Klebsiella trevisanii: a New Species from Water and Soil

C. FERRAGUT,^{1,2*} D. IZARD,² F. GAVINI,² K. KERSTERS,¹ J. DE LEY,¹ AND H. LECLERC²

*Laboratorium voor Microbiologie en Microbiële Genetica, Rijksuniversiteit, B-9000 Gent, Belgium,¹ and
Institut National de la Santé et de la Recherche Médicale, Unité 146, Domaine du Centre d'Enseignement et
de Recherches Techniques en Industrie Alimentaire, 59651 Villeneuve d'Ascq Cédex, France²*

We determined the taxonomic position of 17 strains of bacteria that were previously classified as "*Klebsiella*-like" strains, corresponding to group K of Gavini et al. [Ann. Microbiol. (Paris) **128B**:45-59, 1977]. These strains were isolated from sewage, surface waters, drinking waters, and unpolluted soils. Deoxyribonucleic acid (DNA)-DNA hybridizations and a numerical analysis of electrophoretic protein patterns were used in this study. At least 82% of the group K strains investigated formed a tight protein electrophoretic cluster. The DNA homology values for all of the selected group K strains were higher than 73% and indicated the genetic homogeneity of this group. Two strains, CUETM 77-177 and CUETM 78-134, which were classified as group L strains by Gavini et al., were identified as true members of group K by the above-mentioned methods. The protein gel electrophoretic technique permitted distinction of group K strains from other species of the genus *Klebsiella*. DNA-DNA hybridization experiments revealed relatedness values of 40 to 71% between the reference strain of group K and the species *Klebsiella mobilis*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Klebsiella terrigena*. Phenotypic characteristics, protein electrophoretic patterns, and the results of DNA-DNA hybridizations supported the individuality of group K, and we propose the name *Klebsiella trevisanii* sp. nov. for the strains of this group. These strains were positive in the Voges-Proskauer, urease, and β -galactosidase tests, grew at 4 and 41°C, utilized histamine as a carbon source, did not ferment melezitose, and did not use *m*-hydroxybenzoate; indole was produced by 42% of the isolates. Strain CIP 81-36 (= CUETM 78-120) was designated the type strain of this species.

In a numerical taxonomic study, Gavini et al. (15) investigated 122 strains belonging or related to the genus *Klebsiella*. Two new groups, designated groups K and L, were defined on the basis of phenotypic features. The strains of group K had characteristics of both *Klebsiella pneumoniae* and *Klebsiella oxytoca*; the majority of group K strains were isolated from water. In a recent study IZARD et al. (18) confirmed that group L is a distinct and genetically homogeneous entity within the genus *Klebsiella* and classified this group as a new species, *Klebsiella terrigena*.

The aim of this work was to determine the taxonomic position of group K within the genus *Klebsiella* by comparing the electropherograms of soluble proteins and the results of deoxyribonucleic acid (DNA)-DNA hybridizations and the genetic relationships of the group K strains to other genera in the *Enterobacteriaceae*.

MATERIALS AND METHODS

Bacterial strains. The strains used in this investigation are listed in Table 1. All strains labeled CUETM (Collection de l'Unité d'Ecotoxicologie Microbienne,

59651 Villeneuve d'Ascq, France) were isolates originally studied by Gavini et al. (13-16) by numerical analysis.

Polyacrylamide gel electrophoresis of soluble proteins and numerical analysis of the electropherograms. Cells were grown at 37°C for 16 h on nutrient agar (Oxoid Ltd., London, England) in Roux flasks. Cell-free extracts were prepared by the method of Kersters and De Ley (25), as modified by IZARD et al. (18). Polyacrylamide gel electrophoresis, densitometry, photography of the stained gels, and normalization of the densitometric tracings were performed by the method of Kersters and De Ley (25). Each protein extract was investigated in at least three different electrophoretic experiments. Each normalized densitometric tracing was converted into a sequence of 120 numbers, representing the optical density (expressed as height in millimeters) of each position on the scan. We selected the values from positions 8 to 120 for numerical analysis. Computer-assisted calculations of the Pearson product moment correlation coefficient (*r*) and clustering by the unweighted average-pair group method were carried out by using the Clustan program (version 1C) of Wishart (38) and a Siemens model 7541 (BS2000) computer at the Centraal Digitaal Rekencentrum, Rijksuniversiteit, Gent, Belgium. The most typical electropherogram of each strain was calculated as described previously (35).

TABLE 1. Strains used and relative binding values of DNAs from members of the *Enterobacteriaceae* to [³H]DNAs of group K strain CUETM 78-120 and *K. terrigena* CUETM 77-176^a

