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Anne Le Fleche-Mateos, Marion Levast, Fabienne Lomprez, Yolande Arnoux, Clément Andonian, Michel Perraud, Véronique Vincent, Meriadeg Ar Gouilh, Jean-Michel Thiberge, Mathias Vandenbogaert, et al.

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**International Journal of Systematic and Evolutionary Microbiology**  
**Rouxiella chamberiensis gen. nov., sp. nov., a new Enterobacteriaceae isolated from**  
**parenteral nutrition bags**  
 --Manuscript Draft--

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<b>Abstract:</b>	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%.

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

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12 Running title: *Rouxiella chamberiensis* gen.nov., sp. nov.

13 Contents Category: New Taxa – *Proteobacteria* – *Enterobacteriaceae*.

14 The GenBank/EMBL accession numbers for the *rrs* gene sequences of strain 130333<sup>T</sup> is  
15 KJ526379. The accession numbers for the *rpoB* gene sequence of strain 130333<sup>T</sup> and isolates  
16 140001, 140002, 140003, 140004 and 140005 as determined in this study are KJ526372,  
17 KJ526373, KJ526374, KJ526375, KJ526376 and KJ526377. The GenBank/EMBL accession  
18 numbers for the sequences presented in this study are: KJ774531- KJ774537 (*fusA* gene),  
19 KJ774538- KJ774544 (*pyrG* gene), KJ774545- KJ774551 (*rplB* gene), KJ774553- KJ774559  
20 (*sucA* gene).

21

22 **Abstract**

23 Parenteral nutrition bags for newborns were found contaminated by a previously undescribed

24 *Enterobacteriaceae*. The six isolates studied by *rrs* - (encoding 16S rRNA) and multilocus

25 sequence analysis (MLSA) formed a discrete branch close to genera *Ewingella*, *Rahnella*,

26 *Yersinia*, *Hafnia* and *Serratia*. Phenotypically, the new taxon was distinct from these four

27 genera. The new taxon gave positive Voges-Proskauer, Simmons citrate, *o*-nitrophenyl- $\beta$ -

28 galactoside hydrolysis tests; fermented D-glucose, D-mannitol, L-rhamnose, D-melibiose, L-

29 arabinose, D-xylose, and hydrolyzed esculin; did not ferment maltose, trehalose, raffinose, D-

30 sorbitol, sucrose and D-cellobiose. The following tests, motility, gas production, urease,

31 gelatinase, and nitrate reduction were also negative. All isolates failed to grow at 37°C.

32 Therefore, the new taxon is proposed as a new species and genus and named *Rouxiella*

33 *chamberiensis* gen. nov., sp. nov. The type strain is 130333<sup>T</sup> (= CIP 110714<sup>T</sup> = DSM 28324<sup>T</sup>).

34 The G+C content of the type strain DNA was 53 mol%.

35

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

44  
45 Isolates 130333<sup>T</sup>, 140001, 140002, 140003, 140004, and 140005 were recovered from six  
46 different parenteral nutrition bags in December 2013 in a Hospital located in Chambéry,  
47 France. The isolates were recovered on tryptocasein soy agar (TSA) (bioMérieux, Marcy-  
48 l'Etoile, France) at 30°C under aerobic conditions.

49  
50 Gene sequencing was used to determine the phylogenetic position of the isolates. For this,  
51 strains were cultured on tryptocasein soy agar at 30°C. Total DNA was prepared from  
52 bacterial cultures by using the Promega Genomic DNA purification kit (Promega, Madison,  
53 WI, USA).

54  
55 For *rrs* gene (encoding 16S rRNA) sequencing, universal primers were used, 1.5 kb of the *rrs*  
56 gene were amplified by PCR (Janvier *et al.*, 1995). The amplified product was sequenced in  
57 our laboratory with three primers E, rE and D, which are located in conserved regions of the  
58 *E. coli rrs* gene. Primer E (5'-ATTAGATACCCTGGTAGTCC-3') corresponds to positions  
59 787-806, primer rE (5'-GGACTACCAGGGTATCTAAT-3') is complementary to primer E  
60 and primer D (5'-CAGCAGCCGCGGTAATAC-3') corresponds to positions 519-536  
61 (numbering according to Brosius *et al.*, 1978).

