Recognition of *Morganella* Subspecies, with Proposal of *Morganella morganii* subsp. *morganii* subsp. nov. and *Morganella morganii* subsp. *sibonii* subsp. nov.

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The genus name *Morganella* was established within the family *Enterobacteriaceae* in 1978. *Morganella morganii* is the only species described thus far within this genus, and the name *M. morganii* has been accepted by usage in the scientific community for strains previously known as *Proteus morganii*. *M. morganii* isolates differ in their abilities to ferment trehalose and exhibit variable lysine and ornithine decarboxylase patterns, emphasizing the phenotypic heterogeneity within this species. Previous genetic studies failed to reveal separate entities within the genus *Morganella*. We observed some trehalose-fermenting strains with different lysine and ornithine decarboxylase patterns. Two strains were lysine and ornithine positive, 3 were lysine positive and ornithine negative, and 29 were lysine negative and ornithine positive. These strains and 25 non-trehalose-fermenting strains with different lysine and ornithine decarboxylase patterns were investigated. DNA-DNA hybridization studies and phenotypic characterizations revealed that *M. morganii* can be separated into three DNA relatedness groups and seven biogroups. Strains from DNA relatedness group 1 were trehalose negative, and strains from DNA relatedness groups 2 and 3 were trehalose positive. One biogroup from DNA relatedness group 2 was phenotypically indistinguishable from DNA relatedness group 3. On the basis of these studies, we propose that *M. morganii* be subdivided into *M. morganii* subsp. *morganii* (type strain ATCC 25830) containing biogroups A, B, C, and D (DNA relatedness group 1) and *M. morganii* subsp. *sibonii* (type strain 8103-85; =ATCC 49948) containing biogroups E, F, and G (DNA relatedness groups 2 and 3).

On the basis of genetic studies performed by Brenner et al. in 1978, strains previously known as *Proteus morganii* were placed in a new genus, *Morganella*, and designated *Morganella morganii* (1). The name *M. morganii* is included in the "Approved Lists of Bacterial Names" (18) and has been accepted by usage in the scientific community. The genus name *Morganella* was first proposed by MacDonald Fulton in 1943 (6). *M. morganii* is the only species.

However, prior to the transfer of *P. morganii* to the genus *Morganella*, the homogeneity of this group of organisms was questioned by Siboni, who showed that *M. morganii* could be divided into two groups based on trehalose fermentation, resistance to tetracycline, and flagellar wavelength (16). Recently, the association between trehalose fermentation and tetracycline resistance in *M. morganii* has been observed by others (15). Additional studies by Hickman et al. also called attention to diversity among strains of *M. morganii* (9). They described two groups of *M. morganii* with atypical lysine and ornithine decarboxylase reactions. One group was lysine positive, and another group was ornithine negative. The two groups were shown to be closely related to *M. morganii*^T by DNA-DNA hybridization studies. Non-motile members of the lysine-positive group were later designated *M. morganii* biogroup 1 by Farmer et al. (5).

Decarboxylation of lysine has been assumed to be associated with the presence of a large plasmid in some strains of *M. morganii*, and the value of using this characteristic for biogrouping has been questioned (3).

We observed three strains that were lysine positive,

ornithine negative, and trehalose fermenting and two strains that were lysine positive, ornithine positive, and trehalose fermenting but otherwise resembled *M. morganii* biochemically. These observations prompted further investigations, including DNA-DNA hybridization. The results of these investigations showed that *M. morganii* can be divided into two subspecies and seven biogroups.

MATERIALS AND METHODS

Bacterial strains. The strains included in the study, their origins, and their sources together with some phenotypic characteristics are shown in Table 1. There are seven phenotypic groups. Group A (three isolates) includes the type strain *M. morganii* ATCC 25830. Strain 4567-84^T is a culture of ATCC 25830^T maintained at the Centers for Disease Control (CDC) since 1973. Group B (15 strains) belongs to the lysine-positive biogroup of Hickman et al. (9). Group C (four strains) and group D (three strains) consist of non-trehalose-fermenting strains with atypical decarboxylase reactions. Group E (two strains) and group F (four strains, six isolates) consist of trehalose-fermenting strains with atypical decarboxylase reactions. Group G-1 (8 strains from the CDC, Atlanta, Ga., and strain 20980/68 from K. Siboni, Odense, Denmark) and group G-2 (20 strains from K. Siboni, Odense, Denmark) are groups of trehalose-fermenting strains that resemble group A with respect to decarboxylase reactions. The strains received from Siboni have been studied previously (16, 17).

Identification. The biochemical test methods used have been described elsewhere (8, 9). The strains received from Siboni were reexamined biochemically.

