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Rufibacter quisquiliarum sp. nov., a new member of the phylum Bacteroidetes isolated
from a bioreactor treating landfill leachate
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Abstract:	A new bacterium, CAI-18bT, was isolated from a bioreactor that treated landfill leachate using an oligotrophic growth medium. Phylogenetic analysis based on the 16S rRNA gene revealed that strain CAI-18bT is a member of the genus Rufibacter, showing 97.1% pairwise similarity value to Rufibacter roseus H359T, 96.4% to Rufibacter tibetensis 1351T, 96.4% to Rufibacter glacialis MDT1-10-3T and 96.0% to Rufibacter immobilis MCC P1T. Strain CAI-18bT was rod-shaped, motile, oxidase and catalase positive. The predominant fatty acids were iso-C15:0 (24.1%) and iso-C17:1 I (22.3%), the major respiratory quinone was MK-7, and the predominant polar lipids were phosphatidylethanolamine and an unknown aminophospholipid. The G + C content of the genomic DNA of strain CAI-18bT was 50.7 mol%. The new bacterium can be distinguished from the related type strains based on its capability for the assimilation of N-acetylglucosamine and gentiobiose. On the basis of the phenotypic, chemotaxonomic and molecular data, strain CAI-18bT is considered to represent a new species, for which the name Rufibacter quisquiliarum sp. nov. is proposed. The type strain is CAI-18bT (=DSM 29854T=NCAIM B.02614T).

1 ***Rufibacter quisquiliarum* sp. nov., a new member of the phylum *Bacteroidetes* isolated from a**
2 **bioreactor treating landfill leachate**

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21
22 The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CAI-18b^T
23 is KM083132.

24

25 A new bacterium, CAI-18b^T, was isolated from a bioreactor that treated landfill leachate
26 using an oligotrophic growth medium. Phylogenetic analysis based on the 16S rRNA gene
27 revealed that strain CAI-18b^T is a member of the genus *Rufibacter*, showing 97.1% pairwise
28 similarity value to *Rufibacter roseus* H359^T, 96.4% to *Rufibacter tibetensis* 1351^T, 96.4% to
29 *Rufibacter glacialis* MDT1-10-3^T and 96.0% to *Rufibacter immobilis* MCC P1^T. Strain CAI-
30 18b^T was rod-shaped, motile, oxidase and catalase positive. The predominant fatty acids were
31 iso-C_{15:0} (24.1%) and iso-C_{17:1} I (22.3%), the major respiratory quinone was MK-7, and the
32 predominant polar lipids were phosphatidylethanolamine and an unknown
33 aminophospholipid. The G + C content of the genomic DNA of strain CAI-18b^T was 50.7
34 mol%. The new bacterium can be distinguished from the related type strains based on its
35 capability for the assimilation of N-acetylglucosamine and gentiobiose. On the basis of the
36 phenotypic, chemotaxonomic and molecular data, strain CAI-18b^T is considered to represent
37 a new species, for which the name *Rufibacter quisquiliarum* sp. nov. is proposed. The type
38 strain is CAI-18b^T (=DSM 29854^T=NCAIM B.02614^T).

39

40 Members of the phylum *Bacteroidetes* colonize various types of habitats, including saline and
41 freshwater, soil, compost, activated sludge, dairy products and gastrointestinal tract of animals
42 (Kirchman, 2002; McBride *et al.*, 2014; Thomas *et al.*, 2011). These diverse, Gram-negative, rod-
43 shaped bacteria are also known as degraders of high molecular weight organic matter, such as
44 proteins and polysaccharides (Kirchman, 2002). The number of species within this phylum has been
45 doubled between 2009 and 2014 (Munoz *et al.*, 2016), while the increase in the prokaryotic species
46 names validly published was only 30% in the same period (Parte, 2014), which indicates that strains
47 belonging to *Bacteroidetes* gained relatively increased interest in the last few years.

48

49 A recent cultivation-based analysis of various aquatic habitats in Romania using oligotrophic media
50 resulted in the isolation of bacterial strains representing potentially new species (Felföldi *et al.*,
51 2015). One of the new isolates, strain CAI-18b^T, showed low pairwise similarity values of partial
52 16S rRNA gene sequences to members of the genera *Nibribacter* and *Rufibacter*, which represent a
53 new branch within the family *Cytophagaceae* (order *Cytophagales*, phylum *Bacteroidetes*). Both
54 genera were described in the last few years and contained only a single species. *Nibribacter*
55 *koreensis* was isolated from estuarine water (Kang *et al.*, 2013), while *Rufibacter tibetensis* was
56 isolated from soil (Abaydulla *et al.*, 2012). However, very recently, three additional *Rufibacter*
57 species have been described, *Rufibacter immobilis* from a saline lake (Polkade *et al.*, 2015),
58 *Rufibacter roseus* from radiation-polluted soil (Zhang *et al.*, 2015) and *Rufibacter glacialis* from
59 glacier soil (Liu *et al.*, 2016). This study is aimed the polyphasic characterization of the *Rufibacter*-
60 related bacterial strain CAI-18b^T. Based on the obtained results, this strain is supposed to represent
61 a novel species of the genus *Rufibacter* for which the name *Rufibacter quisquiliarum* sp. nov. is
62 proposed.