Species or group, as received	Refer- ences	Strain no. ^b	Ori- gin ^c	% Relative DNA binding with:			
				CUETM 78-120	CUETM 77-176		
Group K		CUETM 78-120 (K70)	D	100	65		
		CUETM 78-117 (K110)	So	86	63		
		CUETM 78-113 (K47)	Su	86	65		
		CUETM 78-111 (K111)	So	84	68		
		CUETM 78-114 (K24)	Se	84	69		
		CUETM 78-115 (K42)	Se	84	68		
		CUETM 78-121 (K31)	Se	84	65		
		CUETM 78-92 (K26)	Se	83	63		
		CUETM 78-91 (K33)	Se	80	68		
		CUETM 78-116 (K48)	Su	80	70		
		CUETM 78-112 (K120)	So	79	74		
		CUETM 78-118 (K58)	Su	78	61		
		CUETM 78-123 (K76)	D	77	62		
		CUETM 78-122 (K79)	D	75	62		
		CUETM 77-179 (K43)	Su	75	60		
		CUETM 78-119 (K117)	So	74	64		
		CUETM 77-175 (K44) ^d	Su	73	67		
<i>K. terrigena</i> (group L)	15, 18	CUETM 78-141 (L80)	D	71	99		
		CUETM 78-140 (L27)	Se	71	87		
		CUETM 78-144 (L83)	D	69	94		
		CUETM 78-159 (L98)	D	68	96		
		CUETM 78-154 (L108)	So	67	94		
		CUETM 78-139 (L94)	D	63	94		
		CUETM 78-125 (L67)	D	61	97		
		CUETM 78-138 (L78)	D	59	93		
		CUETM 78-132 (L82)	D	59	95		
		CUETM 78-131 (L69)	D	59	93		
		CUETM 77-176 (L84)	D	58	100		
		(= CIP 80-07) ^{d,e}					
		CUETM 78-143 (L50)	Su	58	89		
		CUETM 78-133 (L113)	So	58	88		
		CUETM 78-155 (L66)	D	57	99		
		CUETM 78-129 (L95)	D	56	95		
		CUETM 78-157 (L68)	D	54	95		
		CUETM 78-145 (L63)	D	54	96		
		CUETM 78-151 (L81)	D	54	96		
		CUETM 78-150 (L102)	D	51	95		
		CUETM 78-148 (L87)	D	51	87		
		CUETM 78-156 (L72)	D	50	97		
		Group L		CUETM 77-177 (L28)	Se	86	69
				CUETM 78-134 (L118)	So	77	63
				CUETM 78-127 (L91)	D	71	87
				CUETM 78-149 (L101)	D	41	46
	CUETM 78-180 (M115)		So	65	62		
<i>K. oxytoca</i>		ATCC 13182 (M2) ^e		62	58		
		LM 25-70		60	57		
		CUETM 78-185 (M104)		59	49		
		CUETM 78-177 (M99)	D	57	60		
		CUETM 78-184 (M92) ^d	D	55	57		
		CUETM 78-178 (M114)	So	51	64		
		CUETM 78-183 (M75)	D	50	57		
		CUETM 79-243 (M30)	Se	50	54		
		CUETM 78-179 (M105)	D	50	58		
		CUETM 80-97 (J11)	F	61	58		
<i>K. pneumoniae</i>		CUETM 77-169 (J22) ^d	F	57	61		
		CUETM 77-170 (J21)	F	57	56		
		ATCC 13882 (J1)		53	50		
		CUETM 78-258 (J9)	F	53	61		
		CUETM 78-271 (J38)	Se	53	52		
		CUETM 77-155 (D63)	Su	53	49		

TABLE 1—Continued

Species or group, as received	References	Strain no. ^b	Origin ^c	% Relative DNA binding with:	
				CUETM 78-120	CUETM 77-176
		CUETM 78-51 (D30)	Se	53	54
		CUETM 78-24 (D76)	Su	53	58
		CUETM 77-174 (J8)	F	52	48
		CUETM 78-272 (J7)	F	51	63
		LM 47-70		50	56
		CUETM 78-288 (J25)	Se	48	57
		CUETM 78-275 (J32)	Se	45	59
		ATCC 13883 ^e		ND	ND
<i>K. rhinoscleromatis</i>		ATCC 13884 ^e		ND	ND
		ATCC 6908		ND	ND
<i>K. ozaenae</i>		NCTC 5050 ^e		ND	ND
		ATCC 25926		ND	ND
<i>E. aerogenes</i> (= <i>K. mobilis</i>)	3, 21	CDC 3006-87		54	51
		CDC 1627-66		49	51
		R 7-77		47	54
		CUETM 78-186 (C5)		45	63
		CUETM 77-30		45	ND
		CUETM 79-235		44	53
		CDC 2979-69		44	52
		NCTC 9735		42	58
		ATCC 13048 ^e		42	59
		CDC 3432-70		40	59
<i>E. gergoviae</i>	5, 6, 17, 31	R 2-77		51	38
		CIP 76-02		48	47
		CIP 76-01		42	ND
<i>E. cloacae</i>		CUETM 78-2 (E29)	Se	47	39
		CUETM 78-42 (E91)	D	47	35
		CDC 1347-71		39	34
		ATCC 13047 ^e		37	29
		CUETM 77-125 (G132)	D	37	41
		CUETM 77-116 (G42)	F	35	44
<i>E. amnigenus</i>	14, 19, 24	CUETM 78-100 (H ₃ 162)	So	43	42
		CUETM 78-79 (H ₃ 156)	So	39	ND
		CUETM 78-97 (H ₃ 144)	D	39	ND
		CUETM 78-89 (H ₃ 165)	So	34	39
		CUETM 78-75 (H ₃ 151)	So	32	36
<i>E. intermedium</i>	14, 19, 20, 23	CUETM 77-123 (H ₁ 99)	D	37	34
		CUETM 77-140 (H ₁ 100)	D	26	
		CUETM 77-147 (H ₁ 159)	So	26	27
		CUETM 77-130 (H ₁ 86) (= CIP 79-27) ^d	Su	20	35
<i>E. sakazakii</i>	5, 8, 34	R 20-73		34	29
		R 2-78		29	26
		R 20-77		18	35
<i>Citrobacter diversus</i>		CDC 3613-63		35	ND
<i>Citrobacter freundii</i>		ATCC 6750		32	26
		ATCC 8090 ^e		32	27
		CDC 460-61		21	23
<i>Levinea malonatica</i>		CDC 25-408		29	37
<i>Levinea amaltonatica</i>		CUETM 78-290 (A ₂ 36)	Se	27	ND
		CDC 25-406		22	30
<i>Serratia marcescens</i>		LM 1-63		33	26
		ATCC 13880 ^e		26	28
<i>Serratia fonticola</i>		CUETM 78-11 (C89)	D	33	25
		ATCC 29844 (C95) ^d	D	22	ND