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TABLE 1. Sources, origins, and phenotypic characteristics of strains studied^a

Strain	Biogroup	Source	Origin	Lys	Orn	Tre	Gly	Tet	Mot
25830 ^T	A	ATCC	Stool	0	+	0	(+)	S	(+)
4567-84 ^T		CDC	Stool	0	+	0	(+)	S	(+)
1881-73		CDC	Stool	0	+	0	+	S	0
1939-76	B	CDC	Urine	+	+	0	+	S	0
1274-75		CDC	Neck abscess	+	+	0	+	S	0
2325-70		CDC	Stool	+	+	0	0	S	+
1143-76		CDC	Stool	+	+	0	(+)	S	+
1072-76		CDC	Stool	+	+	0	+	S	0
392-75		CDC	Gastric ulcer	+	+	0	+	S	0
3528-75		CDC	Urine	+	+	0	+	I	0
632-76		CDC	Sputum	+	+	0	+	I	0
1753-71		CDC	Wound	+	+	0	0	S	+
4510-74		CDC	Sputum	+	+	0	+	S	0
420-75		CDC	Wound	+	+	0	+	S	0
2432-75		CDC	Stool	+	+	0	+	S	0
1126-76		CDC	Tracheostomy wound	+	+	0	+	S	0
3544-74		CDC	Groin wound	+	+	0	+	S	0
3566-75		CDC	Eye	+	+	0	+	S	0
1427-73	C	CDC	Sputum	0	0	0	+	S	0
4564-73		CDC	Urine	0	0	0	(+)	R	0
4195-69		CDC	Stool	0	0	0	(+)	R	+
3483-75		CDC	Bile	0	0	0	+	R	+
2866-78	D	CDC	Sputum	+	0	0	+	I	0
728-77		CDC	Tracheal secretion	+	0	0	+	S	0
1584-68		CDC	Wound	+	0	0	+	S	0
AB 2429	E	SSI	Wound	+	+	+	0	R	+
AB 2174		SSI	Wound	+	+	+	0	R	+
13-82	F	von Graevenitz ^b	Unknown	+	0	+	(+)	R	+
13-82 (c2) (AB 2304)		CDC	Single colony from 13-82	0	0	+	0	R	+
13-82 (c3) (AB 2305)		CDC	Single colony from 13-82	+	0	+	0	R	+
8103-85 (AB 2048)		SSI	Sputum	+	0	+	(+)	R	+
8104-85 (AB 2230)		SSI	Anus	+	0	+	(+)	R	+
2134-79		CDC	Urine	0	0	+	+	R	0
3522-75	G-1	CDC	Urine	0	+	+	(+)	R	+
212-83		CDC	Stool	0	+	+	(+)	S	+
336-77		CDC	Ankle	0	+	+	0	R	+
1721-75		CDC	Leg wound	0	+	+	0	S	+
4920-84		CDC	Urine	0	+	+	(+)	R	0
2248-79		CDC	Stool	0	+	+	0	S	+
1629-78		CDC	Stool	0	+	+	0	S	+
4919-84		CDC	Stool	0	0	+	(+)	R	+
20980/68		Siboni ^d	Urine	0	+	+	(+)	S	+
Perch 325 (O 4a,4b)	G-2	Siboni	Unknown	0	+	+	(+)	R	+
Perch 524 (O 4a)		Siboni	Unknown	0	+	+	(+)	R	+
Perch 587 (O 9)		Siboni	Unknown	0	+	+	(+)	R	+
44031/66		Siboni	Pus	0	+	+	(+)	R	+
46428/66		Siboni	Urine	0	+	+	(+)	R	+
47728/66		Siboni	Pus	0	+	+	(+)	R	+
16072/68		Siboni	Urine	0	+	+	+	R	0
18635/68		Siboni	Abscess on foot	0	+	+	(+)	R	+
18862 II/68		Siboni	Clinical isolate	0	+	+	(+)	R	0
18866/68		Siboni	Pus from leg	0	+	+	(+)	R	+
21239/68		Siboni	Cecal abscess	0	+	+	(+)	R	+
21043/68		Siboni	Urine	0	+	+	(+)	R	+
19617/68		Siboni	Urine	0	+	+	(+)	R	+
19504/68		Siboni	Peritonitis	0	+	+	(+)	R	+
35234/76		Siboni	Urine	0	+	+	(+)	S	+
660 III/75		Siboni	Urine	0	+	+	+	R	+
47545/66		Siboni	Clinical isolate	0	+	+	(+)	R	+
18237/68		Siboni	Blood	0	+	+	(+)	R	+
Perch F 5706 (O 15)		Siboni	Unknown	0	+	+	+	R	+
Perch F 17925 (O 16)		Siboni	Unknown	0	+	+	(+)	R	+

^a Lys, lysine; Orn, ornithine; Tre, trehalose; Gly, glycerol; Tet, tetracycline; Mot, motility; S, susceptible; I, intermediately susceptible; R, resistant; +, positive reaction after 48 h; 0, negative reaction after 48 h. Delayed positive results (3 to 7 days) are given in parentheses. SSI, Statens Seruminstitut, Copenhagen, Denmark.

^b A. von Graevenitz, Department of Medical Microbiology, University of Zürich, Switzerland.

^c Swarming.

^d K. Siboni, Department of Clinical Microbiology, Odense University Hospital, Odense, Denmark.

TABLE 2. Phenotypic characteristics differentiating *M. morganii* biogroups^a

Biogroup	No. of strains	Lys	Orn	Tre	Tet (% susceptible)	Mot
A, ATCC 25830 ^T	1	—	+	—	100	+
B	22 ^b	+	+	—	100 ^c	—
C	14 ^b	—	—	—	14	d+
D	3	+	—	—	100 ^d	—
E	2	+	+	+	0	+
F	4	d+	—	+	0	d+
G ^e	29	—	+	+	21	+

^a Lys, lysine; Orn, ornithine; Tre, trehalose; Tet, tetracycline; Mot, motility; +, ≥90% positive within 48 h; —, ≤10% positive within 48 h; d+ = 50 to 89% positive within 48 h.

