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Phylogenetic analyses of *Klebsiella* species delineate *Klebsiella* and *Raoultella* gen. nov., with description of *Raoultella ornithinolytica* comb. nov., *Raoultella terrigena* comb. nov. and *Raoultella planticola* comb. nov.

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The phylogenetic relationships of the type strains of 9 *Klebsiella* species and 20 species from 11 genera of the family *Enterobacteriaceae* were investigated by performing a comparative analysis of the sequences of the 16S rRNA and *rpoB* genes. The sequence data were phylogenetically analysed by the neighbour-joining and parsimony methods. The phylogenetic inference of the sequence comparison confirmed that the genus *Klebsiella* is heterogeneous and composed of species which form three clusters that also included members of other genera, including *Enterobacter aerogenes, Erwinia* clusters I and II and *Tatumella*. Cluster I contained the type strains of *Klebsiella pneumoniae* subsp. *pneumoniae, Klebsiella pneumoniae* subsp. *rhinoscleromatis* and *Klebsiella pneumoniae* subsp. *ozaenae*. Cluster II contained *Klebsiella ornithinolytica, Klebsiella planticola, Klebsiella trevisanii* and *Klebsiella terrigena*, organisms characterized by growth at 10 °C and utilization of L-sorbose as carbon source. Cluster III contained *Klebsiella oxytoca*. The data from the sequence analyses along with previously reported biochemical and DNA–DNA hybridization data support the division of the genus *Klebsiella* into two genera and one genogroup. The name *Raoultella* is proposed as a genus name for species of cluster II and emended definitions of *Klebsiella* species are proposed.

Keywords: *Klebsiella*, *Raoultella*, taxonomy, 16S rRNA, *rpoB*

INTRODUCTION

Until recently, members of the *Enterobacteriaceae* have not been subjected to extensive 16S rDNA analysis (Spröer *et al*., 1999). While some genera have been investigated in detail, e.g. *Yersinia* (Ibrahim *et al*., 1994), *Salmonella* (Chang *et al*., 1997; Christensen *et al*., 1998), *Serratia* (Dauga *et al*., 1990; Harada *et al*., 1996) and *Erwinia* (Kwon *et al*., 1997; Hauben *et al*., 1998), the genus *Klebsiella* has not been analysed extensively (Carter *et al*., 1999). The taxonomy of the genus *Klebsiella* was characterized by a nomenclature reflecting its colourful history. Originally, the medical importance of the genus *Klebsiella* led to its subdivision into three species corresponding to the diseases they caused: *Klebsiella pneumoniae*, *Klebsiella ozaenae* and *Klebsiella rhinoscleromatis*. The 'Oxytocum' group was further individualized (Jain *et al*., 1974) and environmental *Klebsiella* isolates previously classified as '*Klebsiella*-like organisms' (groups J, K, L and M; Gavini *et al*., 1977) were eventually proposed as four new species: *Klebsiella terrigena* (Izard *et al*., 1981), *Klebsiella ornithinolytica* (Sakazaki *et al*., 1989), *Klebsiella planticola* (Bagley *et al*., 1981) and *Klebsiella trevisanii* (Ferragut *et al*., 1983). The last two of these species were subsequently combined as *K. planticola* on the basis of their extensive DNA–DNA homology (Gavini *et al*., 1986). Finally, *Calymmatobacterium granulomatis*, the presumed causative agent of donovanosis, was recently reclassified as *Klebsiella granulomatis* on the basis of phylogenetic data (Kharsany *et al*., 1999; Carter *et al*., 1999). The latest edition of

The GenBank accession number for the 16S rDNA and *rpoB* sequences of *Klebsiella* strains in this paper are given in Methods.

Bergey's Manual of Systematic Bacteriology (Ørskov, 1984) classified the genus *Klebsiella* into five species, namely *K. pneumoniae*, *Klebsiella oxytoca*, *K. terrigena*, *K. ornithinolytica* and *K. planticola*. The species *K. pneumoniae* comprises three subspecies, *K. pneumoniae* subsp. *pneumoniae*, *K. pneumoniae* subsp. *ozaenae* and *K. pneumoniae* subsp. *rhinoscleromatis*. This classification was based on numerical taxonomy using phenotypic and biochemical characters and data derived from DNA–DNA hybridization studies (Brenner & Falkow, 1971; Wayne *et al*., 1987).