TABLE 1—Continued

Species or group, as received	Refer-ences	Strain no. ^b	Ori-gin ^c	% Relative DNA binding with:	
				CUETM 78-120	CUETM 77-176
<i>Serratia rubidaea</i>		CDC 2199-72 (= ATCC 27593) ^e		32	25
<i>Serratia liquefaciens</i>		CUETM 78-197 (SB ₂ 70)	D	19	18
"Buttiauxella agrestis"	10, 16	CUETM 77-74		17	28
		CUETM 78-18 (F42)	So	30	ND
		CUETM 78-31 (F43)	So	27	ND
		CUETM 78-23 (F46)	So	25	ND
		CUETM 78-34 (F38)	So	22	ND
		CUETM 77-167 (F44) (= CIP 80-31) ^d	So	15	ND
<i>Escherichia coli</i>		UWCC K 12-1		20	31
		ATCC 10536		13	23
<i>Shigella sonnei</i>		WRAIR I virulent (= ATCC 29930) ^e		20	27
<i>Shigella flexneri</i>		LM 15-76		16	26
		ATCC 12661		14	29
<i>Shigella dysenteriae</i>		WRAIR A1		11	29
<i>Hafnia alvei</i>		LM 51-70		18	15
<i>Salmonella typhimurium</i>		ATCC 23565		18	30
<i>Rahnella aquatilis</i>	14, 19, 22	CUETM 77-145 (H ₂ 133) (= CIP 78-65)	D	17	20
		CUETM 77-141 (H ₂ 110)	D	13	27
<i>Proteus vulgaris</i>		LM 3-70		14	11
<i>Proteus mirabilis</i>		LM 3-69		13	12
		ATCC 9240		9	11
<i>Proteus rettgeri</i>		LM 7-69		8	ND

^a From reference 18 and unpublished data.

^b Strain numbers in parentheses and beginning with a single letter (K, L, etc.) are strain numbers from Gavini et al. (13–16). Abbreviations: ATCC, American Type Culture Collection, Rockville, Md.; CDC, Centers for Disease Control, Atlanta, Ga.; CIP, Collection de l'Institut Pasteur, Paris, France; CUETM, Collection de l'Unité d'Ecotoxicologie Microbienne, Villeneuve d'Ascq, France; LM, L. Le Minor, Institut Pasteur, Paris, France; NCTC, National Collection of Type Cultures, London, England; R, C. Richard, Institut Pasteur, Paris, France; UWCC, University of Washington Culture Collection, Seattle; WRAIR, Walter Reed Army Institute of Research, Washington, D.C.

^c D, Drinking waters; F, fecal samples; Se, sewage; So, unpolluted soils; Su, surface waters.

^d Phenotypic centrostrain.

^e Type strain.

^f ND, Not determined.

DNA-DNA hybridization. The following two strains were chosen for the preparation of DNA labeled with [³H]thymidine: the type strain of *K. terrigena*, CUETM 77-176 (= L84 [15] = CIP [Collection de l'Institut Pasteur, Paris, France] 80-07 [18]), and the protein electrophoretic centrostrain (i.e., the strain electrophoretically most similar to the others in a taxon) of group K, CUETM 78-120 (= K70).

A modification of the method of Marmur (27), as described by Ferragut and Leclerc (11), was used to prepare both unlabeled and ³H-labeled DNAs. The hybridizations were carried out by the competition method used by De Ley and Tytgat (7), as previously described (12, 13, 22, 23). The mean guanine-plus-cytosine contents of the labeled DNAs of strains CUETM 78-120 and CUETM 77-176 (9, 11, 19) were

56.9 mol% (five determinations) and 56.7 mol% (four determinations), respectively, as determined by the thermal denaturation method. The optimal temperatures of renaturation calculated from the equation of De Ley and Tytgat (7) were 57 and 56.9°C for strains CUETM 78-120 and CUETM 77-176, respectively.