62  
63 It should be noted that the *rrs* gene is not always sufficient to distinguish closely related  
64 species, especially within *Enterobacteriaceae*. Multi-locus sequence analysis (MLSA) was  
65 based on partial sequences of the housekeeping genes *fusA* (634 bp), *pyrG* (307 bp), *rplB* (333  
66 bp), *rpoB* (968 bp) and *sucA* (634 bp) which were identified as best candidates for  
67 *Enterobacteriaceae* most-conserved genes. These genes are single-copy-number genes,  
68 essential and are present in many bacterial lineages. Therefore, they were expected to be  
69 present in all members of the *Enterobacteriaceae* (Deletoile *et al.*, 2009; Paauw *et al.*, 2008;  
70 Achtman *et al.*, 2012 ; Brady *et al.*, 2013). PCR amplification of *fusA*, *pyrG*, *rplB*, *rpoB* and

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

75

76 MLSA was performed with the above genes, for selected *Enterobacteriaceae* strains,  
77 including that of the new taxon (strains 130333<sup>T</sup>, 140001, 140002, 140003, 140004 and  
78 140005). Initial multiple sequence alignment was performed using ClustalW (Thompson *et*  
79 *al.*, 1994), providing as many character matrices. Those matrices were concatenated into a  
80 single character super-matrix. As assessment of the quality of multiple sequence alignment is  
81 important to ensure the accuracy of phylogenetic inference, an additional alignment character  
82 trimming step was carried out to select regions in the matrix that are suited for phylogenetic  
83 inference. Therefore, both sparse columns were excised (stretches of gaps/openings in one or  
84 more sequences) and compositional heterogeneity (ambiguously aligned regions) was  
85 minimized in the alignment matrix, returning a trimmed dataset that allows to reconstruct a  
86 more accurate phylogenetic tree than the initial alignment (Talavera *et al.*, 2007).

87

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

94

95 A whole genome shotgun sequencing experiment and assembly for our type strain 130333<sup>T</sup>  
96 was done using the Next Generation Sequencing (NGS) technique (Illumina MiSeq). Average  
97 nucleotide identities (ANI) (Konstantinidis and Tiedje, 2005a; Konstantinidis and Tiedje,  
98 2005b) were computed on whole genome sequences to measure the genetic and evolutionary  
99 relatedness among strains, and help to consolidate the existing taxonomic ranks of bacterial  
100 strains. Therefore, unequivocal evidence for taxonomic delineation at the species level was  
101 obtained by calculating the ANI of representative genome sequences. The ANI calculations  
102 were performed using the *in silico* DNA–DNA hybridization method (Konstantinidis &  
103 Tiedje, 2005a; Goris *et al.*, 2007) implemented in the JSPECIES software  
104 (<http://www.imedeo.uib-csic.es/jspecies/about.html> ; Richter & Rossello-Mora, 2009) with  
105 default blast parameters.

106

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

115

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

121

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

125

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

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

132

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

139

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

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

148

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

154

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

157

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

166

167 Phenotypic characterization was performed on all isolates. Growth was measured by  
168 spectrophotometer (BioPhotometer, Eppendorf, Le Pecq, France) using brain heart infusion  
169 (bioMérieux, Craaponne, France), Buffered Peptone Water (bioMérieux, Marcy-l'Etoile,  
170 France) and tryptocasein soy agar. Salt tolerance was determined by spectrophotometer at  
171 30°C in buffered peptone water (bioMérieux, Marcy-l'Etoile, France) containing 0-30% (w/v)  
172 NaCl. A medium buffered peptone water without NaCl, contained Bacto-peptone (Difco, New  
173 Jersey, USA) 20 g, distilled water 1 L, pH 7. Biochemical tests were performed by using the  
174 API 20E and API 50CH strips (bioMérieux).