^b Data are from reference 9.

^c Two strains were intermediately susceptible.

^d One strain was intermediately susceptible.

^e Includes G-1 and G-2.

DNA relatedness studies. Strains were grown to log phase in brain heart infusion broth with shaking at $36 \pm 1^\circ\text{C}$ to obtain cells for DNA extraction. The methods used to extract and purify DNA and to determine DNA relatedness between strains have been described elsewhere (2). DNAs were labeled enzymatically in vitro with [³²P]dCTP, using a nick translation reagent kit (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) as directed by the manufacturer.

Antimicrobial susceptibility testing. All strains in groups E, F, and G-2 were tested against tetracycline and colistin by the agar diffusion method using NeoSensitabs (Rosco Diagnostica, Taastrup, Denmark) and Danish blood agar (Statens Seruminstitut, Copenhagen, Denmark). Strains with a zone diameter of ≥28 mm around the tetracycline tablet were considered tetracycline susceptible (MIC correlate, ≤2 µg/ml), and those with a zone diameter of ≤15 mm were considered tetracycline resistant (MIC correlate, ≥32 µg/ml). Strains with a zone diameter of ≥22 mm around the colistin tablet were considered colistin susceptible (MIC correlate, ≤4 µg/ml), and those with a zone diameter of ≤13 mm were considered colistin resistant (MIC correlate, ≥128 µg/ml).

RESULTS

Biochemical tests. *M. morganii* strains were divided into two biogroups on the basis of their abilities to ferment trehalose (Table 2). The trehalose-negative biogroup was subdivided into biogroups A, B, C, and D, and the trehalose-positive biogroup was subdivided into biogroups E, F, and G on the basis of lysine decarboxylase and ornithine decarboxylase reactions (Table 2). Tetracycline resistance and motility were also of some help in distinguishing biogroups A through D. Complete biochemical reactions of these seven biogroups are given in Table 3. Biogroup G strains, although biochemically indistinguishable, are divided into groups G-1 and G-2 because they are distinguishable by DNA hybridization (see below).

DNA relatedness. Initial studies clearly indicated that the strains in biogroup F were distinguishable from those of biogroups A, B, and C (Table 4). When labeled DNA from *M. morganii* 25830^T (biogroup A) was used, biogroup F showed borderline species-level relatedness to biogroups A, B, and C (70% or greater relatedness and 5% or less

divergence in related sequences is used to define strains in a single species [20]).

Further hybridization studies with biogroups A through G revealed three DNA relatedness groups (Table 5). DNA relatedness group 1 contained biogroups A through D. Biogroups E and F and two-thirds of biogroup G strains (biogroup G-2) were in DNA relatedness group 2. The remaining one-third of biogroup G strains (biogroup G-1) constituted DNA relatedness group 3. The three relatedness groups were interrelated at or just below the species level of relatedness. They were separable by the lower level of intergroup relatedness at both optimal (60°C) and stringent (75°C) criteria for DNA reassociation and by their higher divergence in related sequences than was seen with a relatedness group.

Some colonies from strain 13-82 (biogroup F, relatedness group 2) lacked lysine decarboxylase activity [strain 13-82 (c2)], and some colonies swarmed on horse blood agar [strain 13-82 (c3)]. These variants were 94% related to wild-type strain 13-82 (Table 5). Similar intrastrain variation was not demonstrable with biogroup F strains 8103-85 and 8104-85.

Trehalose-positive strains 2134-79 and 4919-84 were lysine negative and ornithine negative, placing them midway between biogroup F (lysine variable and ornithine negative) and biogroup G (lysine negative and ornithine positive). Strain 2134-79 was in DNA relatedness group 2 with the biogroup F strains. Strain 4919-84 was in DNA relatedness group 3.

DISCUSSION

The type strain of *M. morganii* showed average relatedness to strains from biogroups A through D (DNA relatedness group 1) of 89% at the optimal DNA reassociation temperature (60°C), with 1.0% divergence within related sequences, and 86% relatedness at the stringent DNA reassociation temperature (75°C) (Table 5). This high level of relatedness is typical for species whose strains exhibit little heterogeneity. Average relatedness of the *M. morganii* type strain to strains from biogroups E, F, and G-2 (DNA relatedness group 2) was 76% at 60°C with 6% divergence and 62% at 75°C. Relatedness at both optimal and stringent incubation temperatures is at the level of a single species, and the divergence within related sequences is just above the 5% figure used to define a species (20). Similarly, the *M. morganii* type strain shows average relatedness to biogroup G-1 (DNA relatedness group 3) of 78% at 60°C with 4% divergence and 67% at 75°C.

