Rouxiella chamberiensis gen. nov., sp. nov., a new Enterobacteriaceae isolated from parenteral nutrition bags.

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Abstract:	Parenteral nutrition bags for newborns were found contaminated by a previously undescribed Enterobacteriaceae. The six isolates studied by rrs - (encoding 16S rRNA) and multilocus sequence analysis (MLSA) formed a discrete branch close to genera Ewingella, Rahnella, Yersinia, Hafnia and Serratia. Phenotypically, the new taxon was distinct from these four genera. The new taxon gave positive Voges-Proskauer, Simmons citrate, o-nitrophenyl-β-galactoside hydrolysis tests; fermented D-glucose, D-mannitol, L-rhamnose, D-melibiose, L-arabinose, D-xylose, and hydrolyzed esculin; did not ferment maltose, trehalose, raffinose, D-sorbitol, sucrose and D-cellobiose. The following tests, motility, gas production, urease, gelatinase, and nitrate reduction were also negative. All isolates failed to grow at 37°C. Therefore, the new taxon is proposed as a new species and genus and named Rouxiella chamberiensis gen. nov., sp. nov. The type strain is 130333T (= CIP 110714T = DSM 28324T). The G+C content of the type strain DNA was 53 mol%.

Rouxiella chamberiensis gen. nov., sp. nov., a new *Enterobacteriaceae* isolated from parenteral nutrition bags

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The GenBank/EMBL accession numbers for the *rrs* gene sequences of strain 130333^T is KJ526379. The accession numbers for the *rpoB* gene sequence of strain 130333^T and isolates 140001, 140002, 140003, 140004 and 140005 as determined in this study are KJ526372, KJ526373, KJ526374, KJ526375, KJ526376 and KJ526377. The GenBank/EMBL accession numbers for the sequences presented in this study are: KJ774531- KJ774537 (*fusA* gene), KJ774538- KJ774544 (*pyrG* gene), KJ774545- KJ774551 (*rplB* gene), KJ774553- KJ774559 (*sucA* gene).

Abstract

Parenteral nutrition bags for newborns were found contaminated by a previously undescribed *Enterobacteriaceae*. The six isolates studied by *rrs* - (encoding 16S rRNA) and multilocus sequence analysis (MLSA) formed a discrete branch close to genera *Ewingella*, *Rahnella*, *Yersinia, Hafnia* and *Serratia*. Phenotypically, the new taxon was distinct from these four genera. The new taxon gave positive Voges-Proskauer, Simmons citrate, *o*-nitrophenyl-β-galactoside hydrolysis tests; fermented D-glucose, D-mannitol, L-rhamnose, D-melibiose, L-arabinose, D-xylose, and hydrolyzed esculin; did not ferment maltose, trehalose, raffinose, D-sorbitol, sucrose and D-cellobiose. The following tests, motility, gas production, urease, gelatinase, and nitrate reduction were also negative. All isolates failed to grow at 37°C. Therefore, the new taxon is proposed as a new species and genus and named *Rouxiella chamberiensis* gen. nov., sp. nov. The type strain is 130333^T (= CIP 110714^T = DSM 28324^T). The G+C content of the type strain DNA was 53 mol%.

In December 2013, six bacterial isolates were recovered from parenteral nutrition bags used for premature newborns in neonatal intensive care units in Chambéry Hospital (South East of France). The Gram-negative, fermentative, and oxidase negative isolates were not identified by either API-20E strips, VITEK 2 (bioMérieux, Marcy-l'Etoile, France) or matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectrometry (Bruker, Coventry, UK) although *Ewingella americana* and *Pantoea* sp. were suggested. Therefore, molecular methods were required to better characterize the unknown taxon. The outcome is the description of a new genus and species named *Rouxiella chamberiensis* gen. nov., sp. nov.

Isolates 130333^{T}, 140001, 140002, 140003, 140004, and 140005 were recovered from six different parenteral nutrition bags in December 2013 in a Hospital located in Chambéry, France. The isolates were recovered on tryptocasein soy agar (TSA) (bioMérieux, Marcy-l'Etoile, France) at 30°C under aerobic conditions.

Gene sequencing was used to determine the phylogenetic position of the isolates. For this, strains were cultured on tryptocasein soy agar at 30°C. Total DNA was prepared from bacterial cultures by using the Promega Genomic DNA purification kit (Promega, Madison, WI, USA).