While investigating 16S rDNA- and *rpoB* (encoding the bacterial RNA polymerase β-subunit)-based molecular identification of enteric bacteria (Mollet *et al*., 1997), we noticed that 16S rDNA- and *rpoB*-based phylogenetic reconstructions indicated *Klebsiella* to be polyphyletic. This analysis led us to further study the taxonomic relationships among members of *Klebsiella*. 16S rDNA sequence comparison has emerged as one of the most powerful tools for investigating bacterial phylogeny (Woese, 1987; Weisburg *et al*., 1991) and its use has resulted in reclassification of bacterial species and genera (Brenner *et al*., 1993a; Tamura *et al*., 1995; Rikihisa *et al*., 1997), including enteric bacteria (Kwon *et al*., 1997; Spröer *et al*., 1999). The level of 16S rDNA sequence similarity has been proposed as a basis for bacterial species definition (Stackebrandt & Goebel, 1994) and the use of polyphasic taxonomy has been advocated to ensure well-balanced determinations of taxonomic relationships (Vandamme *et al*., 1996). In this study, we determined carbon assimilation patterns, and 16S rDNA and *rpoB* sequences for the eight *Klebsiella* species and the *rpoB* sequence for *K. granulomatis* to clarify the taxonomy of the genus *Klebsiella*.

METHODS

Bacterial strains. Type strains *Klebsiella pneumoniae* subsp. *pneumoniae* ATCC 13883^T, *Klebsiella pneumoniae* subsp. *rhinoscleromatis* ATCC 13884^T, *Klebsiella pneumoniae* subsp. *ozaenae* ATCC 11296^T, *Klebsiella oxytoca* ATCC 13182^T, *Klebsiella ornithinolytica* ATCC 31898^T, *Klebsiella terrigena* ATCC 33257^T, *Klebsiella planticola* ATCC 33531^T and *Klebsiella trevisanii* ATCC 33558^T were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The organisms were cultivated on trypticase soy agar at 32 °C for 2 d. A clinical specimen shown to contain *Klebsiella granulomatis* was kindly provided by Dr S. Hutton (Menzies School of Health Research, Darwin, Australia).

Biochemical tests. Carbon source utilization tests were done by using Biotype strips (BioMérieux) as described by Brenner *et al*. (1993b). Medium no. 2 was used for *K. pneumoniae* subsp. *rhinoscleromatis* and medium no. 1 for the others. After inoculation, the strips were incubated at 32 °C and were read after 2, 3 and 4 d incubation.

16S rDNA and *rpoB* sequencing. Genomic DNA was extracted from each strain and the *K. granulomatis*-positive clinical specimen according to Wilson (1990). 16S rDNA was amplified by PCR (Saiki *et al*., 1985) using oligonucleotide primers fD1 and rP2 under conditions described by Weisburg *et al*. (1991) for each *Klebsiella* strain, but not for the *K. granulomatis*-positive clinical specimen. A portion of the coding region of *rpoB* was PCR-amplified using oligonucleotide primers CM7 (5′-AACCAGTTCCGCGTTGGCCTGG-3′) and CM31b (5′-CCTGAACAACACGCTCGGA-3′) under conditions described by Mollet *et al*. (1997). The success of each PCR was assessed by UV illumination of ethidium-bromide-stained 1% agarose gels after electrophoresis. The resulting amplicons were purified (QIAquick spin PCR purification kit; Qiagen), then sequenced using the reagents of the ABI Prism dRhodamine Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer Applied Biosystems) according to manufacturer's instructions and using the following thermal programme: 25 cycles of denaturation at 95 °C for 20 s, primer-annealing at 50 °C for 10 s and extension at 60 °C for 4 min. Products of sequencing reactions were resolved by electrophoresis in a 0·2 mm 6% polyacrylamide denaturing gel and recorded using an ABI Prism 377 DNA Sequencer (Perkin Elmer Applied Biosystems) following the standard protocol of the supplier. The results obtained were processed into sequence data by sequence analysis software (Applied Biosystems) and partial sequences were combined into a single consensus sequence.