DNA relatedness groups 2 and 3 also exhibit high intra-group relatedness: 92% at 60°C with 1.5% divergence and 89% at 75°C for group 2 and 86% at 60°C with 1% divergence and 84% at 75°C for group 3 (Table 5). Reciprocal relatedness of groups 2 and 3 is at the border of species-level relatedness: 69 and 65% at 60°C with 5% divergence and 55 to 58% at 75°C. Labeled DNAs from both groups 2 and 3 give lower levels of relatedness to DNA relatedness group 1 than was seen in the reciprocal reactions (using labeled DNAs from DNA relatedness group 1). These values, 58 and 50% (at 60 and 75°C for group 2 with group 1) and 65 and 55% (at 60 and 75°C for group 3 with group 1) are below species level, although the percent divergence values (5.0 for group 2 and 3.5 for group 3, respectively, with group 1) are indicative of species-level relatedness and are very similar to those obtained in the reciprocal reactions with labeled DNA from group 1 (6.0 and 4.0, respectively, with groups 2 and 3).

TABLE 3. Phenotypic characteristics of *Morganella* biogroups

Test ^a	Results for biogroup (no. of strains) ^b :							
	A ^c (1)	B (15)	C (4)	D (3)	E (2)	F (4)	G-1 (9)	G-2 (20)
ONPG	0	2 (1)	2 (1)	0	0	0 (1)	0	0
Urea	+	14	+	+	+	+	8 (1)	+
Phenylalanine	+	+	+	+	+	+	8	+
Lysine (Møllers)	0	14	0	+	+	3	0	0
Arginine (Møllers)	0	0	0	0	0	0	0	0
Ornithine (Møllers)	+	15	0	0	+	0	8	+
Indole	+	+	+	+	+	3	5	+
Voges-Proskauer	0	0	0	0	0	0	0	0
Malonate	0	1 (2)	0	0	0	0	0	0
Citrate (Simmons)	0	0	0	0	0	0	0	0
KCN (growth in)	+	13	3	+	+	+	6	+
Gelatin, 22°C	0	0	0	0	0	0	0	0
H ₂ S (TSI)	0	0	1	1	0	0	2 (1)	0
Motility, 22°C	+	4	2	0	+	3	8	18
D-Glucose acid	+	+	+	+	+	+	+	+
D-Glucose gas	+	14	+	+	+	3 (1)	7 (1)	+
Acid from								
L-Arabinose	0	0	0	0	0	0	0	0
D-Xylose	0	0	0	0	0	0	0	0
L-Rhamnose	0	0	0	0	0	0	0	0
Mannose	+	+	+	+	+	+	8 (1)	+
Galactose	+	+	+	+	+	+	+	+
Sucrose	0	0	0	0	0	0	1 (3)	1 (2)
Lactose	0	0	0	0	0	0	0	0
Maltose	0	0	0	0	0	0	0	0
Cellobiose	0	0	0	0	0	0	0	0
Trehalose	0	0	0	0	+	+	+	+
Melibiose	0	0	0	0	0	0	0	0
Raffinose	0	0	0	0	0	0	0	0
Adonitol	0	0	0	0	0	0	0	0
Dulcitol	0	0	0	0	0	0	0	0
D-Sorbitol	0	0	0	0	0	0	0	0
D-Mannitol	0	0	0	0	0	0	0	0
myo-Inositol	0	0	0	0	0	0	0	0
Salicin	0	0	0	0	0	0	0	0
Glycerol	+	12 (1)	2 (2)	+	0	1 (3)	0 (5)	3 (17)
Erythritol	0	0	0	0	0	0	0	0
Alpha-CH ₃ -glycoside	0	0	0	0	0	0	0	0
Esculin	0	0	0	0	0	0	0	0
Nitrate reduction	+	+	+	1	1	+	+	+
Oxidase	0	0	0	0	0	0	0	0
Swarming	0	0	0	0	0	1	0	1
Tetracycline	S	13S/2I	1S/3R	2S/1I	R	R	5S/4R	1S/19R
Colistin	R	R	R	R	R	R	R	R

^a ONPG, *o*-nitrophenyl-β-D-galactopyranoside; TSI, triple sugar iron.^b +, all strains positive after 48 h; 0, all strains negative after 7 days; S, susceptible; R, resistant; I, intermediately susceptible. Results are numbers of strains positive after 48 h of incubation at 35°C. Numbers in parentheses are numbers of strains positive after 3 to 7 days of incubation at 35°C.^c ATCC 25830^T.

A number of factors indicate that all of the *M. morganii* biogroups are a single species. (i) All strains show species-level relatedness to the type strain of *M. morganii*. (ii) DNA relatedness groups 2 and 3 exhibit species-level relatedness. (iii) Divergence within related sequences is similar and at or very close to species level in reciprocal reactions between DNA relatedness groups 1 and 2 and groups 1 and 3. (iv) In reactions between groups 1 and 2 and groups 1 and 3, the level of relatedness obtained at the stringent temperature for DNA reassociation drops only slightly compared with that of the optimal temperature for DNA reassociation. This is indicative of strains of the same species.

We therefore conclude that the relatedness values obtained when labeled DNAs from groups 2 and 3 are reacted with group 1, which are lower than those of the reciprocal

reactions, are due to differences in genome size in the strains of these three DNA relatedness groups. Using this reasoning, 76% of group 1 DNA equals 58% of group 2 DNA, and 78% of group 1 DNA equals 65% of group 3 DNA (Table 5). From these relationships, it follows that 100% of group 1 DNA is equivalent to 76% of group 2 DNA and 83% of group 3 DNA and that 100% of group 3 DNA is equivalent to 92% of group 2 DNA. Actually, 71% of group 3 DNA was equivalent to 69% of group 2 DNA (Table 5), indicating that 100% of group 3 DNA is equivalent to 97% of group 2 DNA.