RESULTS

Comparison of electropherograms of the soluble proteins. Electropherograms of 44 strains belonging to or related to the genus *Klebsiella* were analyzed. The electrophoretic profiles were compared in at least three independent experiments for each strain. The observed reproducibility levels were above $r = 0.95$ for 40

FIG. 1. Matrix of correlation coefficients (r) calculated from a numerical analysis of protein electropherograms of 44 *Klebsiella* strains.

strains and between $r = 0.93$ and 0.95 for the remaining 4 strains. The most typical electropherogram of each strain was used for the matrix of correlation coefficients (Fig. 1). All of the group K strains except CUETM 78-114, CUETM 78-121, and CUETM 78-123 clustered in one homogeneous group and differed from the strains belonging to *K. terrigena*, *K. oxytoca*, *Klebsiella ozaenae*, *K. pneumoniae*, *Klebsiella rhinoscleromatis*, and *Klebsiella mobilis* (= *Enterobacter aerogenes* [3, 23]). The protein patterns of strains CUETM 77-177 and CUETM 78-134 (previously assigned to group L [15]) were indistinguishable from the electropherograms of the majority of strains belonging to group K. Figure 2 shows typical profiles for each group. A visual comparison of the normalized photographs shows that strains CUETM 78-114 and CUETM 78-121 possessed an intense band in the upper half of the gel column and that strain CUETM 78-123 had two heavy protein bands in the same area. However, the rest of the protein pattern was similar to the patterns for the other group K strains. Repeating the experiments did not change the results. Within group K, strain CUETM 78-120 has the highest value of hierarchical order (35) and therefore is considered the most representative strain of group K on the

basis of soluble protein electropherograms (i.e., the electrophoretic centrostrain).

DNA-DNA hybridization. Radioactive DNA from group K electrophoretic centrostrain CUETM 78-116, was hybridized to filter-bound DNAs from 16 isolates belonging to the same group, as well as to DNAs from 113 other organisms belonging to the genus *Klebsiella* and other genera of the *Enterobacteriaceae* (Table 1). The DNA homology values within group K were at least 73%. The levels of DNA relatedness between group K strain CUETM 78-120 and *K. terrigena* (18) (group L) were 50 to 71% (21 strains investigated). The levels of DNA relatedness between the type strain of *K. terrigena*, strain CUETM 77-116, and 17 strains of group K were 60 to 74% (Table 1 and Fig. 3). Strains CUETM 77-177, CUETM 78-134, and CUETM 78-127 (formerly classified as group L strains [15]) shared 86, 77, and 71%, respectively, of their genomes with group K reference strain CUETM 78-120 DNA (Table 1); their homology values with the type strain of group L (*K. terrigena*) were 69, 63, and 87%, respectively (Table 1 and Fig. 3). Low levels of DNA relatedness (41 and 46%) (Table 1 and Fig. 3) were measured between strain CUETM 78-149 (previously classified in group L [15]) and the

FIG. 2. Normalized electropherograms of soluble proteins from 18 *Klebsiella* strains. Lane a, *K. mobilis* ATCC 13048; lane b, *K. rhinoscleromatis* ATCC 13384; lane c, *K. ozaenae* NCTC 5050; lane d, *K. pneumoniae* ATCC 13883; lane e, *K. pneumoniae* CUETM 77-169; lane f, *K. oxytoca* ATCC 13182; lane g, *K. oxytoca* CUETM 78-179; lane h, *K. trevisanii* (group K) CUETM 78-114; lane i, *K. trevisanii* CUETM 78-121; lane j, *K. trevisanii* CUETM 78-123; lane k, *K. trevisanii* CUETM 78-119; lane l, *K. trevisanii* CUETM 78-91; lane m, *K. trevisanii* CUETM 78-120; lane n, *K. trevisanii* CUETM 78-118; lane o, *K. trevisanii* CUETM 78-115; lane p, *K. trevisanii* CUETM 78-113; lane q, *K. terrigena* (group L) CUETM 77-176; lane r, *K. terrigena* CUETM 78-151.

reference strains of both group K and group L.

The other species of the genus *Klebsiella* shared 40 to 65% of their genomes with the genome of the electrophoretic centrostrain of group K, as follows: *K. oxytoca*, 50 to 65% (10 strains); *K. pneumoniae*, 45 to 61% (14 strains); and *K. mobilis*, 40 to 54% (11 strains). Three strains of *Enterobacter gergoviae* (5, 6, 17, 31) exhibited DNA homology values of 42 to 51% with strain CUETM 78-120. Lower levels of relatedness were observed between group K reference DNA and DNAs of strains belonging to the genus *Enterobacter*, as follows: *Enterobacter cloacae*, 35 to 47% (six strains); *Enterobacter amnigenus*, 32 to 43% (five strains); (14, 19, 24); *Enterobacter intermedium*, 20 to 37% (four strains) (14, 19, 20, 23); and *Enterobacter sakazakii*, 18 to 34% (three strains) (5, 8, 34). Hybridizations between the DNAs of group K strain CUETM 78-120 and selected strains of the genera *Citrobacter* and *Levinea* yielded DNA relatedness values of 21 to 35% (seven strains). Homology values indicated significant differences from the genera *Serratia* (17 to 33%, seven strains), *Buttiauxella* (on Validation List No. 8; 15 to 30%, five strains) (10, 16), and *Escherichia-Shigella* (11 to 20%, six strains). Essentially no homology (8 to 18%) was observed with the genera *Hafnia*, *Salmonella*, *Rahnella* (14, 19, 22), and *Proteus* (eight strains).

