L. fem. gen. n. *brennerae* of Brenner, in honor of Frances W. Hickman-Brenner, an American microbiologist, for her contributions to the study of many genera of the *Enterobacteriaceae* [4, 9, 10, 16], including the description of the genus *Kluyvera* [11]) is referred to as DNA hybridization group 4 in this study. Biochemical characteristics are shown in Table 2. Tests that can be used to differentiate *B. brennerae* from other *Buttiauxella* species are the arabinose, arginine dihydrolase, fucose, *myo*-inositol, 5-ketogluconate, lysine decarboxylase, and malonate tests. Isolated from mollusks.

Description of the type strain. *B. brennerae* type strain S1/6-571 (= ATCC 51605 = DSM 9396 = serial no. 145) was isolated from a snail sampled in Braunschweig, Germany. Its colonies are circular, convex, greyish, and smooth on nutrient agar and blood agar. They are 1 to 2 mm in diameter after 1 day and 2 to 3 mm in diameter after 2 days. Good growth occurs at 30 to 36°C, and no growth occurs at 42°C. The type strain has all of the characteristics given for the genus in Table 7 and for the species in Table 2.

Description of *Buttiauxella izardii* sp. nov. *Buttiauxella izardii* (iz.ard'i.i. M. L. masc. gen. n. *izardii*, of Izard, in honor of Daniel Izard, a French microbiologist, for his contribution to the study of the genus *Buttiauxella* [13, 14]) was referred to as DNA hybridization group 5 in this study. Biochemical characteristics are shown in Table 2. No single biochemical characteristic differentiates *B. izardii* from *B. agrestis*, but a combina-

tion of several characteristics differentiates these taxa. Useful differential biochemical tests are the arabinose, citrate, fucose, *myo*-inositol, ornithine decarboxylase, and raffinose tests. Isolated from mollusks.

Description of the type strain. *B. izardii* type strain S3/2-161 (= ATCC 51606 = DSM 9397 = serial no. 151) was isolated from a snail sampled in Braunschweig, Germany. Its colonies are circular, convex, greyish, and smooth on nutrient agar and blood agar. They are 1 to 2 mm in diameter after 1 day and 2 to 3 mm in diameter after 2 days at 30 to 36°C. Weak, slow growth occurs at 42°C. The type strain has all of the biochemical characteristics given for the genus in Table 7 and for the species in Table 2.

Description of *Buttiauxella noackiae* sp. nov. *Buttiauxella noackiae* (no.ack'i.ae. M. L. fem. gen. n. *noackiae*, of Noack, in honor of Katrin Noack, who did most of the physiological tests at the Technische Universität Berlin and spent many years studying the usefulness of miniaturized automated tests for differentiation of various bacterial groups) was previously called enteric group 59 (10, 12) and in this study was referred to as DNA hybridization group 6. Biochemical characteristics of 15 strains isolated from snails are shown in Table 2. These characteristics are almost identical to those described for enteric group 59; the only exception is the result of the lactose test. All snail isolates lack a yellow pigment and are lactose negative, but seven of the eight enteric group 59 strains isolated from humans and food were lactose positive (10, 12). It is possible that there is a correlation between the ability to split and metabolize lactose and the ability to survive in humans. Tests that are useful in differentiating *B. noackiae* from other *Buttiauxella* species are the *N*-acetyl-L-glutamine, L-arginine dihydrolase, melibiose, and L-ornithine decarboxylase tests. In addition, 33% of the strains are phenylalanine deaminase positive and 40% produce indole. A combination of several characteristics is necessary to distinguish *B. noackiae* from other *Buttiauxella* species. Isolated from mollusks, human sputum, human wounds, and food. It is not known whether this species is pathogenic.

Description of the type strain. *B. noackiae* type strain NSW 11 (= ATCC 51607 = DSM 9401 = serial no. 170), an EG59-like strain, was isolated from a snail captured in Sydney, New South Wales, Australia. Good growth occurs at 30 to 36°C. Strain NSW 11^T colonies are circular, convex, greyish, and smooth on nutrient agar and blood agar, 1 to 2 mm in diameter after 1 day, and 2 to 3 mm in diameter after 2 days. Weak growth occurs at 42°C. The type strain has all of the characteristics given for the genus in Table 7 and for the species in Table 2.