Subspecies designations are used to group strains of a single species on the basis of one or more differences deemed of value to microbiologists. Criteria used for subspeciation include differences in biochemical characteristics, pathogenicity, host range, and ability to produce an antibi-

TABLE 4. DNA relatedness of *M. morganii* biogroups A, B, C, and F

Source of unlabeled DNA ^a	% Relative binding with labeled DNA from:					
	Strain 8103-85			ATCC 25830 ^T		
	60°C	%D ^b	75°C	60°C	%D	75°C
Biogroup						
A (2)	56–62	5.5–6.0	44–45	92–100	0.0–1.0	92–100
B (10)	47–69	4.5–6.0	27–51	91–98	1.0–2.0	90–97
C (2)	58–69	5.5	48	90	2.0	92
F (3)	93–100	0.0–1.0	85–100	68–70	5.5	51–59
<i>Proteus</i> spp. (4)	8–11					
<i>Providencia</i> spp. (4)	4–12					
Other <i>Enterobacteriaceae</i> (45)	4–15					

^a Numbers in parentheses are numbers of strains (for biogroups) or species (for genera and family) tested.

^b %D, divergence within related sequences (2) calculated to the nearest 0.5%.

otic. Subspecies may also reflect differences in DNA relatedness. It has been recommended that subspecies designations "be used for genetically close organisms that diverge in phenotype" (20), as is the case in *Salmonella* spp. (4, 10).

The DNA relatedness data are compatible with the designation of three subspecies within *M. morganii*: one representing trehalose-negative biogroups A through D; one representing trehalose-positive biogroups E, F, and G-2; and one representing biogroup G-1, which is also trehalose positive. Since the G-1 and G-2 strains are phenotypically indistinguishable, we propose dividing *M. morganii* into two subspecies.

M. morganii subsp. *morganii* subsp. nov. contains biogroups A, B, C, and D. Biogroup A includes *M. morganii* ATCC 25830^T. Biogroup B is equivalent to the lysine-positive biogroup of Hickman et al. (9) and thus includes *M. morganii* biogroup 1 of Farmer et al. (5). Biogroup C is equivalent to the ornithine-negative biogroups of Hickman et al. (9).

M. morganii subsp. *sibonii* subsp. nov. contains biogroups E, F, and G. Trehalose-positive strains belong to *M. morganii* subsp. *sibonii*. They are usually also characterized by their tetracycline resistance. Siboni found 18 (90%) of 20 trehalose-positive strains to be tetracycline resistant (17). In other studies, frequencies of 35% (19) and 48% (7) tetracycline-resistant *M. morganii* were observed, but the proportions of trehalose-positive strains were not noted in these studies. Tetracycline susceptibility in *M. morganii* subsp. *sibonii* seems to be rare (17). Only 1 (6%) of 16 trehalose-positive strains reported by Senior and Vörös was susceptible to tetracycline (15). There are not many reports on trehalose fermentation in *M. morganii*. Richard observed one of 27 lysine-positive strains to be trehalose positive and tetracycline resistant (14). In other studies, frequencies of trehalose-positive strains of 10% (5) and 14% (9) were observed in strains with lysine or ornithine decarboxylase patterns compatible with those of biogroup A. However, no information regarding tetracycline susceptibility is available for these strains. According to the proposed classification, they belong to *M. morganii* subsp. *sibonii* biogroup G.

Trehalose-negative strains belong to *M. morganii* subsp. *morganii*. Tetracycline resistance also occurs in this subspecies and has a very high frequency in biogroup C. Hickman et al. found 12 (86%) of 14 ornithine-negative strains to be tetracycline resistant (9). The real frequency of tetracycline resistance in biogroup A is unknown. Siboni found 21 (10%) of 203 trehalose-negative strains to be tetracycline resistant (17).

Biogrouping within the two subspecies is justified by the differences in lysine and ornithine decarboxylase patterns (Table 2). However, the value of lysine as a characteristic suitable for biogrouping *M. morganii* strains was questioned by Cornelis et al. (3), who were able to demonstrate an association between lysine decarboxylation and the presence of a large plasmid in 8 of 14 strains of *M. morganii*. These 8 strains yielded lysine-negative segregants after plasmid curing, and 10 of the 14 strains were able to transfer their lysine-positive characteristic to a recipient *M. morganii* strain. Cornelis et al. used a different lysine decarboxylase test medium than we did but were able to confirm the decarboxylation of lysine by demonstrating cadaverine in the test medium. They argued that lysine could be a plasmid-mediated trait in the lysine-positive groups of Hickman et al. (our biogroup B). However, Cornelis et al. did not investigate any of the strains of Hickman et al., and the strains of Cornelis et al. differed from our strains of biogroup B in several aspects, including motility and inability to ferment glycerol (3).

Even though lysine decarboxylation may be plasmid mediated in some strains, it appears to be a stable characteristic, as reported previously (3, 9). In addition, none of the strains in biogroups E and F had lost their abilities to decarboxylate lysine when retested after a 5-year period, although lysine-negative variants segregated in strain 13-82.