For *rrs* gene (encoding 16S rRNA) sequencing, universal primers were used, 1**.**5 kb of the *rrs* gene were amplified by PCR (Janvier *et al.*, 1995). The amplified product was sequenced in our laboratory with three primers E, rE and D, which are located in conserved regions of the *E. coli rrs* gene. Primer E (5'-ATTAGATACCCTGGTAGTCC-3') corresponds to positions 787-806, primer rE (5'-GGACTACCAGGGTATCTAAT-3') is complementary to primer E and primer D (5'-CAGCAGCCGCGGTAATAC-3') corresponds to positions 519-536 (numbering according to Brosius *et al.*, 1978).

It should be noted that the *rrs* gene is not always sufficient to distinguish closely related species, especially within *Enterobacteriaceae*. Multi-locus sequence analysis (MLSA) was based on partial sequences of the housekeeping genes *fusA* (634 bp), *pyrG* (307 bp), *rplB* (333 bp), *rpoB* (968 bp) and *sucA* (634 bp) which were identified as best candidates for *Enterobacteriaceae* most-conserved genes. These genes are single-copy-number genes, essential and are present in many bacterial lineages. Therefore, they were expected to be present in all members of the *Enterobacteriaceae* (Deletoile *et al.*, 2009; Paauw *et al.*, 2008; Achtman *et al.*, 2012 ; Brady *et al.*, 2013). PCR amplification of *fusA*, *pyrG*, *rplB*, *rpoB* and

sucA was performed with primers fusA3 and fusA4, pyrG3 and pyrG4, rplB3 and rplB4, VIC4 and VIC6, sucA-R and sucA-F respectively as published (Mollet *et al.*, 1997; Tayeb *et al.*, 2008; Deletoile *et al.*, 2009; Achtman *et al.*, 2012). Sanger's method with the ABI 3730 XL (Applied Biosystems Inc., CA, USA) was used.

MLSA was performed with the above genes, for selected *Enterobacteriaceae* strains, including that of the new taxon (strains 130333^{T}, 140001, 140002, 140003, 140004 and 140005). Initial multiple sequence alignment was performed using ClustalW (Thompson *et al.*, 1994), providing as many character matrices. Those matrices were concatenated into a single character super-matrix. As assessment of the quality of multiple sequence alignment is important to ensure the accuracy of phylogenetic inference, an additional alignment character trimming step was carried out to select regions in the matrix that are suited for phylogenetic inference. Therefore, both sparse columns were excised (stretches of gaps/openings in one or more sequences) and compositional heterogeneity (ambiguously aligned regions) was minimized in the alignment matrix, returning a trimmed dataset that allows to reconstruct a more accurate phylogenetic tree than the initial alignment (Talavera *et al.*, 2007).

Multi-locus sequence typing (MLST) analysis is becoming a common typing method to characterize isolates. In contrast to MLSA, MLST relies on the comparison of allelic profiles of isolates within species (Maiden *et al.*, 1998). A neighbouring genus, *Pantoea*, showed that the MLST is a powerful typing method. The clonal relationship between the six *Rouxiella chamberiensis* isolates was further studied using five of the six genes (*fusA, pyrG, rplB, rpoB* and *sucA)* of the *Pantoea* MLST scheme described by Deletoile *et al.*, 2009.

A whole genome shotgun sequencing experiment and assembly for our type strain 130333^{T} was done using the Next Generation Sequencing (NGS) technique (Illumina MiSeq). Average nucleotide identities (ANI) (Konstantinidis and Tiedje, 2005a; Konstantinidis and Tiedje, 2005b) were computed on whole genome sequences to measure the genetic and evolutionary relatedness among strains, and help to consolidate the existing taxonomic ranks of bacterial strains. Therefore, unequivocal evidence for taxonomic delineation at the species level was obtained by calculating the ANI of representative genome sequences. The ANI calculations were performed using the *in silico* DNA–DNA hybridization method (Konstantinidis & Tiedje, 2005a; Goris *et al.*, 2007) implemented in the JSPECIES software (http://www.imedea.uib-csic.es/jspecies/about.html ; Richter & Rossello-Mora, 2009) with default blast parameters.