The hybridization data with ^3H -labeled DNA fragments from strain CUETM 77-176^T (= L84)

FIG. 3. Bidimensional representation of DNA relatedness values obtained with 53 *Klebsiella* strains versus reference strain CUETM 77-176 of group L (*K. terrigena*) and reference strain CUETM 78-120 of group K. Symbols: ●, *K. pneumoniae*; *, *K. oxytoca*; ○, *K. terrigena* (group L); ◐, *K. trevisanii* (group K).

are also shown in Table 1 (18; unpublished data). Figure 3 shows a bidimensional representation of the DNA relatedness values obtained with strain CUETM 77-176 (type strain of *K. terrigena*) (18) versus the DNA relatedness values obtained with strain CUETM 78-120 (group K strain). The strains analyzed belong to the species *K. terrigena*, *K. pneumoniae*, and *K. oxytoca* and to group K. Three groups could be distinguished. One group is composed solely of strains belonging to *K. terrigena*; the second group consists of all of the strains belonging to group K and two formerly classified group L strains, CUETM 77-177 (= L28) and CUETM 78-134 (= L118); and the third group consists of strains belonging to *K. pneumoniae* and *K. oxytoca*. Strain CUETM 78-149 (= L101) occupies a separate position.

DISCUSSION

Klebsiella-like group K was previously described by Gavini et al. (15) and included strains isolated from sewage, surface waters, drinking waters, and unpolluted soils. This group was considered to be related to *K. pneumoniae* and *K. oxytoca* but to be distinct from these species with regard to a number of phenotypic properties (15). Later, DNA-DNA hybridizations (12) showed that two strains of group K were only 49 to 57% related to *K. pneumoniae*. In the present study we determined the correct taxonomic position of this group within the genus *Klebsiella*.

All of the group K strains analyzed had DNA homology values higher than 73% when they were compared with electrophoretic centro-strain CUETM 78-120 (Table 1). Two strains, CUETM 77-177 and CUETM 78-134, which were formerly classified as group L strains, showed high levels of DNA relatedness with strain CUETM 78-120 (86 and 77%, respectively), indicating that these strains genetically belong in group K. Of the 17 group K strains investigated, 14 exhibited similar protein patterns and formed a tight cluster, as shown in Fig. 1. Likewise, electrophoresis of soluble proteins placed both above-mentioned group L strains (CUETM 77-177 and CUETM 78-134) in group K. The combined results of DNA-DNA hybridization and protein electrophoresis indicate that the majority of the group K strains constitute a genetically homogeneous group. However, three group K strains (CUETM 78-114, CUETM 78-121, and CUETM 78-123) remained isolated, as determined by protein electrophoresis (Fig. 1), although the DNA homology values for these strains were similar to those observed with the other group K strains (Table 1 and Fig. 3). Strains CUETM 78-114 and CUETM 78-121 had a very strong band in the upper part of the electropherogram, whereas strain CUETM 78-

123 had two bands at the same place. Although quantitative numerical comparisons appeared to remove these three strains from group K (Fig. 1), visual inspection of their gels indicated qualitative overall similarities with the protein band patterns of the other group K strains (Fig. 2).

In a previous paper on group L (*K. terrigena*) (18), we did not determine the level of DNA relatedness of group L strains CUETM 78-127, CUETM 78-154, and CUETM 78-149 to the DNA reference strain of *K. terrigena*. Our present data on DNA-DNA hybridizations (Table 1 and Fig. 3) clearly indicate that strains CUETM 78-127 and CUETM 78-154 belong genetically in group L; thus, classification of these strains as *K. terrigena* is justified. However, strain CUETM 78-149 (= L101) occupies a separate position in Fig. 3, and its correct taxonomic position remains unknown.

Our bidimensional representation of the DNA homology values (Fig. 3) indicates that the level of genomic relationship between groups K and L (*K. terrigena*) is higher than that observed between each of these groups and the species *K. pneumoniae* and *K. oxytoca*. The protein patterns of the strains examined reflect the genetic differences among the species of the genus *Klebsiella* (Fig. 1 and 2).

A numerical analysis of the phenotypic features (15), protein electropherograms, and the results of DNA-DNA hybridizations permit the separation of group K from *K. oxytoca*, *K. pneumoniae*, *K. terrigena*, and *K. mobilis*. Table 1 shows that the levels of DNA homology between group K and strains of other species of the genus *Klebsiella* are 40 to 71% and that the levels of DNA homology between group K and strains belonging to *E. gergoviae* and *E. cloacae* are 35 to 51%. The level of DNA relatedness with the other species studied was less than 43%. Our results support the individuality of group K within the genus *Klebsiella*. The genomic homogeneity of this taxon was confirmed by the high homology values observed (73 to 86%) and by the great similarity of the protein patterns of the group K strains. Therefore, we propose group K as a new species, *Klebsiella trevisanii* (tre.vi.san'ii, L. adj. after V. Trevisan, who originally named and described the genus *Klebsiella*) (36).

The isolates of *K. trevisanii* were phenotypically fairly similar to *Klebsiella* sp. 2 (group 4) of Naemura et al. (28). In a recent study Bagley et al. (2) named the latter group *Klebsiella planticola*. DNA-DNA hybridizations will be necessary to measure the genetic relatedness of these two species.

We suggest that the genus *Klebsiella* should contain the following eight species: (i) *K. pneumoniae* (Schroeter 1886) Trevisan 1887 (32, 27);

(ii) *K. ozaenae* (Abel 1893) Bergey, Harrison, Breed, Hammer and Huntoon 1925 (1, 4); (iii) *K. rhinoscleromatis* Trevisan 1887 (37); (iv) *K. oxytoca* (Flügge 1886) Lautrop 1956 (27); (v) *K. mobilis* Bascomb, Lapage, Willcox and Curtis 1971 (3); (vi) *K. terrigena* Izard, Ferragut, Gavini, Kersters, De Ley and Leclerc 1981 (18); (vii) *K. planticola* Bagley, Seidler and Brenner 1981 (2); and (viii) *K. trevisanii* sp. nov.