63

64 Strain CAI-18b^T was isolated from a bioreactor, which treated the leachate of a landfill site located
65 in Odorheiu Secuiesc (Harghita County, Transylvania, Romania). For isolation, a diluted R2A-

66 based medium was used, which contained 360 mL R2A medium (DSMZ medium 830,
67 www.dsmz.de), 1.33 g CaCl₂ and 1.81 g NH₄Cl in 1 l final volume (pH 8.0) and was solidified with
68 20 g l⁻¹ agar. The standard dilution plating technique was applied to obtain isolates from the
69 samples with incubation at room temperature (20-22 °C). Subsequently, strain CAI-18b^T was
70 maintained on normal R2 agar medium (pH 8.5-8.8) at 28 °C. The type strains for side-by-side
71 analyses, *Rufibacter tibetensis* CCTCC AB 208084^T and *Nibrubacter koreensis* JCM 17917^T, were
72 maintained on the same medium and at the same temperature. Temperature, pH and salt
73 concentration optima were determined at 4, 10, 20, 25, 30, 37 and 45 °C, at pH from 4 to 11 (with
74 intervals of 1) and with NaCl concentration from 0 to 5% (w/v, with intervals of 1%), respectively,
75 as described previously by Felföldi *et al.* (2014).

76

77 Motility of strain CAI-18b^T was studied by native preparation, cell morphology was observed after
78 Gram staining according to Claus (1992). Oxidase activity was examined as given by Tarrand &
79 Gröschel (1982), while catalase reaction was checked as described by Barrow & Feltham (2004).
80 Caseinase and phosphatase activities were determined as described by Smibert & Krieg (1994).
81 Acid production from D-glucose was checked by the oxidative and fermentative test according to
82 Hugh & Leifson (1953). Additional metabolic tests were performed with API 50 CH, API 20 NE
83 and API ZYM (bioMérieux) systems following the instructions given by the manufacturer.
84 Susceptibility of the strains to antibiotics was studied on R2A plates using antibiotic-containing
85 discs (Bio-Rad) after 3 days of incubation at 28 °C. Growth under anaerobic condition was
86 examined using agar slant cultures on R2A medium incubated for one week in an anaerobic
87 chamber (Forma Scientific) at room temperature.

88

89 Analyses of isoprenoid quinones, cellular fatty acids, polar lipids and the determination of DNA
90 base composition were performed as given in Felföldi *et al.* (2011).

91

92 The 16S rRNA gene sequence of strain CAI-18b^T was amplified as described previously (Máthé *et*
93 *al.*, 2014). Purification and sequencing of PCR products were carried out by the LGC Genomics Ltd
94 (Berlin, Germany). Sequence alignment with the closest related type strains and clones was
95 conducted with SINA (Pruesse *et al.*, 2012). Phylogenetic analysis (which included the search for
96 the best-fit models) was performed with the MEGA 6.0 software (Tamura *et al.*, 2013).

97

98 Sequencing the 16S rRNA gene of strain CAI-18b^T resulted in 1434 nucleotides. Based on this data,
99 the most closely related species (represented by type strains) were identified by the EzTaxon-e
100 server (Kim *et al.*, 2012). *Rufibacter roseus* H359^T (=CPCC 100615^T=KCTC 42217^T) shared
101 97.1%, *Rufibacter tibetensis* 1351^T (=CCTCC AB 208084^T=NRRL B-51285^T, type species of the
102 genus) 96.4%, *Rufibacter glacialis* MDT1-10-3^T (=CGMCC 1.9789^T=NBRC 109705^T) 96.4%
103 (since the 16S rRNA gene sequence of the type strain is currently not available in EzTaxon-e,
104 comparison was performed with Blast, Zhang *et al.*, 2000), *Rufibacter immobilis* MCC P1^T (=MCC
105 2268^T=CCTCC AB 2013351^T) 96.0% and *Nibribacter koreensis* GSR3061^T (=KACC
106 16450^T=JCM 17917^T) 94.4% pairwise similarity value based on the 16S rRNA gene, while
107 members of all other genera showed lower than 92.2% similarities to strain CAI-18b^T. The
108 phylogenetic analysis of the 16S rRNA gene (Fig. 1) also confirmed the distinct position of strain
109 CAI-18b^T within the order *Cytophagales* (phylum *Bacteroidetes*). Incorporating environmental
110 clones in the 16S rRNA gene similarity analysis (Supplementary Fig. S1, available in IJSEM
111 Online), it has been shown that this group of bacteria (i.e. members of the genera *Rufibacter* and
112 *Nibribacter*) is rather versatile, since they were detected from many different habitats, including
113 various aquatic and soil types, air samples, plant-, animal- and human-associated environments.

114

115 Cells of strain CAI-18b^T were Gram-negative, rod-shaped (Supplementary Fig. S2, available in
116 IJSEM Online), motile, aerobic and mesophilic with a characteristic heterotrophic metabolism
117 (Table 1). Based on the results of the phenotypic and biochemical investigations, strain CAI-18b^T

118 could be distinguished from the studied type strains, since the new strain was capable for the
119 assimilation of N-acetylglucosamine, D-lactose and gentiobiose in contrast to the negative results
120 observed with related type strains. Moreover, according to antibiotic sensitivity tests
121 (Supplementary Table S1, available in IJSEM Online; Kang *et al.*, 2013; Polkade *et al.*, 2015;
122 Zhang *et al.*, 2015), all studied related strains were susceptible to ampicillin, while strain CAI-18b^T
123 showed resistance to this antibiotic.