A total of 1463 bp for *rrs* gene were determined for strain 130333^T and 949 nucleotides for *rpo*B gene were determined for all six isolates. Sequences were compared to all bacterial sequences available from the GenBank database by using the BLAST program http://www.ncbi.nlm.nih.gov/blast/Blast.cgi. Related sequences were downloaded, compared and phylogenetic trees were generated with the MegAlign module of the Lasergene software (DNASTAR), using the neighbour-joining (NJ) algorithm (Saitou & Nei, 1987). Bootstrap analysis with 1000 replicates was performed to assess the reliability of tree branching. The accession numbers of the sequences used in this study are listed in Figs 1 and 2.

The NJ tree derived from *rrs* gene sequences (1413 bp) (Fig.1) showed strains of the new taxon (referred to as *Rouxiella chamberiensis*) to constitute a discrete branch. Strain 130333^T shared 97.0% similarity with *Ewingella americana* CIP 81.94^T and 96.3% similarity with *Rahnella aquatilis* CIP 78.65^T. Table S1 gives nucleotide substitution ratio (phylogenetic distance) among strains.

The NJ tree derived from *rpoB* gene sequences (631 bp) showed *Rouxiella chamberiensis* strains to constitute a new taxon (Supplementary Fig. S1, available in IJSEM Online). The closest species were *Ewingella americana* and *Rahnella aquatilis*.

An MLSA scheme was used to include 5 representative genes (*fusA, pyrG, rplB, rpoB,* and *sucA*) yielded an unrooted tree displaying 17 genera (Fig.2). A total of 2876 bp for MLSA scheme were determined for each species representative genus. Table S2 gives nucleotide substitution ratio among strains.

All six isolates were 100% identical for each internal portion of selected housekeeping genes. Thus, MLST could not differentiate sequence types among the six isolates.

The ANI values computed on the assembled whole genome shotgun sequence of the *Rouxiella* strain as compared with *Rahnella* strain were ANI=76.42%, those with *Yersinia* strains were ANI=72.52%, those with *Hafnia* were ANI=72.7%, those with *Serratia* strains were ANI=75.16%. This whole genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession JRWU00000000. The version described in this paper is version JRWU01000000.

Ideally, a genus constitutes a discrete phylogenetic branch formed with species sharing common characters. Up to now, no upper limit has been set to between-species phylogenetic distances because well-known genera show different levels of homogeneity.

If we consider the *rrs* rootless tree (Fig. 1), the shortest distances from the new taxon (referred to as *Rouxiella chamberiensis*) are 0.0237 with *Obesumbacterium proteus*, 0.0245 with *Hafnia alvei*, and 0.0266 with *Ewingella americana*. On the same tree, distances among *Leclercia adecarboxylata*, *Pantoea agglomerans*, *Enterobacter cloacae*, and *Klebsiella pneumoniae* are shorter, ranging from 0.0043 to 0.0246.

If we consider the MLSA rootless tree (Fig. 2), the shortest distances from the new taxon (*Rouxiella*) are 0.0639 with *Rahnella aquatilis*, 0.0671 with *Ewingella americana*, and 0.0796 with *Serratia marcescens*. On the same tree, distances among *Kluyvera ascorbata*, *Citrobacter freundii*, *Leclercia adecarboxylata*, *Enterobacter cloacae*, *Escherichia coli*, and *Klebsiella pneumoniae* are shorter, ranging from 0.0275 to 0.0449.

These trees provide no support for including the new taxon in a known genus. Therefore, there is no other option than creating a new genus, *Rouxiella*.

Electron microscopy was used to determine cell morphology and size. Both isolated colonies on agar plate (diluted in 10 µl phosphate buffered saline) and liquid culture aliquots (i.e. peptone water) were sampled and investigated by electron microscopy. Carbon copper grids were covered with 10 µl of bacterial suspension and left at room temperature for 10 minutes. The preparations were fixed with 10% para-formaldehyde for 10 minutes and rinsed with distilled water before adding 5 µl of phospho-tungstic acid. Grids were then rinsed with distilled water, dried and then observed with a Phillips CM10 transmission electron microscope.