The phenotypic features useful in differentiating the species of the genus *Klebsiella* are listed in Table 2. *K. trevisanii* is distinguishable from *K. pneumoniae* and *K. oxytoca* by its ability to grow at 4°C and to utilize histamine as a carbon source. Indole-positive strains of *K. trevisanii* also differ from *K. oxytoca* by the utilization of *m*-hydroxybenzoate. The latter test and different growth responses at 41°C differentiate *K. trevisanii* from *K. terrigena* (Table 2).

Description of *Klebsiella trevisanii* sp. nov. The following description is based on 17 strains belonging to group K of Gavini et al. (15) and two strains belonging to group L (CUETM 77-177 and CUETM 78-134), all of which belong in this new species, as explained above.

Cell characteristics. Cells are gram negative, nonmotile, 0.3 to 1.5 by 0.6 to 6.0 µm, non-sporeforming, straight, and rod-shaped, resembling the cells of other members of the *Enterobacteriaceae*.

Colonial characteristics. Colonies on nutrient agar are smooth, more or less dome-shaped, and of varying degrees of stickiness.

Relationship to oxygen. Facultatively anaerobic.

Biochemical and physiological characteristics. As described by Gavini et al. (15), all 19 *K. trevisanii* strains are positive for citrate (Simmons, at 30 and 37°C), β-galactosidase, growth at 41°C, growth on KCN, lysine decarboxylase, malonate, the methyl red test (at 30 and 37°C),

mucate (Kauffmann), nitrate reduction to nitrite, tartrate (Jordan), urease, the Voges-Proskauer reaction (at 30 and 37°C), and fermentation of adonitol, L-arabinose, cellobiose (acid and gas), dextrin, esculin, D-fructose, D-galactose, D-glucose (gas), glycerol (gas), *meso*-inositol, lactose, maltose, D-mannitol, D-mannose, melibiose, α-methyl-D-glucoside, raffinose, L-rhamnose, D-ribose, salicin, sorbitol, L-sorbose, sucrose, trehalose, and D-xylose.

All strains are negative for arginine dihydro-lase, deoxyribonuclease, gelatinase (at 20°C), growth at 44.5°C, hydrolysis of chitin, starch, and Tween 80, motility (at 30 and 37°C), ornithine decarboxylase, phenylalanine deaminase, production of H₂S, tetrathionate reductase, and fermentation of *meso*-erythritol, glycogen, and melezitose. Additional characteristics are given in Table 3.

Nutritional characteristics. The following carbon sources are utilized by 80 to 100% of the strains (15): L-arabinose, D-ribose, D-xylose, L-rhamnose, D-fructose, D-galactose, D-glucose, D-mannose, cellobiose, lactose, maltose, sucrose, trehalose, starch, gluconate, mucate, salicin, glycerol, adonitol, D-mannitol, *meso*-inositol, sorbitol, acetate, DL-lactate, DL-glycerate, D-malate, L-malate, pyruvate, citrate, malonate, succinate, phenylacetate, benzoate, *p*-hydroxybenzoate, D-quininate, L-α-alanine, D-α-alanine, L-glutamate, L-proline, histamine, and putrescine.

The following carbon sources are not utilized by 80 to 100% of the strains within 5 days: D-arabinose, D-fucose, inulin, ethanol, methanol, *n*-propanol, isopropanol, geraniol, 2,3-butylene-glycol, *meso*-erythritol, dulcitol, ethylene glycol, propylene glycol, propionate, D-tartrate, *meso*-tartrate, citraconate, itaconate, oxalate, pimelate, azelate, suberate, levulinate, mesaconate, glutarate, sebacate, salicylate, adipate,

TABLE 2. Phenotypic characteristics useful in differentiating *Klebsiella* species^a

Species	Growth at:		Presence of:		Formation of indole	Voges-Proskauer reaction	<i>m</i> -Hydroxybenzoate as carbon source	Histamine as carbon source
	4°C	41°C	Urease	β-Galactosidase				
<i>K. pneumoniae</i>	— ^b	+	+	+	—	+	—	—
<i>K. ozaenae</i>			d	+	—	—		
<i>K. rhinoscleromatis</i>			—	—	—	—		
<i>K. oxytoca</i>	—	+	+	+	+	+	+	—
<i>K. mobilis</i>	(+)	+	—	+	—	+	+	+
<i>K. terrigena</i>	+	—	+	+	—	+	+	+
<i>K. planticola</i>			+		— or +	+	— or +	
<i>K. trevisanii</i>	+ or (+)	+	+	+	— or +	+	—	+

^a See references 2, 3, 15, 29, and 30.

^b +, Positive reaction for at least 90% of the strains within 24 to 48 h; —, negative reaction for at least 90% of the strains within 24 to 48 h; (+), slow positive reaction after more than 48 h; d, different reactions. Where two results are given, the first is the more frequent result.