175  
176 Susceptibilities to a panel of 39 antibiotics including ampicillin, amoxicillin, amoxicillin +  
177 clavulanic acid, ticarcillin, ticarcillin + clavulanic acid, piperacillin, piperacillin + tazobactam,  
178 mecillinam, imipenem, ertapenem, aztreonam, cefalotin, cefuroxim, cefamandol, ceftazidime,  
179 cefotaxime, cefepime, cefixime, ceftazidime, gentamicin, tobramycin, kanamycin, netilmicin,  
180 amikacin, tetracycline, minocycline, tigecycline, azithromycin, colistin, sulfamide,  
181 trimethoprim, cotrimoxazole, nitrofurantoin, norfloxacin, pefloxacin, ciprofloxacin, nalidixic  
182 acid, fosfomycin and chloramphenicol, were determined by disk diffusion method on  
183 Mueller-Hinton agar (bioMérieux, Marcy-l'Etoile, France) by the Antimicrobial Agents Unit  
184 (Institut Pasteur, Paris, France) (Hombach *et al.*, 2014).

185  
186 The guanine-plus-cytosine (G+C) content of the DNA of *Rouxiella chamberiensis* 130333<sup>T</sup>  
187 was obtained from whole genome sequence.

188  
189 Supplementary Fig. S2 shows an electron micrograph of strain 130333<sup>T</sup>. Cells from brain  
190 heart infusion medium measured 0.5 to 0.7 µm wide and 1.8 to 2 µm long without flagella.  
191 The phenotypic features of the taxon under study are given in the species description. All  
192 isolates of *Rouxiella chamberiensis* were phenotypically identical. Tests useful to  
193 differentiating *Rouxiella* from closely related genera (*Ewingella*, *Rahnella*, *Yersinia*, *Serratia*,  
194 *Obesumbacterium* and *Hafnia*) are shown in table 1 (Grimont *et al.*, 1983 ; Grimont *et al.*,  
195 2006; Brenner Don J. *et al.*, 2005). Results of API 20E and API 50CH strips showed that the  
196 six isolates shared the same phenotype. Identification attempts with API 20E yielded code  
197 1205353 which translated as *Pantoea* sp. with 48.4% probability. The results obtained using  
198 the MALDI-TOF (bioMérieux, Marcy-l'Etoile, France) did not match with any record in the  
199 database (Brucker, Daltonics, Germany). Some characteristics were common to the genera  
200 listed in table 1 (Brenner *et al.*, 2005). All genera in Table 1 were ONPG test, D-glucose, D-  
201 mannitol, D-mannose, and catalase positive. All genera in Table 1 were arginine dihydrolase,  
202 H<sub>2</sub>S, L-tryptophan, α-methylglucoside, fermentation of erythritol and oxydase negative. All  
203 genera were resistant to the vibriostatic O/129.  
204 *Rouxiella* is different from *Ewingella* by the lemon yellow colony colour, growth at 4°C, no  
205 growth at 37°C, not motile, nitrate reduced, D-lactose, D-trehalose, D-arabinose, D-cellobiose  
206 were negative tests and L-arabinose, D-xylose, inositol and D-melibiose were positive tests.



207 *Rouxiella* is different from *Rahnella* by the nitrate reduction, D-lactose, D-trehalose, D-  
208 cellobiose, maltose, raffinose, D-sorbitol, sucrose, D-adonitol, dulcitol and inositol  
209 fermentation.

210 *Rouxiella* is different from *Yersinia* by the lemon colony colour, no growth at 37°C, Simmons  
211 citrate, Voges-Proskauer reaction, D-sorbitol and inositol fermentation.

212 *Rouxiella* is different from *Serratia* by the lemon yellow colony colour, nitrate reduction, D-  
213 trehalose, maltose fermentation, lack of gelatin hydrolysis.

214 *Rouxiella* is different from *Obesumbacterium* by the lemon colony colour, no growth at 37°C,  
215 lack of lysine decarboxylase, ornithine decarboxylase, Simmons citrate utilization, nitrate not  
216 reduced, D-trehalose, L-arabinose, D-sorbitol, D-xylose, glycerol, inositol and D-melibiose  
217 fermentation.