Sequence data analyses. 16S rDNA sequences and *rpoB* sequences of non-*Klebsiella* species were obtained from the GenBank database. Pairwise sequence comparisons were determined using the GCG program (Infobiogen). The sequences were aligned by using the multisequence alignment program CLUSTAL (Higgins & Sharp, 1989) in the BISANCE software package (Dessen *et al*., 1990). Phylogenetic relationships were inferred from this alignment by using programs within version 3.4 of the PHYLIP software package (Felsenstein, 1989, 1993). A distance matrix was generated using DNADIST under the assumptions of Jukes & Cantor (1969) and Kimura (1980). Phylogenetic trees were derived from theses matrices using neighbour-joining. For parsimony analysis DNAPARS was used. Evaluation of individual node strength used the SEQBOOT bootstrapping method with 100 samples.

Nucleotide sequence accession numbers. Partial *rpoB* and 16S rDNA sequences obtained in this study were deposited in the GenBank database under the following numbers. 16S rDNA: *K. pneumoniae* subsp. *pneumoniae*, AF130981; *K. pneumoniae* subsp. *rhinoscleromatis*, AF130983; *K. pneumoniae* subsp. *ozaenae*, AF130982; *K. oxytoca*, AF129440; *K. ornithinolytica*, AF129441; *K. planticola*, AF129443; *K. trevisanii*, AF129444; *K. terrigena*, AF129442. *rpoB*: *K. pneumoniae* subsp. *pneumoniae*, AF253134; *K. pneumoniae* subsp. *rhinoscleromatis*, AF129446; *K. pneumoniae* subsp. *ozaenae*, AF129445; *K. oxytoca*, 253153; *K. ornithinolytica*, AF129447; *K. planticola*, AF129449; *K. trevisanii*, AF129450; *K. terrigena*, AF129448; *Klebsiella granulomatis*, AF218573.

RESULTS

Biochemical tests

Major differential characteristics of the eight species and subspecies currently belonging to the genus *Klebsiella* are reported in Table 1. All strains of these species are Gram-negative, non-motile and encapsulated. Among the differential characters, growth at 10 °C and unique utilization of L-sorbose as carbon

Table 1. Phenotypic differential characteristics of *Klebsiella* species

K. granulomatis was not available for this study.

Test	*K. pneumoniae* subsp. *pneumoniae*	*K. pneumoniae* subsp. *ozaenae*	*K. pneumoniae* subsp. *rhinoscleromatis*	*K. oxytoca*	*K. planticola**	*K. terrigena*	*K. ornithinolytica*
Growth at 10 °C	−	−	−	+	+	+	+
Indole	−	−	−	+	+	−	+
Ornithine decarboxylase	D	−	−	−	−	−	+
Voges–Proskauer reaction	+	−	−	+	+	+	D
Utilization of the following as a carbon source:							
4-Aminobutyrate	+	−	−	+	+	−	+
Benzoate	−	−	−	+	+	+	−
m-Coumarate	+	−	−	+	+	−	+
Dulcitol	−	−	−	+	−	−	−
L-Fucose	−	+	+	+	+	+	+
D-Glucosamine	+	−	+	+	+	+	−
3-*O*-Methyl-D-glucose	−	−	−	−	+	+	+
Histamine	−	−	−	−	+	+	−
5-Ketogluconate	−	−	−	+	+	+	−
D-Melezitose	−	−	−	+	+	+	−
Palatinose	+	−	−	+	+	+	+
Phenylacetate	−	−	−	+	+	+	−
Putrescine	−	−	−	+	+	+	−
Quinate	+	−	−	+	+	+	−
L-Rhamnose	−	+	+	+	+	+	+
L-Sorbose	−	−	−	+	+	+	+
D-Tagatose	−	−	−		−	−	−
L-Tartrate	−	+	+	+	−	−	−
Meso-Tartrate	−	−	+	+	+	+	+
D-Turanose	−	+	−	−	+	+	−

* Including *K. trevisanii* isolate.

source were negative for the three *K. pneumoniae* subspecies, but positive for the five other *Klebsiella* isolates.

16S rDNA and *rpoB* amplification and sequencing

The almost complete 16S rDNA sequence, about 1438 bp corresponding to nt 50–1486 (*Escherichia coli* numbering; Brosius *et al.*, 1978) was determined for the type strains of eight *Klebsiella* species and deposited in the GenBank database. A 512 bp sequence corresponding to codons 500–670 of the 1342 aa coding region in *Escherichia coli rpoB* (Ovchinnikov *et al.*, 1981) was obtained for these eight type strains and *K. granulomatis*.

