Klebsiella terrigena, a New Species from Soil and Water

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Deoxyribonucleic acid (DNA)-DNA hybridization and numerical analysis of electrophoretic protein patterns were carried out to determine the taxonomic position of 37 "*Klebsiella pneumoniae*-like" strains from nonclinical origin (soil and water) and previously studied by numerical taxonomy (group L of Gavini et al., Ann. Microbiol. [Inst. Pasteur] **128B**:45-59, 1977). The DNA interrelatedness for 20 selected strains of group L was at least 87%, except for two strains (63 and 69%). Protein electrophoretograms indicated likewise that at least 85% of the L strains constitute a genetically homogeneous group. The latter technique enabled the distinction of strains of group L from those of *K. pneumoniae*, *K. oxytoca*, and an unnamed *Klebsiella* cluster (group K) related to *K. pneumoniae* and *K. oxytoca*. On the basis of their phenotypic characters, protein electrophoretic patterns, and DNA-DNA relatedness, we propose for these strains the name *Klebsiella terrigena* sp. nov., with strain CIP 80-07 (= CUETM 77-176) as the type strain. The DNA of this strain was found to be 49 to 64% homologous to *K. oxytoca* DNA, 48 to 63% to *K. pneumoniae* DNA, and 51% to *K. ozaenae* DNA.

In 1977 Gavini et al. (14) showed, by a numerical analysis of the phenotypic properties of 122 strains belonging or related to the genus *Klebsiella*, the existence of two new groups. (i) Group K was related to *Klebsiella pneumoniae* and to *K. oxytoca*; the majority of the 24 group K strains were isolated from water. (ii) Group L was found to be related to *K. pneumoniae*, although the 37 strains (isolated from water or soil) possessed tetrathionate reductase and utilized *m*-hydroxybenzoate. In a more recent numerical taxonomy study of 180 strains isolated from clinical and nonclinical sources, Naemura et al. (28) confirmed the existence of group L.

The purpose of this study was to determine the homogeneity and the taxonomic position of group L (by analysis of electrophoretograms of soluble proteins and deoxyribonucleic acid [DNA]-DNA hybridization) and its genetic relationships to other genera of the family "*Enterobacteriaceae*" (not on Approved Lists of Bacterial Names [36]).

MATERIALS AND METHODS

Bacterial strains and growth media. The 177 strains used in this study are listed in Table 1. All strains labeled CUETM (Collection de l'Unité d'Ecotoxicologie Microbienne, Villeneuve d'Ascq, France) were isolates originally studied by Gavini et al. (12-15) by numerical analysis. For the preparation of DNA, cells were grown in nutrient broth at 30°C for 24 h with shaking. For the electrophoresis of soluble proteins, cells (about 2 g, wet weight) were grown at 37°C for 16 h on nutrient agar (Oxoid) in Roux flasks.

Preparation of cell-free extracts for electrophoresis. Cell-free extracts were prepared according to the method of Kersters and De Ley (24) with the following modifications. After French pressure cell treatment, intact bacteria and debris were removed by centrifugation for 15 min at 30,000 rpm and 4°C in a Beckman model L centrifuge (rotor type 30). Up to 4 ml of the turbid supernatant was centrifuged in a Beckman type 40 rotor for 1 h at 4°C and 35,000 rpm. A 2-ml portion of the supernatant (containing <25 mg of protein per ml) was again centrifuged for 4 h at 4°C and 35,000 rpm. The supernatant after the last centrifugation step contained the soluble proteins; it was dialyzed overnight at 4°C against 1 liter of 6.4 mM tris(hydroxymethyl)aminomethane-hydrochloride buffer, pH 9.1, and then filtered at 4°C through a VCPW membrane filter (0.1- μ m pore size, 25-mm diameter; Millipore Corp.) in an Amicon 8MC apparatus under 1.5-kg/cm² nitrogen pressure.