124
125 The major respiratory quinone of CAI-18b^T was menaquinone MK-7, which is the characteristic
126 respiratory quinone of the family *Cytophagaceae* (McBride *et al.*, 2014). The fatty acid pattern of
127 strain CAI-18b^T was dominated by iso-C_{15:0} (24.1%) and iso-C_{17:1} I (22.3%), and in lower amounts
128 C_{17:1}ω6c (6.9%), C_{16:1}ω7c (5.7%), anteiso-C_{15:0} (5.6%) and several other components have been
129 detected (Table 2). Comparing these data with related strains analyzed in this study (Table 2) and
130 with those obtained by others (Polkade *et al.*, 2015; Zhang *et al.*, 2015; Liu *et al.*, 2016), fatty acids
131 contributing >10% could be very variable within the genus *Rufibacter* even if the same or similar
132 cultivation conditions were applied. A major fatty acid of strain CAI-18b^T, iso-C_{15:0}, was present in
133 less than half of the amount found in *R. tibetensis* CCTCC AB 208084^T and *R. glacialis* MDT1-10-
134 3^T (9.8% and 8.9%, respectively), while some important fatty acids detected in the new strain were
135 completely missing in other *Rufibacter* species: e.g. C_{17:1}ω6c (6.9% in CAI-18b^T) was not present
136 in strain *R. roseus* H359^T (strain showing the highest 16S rRNA gene similarity to our novel
137 bacterium), and the fatty acid iso-C_{15:0} 2-OH (3.3% in CAI-18b^T) was not detected in *R. roseus*
138 H359^T, *R. tibetensis* CCTCC AB 208084^T and *R. glacialis* MDT1-10-3^T, while C_{15:0} (3.4% in CAI-
139 18b^T) was not detected in *R. roseus* H359^T, *R. glacialis* MDT1-10-3^T and *R. immobilis* MCC P1^T.

140
141 The polar lipid pattern of strain CAI-18b^T was complex with the dominance of
142 phosphatidylethanolamine and an unknown aminophospholipid, and additionally two other
143 unknown aminophospholipids, three unknown phospholipids and two unknown lipids were detected

144 as minor components (Supplementary Fig. S3, available in IJSEM Online). Similar polar lipid
145 compositions have been reported for other *Rufibacter* species (Kang *et al.*, 2013; Polkade *et al.*,
146 2015; Zhang *et al.*, 2015; Liu *et al.*, 2016).

147

148 The genomic G+C content value of strain CAI-18b^T is 50.7 mol% (HPLC), which falls within the
149 range reported for the type species and other members of the genus *Rufibacter* (43.9-52.6 mol%;
150 Abaydulla *et al.*, 2012; Polkade *et al.*, 2015; Zhang *et al.*, 2015; Liu *et al.*, 2016), but is higher by 7
151 mol% than this of *R. roseus* H359^T (43.9 mol%), the strain having the highest 16S rRNA gene
152 similarity to CAI-18b^T. According to Mesbah *et al.* (2011), the mol% G+C range within a species is
153 less than 3% and is not higher than 10% within a genus, therefore the genomic G+C content of
154 strain CAI-18b^T supported the view that the new strain belongs to the genus *Rufibacter*.

155

156 Based on the comparative data presented in this study, strain CAI-18b^T is considered to represent a
157 novel species within the genus *Rufibacter*, for which the name *Rufibacter quisquiliarum* sp. nov. is
158 proposed.

159

160

161 **Description of *Rufibacter quisquiliarum* sp. nov.**

162

163 *Rufibacter quisquiliarum* (quis.qui.li.a'rum. L. gen. fem. pl. n. *quisquiliarum* of waste, of rubbish).

164

165 Cells are short, rod-shaped (0.3-0.5 x 0.7-1.6 µm) and motile. Colonies on R2A agar medium (pH
166 8.8) are pinkish-red-colored, circular and raised with an average diameter of 2 mm. Growth occurs
167 at 4-45 °C (optimum, 20-37 °C), at pH 7-11 (optimum, pH 8-10) and 0-2% (w/v) NaCl
168 concentration. Positive for oxidase, catalase and caseinase activities. Negative for oxidative and
169 fermentative acid production from D-glucose, nitrate reduction, indole production, urease and

170 phosphatase enzyme activities. D-Galactose, D-glucose, D-mannose, N-acetylglucosamine,
171 esculine, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-trehalose, starch (amidon), glycogen
172 and gentiobiose are assimilated, while all other carbon sources in the API 50 CH and API 20NE
173 tests are not assimilated. According to API 20NE and API ZYM tests, positive for gelatinase,
174 alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase,
175 cystine arylamidase (weak), trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -
176 galactosidase, α -glucosidase, *N*-acetyl- β -glucosaminidase; and negative for arginine dihydrolase,
177 lipase (C14), α -chymotrypsin, β -galactosidase, β -glucuronidase, β -glucosidase, α -mannosidase, α -
178 fucosidase enzyme activities. The major fatty acids are iso-C15:0 (24.1%) and iso-C17:1 I (22.3%),
179 the predominant polar lipids are phosphatidylethanolamine and an unknown aminophospholipid.

180

181 The G + C content of the genomic DNA is 50.7 mol%.

182

183 The type strain is CAI-18b^T (=DSM 29854^T=NCAIM B.02614^T) which was isolated from a
184 bioreactor treating landfill leachate.

185

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192

193 REFERENCES

194

195 **Abaydulla, G., Luo, X., Shi, J., Peng, F., Liu, M., Wang, Y., Dai, J. & Fang, C. (2012).**

196 *Rufibacter tibetensis* gen. nov., sp. nov., a novel member of the family *Cytophagaceae*

197 isolated from soil. *Antonie van Leeuwenhoek* **101**, 725-731.

198 **Barrow, G. I. & Feltham, R. K. A. (2004).** *Cowan and Steel's Manual for the Identification of*

199 *Medical Bacteria*, 3rd edn. Cambridge: Cambridge University Press.