218 *Rouxiella* is different from *Hafnia* by the lemon yellow colony colour, no growth at 37°C, no  
219 lysine decarboxylase and ornithine decarboxylase, nitrate not reduced, inositol and D-  
220 melibiose not fermented.

221

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

231

232 The isolates under study constitute a new bacterial taxon that could not be assigned to any  
233 known genus.

234 Based on sequence comparisons and phenotypic characterization, the novel genus *Rouxiella*  
235 gen. nov. with a single species *Rouxiella chamberiensis* sp. nov. is proposed, with 130333<sup>T</sup> as  
236 type strain.

237

238 **Description of *Rouxiella* gen. nov.**

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

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

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

256

257 **Description of *Rouxiella chamberiensis* sp. nov.**

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

260 Grows in tryptocasein soy agar (or broth) at temperatures between 4 (3-day) and 30°C (1-  
261 day). Optimum temperature for growth is 30°C. Colonies on tryptocasein soy agar after 24h  
262 incubation are circular, 0.5 to 1.0 mm in diameter and 1 mm or above in 48h, smooth, convex,  
263 and lemon yellow. Grows on Drigalski and McConkey agar as lactose negative colonies.

264 Grows at 30°C in peptone water containing 0 to 7% NaCl. Optimum growth occurs with 0.5%  
265 NaCl. Facultatively anaerobic. Non-hemolytic in tryptocasein soy agar supplemented with 5%  
266 horse blood. Oxidase test negative, catalase positive. Nitrate not reduced to nitrite. L-  
267 tryptophane not deaminated. o-Nitrophenyl- $\beta$ -D-galactoside hydrolyzed. Gas was not  
268 produced from Meat Liver agar (Bio-Rad, Marnes-la-Coquette, France). Susceptible to the  
269 following antimicrobial agents: ampicillin, amoxicillin, amoxicillin + clavulanic acid,  
270 ticarcillin, ticarcillin + clavulanic acid, piperacillin, piperacillin + tazobactam, mecillinam,  
271 imipenem, ertapenem, aztreonam, cefalotin, cefuroxim, cefamandol, cefoxitin, cefotaxime,  
272 cefepime, cefixime, ceftazidime, gentamicin, tobramycin, kanamycin, netilmicin, amikacin,  
273 tetracycline, minocycline, tigecycline, azithromycin, colistin, sulfamide, trimethoprim,  
274 cotrimoxazole, nitrofurantoin, norfloxacin, pefloxacin, ciprofloxacin, nalidixic acid,  
275 fosfomicin and chloramphenicol.

276

277 The DNA G+C content of the type-strain is 53 mol%. The type-strain is 130333<sup>T</sup> (= CIP  
278 110714<sup>T</sup> = DSM 28324<sup>T</sup>). Contaminant of parenteral nutrition bags.

279

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285

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296 *Lelliottia nimipressuralis* comb. nov. and *Lelliottia amnigena* comb. nov., respectively, *E.*  
297 *gergoviae* and *E. pyrinus* into *Pluralibacter* gen. nov. as *Pluralibacter gergoviae* comb. nov.  
298 and *Pluralibacter pyrinus* comb. nov., respectively, *E. cowanii*, *E. radicincitans*, *E. oryzae*  
299 and *E. arachidis* into *Kosakonia* gen. nov. as *Kosakonia cowanii* comb. nov., *Kosakonia*  
300 *radicincitans* comb. nov., *Kosakonia oryzae* comb. nov. and *Kosakonia arachidis* comb. nov.,  
301 respectively, and *E. turicensis*, *E. helveticus* and *E. pulveris* into *Cronobacter* as *Cronobacter*  
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378

379 **Figures :**

380

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

385

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

390

391 **Tables**

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

393

394 **Supplementary data:**

395

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

400

401 **Fig. S2.** Transmission electron microscopy of *Rouxiella chamberiensis* 130333T. Negative  
402 stain. Bar = 0.5  $\mu$ m.

403

404 **Table S1.** Square table with *rrs* distances (proportions of nucleotide substitution).