Phenotypic characterization was performed on all isolates. Growth was measured by spectrophotometer (BioPhotometer, Eppendorf, Le Pecq, France) using brain heart infusion (bioMérieux, Craponne, France), Buffered Peptone Water (bioMérieux, Marcy-l'Etoile, France) and tryptocasein soy agar. Salt tolerance was determined by spectrophotometer at 30°C in buffered peptone water (bioMérieux, Marcy-l'Etoile, France) containing 0-30% (w/v) NaCl. A medium buffered peptone water without NaCl, contained Bacto-peptone (Difco, New Jersey, USA) 20 g, distilled water 1 L, pH 7. Biochemical tests were performed by using the API 20E and API 50CH strips (bioMérieux).

Susceptibilities to a panel of 39 antibiotics including ampicillin, amoxicillin, amoxicillin + clavulanic acid, ticarcillin, ticarcillin + clavulanic acid, piperacillin, piperacillin + tazobactam, mecillinam, imipenem, ertapenem, aztreonam, cefalotin, cefuroxim, cefamandol, cefoxitin, cefotaxime, cefepime, cefixime, ceftazidime, gentamicin, tobramycin, kanamycin, netilmicin, amikacin, tetracycline, minocycline, tigecycline, azithromycin, colistin, sulfamide, trimethoprim, cotrimoxazole, nitrofurantoin, norfloxacin, pefloxacine, ciprofloxacin, nalidixic acid, fosfomycin and chloramphenicol, were determined by disk diffusion method on Mueller-Hinton agar (bioMérieux, Marcy-l'Etoile, France) by the Antimicrobial Agents Unit (Institut Pasteur, Paris, France) (Hombach *et al.,* 2014).

The guanine-plus-cytosine (G+C) content of the DNA of *Rouxiella chamberiensis* 130333^{T} was obtained from whole genome sequence.

Supplementary Fig. S2 shows an electron micrograph of strain 130333^{T}. Cells from brain heart infusion medium measured 0.5 to 0.7 μm wide and 1.8 to 2 μm long without flagella. The phenotypic features of the taxon under study are given in the species description. All isolates of *Rouxiella chamberiensis* were phenotypically identical. Tests useful to differentiating *Rouxiella* from closely related genera (*Ewingella, Rahnella, Yersinia, Serratia, Obesumbacterium* and *Hafnia)* are shown in table 1 (Grimont *et al.,* 1983 ; Grimont *et al.,* 2006; Brenner Don J. *et al.,* 2005). Results of API 20E and API 50CH strips showed that the six isolates shared the same phenotype. Identification attempts with API 20E yielded code 1205353 which translated as *Pantoea* sp. with 48.4% probability. The results obtained using the MALDI-TOF (bioMérieux, Marcy-l'Etoile, France) did not match with any record in the database (Brucker, Daltonics, Germany). Some characteristics were common to the genera listed in table 1 (Brenner *et al.,* 2005). All genera in Table 1 were ONPG test, D-glucose, D-mannitol, D-mannose, and catalase positive. All genera in Table 1 were arginine dihydrolase, H2S, L-tryptophan, α-methylglucoside, fermentation of erythritol and oxydase negative. All genera were resistant to the vibriostatic O/129.

Rouxiella is different from *Ewingella* by the lemon yellow colony colour, growth at 4°C, no growth at 37°C, not motile, nitrate reduced, D-lactose, D-trehalose, D-arabinose, D-cellobiose were negative tests and L-arabinose, D-xylose, inositol and D-melibiose were positive tests.

Rouxiella is different from *Rahnella* by the nitrate reduction, D-lactose, D-trehalose, D-cellobiose, maltose, raffinose, D-sorbitol, sucrose, D-adonitol, dulcitol and inositol fermentation.
Rouxiella is different from *Yersinia* by the lemon colony colour, no growth at 37°C, Simmons citrate, Voges-Proskauer reaction, D-sorbitol and inositol fermentation.
Rouxiella is different from *Serratia* by the lemon yellow colony colour, nitrate reduction, D-trehalose, maltose fermentation, lack of gelatin hydrolysis.
Rouxiella is different from *Obesumbacterium* by the lemon colony colour, no growth at 37°C, lack of lysine decarboxylase, ornithine decarboxylase, Simmons citrate utilization, nitrate not reduced, D-trehalose, L-arabinose, D-sorbitol, D-xylose, glycerol, inositol and D-melibiose fermentation.
Rouxiella is different from *Hafnia* by the lemon yellow colony colour, no growth at 37°C, no lysine decarboxylase and ornithine decarboxylase, nitrate not reduced, inositol and D-melibiose not fermented.