Data analysis

Among *Klebsiella* strains, overall 16S rDNA sequence similarities varied from 96·3 to 99·9 % and *rpoB* sequence similarities varied from 93·9 to 99·9 %. The sequences of *K. pneumoniae* subsp. *pneumoniae*, *K. pneumoniae* subsp. *rhinoscleromatis*, *K. pneumoniae* subsp. *ozaenae* and *K. granulomatis* isolates exhibited a 98·2–99·7 % 16S rDNA sequence similarity and 99·4–100 % *rpoB* sequence similarity. Likewise, the 16S rDNA sequences of *K. planticola*, *K. trevisanii* and *K. terrigena* isolates exhibited 97·0–99·9 % similarity, whereas *rpoB* sequence similarities ranged from 93·9 to 99·8 %. Indeed, *K. planticola* and *K. trevisanii* sequences were almost identical and shared a 99·9 % 16S rDNA sequence similarity and a 99·8 % *rpoB* sequence similarity. Dendrograms inferred from the 16S rDNA alignment by using neighbour-joining (Fig. 1) and parsimony methods yielded similar topologies in which klebsiellae formed three clusters. The three *K. pneumoniae* subspecies, *pneumoniae*, *rhinoscleromatis* and *ozaenae*, and *K. granulomatis* clustered together, as did *K. planticola*, *K. trevisanii*, *K. ornithinolytica* and *K. terrigena*, with *K. oxytoca* being only distantly related. Dendrograms inferred from the *rpoB* alignment using the same methods yielded three clusters mixing with representatives of other *Enterobacteriaceae* genera (Fig. 2). Bootstrap values > 70 % were calculated for the *K. planticola*/*K. trevisanii*/*K. ornithinolytica* cluster and for the *K. pneumoniae*/*K. granulomatis* cluster based on both 16S rDNA- and *rpoB*-based topologies.

DISCUSSION

Phylogenetic relationships among *Klebsiella* and other *Enterobacteriaceae* genera

The results of the analyses of 16S rDNA and *rpoB* sequences support the heterogeneous taxonomic structure of the genus *Klebsiella* (Figs 1 and 2). The genus *Klebsiella* consists of three phyletic lines which were shared with other members of the *Enterobacteriaceae*, including *Enterobacter aerogenes*, *Erwinia* and *Tatumella* (Figs 1 and 2). Cluster I comprises *K. pneumoniae* subspecies *pneumoniae*, *rhinoscleromatis* and *ozaenae*, and *K. granulomatis*; cluster II contains *K. ornithinolytica*, *K. planticola*, *K. trevisanii* and *K. terrigena*; and cluster III contains *K. oxytoca*. The