Polyacrylamide gel electrophoresis and numerical analysis of the normalized electrophoretograms. Electrophoresis of soluble proteins, densitometry, normalization of densitometric tracings, and photography were carried out as described previously (24). Each protein extract was investigated in at least three and mostly in five independent electrophoretic runs. Each normalized densitometric tracing was converted into a sequence of 130 numbers, representing the optical densities (expressed as height in millimeters) of each position on the scan. The Pearson product-moment correlation coefficient (*r*) between each pair of densitometric tracings was calculated, and the electrophoretograms were clustered by the unweighted average pair group method, using a Siemens 7755 (BS2000) computer (24). The most typical electrophoretogram of each strain was selected as the one with the highest product of *z* values. The *z* transform-

TABLE 1. *Strains used and relative binding of their DNA to [³H]DNA of the phenotypic centrostrain CUETM 77-176*

Species (name as received) or group	Strain no. ^a	Strain no. from Gavini et al. (12-15)	Origin of strains ^b	% Relative DNA binding at 56.9°C with DNA of strain CUETM 77- 176
Group L	CUETM 77-176 ^c	L 84	D	100
	CUETM 78-141	L 80	D	99
	CUETM 78-155	L 66	D	99
	CUETM 78-156	L 72	D	97
	CUETM 78-125	L 67	D	97
	CUETM 78-145	L 63	D	96
	CUETM 78-151	L 81	D	96
	CUETM 78-132	L 82	D	95
	CUETM 78-129	L 95	D	95
	CUETM 78-157	L 68	D	95
	CUETM 78-150	L 102	D	95
	CUETM 78-144	L 83	D	94
	CUETM 78-139	L 94	D	94
	CUETM 78-131	L 69	D	93
	CUETM 78-138	L 78	D	93
	CUETM 78-143	L 50	Su	89
	CUETM 78-133	L 113	So	88
	CUETM 78-148	L 87	D	87
	CUETM 77-177	L 28	Se	69
	CUETM 78-134	L 118	So	63
	CUETM 77-178	L 97	D	ND ^d
	CUETM 78-124	L 45	Su	ND
	CUETM 78-126	L 89	D	ND
	CUETM 78-127	L 91	D	ND
	CUETM 78-128	L 119	Su	ND
	CUETM 78-130	L 73	D	ND
	CUETM 78-135	L 85	D	ND
	CUETM 78-136	L 109	So	ND
	CUETM 78-140	L 27	Se	ND
	CUETM 78-142	L 74	D	ND
	CUETM 78-147	L 49	Su	ND
	CUETM 78-149	L 101	D	ND
	CUETM 78-152	L 52	Su	ND
	CUETM 78-153	L 112	So	ND
	CUETM 78-154	L 108	So	ND
	CUETM 78-158	L 86	D	ND
	CUETM 78-159	L 98	D	ND
Group K (14)	CUETM 78-120	K 70	D	65
	CUETM 77-179	K 43	Su	60
	CUETM 77-175 ^c	K 44	Su	ND
	CUETM 78-115	K 42	Se	ND
	CUETM 78-118	K 58	Su	ND
	CUETM 78-119	K 117	So	ND
<i>Klebsiella oxytoca</i>	CUETM 78-178	M 114	So	64
	CUETM 78-180	M 115	So	62
	CUETM 78-177	M 99	D	60
	ATCC 13182 ^e	M 2		58
	CUETM 78-179	M 105	D	58
	LM 25-70			57
	CUETM 78-184 ^c	M 92	D	57
	CUETM 78-183	M 75	D	57
	CUETM 79-243	M 30	Se	54
	CUETM 78-182	M 93	D	54
	CUETM 78-185	M 104	D	49