200 **Claus, M. (1992).** A standardised Gram staining procedure. *World J Microbiol Biotechnol* **8**, 451-

201 452.

202 **Felföldi, T., Kéki, Zs., Sipos, R., Márialigeti, K., Tindall, B. J., Schumann, P. & Tóth, E. M.**

203 **(2011).** *Ottowia pentelensis* sp. nov., a floc-forming betaproteobacterium isolated from an

204 activated sludge system treating coke plant effluent. *Int J Syst Evol Microbiol* **61**, 2146-

205 2150.

206 **Felföldi, T., Vengring, A., Kéki, Zs., Márialigeti, K., Schumann, P. & Tóth, E.M. (2014).**

207 *Eoetvoesia caeni* gen. nov., sp. nov., isolated from an activated sludge system treating coke

208 plant effluent. *Int J Syst Evol Microbiol* **64**, 1920-1925.

209 **Felföldi, T., Kovács, E., Fikó, D. R., Tankó, Gy., Szabó, A., Nagymáté, Zs., Szilveszter, Sz. &**

210 **Máthé, I. (2015).** Unconventional strategies for the cultivation of new bacterial strains from

211 aquatic environments. *Acta Microbiol Immunol Hung* **62 (suppl.)**, 150-151.

212 **Hugh, R. & Leifson, E. (1953).** The taxonomic significance of fermentative versus oxidative

213 metabolism of carbohydrates by Gram negative bacteria. *J Bacteriol* **66**, 24-26.

214 **Kang, J. Y., Chun, J. & Jahng, K. Y. (2013).** *Nibribacter koreensis* gen. nov., sp. nov., isolated

215 from estuarine water. *Int J Syst Evol Microbiol* **63**, 4663-4668.

216 **Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J.**

217 **H., Yi, H., Won, S. & Chun, J. (2012).** Introducing EzTaxon-e: a prokaryotic 16S rRNA

218 Gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol*

219 *Microbiol* **62**, 716-721.

220 **Kirchman, D. L. (2002).** The ecology of Cytophaga-Flavobacteria in aquatic environments. *FEMS*
221 *Microbiol Ecol* **39**, 91-100.

222 **Liu, Q., Liu, H-C., Zhang, J-L., Zhou, Y-G. & Xin, Y-H. (2016).** *Rufibacter glacialis* sp. nov., a
223 psychrotolerant bacterium isolated from glacier soil. *Int J Syst Evol Microbiol* **66**, 315-318.

224 **Máthé, I., Borsodi, A. K., Tóth, E. M., Felföldi, T., Jurecska, L., Krett, G., Kelemen, Zs.,**
225 **Elekes, E., Barkács, K. & Márialigeti, K. (2014).** Vertical physico-chemical gradients
226 with distinct microbial communities in the hypersaline and heliothermal Lake Ursu (Sovata,
227 Romania). *Extremophiles* **18**, 501-514.

228 **McBride, M. J., Liu, W., Lu, X., Zhu, Y. & Zhang, W. (2014).** The Family *Cytophagaceae*. In
229 *The Prokaryotes - Other Major Lineages of Bacteria and the Archaea*, pp. 577-593. Edited
230 by E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt & F. Thompson. Berlin: Springer.

231 **Mesbah, N. M., Whitman, W. B. & Mesbah, M. (2011).** Determination of the G+C content of
232 Prokaryotes. In *Methods in Microbiology, Volume 38, Taxonomy of Prokaryotes*, pp. 298-
233 324. Edited by F. Rainey & A. Oren. London, United Kingdom: Academic Press.

234 **Munoz, R., Rosselló-Móra, R. & Amann, R. (2016).** Revised phylogeny of *Bacteroidetes* and
235 proposal of sixteen new taxa and two new combinations including *Rhodothermaeota* phyl.
236 nov. *Syst Appl Microbiol* (in press)

237 **Parte, A. C. (2014).** LPSN - list of prokaryotic names with standing in nomenclature. *Nucl Acids*
238 *Res* **42**, D613-D616.

239 **Polkade, A. V., Ramana, V. V., Joshi, A., Pardesi, L. & Shouche, Y. S. (2015).** *Rufibacter*
240 *immobilis* sp. nov., isolated from a high-altitude saline lake. *Int J Syst Evol Microbiol* **65**,
241 1592-1597.

242 **Pruesse, E., Peplies, J. & Glöckner, F. O. (2012).** SINA: accurate high throughput multiple
243 sequence alignment of ribosomal RNA genes. *Bioinformatics* **28**, 1823-1829.

244 **Smibert, R. M. & Krieg, N. R. (1994).** Phenotypic characterization. In *Methods for General and*

245 *Molecular Bacteriology*, pp. 607-654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood
246 & N. R. Krieg. Washington, DC: American Society for Microbiology.

247 **Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013).** MEGA6: Molecular
248 Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* **30**, 2725-2729.

249 **Tarrand, J. J. & Gröschel, D. H. M. (1982).** Rapid, modified oxidase test for oxidase-variable
250 bacterial isolates. *J Clin Microbiol* **16**, 772-774.

251 **Thomas, F., Hehemann, J-H, Rebuffet, E., Czjzek, M. & Michel, G. (2011).** Environmental and
252 gut Bacteroidetes: The food connection. *Front Microbiol* **2**, 93.

253 **Zhang, Z., Schwartz, S., Wagner, L. & Miller, W. (2000).** A greedy algorithm for aligning DNA
254 sequences. *J Comput Biol* **7**, 203-214.