405 Numerals given in the first line correspond to species listed in the first column.

406

407 **Table S2.** Square table with MLSA distances (proportions of nucleotide substitution).

408 Numerals given in the first line correspond to species listed in the first column.

409

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

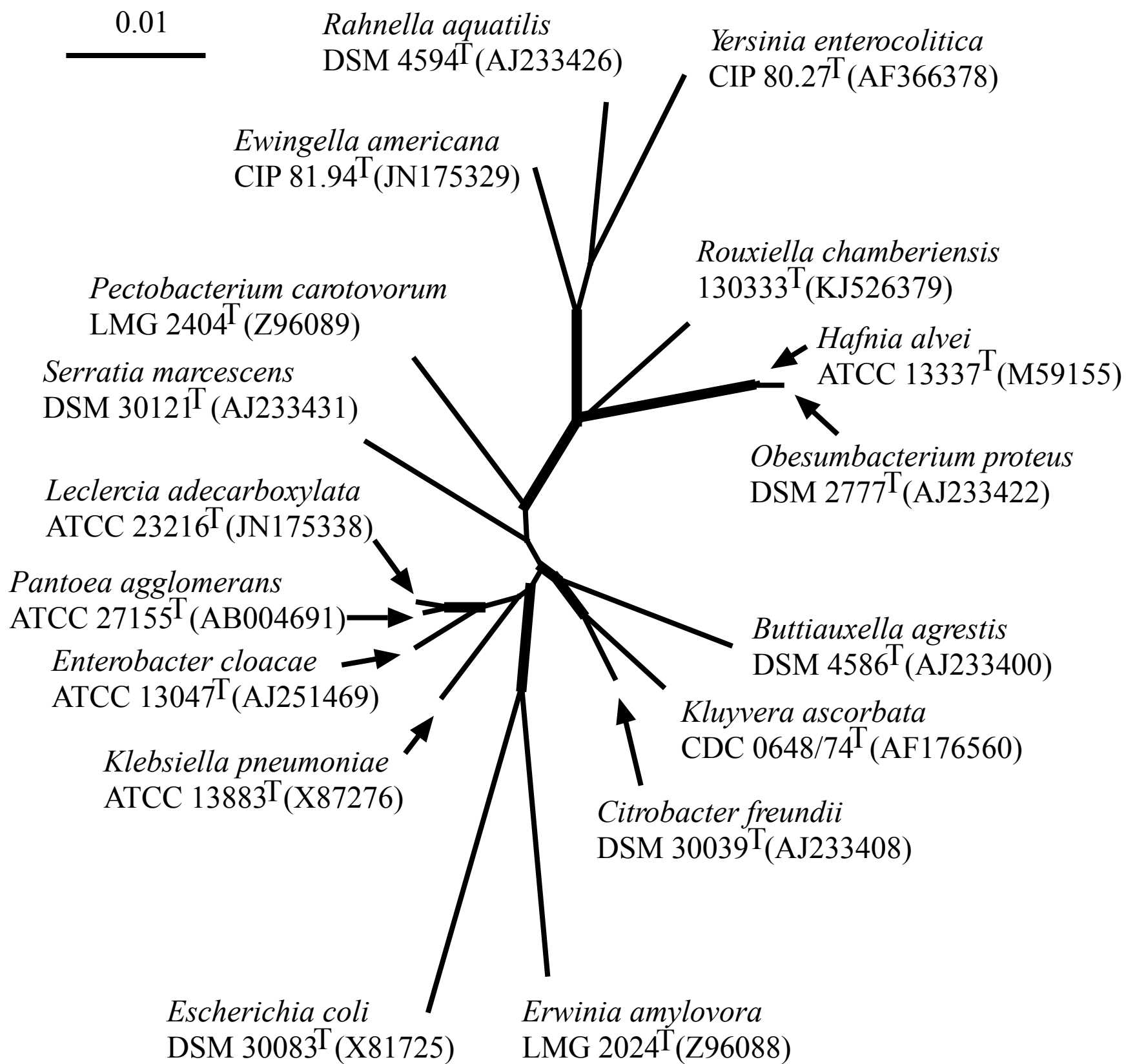
418 (other tests) : -, less than 9% positive.