TABLE 1—*Continued*

Species (name as received) or group	Strain no. ^a	Strain no. from Gavini et al. (12-15)	Origin of strains ^b	% Relative DNA binding at 56.9°C with DNA of strain CUETM 77- 176
<i>Klebsiella pneumoniae</i>	CUETM 78-272	J 7	F	63
	CUETM 77-169 ^c	J 22	F	61
	CUETM 78-258	J 9	F	61
	CUETM 78-275	J 32	Se	59
	CUETM 80-97	J 11	F	58
	CUETM 78-265	J 16	F	58
	CUETM 78-146	J 77	D	58
	CUETM 78-24	D 76	Su	58
	CUETM 78-288	J 25	Se	57
	CUETM 78-289	J 59	Su	57
	CUETM 77-170	J 21	F	56
	LM 47-70			56
	CUETM 77-180	J 56	Su	55
	CUETM 78-284	J 20	F	55
	CUETM 78-51	D 30	Se	54
	CUETM 77-181	J 3	F	53
	CUETM 78-264	J 61	Su	53
	CUETM 78-271	J 38	Se	52
	ATCC 13882	J 1		50
	CUETM 78-13	D 64	Su	50
	CUETM 77-155	D 63	Su	49
	CUETM 77-174	J 8	F	48
<i>Enterobacter aerogenes</i>	CUETM 78-186	c 5		63
	ATCC 13048 ^c			59
	CDC 3432-70			59
	R 9-77			59
	NCTC 9735			58
	LM 3-70			58
	R 10-77			58
	R 6-77			56
	R 7-77			54
	R 1-78			54
	CUETM 79-235			53
	CDC 2979-69			52
	CDC 3006-67			51
	CDC 1627-66			51
	R 8-77			50
<i>Klebsiella ozaenae</i>	CDC 276-71			51
"Enterobacter gergoviae" (5, 6, 32)	R 3-77			48
	CIP 76-02			47
	R 2-77			38
	R 6-77			34
<i>Enterobacter cloacae</i>	CUETM 77-116	G 42	F	44
	CUETM 77-125	G 132	D	41
	CUETM 77-120 ^c	G 45	F	39
	CUETM 78-2	E 29	Se	39
	CUETM 78-47	E 74	Su	38
	CUETM 78-42	E 91	D	35
	CUETM 77-126	G 127	D	35
	Paris G1	G 13		34
	CDC 1347-71			34
	CUETM 77-135	G 137	D	31
	ATCC 13047 ^c			29

TABLE 1—Continued

Species (name as received) or group	Strain no. ^a	Strain no. from Gavini et al. (12-15)	Origin of strains ^b	% Relative DNA binding at 56.9°C with DNA of strain CUETM 77- 176
"Enterobacter amnigenus" (18, 23)	CUETM 78-96	H ₃ 79	Su	42
	CUETM 78-100	H ₃ 162	So	40
	CUETM 78-89	H ₃ 165	So	39
	CUETM 78-75	H ₃ 151	So	36
	CUETM 78-73	H ₃ 118	D	34
	ATCC 33072 (=CUETM 77-118) ^c	H ₃ 145	Su	33
	CUETM 77-132	H ₃ 130	D	31
	CUETM 77-137	H ₃ 111	D	28
"Enterobacter intermedium" (18, 19, 22)	CUETM 77-145	H ₁ 103	D	42
	ATCC 33110 (=CIP 79-27) ^c	H ₁ 86	Su	35
	CUETM 77-123	H ₁ 99	D	34
	CUETM 78-87	H ₁ 141	D	30
	CUETM 77-147	H ₁ 159	So	27
	CUETM 77-140	H ₁ 100	D	22
<i>Levinea malonatica</i>	LM 2-70			39
	CDC 25-408			37
	R 35380/73			30
<i>Levinea amalonatica</i>	R 17996/73			38
	CDC 25-406			30
<i>Escherichia coli</i>	CUETM 79-193			37
	UWCC K12-1			31
	CUETM 79-197			28
	ATCC 10536			23
"Rahnella aquatilis" (18, 21)	CUETM 77-141	H ₂ 110	D	27
	ATCC 33071 (=CIP 78-65) ^c	H ₂ 133	D	20
	CUETM 77-108	H ₂ 136	D	13
	CUETM 78-68	H ₂ 163	So	13
	CUETM 77-143	H ₂ 106	D	10
	CUETM 77-146	H ₂ 147	So	9
<i>Shigella sonnei</i>	WRAIR I virulent ^c (=ATCC 29930)			27
	ATCC 9290			22
<i>Citrobacter freundii</i>	ATCC 8090 ^c			27
	ATCC 6750			26
	CDC 460-61			23
<i>Serratia rubidaea</i>	CDC 2199-72 ^c (=ATCC 27593)			25
	CDC 934-72			18
<i>Serratia fonticola</i> (9)	ATCC 29846 (=CUETM 78-11)	C 89	D	25
	ATCC 29845	C 81	D	14
<i>Providencia alcalifaciens</i>	LM 5-70			25
<i>Proteus rettgeri</i>	LM 7-69			23