TABLE 3. Phenotypic characteristics of *K. trevisanii* strains (group K)^a

Characteristic	Group K (19 strains)		Type strain CIP 81-36 (= CUETM 78-120)
	Reaction ^b	% Positive	
Fermentation of:			
Dulcitol	— or +	11	—
Inulin	— or +	21	—
Growth at 4°C	+ or (+)	63	+
Indole	— or +	42	—
D-Tartrate (Kauffmann)	— or +	42	—
L-Tartrate (Kauffmann)	— or +	42	+
meso-Tartrate (Kauffman)	— or +	21	—
β-Xylosidase	+ or —	95	+

^a From reference 15 and unpublished data.^b +, Positive reaction within 24 to 48 h; (+), slow positive reaction after more than 48 h; —, negative reaction. Where two results are given, the first is the more frequent result.

butyrate, isobutyrate, *n*-valerate, isovalerate, *n*-caproate, heptanoate, caprylate, pelargonate, caprate, *m*-hydroxybenzoate, D-mandelate, L-mandelate, phthalate, isophthalate, terephthalate, phenol, benzylformate, glycine, β-alanine, L-leucine, L-isoleucine, L-valine, L-citrulline, L-threonine, L-cysteine, L-methionine, DL-2-aminobutyrate, DL-3-aminobutyrate, DL-5-aminovalerate, L-phenylalanine, L-tyrosine, 2-aminobenzoate, 3-aminobenzoate, 4-aminobenzoate, L-tryptophan, D-tryptophan, spermine, amylamine, ethanolamine, butylamine, diphenylamine, urea, urate, sarcosin, betaine, creatin, pantothenate, trigonelline, nicotinate, acetamide, naphthalene, dodecane, and hexadecane.

Genetic data. The mean guanine-plus-cytosine content of the DNA of the type strain, CIP 81-36, is 56.9 mol%. DNA-DNA hybridization values for the most related species are as follows: *K. terrigena*, 50 to 71% (20 strains tested); *K. oxytoca*, 50 to 65% (10 strains tested); *K. pneumoniae*, 45 to 61% (14 strains tested); and *K. mobilis*, 40 to 54% (11 strains tested).

Habitat. Found in unpolluted soils and water (drinking water, surface water, sewage).

Type strain. Gavini K70 = CUETM 78-120 = CIP 81-36. Isolated from drinking water. The characteristics of the type strain are those given above for the species, and the biochemical characteristics are shown in Table 3.

ACKNOWLEDGMENTS

We thank A. Bernigaud, E. Dewailly, D. Dewettinck, and U. Torck for technical assistance.

J.D.L. is indebted to the Fonds voor Kollektief Fundamenteel Onderzoek for research and personnel grants.

LITERATURE CITED

- Abel, R. 1893. Bakteriologische Studien über *Ozaena simplex*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. 13:161-173.
- Bagley, S. T., R. J. Seidler, and D. J. Brenner. 1981. *Klebsiella planticola* sp. nov.: a new species of *Enterobacteriaceae* found primarily in nonclinical environments. Curr. Microbiol. 6:105-109.
- Bascomb, S., S. P. Lapage, W. R. Willcox, and M. A. Curtis. 1971. Numerical classification of the tribe *Klebsiellae*. J. Gen. Microbiol. 66:279-295.
- Bergey, D. H., F. C. Harrison, R. S. Breed, B. W. Hammer, and F. M. Huntoon. 1925. Bergey's manual of determinative bacteriology, 2nd ed. The Williams & Wilkins Co., Baltimore.
- Brenner, D. J., J. J. Farmer III, F. W. Hickman, M. A. Asbury, and A. G. Stelgerwalt. 1977. Taxonomic and nomenclature changes in *Enterobacteriaceae*. Publication 78-8356. Centers for Disease Control Publications, Atlanta, Ga.
- Brenner, D. J., C. Richard, A. G. Stelgerwalt, M. A. Asbury, and M. Mandel. 1980. *Enterobacter gergoviae* sp. nov.: a new species of *Enterobacteriaceae* found in clinical specimens and the environment. Int. J. Syst. Bacteriol. 30:1-6.
- De Ley, J., and R. Tytgat. 1970. Evaluation of membrane filter methods for DNA:DNA hybridization. Antonie van Leeuwenhoek J. Microbiol. Serol. 36:461-474.
- 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.
- Ferragut, C., F. Gavini, D. Izard, and H. Leclerc. 1978. Etude du % GC dans un groupe d'entérobactéries H₂S⁺, apparentées au genre *Citrobacter*. Can. J. Microbiol. 24:473-479.
- 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 C2:33-44.
- Ferragut, C., and H. Leclerc. 1976. Etude comparative des méthodes de détermination du Tm de l'ADN bactérien. Ann. Microbiol. (Paris) 127A:223-235.
- Ferragut, C., and H. Leclerc. 1978. Characterization of motile and acetoin-negative *Klebsiella pneumoniae* strains by DNA:DNA hybridization. Antonie van Leeuwenhoek J. Microbiol. Serol. 44:407-424.
- Gavini, F., C. Ferragut, D. Izard, P. A. Trinel, H. Leclerc, B. Lefebvre, and D. A. A. Mossel. 1979. *Serratia fonticola*, a new species from water. Int. J. Syst. Bacteriol. 29:92-101.
- Gavini, F., C. Ferragut, and H. Leclerc. 1976. Etude taxonomique d'entérobactéries appartenant ou apparentées au genre *Enterobacter*. Ann. Microbiol. (Paris) 127B:317-335.
- Gavini, F., H. Leclerc, B. Lefebvre, C. Ferragut, and D. Izard. 1977. Etude taxonomique d'entérobactéries appartenant ou apparentées au genre *Klebsiella*. Ann. Microbiol. (Paris) 128B:45-59.
- Gavini, F., B. Lefebvre, and H. Leclerc. 1976. Positions