Strain 130333^{T} was susceptible to 39 antibiotics agents including ampicillin, amoxicillin, amoxicillin + clavulanic acid, ticarcillin, ticarcillin + clavulanic acid, piperacillin, piperacillin + tazobactam, mecillinam, imipenem, ertapenem, aztreonam, cefalotin, cefuroxim, cefamandol, cefoxitin, cefotaxime, cefepime, cefixime, ceftazidime, gentamicin, tobramycin, kanamycin, netilmicin, amikacin, tetracycline, minocycline, tigecycline, azithromycin, colistin, sulfamide, trimethoprim, cotrimoxazole, nitrofurantoin, norfloxacin, pefloxacine, ciprofloxacin, nalidixic acid, fosfomycin and chloramphenicol according to CASFM-EUCAST (Comité de l'Antibiogramme de la Société Française de Microbiologie, European Committee on Antimicrobial Susceptibility Testing, 2013).

The isolates under study constitute a new bacterial taxon that could not be assigned to any known genus.
Based on sequence comparisons and phenotypic characterization, the novel genus *Rouxiella* gen. nov. with a single species *Rouxiella chamberiensis* sp. nov. is proposed, with 130333^{T} as type strain.

Description of *Rouxiella* gen. nov.
Rouxiella (Roux.i.el'la. N.L. fem. dim. n. *Rouxiella*, named after Pierre Paul Emile Roux, French physician, bacteriologist and immunologist who was one of the closest collaborator of Louis Pasteur and co-founder of the Institut Pasteur).

Straight rods, 0.5-0.7 μm wide and 1.8-2 μm long. Non-encapsulated. Non-spore-forming. Non motile. Non-hemolytic. Lemon yellow colony colour. Gram-negative. Growth is facultatively anaerobic and occurs at 4-30°C. No growth at 37°C (21-day). Growth occurs with 0-7% NaCl (optimum, 0.5% NaCl). Oxidase negative. Glucose fermented, Voges-Proskauer and Simmons citrate tests positive. Nitrate not reduced to nitrite, H2S and indol not produced. Gelatin and urea not hydrolysed. Arginine dihydrolase, lysine and ornithine decarboxylase tests negative. Esculin hydrolysed. In API50 CH strips, after 48h, acid is produced from L-arabinose, D-glucose, *myo*-inositol, D-mannitol, D-mannose, D-melibiose, L-rhamnose, salicin, D-xylose. Acid not produced from D-arabitol, D-cellobiose, D-lactose, maltose, α-methyl-D-glucoside, D-raffinose, D-sorbitol, sucrose, D-trehalose, glycerol, D-adonitol and dulcitol. The G+C content of DNA from the type strain of the type species is 53 mol%.

The genus belongs to the family *Enterobacteriaceae*. The type species is *Rouxiella chamberiensis.*

Description of *Rouxiella chamberiensis* sp. nov.

Rouxiella chamberiensis (cham.be.ri.en'sis. N.L. fem. adj. *chamberiensis* of, or belonging to Chambéry, referring to the city of isolation).

Grows in tryptocasein soy agar (or broth) at temperatures between 4 (3-day) and 30°C (1-day). Optimum temperature for growth is 30°C. Colonies on tryptocasein soy agar after 24h incubation are circular, 0.5 to 1.0 mm in diameter and 1 mm or above in 48h, smooth, convex, and lemon yellow. Grows on Drigalski and McConkey agar as lactose negative colonies. Grows at 30°C in peptone water containing 0 to 7% NaCl. Optimum growth occurs with 0.5% NaCl. Facultatively anaerobic. Non-hemolytic in tryptocasein soy agar supplemented with 5% horse blood. Oxidase test negative, catalase positive. Nitrate not reduced to nitrite. L-tryptophane not deaminated. o-Nitrophenyl-β-D-galactoside hydrolyzed. Gas was not produced from Meat Liver agar (Bio-Rad, Marnes-la-Coquette, France). Susceptible to the following antimicrobial agents: ampicillin, amoxicillin, amoxicillin + clavulanic acid, ticarcillin, ticarcillin + clavulanic acid, piperacillin, piperacillin + tazobactam, mecillinam, imipenem, ertapenem, aztreonam, cefalotin, cefuroxim, cefamandol, cefoxitin, cefotaxime, cefepime, cefixime, ceftazidime, gentamicin, tobramycin, kanamycin, netilmicin, amikacin, tetracycline, minocycline, tigecycline, azithromycin, colistin, sulfamide, trimethoprim, cotrimoxazole, nitrofurantoin, norfloxacin, pefloxacine, ciprofloxacin, nalidixic acid, fosfomycin and chloramphenicol.