TABLE 1—Continued

Species (name as received) or group	Strain no. ^a	Strain no. from Gavini et al. (12-15)	Origin of strains ^b	% Relative DNA binding at 56.9°C with DNA of strain CUETM 77-176
<i>Erwinia amylovora</i>	ATCC 15580 ^c			37
"Enterobacter sakazakii" (5, 37)	R 20-77			35
	R 16-76			31
	R 20-73			29
	R 2-78			26
	R 3-74			20
<i>Serratia plymuthica</i> (16, 17)	CUETM 78-218	SB ₁ 62	D	34
	B 3617			12
<i>Serratia marcescens</i>	CDC 868-57			31
	ATCC 13880 ^c			28
	LM 1-63			26
<i>Salmonella typhimurium</i>	ATCC 23565			30
	UWCC LT2			25
<i>Shigella dysenteriae</i>	WRAIR A1			29
<i>Shigella flexneri</i>	ATCC 12661			29
	LM 15-76			26
<i>Serratia liquefaciens</i>	LM 13-70			28
	CUETM 78-197	SB ₂ 70	D	18
<i>Erwinia dissolvens</i>	ATCC 23373 ^c			17
<i>Hafnia alvei</i>	LM 51-70			15
	CUETM 78-268	I 120	D	11
	CUETM 78-267	I 71	Se	9
	CUETM 77-171	I 94	Su	9
	CUETM 77-144 ^c	I 114	D	8
	ATCC 13337 ^c			8
<i>Proteusmorganii</i>	LM 11-70			15
<i>Proteus mirabilis</i>	LM 3-69			12
	ATCC 9240			11
<i>Proteus vulgaris</i>	LM 3-70			11
	ATCC 13315 ^c			8

^a Abbreviations: ATCC, American Type Culture Collection, Rockville, Md., U.S.A.; B, J. Brisou, Université de Poitiers, Poitiers, France; CDC, Center for Disease Control, Atlanta, Ga., U.S.A.; 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; Paris, Collection des Entérobactéries, Institut Pasteur, Paris, France; R. C. Richard, Institut Pasteur, Paris, France; UWCC, University of Washington Culture Collection, Seattle, Wash., U.S.A.; WRAIR, Walter Reed Army Institute of Research, Washington, D.C., U.S.A.

^b Origin: D, drinking waters; F, fecal samples; Se, sewage; So, unpolluted soils; Su, surface waters.

^c Phenotypic centrostrain.

^d ND, Not determined.

^e Type strain.

mation is the inverse hyperbolic tangent of the correlation coefficient r (33). However, in a few cases we selected the gel with the second highest product of z values as the most typical one, after visual comparison

of the normalized photographs. In the final numerical analysis, only the most representative electrophoretogram per strain was used. The homogeneity of group L was determined by calculation of the hierarchical

order, H , of each electrophoretogram, H being the product of all of the z values of each strain (35, 38).