Two trehalose-positive strains (2134-79 and 4919-84) that were lysine and ornithine negative belonged to different hybridization groups but could not be separated biochemically. Such strains should be reported as *M. morganii* subsp. *sibonii*, lysine- and ornithine-negative variants.

Although ATCC 25830^T is glycerol positive, glycerol fermentation in biogroup A seems to be rare. The reported frequencies of glycerol fermentation in *M. morganii* have been 2.7% and 5% (5, 15).

Strains of biogroup G-1 formed DNA relatedness group 3. Phenotypically, these strains are indistinguishable from those of group G-2. For this reason, we do not propose biogroup G-1 as a separate subspecies (20). If useful tests to identify it become available, it will become a third subspecies.

Characteristics useful for differentiating the two *M. morganii* subspecies from *Proteus* and *Providencia* species are shown in Table 6. *M. morganii* subsp. *morganii* biogroup C can be differentiated from *Providencia rustigianii* only by the ability of *M. morganii* subsp. *morganii* to hydrolyze urea.

Sources of *M. morganii* strains are given in Table 1. No effort was made to obtain additional clinical information. It is

TABLE 5. DNA relatedness of *M. morganii* biogroups A through G

Source of unlabeled DNA	% Relative binding with labeled DNA from strain:																	
	25830 ^T			4567-84 ^T			8103-85			13-82			3522-75			44031/66		
	60°C	%D ^a	75°C	60°C	%D	75°C	60°C	%D	75°C	60°C	%D	75°C	60°C	%D	75°C	60°C	%D	75°C
Biogroup A																		
25830 ^T	100	0.0	100	100	1.0	97	62	4.0	50	53	6.5	45	67	3.5	54	77	5.0	60
4567-84 ^T	100	0.5	98	100	0.0	100	68	4.5	56	62	5.5	47	65	3.5	55	80	5.0	65
1881-73				85	1.0	86				56	5.0	44						
Biogroup B																		
1939-76				97	2.0	93				57	5.5	47						
1274-75				95	0.5	92				47	5.0	47	63	3.5	55			
2325-70				94	2.5	87				61	5.5	48						
1143-76	100	1.0	100	93	0.5	95	72	4.5	49	57	4.5	50	70	3.5	60	82	5.5	61
1072-76				92	1.5	94				57	6.0	48						
392-75				92	0.5	88				50	5.0	48						
3528-75				92	1.0	85				52	6.0	47						
632-76				90	1.0	85				50	6.0	47						
1753-71				87	1.5	85				51	6.0	41						
4510-74				86	1.0	80				52	5.0	42						
420-75				85	1.0	88				51	5.0	47	64	4.0	54			
2432-75				82	0.5	81				49	5.0	45						
1126-76				81	0.5	81				48	5.0	46						
3544-74				80	0.5	71				45	5.5	42						
3566-75				76	0.5	81				45	4.5	43	54	3.5	50			
Biogroup C																		
1427-73				94	0.5	88				62	4.5	54						
4564-73				88	1.5	82				67	4.0	63						
4195-69				87	1.5	86				59	5.0	55						
3483-75				87	1.5	76				59	6.5	48						
Biogroup D																		
2866-78				93	1.0	84				64	5.5	56	70	3.5	60			
728-77				87	1.0	94				58	3.5	53						
1584-68				84	1.0	90				53	4.5	52						
Biogroup E																		
AB 2429																91	2.5	90
AB 2174	79	5.5	61				83	1.0	84				72	5.0	61	95	2.5	90
Biogroup F																		
13-82	74	6.0	60	76	5.5	60	97	0.0	99	100	0.0	100	75	4.5	62	89	1.5	86
13-82 (c2) (AB 2304)				72	5.5	60				97	0.5	92						
13-82 (c3) (AB 2305)				77	5.5	59				94	1.0	100						
8103-85 (AB 2048)	77	5.5	62	78	6.0	67	100	0.0	100	98	0.5	89	74	5.0	60	88	2.0	86
8104-85 (AB 2230)				76	6.0	62				99	0.5	89	69	5.5	51			
2134-79				73	6.0	56				81	2.5	74						
Biogroup G-1																		
3522-75	80	4.0	65	79	4.0	70	61	4.0	55	56	5.5	53	100	0.0	100	77	5.0	64
212-83				83	3.5	77				66	6.0	54	89	1.0	87			
336-77				78	3.5	67				61	5.5	49	83	1.0	84			
1721-75				74	3.5	66				63	5.0	49	83	1.5	78			
4920-84													85	1.0	82			
2248-79	76	4.0	65				70	4.0	61				87	1.0	90	82	5.5	67
1629-78													81	1.0	76			
4919-84	77	4.0	62				69	4.0	60				90	0.5	91	78	5.0	64
20980/68	74	5.0	65				68	5.0	55				88	1.0	86	82	5.0	65
Biogroup G-2																		
Perch 325																94	0.5	90
Perch 524																95	0.5	90
Perch 587																95	1.0	86
44031/66	75	6.0	58				84	1.5	87				71	5.0	59	100	0.0	100
46428/66																96	0.5	92
47728/66	78	5.5	60				83	1.5	84				72	4.5	56	93	0.5	93
16072/68																91	0.5	89
18635/68																93	1.5	87
18862 II/68																94	1.0	91
18866/68	74	6.0	57				82	1.0	83				71	4.5	56	92	1.5	88
21239/68																92	2.5	90
21043/68	72	6.0	60				79	1.5	82				65	5.0	53	93	1.0	92
19617/68																92	2.0	89
19504/68																90	2.0	88
35234/76																90	2.5	87
660 III/75																95	1.0	92
47545/66																98	2.0	95
18237/68																94	0.5	95
Perch F 5706																89	1.0	87
Perch F 17925																95	0.5	93

^a %D, divergence within related sequences (2) calculated to the nearest 0.5%.