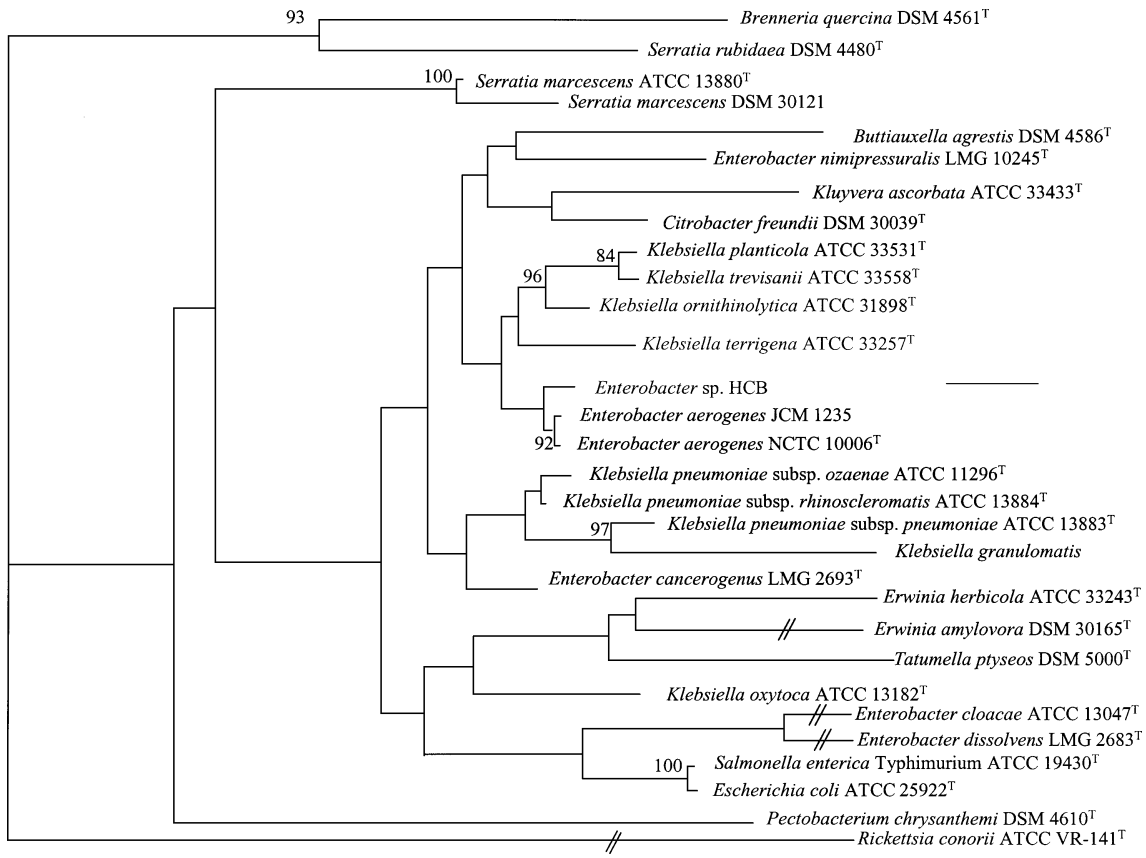

Fig. 1. Phylogenetic tree derived from the 16S rDNA sequence of members of various genera of the family *Enterobacteriaceae*, including nine *Klebsiella* (*Raoultella*) species, determined by neighbour-joining from a Jukes & Cantor DNA distance matrix with *Rickettsia conorii* as outgroup. Numbers within the dendrogram indicate the occurrence (%) of the branching in 100 bootstrapped trees (only values of 70 and above are shown). The scale bar indicates 1% divergence.

validity of the three-cluster organization is supported by the fact that consensus topologies were obtained using two methods of analysis of two different molecule sequences and the fact that bootstrap values for these topologies were greater than 70%. Although moderate, these bootstrap values are in the range of those previously reported by 16S rDNA analysis of *Enterobacteriaceae* (Spröer *et al.*, 1999; Kwon *et al.*, 1997). Bootstrap values as low as 40% (Kwon *et al.*, 1997) and 60% (Spröer *et al.*, 1999) have been cited in previous 16S rDNA phylogenetic analyses of *Enterobacteriaceae* genera.

The phylogenetic relationships of the genus *Klebsiella* to other genera of the family *Enterobacteriaceae* have been previously studied by genomic DNA relatedness, although initial studies were restricted to *K. pneumoniae* subspecies (Brenner & Falkow, 1972; Murata & Starr, 1974; Woodward *et al.*, 1979). Based on the percentage of DNA–DNA reassociation with *Escherichia coli* K-12, Brenner & Falkow (1972) distinguished three groups among the *Enterobacter*/*Klebsiella* species, consistent with the three clusters we delineated in this study. One group contained one strain of *Enterobacter cloacae*, exhibiting 48% homology with *Escherichia coli* K-12, the second group contained *K. pneumoniae*, *K. ozaenae* and *Enterobacter aerogenes* and exhibited 35–40% homology with *Escherichia coli* K-12 and the third group contained '*Enterobacter liquefaciens*' and '*Enterobacter hafniae*' and exhibited 15–21% homology with *Escherichia coli* K-12. Brenner & Falkow (1972) noted that intrageneric relatedness had to be tested. Further analysis of DNA–DNA reassociation between five strains of *K. pneumoniae* subsp. *pneumoniae*, *K. pneumoniae* subsp. *ozaenae* and *K. pneumoniae* subsp. *rhinoscleromatis* disclosed 80–91% homology and led to the conclusion that three subspecies existed among *K. pneumoniae* strains (Brenner *et al.*, 1972). Although Murata & Starr (1974) stated that, on the basis of DNA segmented homology test results, the genera *Erwinia*, *Escherichia*, *Klebsiella*, *Enterobacter* and *Serratia* should be integrated into a single genus, this proposition was based on observation of a low genetic homology level of about 10%. Further studies compared DNA–DNA relatedness among newly described *Klebsiella* species. *K. planticola* (including *K. trevisanii*) exhibited 87–100% intraspecific relatedness, 51–63%