DNA-DNA hybridizations. The strain chosen for DNA labeling with [^3H]thymidine was the phenotypic "centrostrain" (i.e., the strain phenotypically most similar to the others in a taxon) CUETM 77-176 (L 84; 14). The extraction and purification of both labeled and unlabeled DNA were performed according to Marmur's method (27) as slightly modified by Ferragut and Leclerc (10). DNA-DNA reassociation was carried out by competition reaction in the conditions used by De Ley and Tytgat (7) and previously described (11, 12, 21, 22). The average guanine-plus-cytosine content of labeled CUETM 77-176 DNA, determined as previously described (9, 10, 18), was 56.7 mol% (four determinations). The optimal temperature of renaturation calculated from De Ley and Tytgat's equation (7) was 56.9°C.

RESULTS

Comparison of electrophoretic protein patterns of 52 *Klebsiella* strains. Electrophoretograms were prepared from the 37 strains belonging to group L, 6 strains of group K, 6 strains of *K. pneumoniae* (group J of Gavini et al. [14]), and 3 strains of *K. oxytoca* (group M of Gavini et al. [14]). More than 200 normalized electrophoretograms of 52 *Klebsiella* strains were compared by numerical analysis. The lower reproducibility limits were above $r = 0.96$ for 42 strains and above $r = 0.94$ for the remaining 10 strains.

The final numerical analysis is represented as a sorted and shaded matrix of correlation coefficients (Fig. 1). The following four groups of *Klebsiella* strains could be distinguished by protein electrophoresis: *K. oxytoca* (group M), *K. pneumoniae* (group J), group K, and group L. Normalized protein electrophoretograms of 15 representative strains of these four *Klebsiella* groups are shown in Fig. 2. The great electrophoretic homogeneity within group L is apparent from Fig. 1, with the exception of the following five strains: CUETM 77-177, 78-134, 78-127, 78-149, and 78-154 (Fig. 1 and 2). Repeating the experiments did not change the results. Both numerical analysis (Fig. 1) and visual comparison of normalized photographs (Fig. 2) showed that strains CUETM 77-177 and CUETM 78-134 belong to group K. Within group L, strain CUETM 78-126 had the highest value of hierarchical order H and can therefore be considered the most representative strain of group L on the basis of protein electrophoretic patterns.

DNA-DNA hybridization. The DNA-DNA hybridization values between the phenotypic centrostrain CUETM 77-176 of group L and 19 other isolates of the same group were determined (Table 1). The strains of group L formed a very tight hybridization group. The relative

binding ratio within the group was at least 87%, except for two strains (CUETM 77-177, 69%; CUETM 78-134, 63%).

To determine the taxonomic position of group L, hybridization values were measured between the centrostrain and a great number of strains belonging to *Klebsiella* and other, related genera (Table 1). Strain CUETM 77-176 shared 60 to 65% of its genome with two strains of group K related to the genus *Klebsiella*, 49 to 64% with *K. oxytoca* (11 strains used), 48 to 63% with *K. pneumoniae* (21 strains used), and 51% with *K. ozaenae* (1 strain). The DNA relatedness values were in the range 8 to 48% for all of the other species investigated except for *Enterobacter aerogenes*. Hybridization between DNA of strain CUETM 77-176 and 15 strains of *E. aerogenes* yielded a degree of reassociation of 50 to 63%. The other organisms belonging to the genus *Enterobacter* yielded moderate to low values: 34 to 48% hybridization with "*E. gergoviae*" (not on Approved Lists [36]) (5, 6, 32), 29 to 44% with *E. cloacae*, 28 to 42% with "*E. amnigenus*" (not on Approved Lists [36]) (13, 18, 23), 22 to 42% with "*E. intermedium*" (not on Approved Lists [36]) (13, 18, 19, 22), and 20 to 35% with "*E. sakazakii*" (not on Approved Lists [36]) (5, 37). The levels of hybridization indicated a significant difference from members of the genera *Levinea* (30 to 39%), *Escherichia*, and *Shigella* (22 to 37%), *Serratia* (12 to 34%), and *Citrobacter* (23 to 27%). The lowest degree of hybridization existed between group L and representative strains of the genera *Providencia* (25%), *Proteus* (8 to 23%), *Hafnia* (8 to 15%), and "*Rahnella*" (not on Approved Lists [36]) (9 to 27%) (13, 18, 21).