Description of *Buttiauxella warmboldiae* sp. nov. *Buttiauxella warmboldiae* (warm'bold.i.ae. M. L. fem. gen. *warmboldiae*, of Warmbold, in honor of Sabine Warmbold, who isolated most strains of the new *Buttiauxella* species at the Staatliches Medizinaluntersuchungsamt Braunschweig) was referred to as DNA hybridization group 7 in this study. Biochemical characteristics are shown in Table 2. *myo*-Inositol is utilized and acid is produced by freshly isolated strains for many months, in contrast to all other species belonging to the genera *Buttiauxella* and *Kluyvera*. However, these characteristics are lost after some years of storage; therefore, they are not constitutive. Other biochemical tests that are useful in differentiating *B. warmboldiae* from other *Buttiauxella* species are the arabinose, arginine dihydrolase, citrate, fucose, KCN, malonate, maltitol, ornithine decarboxylase, and palatinose tests. As Table 3 shows, this species is the species that is the most distant from all other *Buttiauxella* species and has the highest phenotypic

probability distance and the lowest level of DNA-DNA relatedness. Isolated from snails.

Description of the type strain. *B. warmboldiae* type strain NSW 326 (= ATCC 51608 = DSM 9404 = serial no. 182), an EG59-like strain, was isolated from a snail sampled in Sydney, New South Wales, Australia. Good growth occurs at 30 to 36°C. Strain NSW 326^T colonies are circular, convex, greyish, and smooth on nutrient agar and blood agar, 1 to 2 mm in diameter after 1 day, and 2 to 3 mm in diameter after 2 days. Moderate growth occurs at 42°C. The type strain has all of the characteristics given for the genus in Table 7 and for the species in Table 2.

Description of *Kluyvera cochleae* sp. nov. *Kluyvera cochleae* (coch'le.ae. L. fem. gen. n. *cochleae*, of a snail) was referred to as DNA hybridization group 8 in this study. Generally less metabolically active than other *Kluyvera* species; e.g., the arabinose, *m*-coumarate, 3-hydroxyphenylacetate, lactulose, and 3-phenylpropionate reactions are negative. Negative for indole production and lysine decarboxylase activity. The negative methyl red reaction and positive Voges-Proskauer reaction distinguish *K. cochleae* from all *Buttiauxella* species and all other *Kluyvera* species. Other characteristics that differentiate *K. cochleae* from other *Kluyvera* species, as well as *Buttiauxella* species, are shown in Table 2. *K. cochleae* exhibits the largest probabilistic distance to other *Kluyvera* species (Tables 3 and 4). Isolated from mollusks.

Description of the type strain. *K. cochleae* type strain S 3/1-49 (= ATCC 51609 = DSM 9406 = serial no. 185) was isolated from a snail sampled in Braunschweig, Germany. Its colonies are circular, convex, greyish, and smooth on nutrient agar and blood agar. They are 1 to 2 mm in diameter after 1 day and 2 to 3 mm in diameter after 2 days at 30 to 36°C. Moderate growth occurs at 42°C. The type strain has all of the characteristics given for the genus in Table 7 and for the species in Table 2.

Description of *Kluyvera georgiana* sp. nov. *Kluyvera georgiana* (georg'i.a.na. M. L. fem. adj. *georgiana*, of Georgia, where important work on *Buttiauxella* and *Kluyvera* was done) was previously called *Kluyvera* species group 3 (11) and in this study was referred to as DNA hybridization group 9. The 3-phenylpropionate and *m*-coumarate reactions differentiate *K. georgiana* from *K. cochleae*. A combination of tests is necessary to differentiate *K. georgiana* from *K. ascorbata* and *K. cryocrescens*. Isolated from human sputum and throat cultures.

Description of the type strain. The type strain of *K. georgiana* is strain CDC 2891-76 (= ATCC 51603 = DSM 9409 = serial no. 189). It was isolated from human sputum (11). Good growth occurs at 30 to 42°C. Strain CDC 2891-76^T colonies are circular, convex, greyish, and smooth on nutrient agar and blood agar, 1 to 2 mm in diameter after 1 day, and 2 to 3 mm in diameter after 2 days. The type strain has all of the characteristics given for the genus in Table 7 and for the species in Table 2.