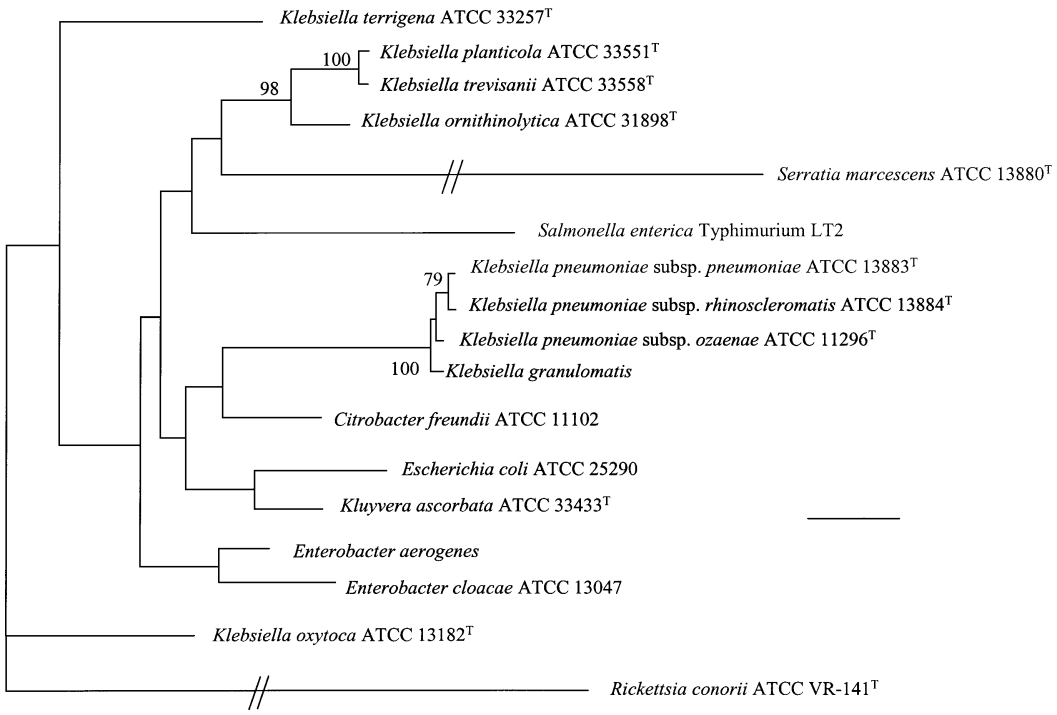

Fig. 2. Phylogenetic tree derived from the partial *rpoB* sequence of members of various genera of the family *Enterobacteriaceae*, including nine *Klebsiella* (*Raoultella*) species, determined by neighbour-joining from a Jukes & Cantor DNA distance matrix with *Rickettsia conorii* as outgroup. Numbers within the dendrogram indicate the occurrence (%) of the branching in 100 bootstrapped trees (only values of 70 and above are shown). The scale bar indicates 1% divergence.

relatedness to *Enterobacter aerogenes*, 48–63% relatedness to *K. pneumoniae*, 49–62% relatedness to *K. oxytoca* and $< 45\%$ relatedness to representatives of other genera (Ferragut *et al.*, 1983). These data are in accordance with the taxonomic conclusions of this study. *K. ornithinolytica* exhibited 69–100% intraspecific relatedness, 31–72% relatedness to *K. pneumoniae*, 34–76% relatedness to *K. oxytoca*, 13–38% relatedness to *K. planticola*, 10–34% relatedness to *K. terrigena* and $\leqslant 20\%$ relatedness to representatives of other genera (Sakazaki *et al.*, 1989). *K. terrigena* exhibited 63–100% intraspecific homology, 50–63% homology with *Enterobacter aerogenes*, 49–64% homology with *K. oxytoca*, 48–63% homology with *K. pneumoniae* and $< 45\%$ homology with other representatives of *Enterobacteriaceae* (Izard *et al.*, 1981), in accordance with our taxonomic propositions. *K. planticola* exhibited 72–100% intraspecific reassociation, 25–62% homology with *K. oxytoca*, 20% homology with *Enterobacter aerogenes* (Seidler *et al.*, 1975) and 7–29% homology with *K. pneumoniae* (Bagley *et al.*, 1981). Except for *K. ornithinolytica*, DNA–DNA data are in accordance with our proposition to divide the currently recognized *Klebsiella* species into two genera. Furthermore, *K. oxytoca* forms a distinct genogroup of uncertain taxonomic position; discrepancies in reported DNA–DNA reassociation data may suggest this taxon is not homogeneous.