255 **Zhang, Z-D., Gu, M-Y., Zhu, J., Li, S-H., Zhang, L-J., Xie, Y-Q., Shi, Y-H., Wang, W. & Li,**
256 **W-J. (2015).** *Rufibacter roseus* sp. nov., isolated from radiation-polluted soil. *Int J Syst Evol*
257 *Microbiol* **65**, 1572-1577.

258

259 FIGURE LEGENDS

260

261

262 **Fig. 1.** Phylogenetic position of CAI-18b^T and related type strains based on the 16S rRNA gene.

263 Phylogenetic tree has been constructed based on 1340 nucleotide positions using the maximum

264 likelihood (ML) method with Kimura 2-parameter nucleotide substitution model. Bootstrap values

265 >50% for the ML (left) and neighbor-joining (right) methods are shown. Branches recovered with

266 both treeing methods are marked with black dots. GenBank accession numbers are given in

267 parentheses. Bar, 0.05 substitutions per nucleotide.

268

271 **Table 1.** Phenotypic and biochemical characteristics of CAI-18b^T and related type strains.

272 Strains: 1, CAI-18b^T (=DSM 29854^T); 2, *Rufibacter roseus* H359^T; 3, *R. tibetensis* CCTCC AB
 273 208084^T; 4, *Nibribacter koreensis* JCM 17917^T. All strains had rod-shaped cells, formed pinkish-
 274 red colonies. All strains were positive for oxidase, catalase, caseinase, alkaline phosphatase,
 275 esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, acid
 276 phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetyl- β -glucosaminidase activities; and
 277 positive for the assimilation of D-glucose, D-maltose, D-trehalose and amidon (starch). All strains
 278 were negative for nitrate reduction (both to nitrite and nitrogen gas), indole production, arginine
 279 dihydrolase and urease activities, for the assimilation of D-mannitol, capric acid, citrate,
 280 phenylacetic acid, glycerol, erythritol, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, β -
 281 methyl-D-xylopyranoside, D-fructose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-
 282 sorbitol, α -methyl-D-mannopyranoside, α -methyl-D-glucopyranoside, amygdalin, arbutin, salicin,
 283 D-melezitose, xylitol, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol,
 284 gluconate, 2-ketogluconate, 5-ketogluconate; and for β -glucuronidase, β -glucosidase, α -
 285 mannosidase, α -fucosidase enzyme activities. Symbols: +, present; -, absent; w, weak reaction. NT,
 286 not tested. Data are from the present study (unless otherwise indicated). API ZYM enzyme activity
 287 data is not available for strain *R. roseus* H359^T.

<i>Characteristic</i>	<i>1</i>	<i>2*</i>	<i>3</i>	<i>4</i>
Motility	+	+	+	-
Temperature range (optimum) (°C)	4-45 (20-37)	4-37 (30)	20-37 (25-30)	10-45 (20-25)
pH range (optimum)	7-11 (8-10)	6-9 (7)	7-11 (8-9)	6-11 (9-10)
NaCl concentration for growth (%)	0-2	0-4	0-2	0-2
Assimilation of (API 50 CH)				
D-Arabinose	-	+	-	-
D-Galactose	+	+	-	-
D-Fructose	-	+	-	-
D-Mannose	+	+	-	-
N-Acetylglucosamine	+	-	-	-

<i>Characteristic</i>	<i>1</i>	<i>2*</i>	<i>3</i>	<i>4</i>
Esculin	+	+	+	-
D-Cellobiose	+	+	+	-
D-Lactose	+	-	-	-
D-Melibiose	+	+	-	-
D-saccharose (sucrose)	-	+	-	-
Inulin	-	+	-	-
D-Raffinose	-	+	-	-
Glycogen	+	+	-	-
Gentiobiose	+	-	-	-
Glucose fermentation (API 20 NE)	-	+	-	-
Gelatine hydrolysis (API 20 NE)	+	-	+	+
Assimilation of (API 20 NE)				
L-Arabinose	-	-	+	-
Adipic acid	-	-	-	+
Malic acid	-	-	-	+
Enzyme activity (API ZYM)				
Lipase (C14)	-	NT	-	+
Cystine arylamidase	w	NT	+	+
α -Chymotrypsine	-	NT	+	-
α -Galactosidase	+	NT	+	-
β -Galactosidase	-	NT	+	-
β -Glucosidase	+	NT	+	-
DNA G + C content (mol%)	50.7	43.9	46.8 [†]	44.9 [‡]

288 *All data from Zhang *et al.* (2015).

289 [†]Data from Abaydulla *et al.* (2012).

290 [‡]Data from Kang *et al.* (2013).

291

292 **Table 2.** Major fatty acids of CAI-18b^T and related type strains.

293 Strains: 1, CAI-18b^T (=DSM 29854^T); 2, *Rufibacter roseus* H359^T; 3, *R. tibetensis* CCTCC AB
 294 208084^T; 4, *Nibribacter koreensis* JCM 17917^T; -, not detected; TR, <1.0%. Data are from the
 295 present study, except fatty acid data of *R. roseus* H359^T. All strains were grown on R2A medium
 296 (pH 8.0) for 3 days at 25 °C, except *R. roseus* H359^T, which was grown on TSA medium for 3 days.