DISCUSSION

Group L was originally described by Gavini et al. (14), and its existence among other *Klebsiella*-like organisms from nonclinical origin was later confirmed by Naemura et al. (28). The 37 strains constituting group L have been isolated from sewage, surface water, drinking water, and unpolluted soils (14). They were considered to be related to *K. pneumoniae* on the basis of phenotypic characteristics (14). Later the DNA-DNA hybridization data of Ferragut and Leclerc (11) showed that two L-strains were 48 to 59% related to *K. pneumoniae*. Our paper clarifies the taxonomic status of group L within the genus *Klebsiella*.

Eighteen of 20 selected L-strains had DNA-DNA reassociation values of at least 87% with the phenotypic centrostrain CUETM 77-176 (Table 1). Thirty-two of the 37 L-strains displayed very similar and sometimes almost identical protein electrophoretograms (Fig. 1 and 2).

Because bacteria with very similar protein patterns are genetically highly related (24, 25), the combined results of phenotypic features, protein electrophoresis, and DNA-DNA hybridization

indicate that the majority of L-strains constitute a genetically homogeneous group.

Five L-strains (CUETM 77-177, 78-134, 78-127, 78-149, and 78-154) exhibited an aberrant

Fig. 1. Sorted and differentially shaded matrix of correlation coefficients based on the numerical analysis of protein electrophoretograms from 52 *Klebsiella* strains. The range of correlation coefficients (r) is symbolized as: S, 0.96 to 1.0; N, 0.92 to 0.95; /, 0.88 to 0.91; -, 0.84 to 0.87; open space, <0.84.

protein pattern. The electrophoretograms of strains CUETM 77-177 and 78-134 were very similar to the patterns of group K strains (Fig. 1 and 2). The hybridization values (63 to 69%) of both strains with the phenotypic centrostrain of group L (CUETM 77-176; Table 1) are in agreement with the classification based on the electrophoretograms, because two authentic K-strains (CUETM 78-120 and 77-179) hybridize

with strain CUETM 77-176 at a level (60 to 65%) comparable to that at which strains CUETM 77-177 and 78-134 hybridize with CUETM 77-176 (Table 1). A reexamination of the results of Gavini et al. (14) indicates that both strains display phenotypic similarities with each other (Fig. 1 of reference 14) and that strain CUETM 77-177 is phenotypically most removed from all of the other strains of group L. The exact taxo-

FIG. 2. Normalized protein electrophoretograms of 15 *Klebsiella* strains: (a) *K. oxytoca* CUETM 78-182; *K. pneumoniae* (b) ATCC 13882 and (c) CUETM 77-181; *K. terrigena* (group L) (d) CUETM 77-176, (e) CUETM 78-155, (f) CUETM 78-151, (g) CUETM 78-159, (h) CUETM 78-125, (i) CUETM 78-136, and (j) CUETM 78-154; *Klebsiella*-like strains (group K) (k) CUETM 78-120, (l) CUETM 78-134, and (m) CUETM 77-177; unclassified *Klebsiella* strains (n) CUETM 78-127 and (o) CUETM 78-149.

TABLE 2. Characteristics useful in differentiating the species of the genus *Klebsiella* (2, 14, 29, 30, 31)

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

^a Symbols: +, Positive reaction for at least 90% of the strains within 24 h to 48 h; —, negative reaction for at least 90% of the strains; d, different biochemical types (+ or —); (+), slow positive reaction.