- taxonomiques d'entérobactéries H_2S^- par rapport au genre *Citrobacter*. Ann. Microbiol. (Paris) 127A:275-295.
17. Goullet, P. H. 1980. Distinctive electrophoretic patterns of esterases from *Klebsiella pneumoniae*, *K. oxytoca*, *Enterobacter aerogenes* and *E. gergoviae*. J. Gen. Microbiol. 117:483-491.
 18. Izard, D., C. Ferragut, F. Gavini, K. Kersters, J. De Ley, and H. Leclerc. 1981. *Klebsiella terrigena*: a new species from soil and water. Int. J. Syst. Bacteriol. 31:116-127.
 19. Izard, D., C. Ferragut, F. Gavini, and H. Leclerc. 1978. Variations of the moles percent guanine plus cytosine within a group of *Enterobacteriaceae* belonging or related to the genus *Enterobacter*. Int. J. Syst. Bacteriol. 28:449-452.
 20. Izard, D., F. Gavini, and H. Leclerc. 1979. Polynucleotide sequence relatedness and genome size among *Enterobacter intermedium* sp. nov. and the species *Enterobacter cloacae* and *Klebsiella pneumoniae*. Zentralbl. Bakteriол. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe C 1:51-60.
 21. Izard, D., F. Gavini, P. A. Trinel, F. Krubwa, and H. Leclerc. 1980. Contribution of DNA-DNA hybridization to the transfer of *Enterobacter aerogenes* to the genus *Klebsiella* as *K. mobilis*. Zentralbl. Bakteriол. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe C 1:257-263.
 22. Izard, D., F. Gavini, P. A. Trinel, and H. Leclerc. 1979. *Rahnella aquatilis*, nouveau membre de la famille des *Enterobacteriaceae*. Ann. Microbiol. (Paris) 130A:163-177.
 23. Izard, D., F. Gavini, P. A. Trinel, and H. Leclerc. 1979. Etude d'un groupe nouveau d'*Enterobacteriaceae* (groupe H_1) apparenté à l'espèce *Enterobacter cloacae*. Can. J. Microbiol. 25:713-718.
 24. Izard, D., F. Gavini, P. A. Trinel, and H. Leclerc. 1981. Deoxyribonucleic acid relatedness between *Enterobacter cloacae* and *Enterobacter amnigenus* sp. nov. Int. J. Syst. Bacteriol. 31:35-42.
 25. Kersters, K., and J. De Ley. 1975. Identification and grouping of bacteria by numerical analysis of their electrophoretic protein patterns. J. Gen. Microbiol. 87:333-342.
 26. Lautrop, H. 1956. Gelatin-liquefying *Klebsiella* strains (*Bacterium oxytoca* [Flügge]). Acta Pathol. Microbiol. Scand. 39:375-384.
 27. Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Mol. Biol. 3:208-218.
 28. Naemura, L. G., S. T. Bagley, R. J. Seidler, J. B. Kaper, and R. R. Colwell. 1979. Numerical taxonomy of *Klebsiella pneumoniae* strains isolated from clinical and nonclinical sources. Curr. Microbiol. 2:175-180.
 29. Ørskov, I. 1974. Genus VI, *Klebsiella* Trevisan 1885, p. 321-324. In R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
 30. Richard, C. 1977. Présence chez *Enterobacter aerogenes* d'antigènes capsulaires apparentés à ceux de *Klebsiella*. Intérêt de l'utilisation de *meta*-hydroxy-benzoate dans le diagnostic différentiel *E. aerogenes*-*K. pneumoniae*. Ann. Microbiol. (Paris) 128A:289-295.
 31. Richard, C., B. Joly, J. Sirot, G. H. Stoleru, and M. Popoff. 1976. Etude de souches de *Enterobacter* appartenant à un groupe particulier proche de *E. aerogenes*. Ann. Microbiol. (Paris) 127A:545-548.
 32. Schroeter, J. 1886. In F. Cohn (ed.), Kryptogamenflora von Schlesien, Band 3, Heft 3, p. 1-814. Pilze. J.U. Kern's Verlag, Breslau.
 33. 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.
 34. Steigerwalt, A. G., G. R. Fanning, M. A. Fife-Asbury, and D. J. Brenner. 1976. DNA relatedness among species of *Enterobacter* and *Serratia*. Can. J. Microbiol. 22:121-137.
 35. Swings, J., K. Kersters, and J. De Ley. 1976. Numerical analysis of electrophoretic protein patterns of *Zymomonas* strains. J. Gen. Microbiol. 93:266-271.
 36. Trevisan, V. 1885. Caratteri di alcuni nuovi generi di Batteriacee. Atti Accad. Fisio-Medico-Stat. Milano Ser. 4 3:92-107.
 37. Trevisan, V. 1887. Sul micrococco della rabbia e sulla possibilità di riconoscere durante il periodo d'incubazione dall'esame del sangue della persona morsicata, se ha contratta l'infezione rabbica. Rend. Ist. Lomb. Accad. Sci. Lett. Ser. 2 20:88-105.
 38. Wishart, D. 1978. Clustan user manual, 3rd ed. Program Library Unit, Edinburgh University, Edinburgh.