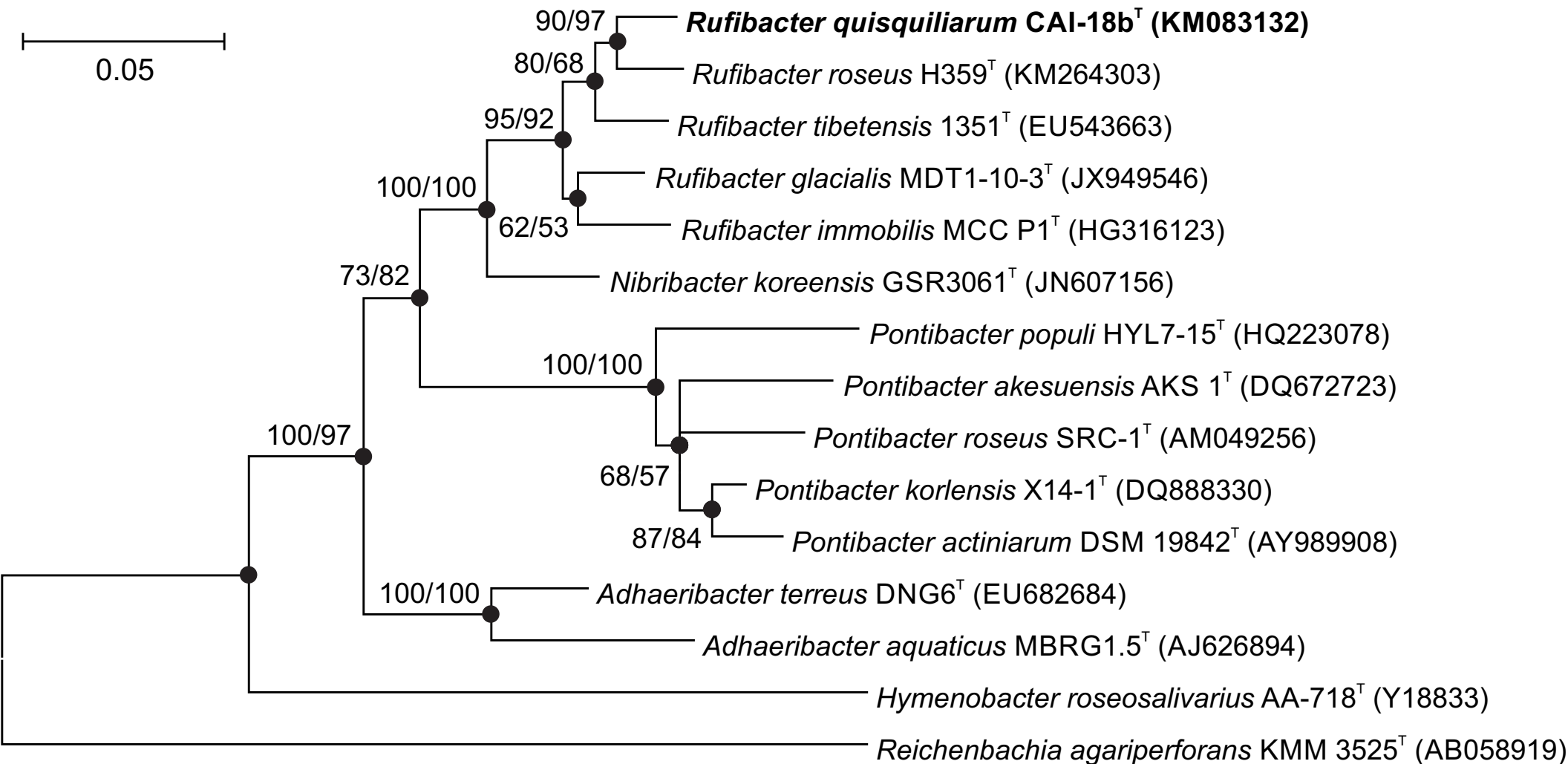
<i>Fatty acid</i>	<i>1</i>	<i>2*</i>	<i>3</i>	<i>4</i>
iso-C _{15:0}	24.1	30.5	9.8	22.9
iso-C _{17:1} I	22.3	32.9‡	16.1	24.8
C _{17:1} ω6c	6.9	-	13.9	6.1
C _{16:1} ω7c	5.7	6.3‡	8.3§	1.7
anteiso-C _{15:0}	5.6	4.5	6.2	4.0
C _{15:0}	3.4	-	9.8	2.1
iso-C _{16:1} H	3.4	-	1.8	3.8
iso-C _{15:0} 2-OH	3.3	-	-§	1.1
C _{15:1} ω6c	3.2	-	6.1	2.1
iso-C _{16:0}	3.1	-	3.0	4.6
iso-C _{17:0} 3-OH	3.1	3.8	2.4	3.8
C _{16:1} ω5c	2.9	5.8	4.3	3.1
iso-C _{15:0} 3-OH	2.5	2.3	1.8	2.5
Summed feature 1†	1.7	2.4	1.3	2.6
iso-C _{14:0}	1.7	-	TR	1.7
iso-C _{16:0} 3-OH	1.5	-	1.7	1.4
iso-C _{15:1} G	0.6	-	1.9	5.4
C _{16:0}	TR	2.1	1.1	TR
C _{17:1} ω8c	TR	-	2.6	TR

297 *Data from Zhang *et al.* (2015).

298 †Summed features represent two or three fatty acids that cannot be differentiated using the MIDI system. Summed
 299 feature 1: iso-C_{15:1} I/H and/or C_{13:0} 3-OH.

300 ‡These two fatty acids were detected as summed feature components (Summed feature 3, C_{16:1}ω7c/C_{16:1}ω6c and
 301 Summed feature 4, iso-C_{17:1} I/anteiso-C_{17:1} B, respectively) in the case of this strain.

302 §These two components were detected as Summed feature 3 in the case of this strain.