TABLE 3. *Phenotypic characteristics of K. terrigena strains (group L) (14; unpublished data)*

Characteristic	Group L (33 strains)			Type strain (CIP 80.07 = CUETM 77-176) reaction ^a
	Reaction ^a	% Positive	% Slowly positive ^b	
Growth at:				
4°C	+	91	6	+4
41°C	—	0		—
44.5°C	—	0		—
Motility (30 and 37°C)	—	0		—
Lysine decarboxylase	+	100		+
Arginine dihydrolase	—	0		—
Ornithine decarboxylase	—	0		—
Gelatinase (20°C)	—	0		—
Urease	+	100		+
β -Galactosidase	+	100		+
β -Xylosidase	+	100		+
Deoxyribonuclease	—	0		—
Tetrathionate reductase	+	94		+
Phenylalanine deaminase	—	0		—
Chitinolysis	—	0		—
Tween 80 hydrolysis	—	0		—
Indole	—	0		—
Methyl red test (30 and 37°C)	+	100		+
Voges-Proskauer reaction (30 and 37°C)	+	100		+
Nitrate to nitrite	+	100		+
H ₂ S production	—	0		—
Citrate (Simmons; 30 and 37°C)	+	100		+2
Malonate	+	100		+
Mucate (Kauffmann)	+	100		+
D-Tartrate (Kauffmann)	— or +	15		—
L-Tartrate (Kauffmann)	— or +	15		—
meso-Tartrate (Kauffmann)	— or +	12		+
Tartrate (Jordan)	+ or —	73		—
Growth on KCN	+	100		+
Fermentation of:				
L-Arabinose	+	100		+
D-Ribose	+	100		+
D-Xylose	+	100		+
D-Galactose	+	100		+
D-Glucose (gas)	+	100		+
D-Mannose	+	100		+
L-Rhamnose	+	100		+
D-Fructose	+	100		+
α -Methyl-D-glucoside	+	100		+
Cellobiose (acid and gas)	+	100		+
Lactose	+	100		+
Maltose	+	100		+
Melibiose	+	100		+
Raffinose	+	100		+
Sucrose	+	100		+
Trehalose	+	100		+
Glycerol (gas)	+	100		+
Adonitol	+	100		+
meso-Inositol	+	100		+
D-Mannitol	+	100		+
Sorbitol	+	100		+
Dextrin	+	100		+
Esculin	+	100		+
Salicin	+	100		+

TABLE 3—Continued

Characteristic	Group L (33 strains)			Type strain (CIP 80.07 = CUETM 77-176) reaction ^a
	Reaction ^a	% Positive	% Slowly positive	
L-Sorbose	+	97		+
Melezitose	+	97		+
Inulin	(+) or —		82	+7
Glycogen	d	18	6	—
Dulcitol	—	6		—
meso-Erythritol	—	0		—
Pectin	—	0		—
Starch	—	0		—

^a Symbols: +, Positive reaction within 24 to 48 h; + or —, — or +, (+) or —, the first sign corresponds to the most frequent result; —, negative reaction. Numbers after symbols indicate number of days for reaction to take place.

^b Positive reaction between 2 and 30 days.

nomic positions of strains CUETM 78-127, 78-149, and 78-154 remain to be determined. Strain CUETM 78-154 probably belongs to group L because its protein pattern displays great overall similarities with those of the other L-strains (Fig. 2). Numerical analysis (Fig. 1) places this strain outside group L because its electrophoretogram contains one heavier protein band in the lower part of the gel (Fig. 2).