The DNA G+C content of the type-strain is 53 mol%. The type-strain is 130333^T (= CIP 110714^T = DSM 28324^T). Contaminant of parenteral nutrition bags.

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Figures :

Fig.1. Neighbour-joining unrooted tree based on *rrs* gene sequences. Bootstrap values >75% (based on 1000 replicates) are indicated by thick lines. GenBank accession numbers are given in parentheses. The distance scale indicates the proportion of substitutions per nucleotide position.

Fig.2. Neighbour-joining unrooted tree based on MLSA including 5 genes (*fusA, pyrG, rplB, rpoB, sucA*). Bootstrap values >75% (based on 1000 replicates) are indicated by thick lines. GenBank accession numbers are given in parentheses. The distance scale indicates the proportion of substitutions per nucleotide position.

Tables

Table 1. Tests of value in differentiating *Rouxiella* from closest genera.

Supplementary data:

Fig. S1. Neighbour-joining tree based on *rpoB* gene sequences. Bootstrap values >50% (based on 1000 replicates) are given at branching points. GenBank accession numbers are given in parentheses. The distance scale indicates the proportion of substitutions per nucleotide position.

Fig. S2. Transmission electron microscopy of *Rouxiella chamberiensis* 130333T. Negative stain. Bar = 0.5 μm.

Table S1. Square table with rrs distances (proportions of nucleotide substitution).
Numerals given in the first line correspond to species listed in the first column.

Table S2. Square table with MLSA distances (proportions of nucleotide substitution).
Numerals given in the first line correspond to species listed in the first column.

410 **Table 1.** Tests of value in differentiating *Rouxiella* from closest genera.

411 Genera : 1, *Rouxiella chamberiensis* gen. nov., sp. nov. (n=6) ; 2, *Ewingella ;* 3, *Rahnella ;*
412 4, *Yersinia ;* 5, *Serratia ;* 6, *Obesumbacterium ;* 7, *Hafnia.*
413 Data were taken from the following sources: taxa 1 (6 strains, this study);
414 2 (Grimont *et al.*, 1983) ; 3, 4, 6, 7 (Brenner and Farmer, 2005);
415 5 (Grimont and Grimont, 2006).
416 Symbols : +, 90-100% strains positive after 2-day (carbon sources) or 1-day incubation
417 (other tests) : d, 11-89% strains positive after 2-day (carbon sources) or 1-day incubation
418 (other tests) : -, less than 9% positive.
419

Characteristic	***1***	***2***	***3***	***4***	***5***	***6***	***7***
Colony colour	Lemon yellow	No pigment	yellow	No pigment	pink, red or no pigment	No pigment	No pigment
Growth at 37°C	-	+	+	+	d	+	+
Growth at 30°C	+	+	+	+	+	+	+
Growth at 4°C	+	-	+	d	d	+	+
Mobility	-	+	d	- 37°C, + 25°C	d	-	d 37°C, + 22°C
Lysine decarboxylase	-	-	-	-	d	+	+
Ornithine decarboxylase	-	-	-	d	d	+	+
Citrate (Simmons)	+	+	+	-	+	-	d
Urease	-	-	-	d	d	-	-
Indol	-	-	-	d	d	-	-
Voges-Proskauer reaction	+	+	+	- 37°C	d	+	d 37°C, + 22°C
Nitrate reduced	-	+	+	d	+	+	+
Esculin hydrolysis	+	+	+	d	d	+	d
Acid from:							
D-Lactose	-	+	+	d	d	-	-
D-Trehalose	-	+	+	d	+	+	d
L-Arabinose	+	-	+	+	d	-	d
D-Arabitol	-	+	-	d	d	-	-
D-cellobiose	-	+	+	d	d	-	d
Maltose	-	-	+	d	+	-	d
Raffinose	-	-	+	d	d	-	-
L-Rhamnose	+	d	+	d	d	+	d
Salicin	+	+	+	d	+	+	d
D-Sorbitol	-	-	+	+	d	+	-
Sucrose	-	-	+	d	d	-	d
D-Xylose	+	-	+	d	d	-	+
Glycerol	-	d	d	d	d	+	d
D-Adonitol	-	-	+	d	d	-	-
Dulcitol	-	-	+	-	d	-	-
Inositol	+	-	-	-	d	-	-
D-Melibiose	+	-	+	d	d	-	-
Gelatinase	-	-	-	-	d	-	-