Supplementary material for

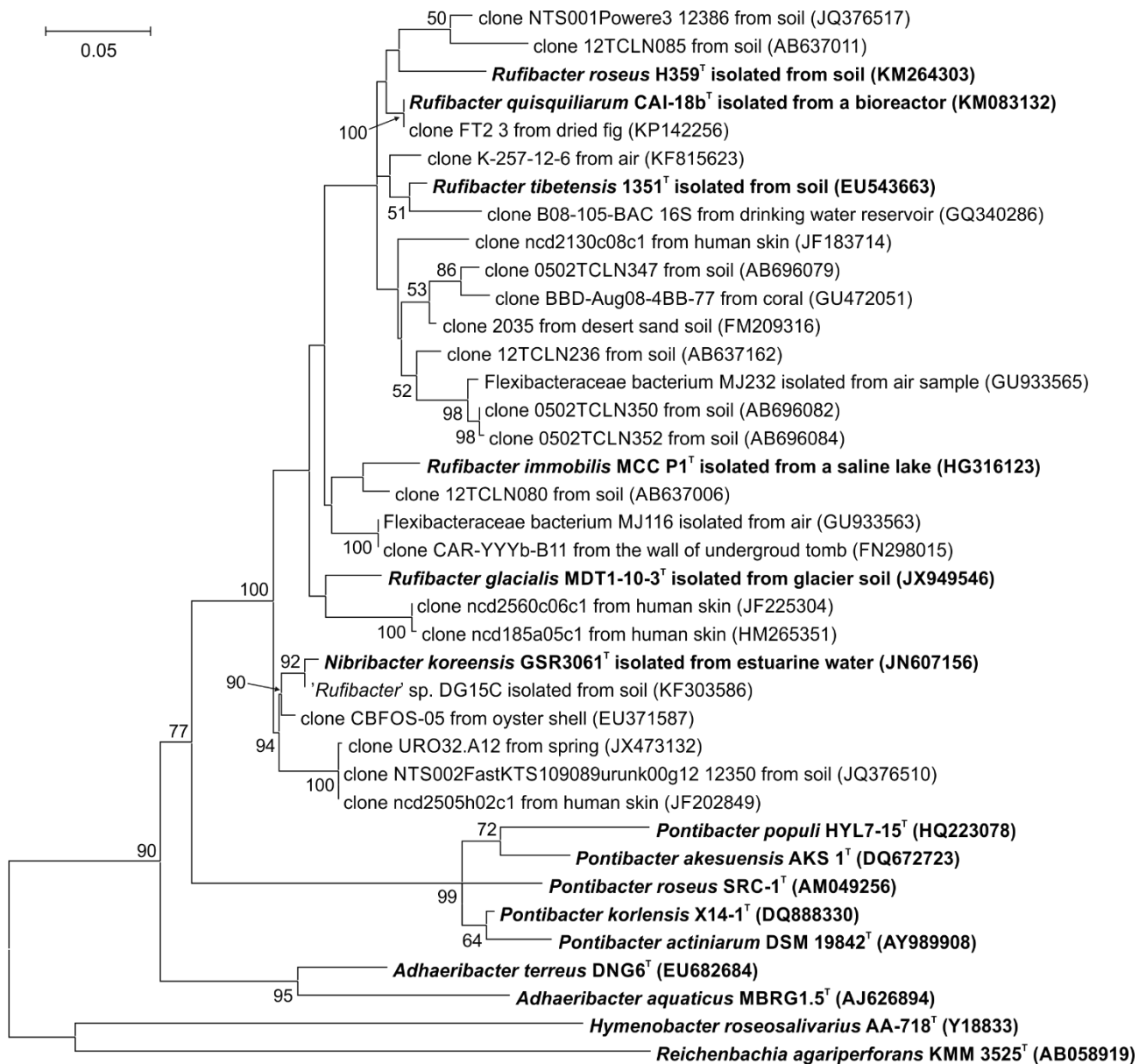
Rufibacter quisquiliarum sp. nov., a new member of the phylum Bacteroidetes isolated from a bioreactor treating landfill leachate

Tamás Felföldi, Anikó Mentés, Peter Schumann, Zsuzsa Kéki, István Máthé, Károly Márialigeti, Erika M. Tóth

Supplementary Table S1. Antibiotic resistance of CAI-18b^T and related type strains.

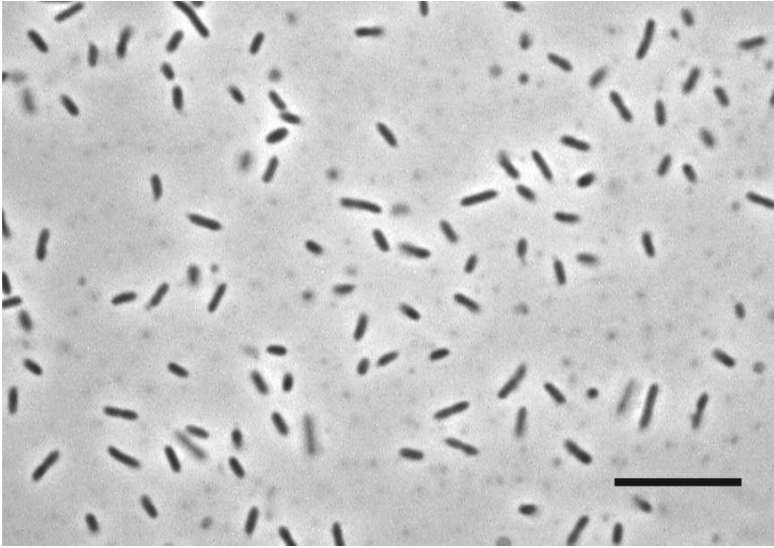
Strains: 1, CAI-18b^T (=DSM 29854^T); 2, *Rufibacter tibetensis* CCTCC AB 208084^T; 3, *Nibrubacter koreensis* JCM 17917^T. Size of inhibition zone is given in cm. Data are from the present study. All strains were grown on R2A medium (pH 8.8) for 3 days at 28 °C.