Previously, most 16S rDNA-based taxonomic analyses of *Klebsiella* species have been limited to one *Klebsiella* species, thus preventing genus-wide taxonomic conclusions to be drawn. For example, the proposition that *Erwinia* clusters I and II, *Escherichia coli*, *Serratia marcescens* and *K. pneumoniae* may form a macrocluster at the genus level (Kwon *et al.*, 1997) was supported by the incorporation of only *K. pneumoniae* 16S rDNA sequence in the analysis, low 16S rDNA sequence similarity ($< 95\%$) and low bootstrap values $< 50\%$ (Kwon *et al.*, 1997). Likewise, although the polyphyletic positions of *Klebsiella* species have been previously noted, this did not lead to taxonomic reappraisals because in most studies *K. pneumoniae* subsp. *pneumoniae* was included as the sole representative of the genus *Klebsiella* (Spröer *et al.*, 1999; Hauben *et al.*, 1998; Kwon *et al.*, 1997). A recent 16S rDNA-based phylogenetic analysis of *K. granulomatis* incorporated *K. pneumoniae* subsp. *pneumoniae*, *K. pneumoniae* subsp. *rhinoscleromatis*, *K. oxytoca*, *K. ornithinolytica* and *K. planticola* and disclosed the three-cluster organization we investigated (Carter *et al.*, 1999). In this study, polyphyletic positions were supported by reasonably high bootstrap values of $> 50\%$. Also, our *rpoB*-based study of enteric bacteria included *K. pneumoniae* subsp. *pneumoniae*, *K. ornithinolytica* and *K. oxytoca* and disclosed the same three-cluster architecture (Mollet *et al.*, 1997). These

data are in accordance with the three-cluster organization we investigated in this study. That these three *Klebsiella* clusters grouped with representatives of other *Enterobacteriaceae* genera clearly indicated that they belong to different genera. Also, representatives of *Klebsiella* exhibited intergenus 16S rDNA sequence similarities higher than intragenus 16S rDNA sequence similarities. In this study, 98% 16S rDNA sequence similarity and 94% *rpoB* sequence similarity appeared reasonable cut-off values to delineate different genera. Although 97% 16S rDNA sequence similarity has been proposed as a lower value to delineate bacterial isolates at the species level (Stackebrandt & Goebel, 1994), numerous bacterial species exhibit more than 99% 16S rDNA sequence similarity with one another (Fox *et al*., 1992; Martinez-Murcia *et al*., 1992; Forsman *et al*., 1994; Roux & Raoult, 1995) and no guidelines have been issued regarding 16S rDNA-based delineation of bacterial genera. A recent 16S rDNA-based phylogenetic analysis of various genera of the family *Enterobacteriaceae* retained 94–97% sequence similarity to delineate genera within this family (Spröer *et al*., 1999). Differentiation at the genus level of the three *Klebsiella* clusters is further supported by growth at low temperature for members of cluster II, consistent with their recovery from plants, soil and water, whereas members of cluster I do not grow at 10 °C and are mainly recovered from mammals mucosae. Also, only members of cluster II grow in the presence of L-sorbose, a source of carbon characteristic of plants. These ecological and phenotypic data reflect the evolutionary separation of these three clusters. Finally, a proposal to classify *K. oxytoca* in a separate, unnamed genus has been made (Jain *et al*., 1974). Although this proposal was not endorsed at that time, our data support this move.