TABLE 6. Characteristics useful for differentiating *M. morganii* subspecies and biogroups from *Proteus* and *Providencia* species^a

Strain	Reference or source	Ur	Lys	Orn	Ind	Cit	KCN	Gel (22°C)	H ₂ S (TSI)	Mot (22°C)	X	Mn	Ma	Tre	Ad	Mt	Er
<i>M. morganii</i> subsp. <i>morganii</i>																	
Biogroup A ^b	11	+	-	+	+	-	+	-	-	+	-	+	-	-	-	-	-
Biogroup B	9	+	+	+	+	-	+	-	d-	-	-	+	-	-	-	-	-
Biogroup C	9	+	-	-	+	-	+	-	d+	d+	-	+	-	-	-	-	-
Biogroup D	This study	+	+	-	+	-	+	-	d-	-	-	+	-	-	-	-	-
<i>M. morganii</i> subsp. <i>sibonii</i>																	
Biogroup E	This study	+	+	+	+	-	+	-	-	+	-	+	-	+	-	-	-
Biogroup F	This study	+	d+	-	d+	-	+	-	-	d+	-	+	-	+	-	-	-
Biogroup G	This study	+	-	+	d+	-	d+	-	d-	+	-	+	-	+	-	-	-
<i>Proteus mirabilis</i>	5	+	-	+	-	d+	+	+	+	+	+	-	-	+	-	-	-
<i>Proteus vulgaris</i>	5	+	-	-	+	d-	+	+	+	+	+	-	+	d-	-	-	-
<i>Proteus penneri</i>	5	+	-	-	-	-	+	d+	d-	d+	+	-	+	d+	-	-	-
<i>Proteus myxofaciens</i>	5	+	-	-	-	d+	+	+	-	+	-	-	+	+	-	-	-
<i>Providencia rettgeri</i>	5	+	-	-	+	+	+	-	-	+	-	+	-	-	+	+	d+
<i>Providencia stuartii</i>	5	d-	-	-	+	+	+	-	-	d+	-	+	-	+	-	-	-
<i>Providencia alcalifaciens</i>	5	-	-	-	+	+	+	-	-	+	-	+	-	-	+	-	-
<i>Providencia rustigianii</i>	5	-	-	-	+	d-	+	-	-	d-	-	+	-	-	-	-	-
<i>Providencia heimbachae</i>	12	-	-	-	-	-	-	-	-	d-	-	+	d+	-	+	-	-

^a Ur, urea; Lys, lysine decarboxylase; Orn, ornithine decarboxylase; Ind, indole; Cit, citrate; KCN, growth in KCN; Gel, gelatin; TSI, triple sugar iron; Mot, motility; X, xylose; Mn, Mannose; Ma, Maltose; Tre, trehalose; Ad, adonitol; Mt, mannitol; Er, erythritol; +, ≥90% positive within 48 h; -, ≤10% positive within 48 h; d+, 50 to 89% positive within 48 h; d-, 11 to 49% positive within 48 h.

^b *M. morganii* ATCC 25830^T.

premature to speculate on any differential clinical significance of the subspecies or the biogroups within them. Nonetheless, some differences in the sources of isolation of the few strains in this study are worth noting. Twenty-five *M. morganii* subsp. *morganii* strains were mostly isolated from stool (32%), wounds (28%), sputum (20%), and urine (12%). Seventeen *M. morganii* subsp. *sibonii* strains from biogroups E, F, and G-2 were isolated predominantly from wounds and urine (41% each), with only a single isolate from stool (6%). The nine biogroup G-1 strains (DNA relatedness group 3) in *M. morganii* subsp. *sibonii* were isolated from stool (44%), urine (33%), and wounds (22%). The only isolate from blood in this study was a biogroup G-2 strain.

Description of *Morganella morganii* subsp. *morganii* subsp. nov. (mor.ga'ni.i. M. L. gen. n. *morganii*, of Morgan; named after H. de R. Morgan, a British bacteriologist who first studied the organism).

The description is based on the strains belonging to biogroups A, B, C, and D of the present study. Characteristics are as given previously for the genus (13), with the following exceptions. Members of biogroup B are lysine positive and mostly nonmotile. Members of biogroup C are ornithine negative. Members of biogroup D are lysine positive and ornithine negative. Biochemical characteristics for biogroup A are as given previously for ATCC 25830^T (11). Complete biochemical characteristics of biogroups A, B, C, and D are listed in Tables 2 and 3. Acid not produced from trehalose. Isolated from human clinical specimens (stool, wound, sputum, eye, bile, gastric ulcer, urine). The type strain is ATCC 25830.

Description of *Morganella morganii* subsp. *sibonii* subsp. nov. (si.bo'ni.i. M. L. gen. n. *sibonii*, of Siboni; named after Knud Siboni, a Danish microbiologist who first recognized trehalose-fermenting *M. morganii* as a separate group).