REFERENCES

- Altwegg, M., A. von Graevenitz, and J. Zollinger-Iten. 1987. Medium and temperature dependence of decarboxylase reactions in *Aeromonas* spp. *Curr. Microbiol.* **15**:1-4.
- Altwegg, M., J. Zollinger-Iten, and A. von Graevenitz. 1986. Differentiation of *Kluyvera cryocrescens* from *Kluyvera ascorbata* by irgasan susceptibility testing. *Ann. Microbiol. (Inst. Pasteur)* **137A**:159-168.
- Brenner, D. J., G. R. Fanning, A. V. Rake, and K. E. Johnson. 1969. Batch procedure for thermal elution of DNA from hydroxyapatite. *Anal. Biochem.* **28**:447-459.
- 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., P. A. D. Grimont, A. G. Steigerwalt, G. R. Fanning, E. Ageron, and C. F. Riddle. 1993. Classification of citrobacteria by DNA hybridization: designation of *Citrobacter farmeri* sp. nov., *Citrobacter youngae* sp. nov., *Citrobacter braakii* sp. nov., *Citrobacter werkmanii* sp. nov., *Citrobacter sedlakii* sp. nov., and three unnamed *Citrobacter* genomospecies. *Int. J. Syst. Bacteriol.* **43**:645-658.
- Brenner, D. J., A. C. McWhorter, J. K. Leete-Knutson, and A. G. Steigerwalt. 1982. *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. *J. Clin. Microbiol.* **15**:1133-1140.
- Dollberg, S., A. Gandacu, and A. Klar. 1990. Acute pyelonephritis due to a *Kluyvera* species in a child. *Eur. J. Clin. Microbiol. Infect. Dis.* **9**:281-283.
- Ewing, W. H. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier, New York.
- Farmer, J. J., III, M. A. Asbury, F. W. Hickman, D. J. Brenner, and the *Enterobacteriaceae* Study Group. 1980. *Enterobacter sakazakii*: a new species of "Enterobacteriaceae" isolated from clinical specimens. *Int. J. Syst. Bacteriol.* **30**:569-584.
- Farmer, J. J., III, B. R. Davis, F. W. Hickman-Brenner, A. McWhorter, G. P. Huntley-Carter, M. A. Asbury, C. Riddle, H. G. Wathen-Grady, C. Elias, G. R. Fanning, A. G. Steigerwalt, C. M. O'Hara, G. K. Morris, P. B. Smith, and D. J. Brenner. 1985. Biochemical identification of new species and biogroups of *Enterobacteriaceae* isolated from clinical specimens. *J. Clin. Microbiol.* **21**:46-76.
- Farmer, J. J., III, G. R. Fanning, G. P. Huntley-Carter, B. Holmes, F. W. Hickman, C. Richard, and D. J. Brenner. 1981. *Kluyvera*, a new (redefined) genus in the family *Enterobacteriaceae*: identification of *Kluyvera ascorbata* sp. nov. and *Kluyvera cryocrescens* sp. nov. in clinical specimens. *J. Clin. Microbiol.* **13**:919-933.
- Farmer, J. J., III, and M. T. Kelly. 1991. *Enterobacteriaceae*, p. 360-383. In A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C.
- Ferragut, C., D. Izard, F. Gavini, B. Lefebvre, and H. Leclerc. 1981. *Buttiauxella*, a new genus of the family *Enterobacteriaceae*. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe C* **2**:33-44.
- Gavini, F., D. Izard, C. Ferragut, J. J. Farmer III, and H. Leclerc. 1983. Separation of *Kluyvera* and *Buttiauxella* by biochemical and nucleic acid methods. *Int. J. Syst. Bacteriol.* **33**:880-882.
- Gavini, F., B. Lefebvre, and H. Leclerc. 1976. Positions taxonomiques d'entérobactéries H₂S—par rapport au genre *Citrobacter*. *Ann. Microbiol. (Inst. Pasteur)* **127A**:275-295.
- Hickman-Brenner, F. W., G. P. Huntley-Carter, Y. Saitoh, A. G. Steigerwalt, J. J. Farmer III, and D. J. Brenner. 1984. *Moellerella wisconsinensis*, a new genus and species of *Enterobacteriaceae* found in human stool specimens. *J. Clin. Microbiol.* **19**:460-463.
- Luttrell, R. E., G. A. Rannick, J. L. Soto-Hernandez, and A. Verghese. 1988. *Kluyvera* species soft tissue infection: case report and review. *J. Clin. Microbiol.* **26**:2650-2651.
- Véron, M. 1975. Nutrition et taxonomie des enterobactéries. I. Méthode d'étude des auxanogrammes. *Ann. Microbiol. (Inst. Pasteur)* **126A**:267-274.
- Wayne, L. G., D. J. Brenner, R. R. Colwell, P. A. D. Grimont, O. Kandler, M. I. Krichevsky, L. H. Moore, W. E. C. Moore, R. G. E. Murray, E. Stackebrandt, M. P. Starr, and H. G. Trüper. 1987. Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics. *Int. J. Syst. Bacteriol.* **37**:463-464.