Interspecific relationships among clusters

Cluster I contains isolates which have been recognized as being subspecies of *K. pneumoniae* on the basis of phenotypic characters and DNA–DNA hybridization values of 80–90% (Brenner *et al*., 1972) and 73–100% (Woodward *et al*., 1979). Our data support this analysis since these isolates share >99% 16S rDNA and *rpoB* sequence similarities, *K. pneumoniae* subsp. *ozaenae* and *K. granulomatis* being indistinguishable on the basis of their *rpoB* sequence. Taxonomic relationships between members of cluster II cannot be firmly derived from our data because of moderate bootstrap values. In this cluster, *K. planticola* and *K. trevisanii* 16S rDNA and *rpoB* sequences shared >99% similarity. These data are in agreement with the previous conclusion that these two taxa are indistinguishable at the species level on the basis of phenotypic characteristics and very high levels of DNA–DNA hybridization and thus with the proposal for their combination under the name *K. planticola* (Gavini *et al*., 1986). *K. ornithinolytica* was more closely related to *K. planticola* than to *K. terrigena* and *K. oxytoca* on the basis of both phenotypic numeric taxonomy and 16S rDNA and *rpoB* sequences. Despite the fact that the first two isolates of *K. ornithinolytica* were reported as ornithine-positive *K. oxytoca*, these data are not in agreement with those derived from a DNA–DNA relatedness study which reported 33–78% relatedness between *K. ornithinolytica* and *K. oxytoca* and 11–49% relatedness between *K. ornithinolytica* and *K. planticola* (Sakazaki *et al*., 1989). In the same study however, 18–45% relatedness was noted between *K. ornithinolytica* and *K. terrigena* which is not in agreement with data presented in our study. The taxonomic relationships of *K. terrigena* warrant further study.

Emendation of the genus *Klebsiella* (Trevisan 1885) Ørskov 1984

On the basis of the evidence presented above, it is proposed that the genus *Klebsiella* should be divided into two genera, *Klebsiella* and *Raoultella* gen. nov. and that '*K. oxytoca*' should be left as a monophyletic species. This proposal requires emending the description of the genus *Klebsiella* (Ørskov, 1984) together with a description of the new genus *Raoultella*.

Straight rods, 0·3–1·0 μm in diameter and 0·6–6·0 μm in length, arranged singly, in pairs or short chains. Conform to the general definition of the family *Enterobacteriaceae*. Capsulated. Gram-negative. Nonmotile. Facultatively anaerobic, having both a respiratory and a fermentative type of metabolism. Grow on meat extract medium, producing more or less dome-shaped, glistering colonies of varying degrees of stickiness, depending on the strain and the composition of the medium. Does not grow at 10 °C. There are no special growth factor requirements. Oxidase-negative, catalase-positive. Most strains can use citrate and glucose as sole carbon source, but cannot use L-sorbose as sole carbon source. Glucose is fermented with the production of acid and gas and most strains produce 2,3-butanediol as a major end product of glucose fermentation. Voges–Proskauer test is usually positive. Recovered from mammal mucosae, including human specimens. Type species is *K. pneumoniae* subsp. *pneumoniae* and the type strain is *K. pneumoniae* subsp. *pneumoniae* ATCC 13883^T (= CIP 82.91^T).

Description of *Raoultella* gen. nov.

Raoultella (Ra.oul.tel'la. M.L. dim. suffix *tella*; M.L. fem. n. *Raoultella* named after the French bacteriologist Didier Raoult, Université de la Méditerranée, Marseille, France).

The genus *Raoultella* is composed of Gram-negative, oxidase-negative, aerobic, non-motile, capsulated rods. Facultatively anaerobic, having both a respiratory and a fermentative type of metabolism. Cells grow on meat extract medium; growth at 10 °C is a hallmark of the genus. Oxidase-negative, catalase-positive. Most strains can use citrate and glucose as sole carbon source. Glucose is fermented with the

production of acid and gas, and most strains produce 2,3-butanediol as a major end product of glucose fermentation. Voges–Proskauer test is always positive. Recovered from water, soil, plants and occasionally mammal mucosae, including human specimens. Type species is *Raoultella planticola* comb. nov. and the type strain is *Raoultella planticola* ATCC 33531^T (CIP 100751^T).

Description of *Raoultella planticola* comb. nov.

The most recent description is that by Gavini *et al.* (1986). The 16S rDNA nucleotide sequence of the type strain, ATCC 33531^T (CIP 100751^T), is deposited in the GenBank database under accession number AF129443.

Description of *Raoultella ornithinolytica* comb. nov.

The most recent description is that by Sakazaki *et al.* (1989). The 16S rDNA nucleotide sequence of the type strain, ATCC 31898^T (CIP 103576^T), is deposited in the GenBank database under accession number AF129441.

Description of *Raoultella terrigena* comb. nov.

The most recent description is that by Izard *et al.* (1981). The 16S rDNA nucleotide sequence of the type strain, ATCC 33257^T (CIP 80.7^T), is deposited in the GenBank database under accession number AF129442.

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