420

Figure
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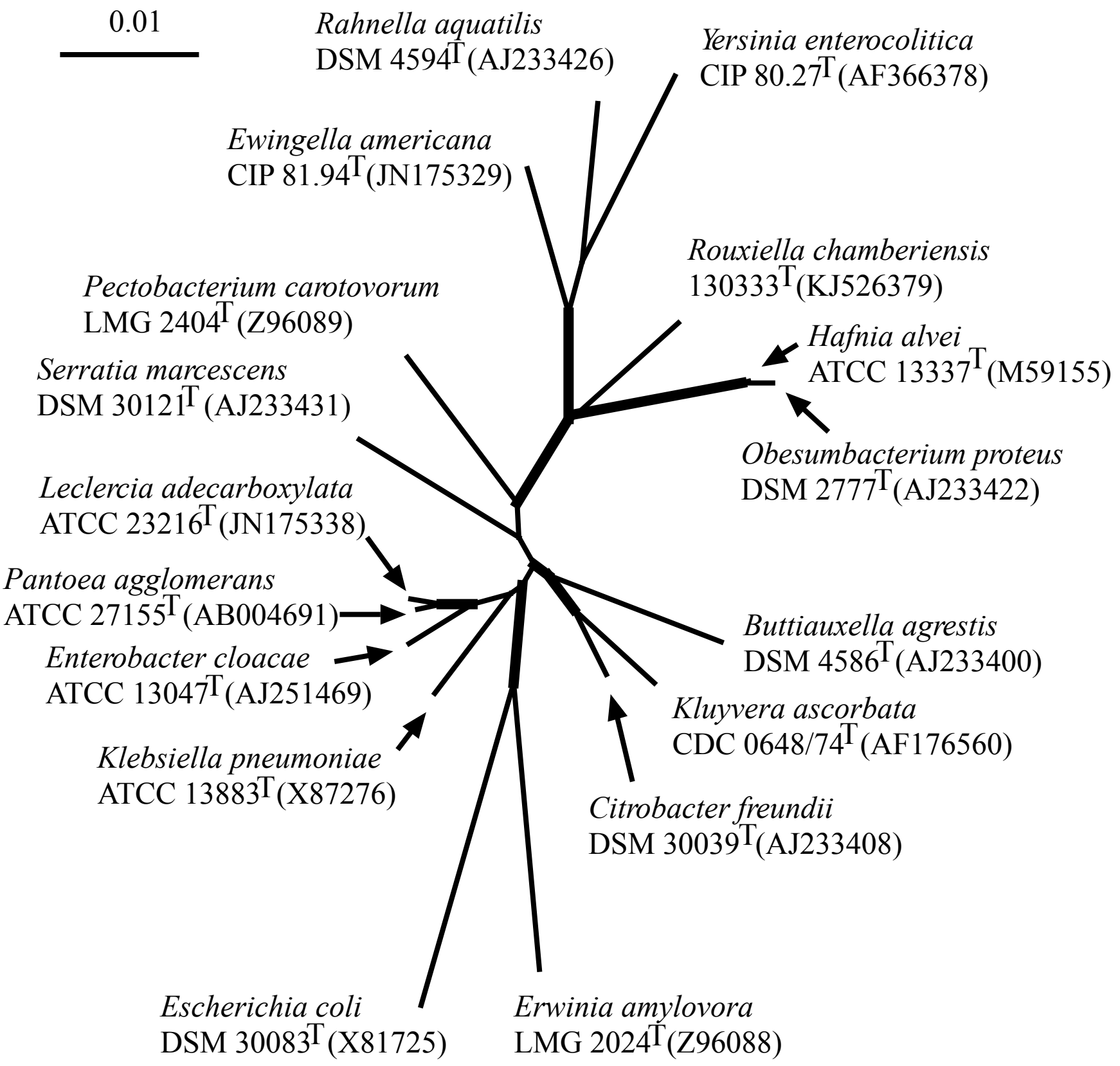