The description is based on studies of 35 strains (groups E, F, G-1, and G-2 of the present study). Characteristics are as given previously for the genus (13) with the following excep-

tions. Acid is produced from trehalose, and two strains are swarming. Members of biogroup E are lysine positive and ornithine positive. Members of biogroup F are lysine variable and ornithine negative. Members of biogroup G are lysine negative and ornithine positive. Complete biochemical reactions are listed in Tables 2 and 3. Most strains are tetracycline resistant. All strains are colistin resistant. Isolated from human clinical specimens (sputum, urine, stool, wound). The type strain is 8103-85 (=ATCC 49948).

Description of the type strain. The type strain of *M. morganii* subsp. *sibonii* is 8103-85 (=AB 2048^T, =ATCC 49948). This strain was isolated from human sputum. Colonies on nutrient agar are circular, convex, and smooth with entire edges. Colonies are 1 to 2 mm in diameter, greyish, and opaque after 24 h at 35°C. Good growth occurs at 22°C. Motile. Urease, phenylalanine, and indole positive. Lysine decarboxylase positive, ornithine and arginine decarboxylase negative. Voges-Proskauer and malonate negative. No growth occurs in citrate. KCN resistant. Gelatin not liquefied. H₂S negative. β-Galactosidase and β-glucuronidase negative. Acid and gas produced from glucose. Acid produced from mannose, galactose, and trehalose. Acid not produced from arabinose, xylose, rhamnose, sucrose, lactose, maltose, cellobiose, melibiose, raffinose, adonitol, dulcitol, sorbitol, mannitol, inositol, salicin, erythritol, glycerol, and esculin. α-Methyl glycoside negative. Nitrate reductase positive. Oxidase negative. Tetracycline and colistin resistant.

REFERENCES

- 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 Knutson, and A. G. Steigerwalt. 1982. *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. J. Clin. Microbiol. 15:1133-1140.

3. Cornelis, G., M. van Bouchaute, and G. Wauters. 1981. Plasmid-encoded lysine decarboxylation in *Proteus morganii*. J. Clin. Microbiol. 14:365-369.
4. Crosa, J. G., D. J. Brenner, W. H. Ewing, and S. Falkow. 1973. Molecular relationships among the *Salmonellae*. J. Bacteriol. 115:307-315.
5. 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.
6. Fulton, M. 1943. The identity of *Bacterium columbensis* Castellani. J. Bacteriol. 46:79-82.
7. Hedges, R. W., N. Datta, J. N. Coetzee, and S. Dennison. 1973. R factors from *Proteus morganii*. J. Gen. Microbiol. 77:249-259.
8. Hickman, F. W., and J. J. Farmer III. 1978. *Salmonella typhi*: identification, antibiograms, serology, and bacteriophage typing. Am. J. Med. Technol. 44:1149-1159.
9. Hickman, F. W., J. J. Farmer III, A. G. Steigerwalt, and D. J. Brenner. 1980. Unusual groups of *Morganella* ("Proteus") *morganii* isolated from clinical specimens: lysine-positive and ornithine-negative biogroups. J. Clin. Microbiol. 12:88-94.
10. Le Minor, L., M. Véron, and M. Popoff. 1982. Taxonomie des *Salmonella*. Ann. Microbiol. (Inst. Pasteur) 133B:223-243.
11. Lessel, E. F. 1971. Status of the name *Proteus morganii* and designation of the neotype strain. Int. J. Syst. Bacteriol. 21:55-57.
12. Müller, H. E., C. M. O'Hara, G. R. Fanning, F. W. Hickman-Brenner, J. M. Swenson, and D. J. Brenner. 1986. *Providencia heimbachae*, a new species of *Enterobacteriaceae* isolated from animals. Int. J. Syst. Bacteriol. 36:252-256.
13. Penner, J. L. 1984. Genus XIII *Morganella* Fulton 1943, p. 497. In N. R. Krieg and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 1. The Williams & Wilkins Co., Baltimore.
14. Richard, C. 1979. Entérobactéries inhabituelles. Bull. Inst. Pasteur 77:83-98.
15. Senior, B. W., and S. Vörös. 1990. Protein profile typing—a new method of typing *Morganella morganii* strains. J. Med. Microbiol. 33:259-264.
16. Siboni, K. 1976. Correlation of the characters fermentation of trehalose, non-transmissible resistance to tetracycline, and flagellar wavelength in *Proteus morganii*. Acta Pathol. Microbiol. Scand. Sect. B 84:421-427.
17. Siboni, K. 1980. The occurrence of the trehalose fermenting, tetracycline and polymyxin resistant phenotype among the *Enterobacteriaceae*. Acta Pathol. Microbiol. Scand. Sect. B 88:185-188.
18. Skerman, V. B. D., V. McGowan, and P. H. A. Sneath (ed.). 1980. Approved lists of bacterial names. Int. J. Syst. Bacteriol. 30:225-420.
19. von Graevenitz, A., and M. Nourbakhsh. 1972. Antimicrobial resistance of the genera *Proteus*, *Providencia* and *Serratia* with special reference to multiple resistance patterns. Med. Microbiol. Immunol. 157:142-148.
20. 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.