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
<b>Acid from:</b>							
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	-	-

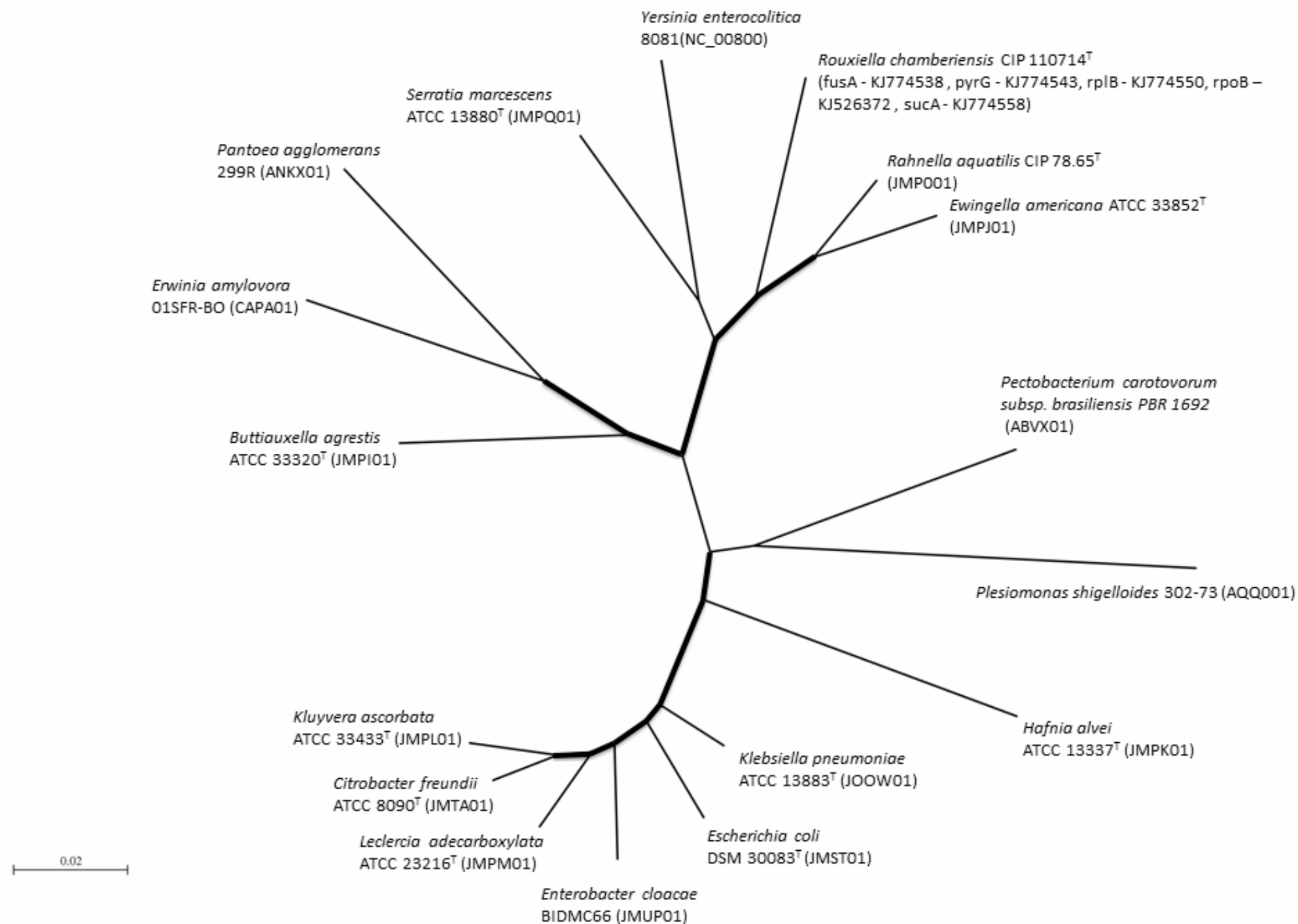






Figure

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