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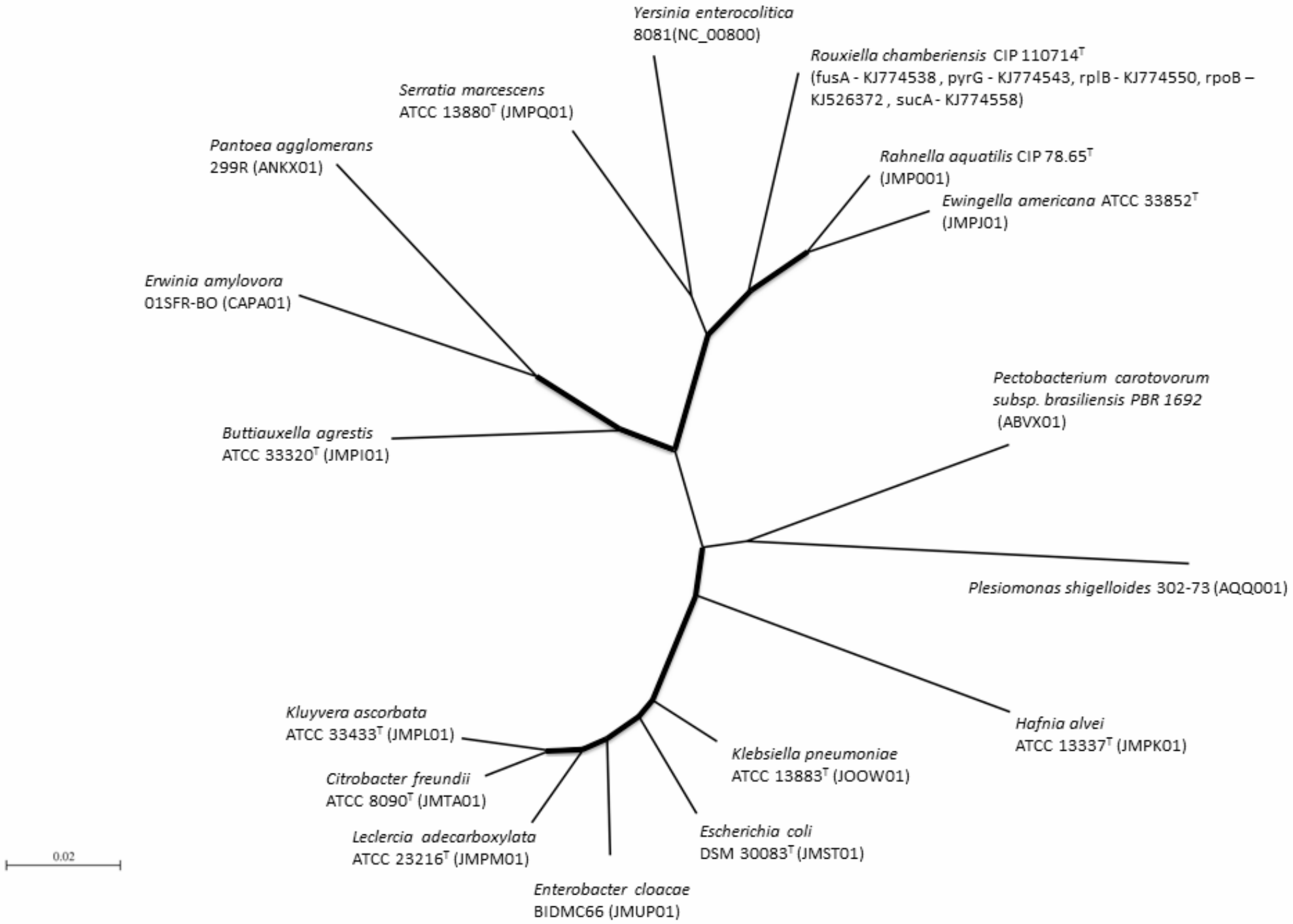

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