<i>Antibiotic</i>	<i>Disk Content</i>	<i>1</i>	<i>2</i>	<i>3</i>
Amoxicillin + Clavulanic acid	20 + 10 µg	0.1	1.6	0.7
Ampicillin	10 µg	0.3	1.7	1.7
Cefoxitin	30 µg	1	2.6	1.8
Cefuroxime	30 µg	0.2	0.4	0.8
Clindamycin	2 µg	1.3	2.2	1.6
Erythromycin	15 µg	0.8	1.8	1.5
Gentamycin	120 µg	0.4	0.6	0.7
Gentamycin	10 µg	0	0.2	0.2
Imipenem	10 µg	3.5	3.8	3
Meropenem	10 µg	1.5	2	2
Neomycin	30 UI	0.1	0.2	0.2
Netilmicin	30 µg	0.2	0.3	0.3
Piperacillin + Tazobactam	100 + 10 µg	1.2	2.8	1.8
Polymyxin B	300 UI	0	0.1	0.1
Vancomycin	30 µg	1.3	1.2	0.5

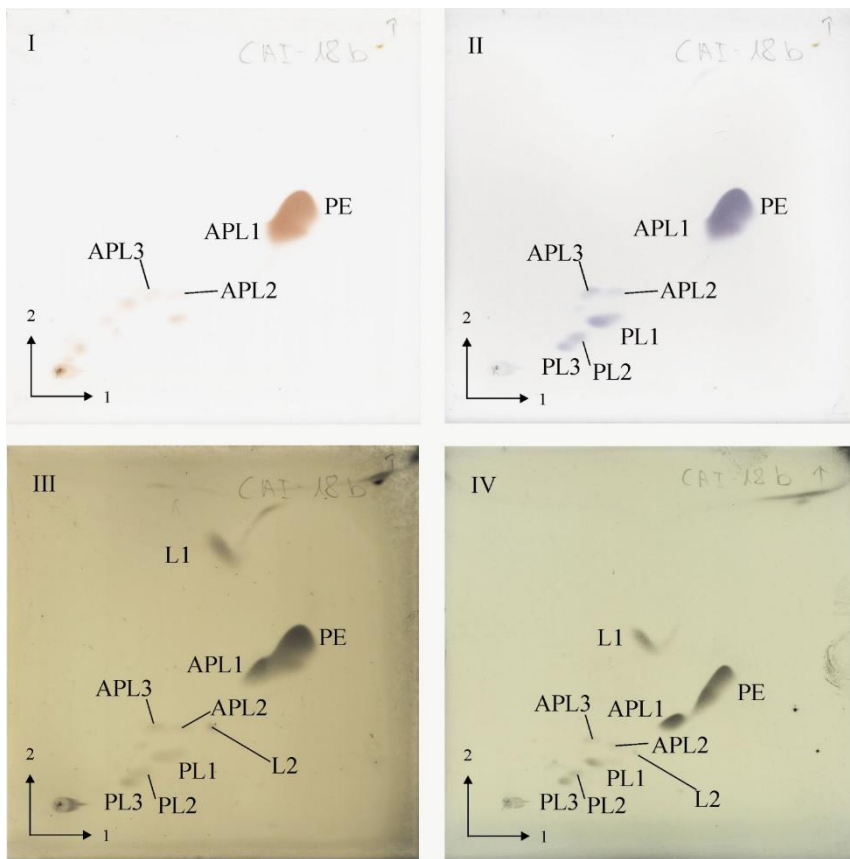


Supplementary Fig. S1. Phylogenetic distribution of *Rufibacter*- and *Nibribacter*-related environmental 16S rRNA gene clone sequences.

Phylogenetic tree has been constructed based on 600 nucleotide positions using the maximum likelihood (ML) method with Kimura 2-parameter nucleotide substitution model. Bootstrap values >50% are shown. Type strains (according to Fig. 1.) are highlighted with bold letters. GenBank accession numbers are given in parentheses. Bar, 0.05 substitutions per nucleotide.



Supplementary Fig. S2. Phase-contrast micrograph from cells of strain CAI-18b^T. Native preparation, after 3 days of incubation on R2A agar. Bar, 5 μ m.



Supplementary Fig. S3. Polar lipid profile of strain CAI-18b^T.

Two-dimensional TLC of polar lipids after spraying with ninhydrin and heating at 100 °C for 10 minutes (I, aminolipids), after spraying with molybdenum blue (II, phospholipids), after spraying with molybdenum blue (Sigma) and subsequent heating at 200 °C for 15 min (III, total lipids) and after spraying with 20% (w/v) ethanolic phosphomolybdic acid (Sigma) and subsequent heating at 200 °C for 15 min (IV, total lipids). Chloroform/methanol/water (65:25:4, by vol.) was used in the first direction (1), followed by chloroform/acetic acid/methanol/water (80:15:12:4, by vol.) in the second direction (2). Abbreviations: PE, phosphatidylethanolamine; APL1-3, unknown aminophospholipids; PL1-3, unknown phospholipids; L1-2, unknown lipids.