A numerical analysis of the phenotypic features (14), the protein electrophoretograms, and the results of DNA-DNA hybridizations permit the differentiation of group L from *K. oxytoca*, *K. pneumoniae*, and the *Klebsiella*-like group K. The DNA relatedness with the other species studied was lower than 48%, except for *E. aerogenes* (50 to 63%). According to Bascomb et al. (2) and Izard et al. (20), *E. aerogenes* should be classified in the genus *Klebsiella*. The latter species can be found on the Approved Lists (36) as both *E. aerogenes* and *K. mobilis*, with the same type strain, ATCC 13048. The range of DNA reassociation values between group L and the *Klebsiella* species (48 to 63%, Table 1) is higher than the intragenic relatedness level (35 to 55%) proposed by Brenner et al. (4) for the family.

The three methods combined indicate that, on phenotypic and genotypic grounds, group L should be considered a homogeneous and distinct taxon within the genus *Klebsiella*. We therefore propose group L as a new species, *Klebsiella terrigena* (ter.ri.ge'na. L.n. terra soil; L. suffix *gena* origin; M.L.n. *terrigena* from soil) because of the environmental origin of the isolates (soil, water). We now suggest that the genus *Klebsiella* should contain the following six species: (i) *K. pneumoniae* (Schroeter 1886) Trevisan 1887 (34, 39), (ii) *K. ozaenae* (Abel 1893) Bergey, Harrison, Breed, Hammer, and Huntton 1925 (1, 3), (iii) *K. rhinoscleromatis* Trevisan 1887 (39), (iv) *K. oxytoca* (Flügge 1886)

Lautrop 1956 (26), (v) *K. mobilis* Bascomb, Lapage, Willcox, and Curtis 1971 (2), and (vi) *K. terrigena* sp. nov.

The phenotypic features used in differentiating the species of the genus *Klebsiella* are listed in Table 2.

Description of *Klebsiella terrigena* sp. nov. The description of this new species is based on 33 strains of group L from Gavini et al. (14); their L-strains CUETM 78-134, CUETM 77-177, CUETM 78-127, and 78-149 do not belong in this new species (see above).

Cell characteristics. Gram-negative, non-motile, nonsporeforming, straight rods measuring 0.3 to 1.5 by 0.6 to 6.0 μ m.

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

Culture conditions. Growth occurs on nutrient agar at 30 and 37°C but not at 41°C.

Relationships to oxygen. Facultatively anaerobic.

Biochemical characteristics. The biochemical features (14) are summarized in Table 3.

Nutritional characteristics. The following carbon sources are utilized (by 80 to 100% of the strains) (14): 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, fumarate, phenylacetate, benzoate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, D-quinic acid, L-aspartate, L-glutamate, L- α -alanine, D- α -alanine, L-serine, L-proline, L-histidine, 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, itaconate, aconitate, oxalate, pimelate, suberate, azelate, levulinate, 2-keto-glutarate, glutarate, adipate, sebacate, salicylate, butyrate, isobutyrate, *n*-valerate, isovalerate, *n*-caproate, heptanoate, pelargonate, caprate, D-mandelate, L-mandelate, phthalate, isophthalate, terephthalate, phenol, benzyl formate, glycine, β -alanine, L-leucine, L-isoleucine, L-valine, L-lysine, 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, D-tryptophan, L-tryptophan, spermine, amylamine, ethanolamine, butylamine, benzylamine, diphenylamine, urea, urate, sarcosine, betaine, creatine, pantothenate, nicotinate, trigonelline, naphthalene, dodecane, and hexadecane.

Genetic data. The average guanine-plus-cytosine content of the DNA of the type strain, CIP 80-07, is 56.7 mol%. Genetic relatedness (DNA-DNA hybridization) with the most related species is as follows: *K. oxytoca*, 49 to 64% (11 strains used); *K. pneumoniae*, 48 to 63% (21 strains used); *E. aerogenes*, 50 to 63% (15 strains used); and *K. ozaenae*, 51% (1 strain used).

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

Type strain. Gavini L 84 = CUETM 77-176 = CIP (Collection de l'Institut Pasteur, Paris, France) 80-07. Isolated from drinking water. The characteristics of the type strain are those given above for the species; the biochemical characteristics are presented in Table 3.

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