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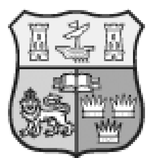

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Ruthenibacterium lactatiformans gen. nov., sp. nov., a new anaerobic, lactate-producing member of the family Ruminococcaceae isolated from human feces

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1 ***Ruthenibacterium lactatiformans* gen. nov., sp. nov., a new anaerobic, lactate-producing**
2 **member of the family *Ruminococcaceae* isolated from human feces**
3
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21 The GenBank accession number for the 16S rRNA gene sequence of strains 585-1^T and 668
22 are KM098109 and KM098110, respectively.
23

Abstract

Two novel strains of Gram-negative staining, rod-shaped, obligately anaerobic, non-sporeforming, non-motile bacteria were isolated from the feces of healthy human subjects. The strains designated as 585-1^{T} and 668 are characterized by mesophilic fermentative metabolism, production of D-lactic, succinic, and acetic acids as end products of D-glucose fermentation, prevalence of $C_{18:1}\omega9$, $C_{18:1}\omega9a$, $C_{16:0}$, and $C_{16:1}\omega7$-cis fatty acids, presence of glycine, glutamic acid, lysine, alanine, and aspartic acid in petidoglycan peptide moiety and lack of respiratory quinones. Whole genome sequencing revealed the DNA G+C content was 56.4-56.6 mol%. Complete 16S rRNA gene sequences shared 91.7/91.6% identity with *Anaerofilum pentosovorans* Fae^{T}, 91.3/91.2% with *Gemmiger formicilis* ATCC 27749^{T}, and 88.9/88.8% with *Faecalibacterium prausnitzii* ATCC 27768^{T}. On the basis of chemotaxonomic and genomic properties it was concluded that the strains represent a new species in a new genus within the family *Ruminococcaceae*, for which the name *Ruthenibacterium lactatiformans* gen. nov., sp. nov. is proposed. The type strain is 585-1^{T} (= DSM 100348^{T}, = VKM B-2901^{T}).

The family *Ruminococcaceae* (Rainey, 2009) comprises a morphologically, physiologically, and ecologically divergent group of microorganisms within the order *Clostridiales*, the class *Clostridia* and the phylum *Firmicutes*, and was first described in the 2nd edition of Bergey's Manual of Systematic Bacteriology (De Vos *et al.*, 2009) on the basis of 16S rRNA gene sequence homology as opposed to the phenotypic classification used earlier. Historically, members of this group belonged to 'clostridial clusters' III and IV according to classification introduced by Collins *et al.* (1994).

Despite the recent advances in resolving the 'taxonomic conundrum' within the *Clostridia* class, significant discrepancies still exist between the major DNA databases (Yutin & Galperin, 2013; Lawson & Rainey, 2016). According to the List of Prokaryotic Names with Standing in Nomenclature (http://www.bacterio.net), the family *Ruminococcaceae* comprises 14 genera (*Acetanaerobacterium*, *Acetivibrio*, *Anaerofilum*, *Anaerotruncus*, *Ethanoligenens*, *Faecalibacterium*, *Fastidiosipila*, *Hydrogenoanaerobacterium*, *Oscillibacter*, *Oscillospira*, *Papillibacter*, *Ruminococcus*, *Sporobacter*, and *Subdoligranulum*) of which 12 were included into the original description of the family (Rainey, 2009). By contrast, the Ribosomal Database Project (RDP, http://rdp.cme.msu.edu) lists 21 genera within this family including *Butyricicoccus*, *Saccharofermentans*, *Flavonifractor*, *Pseudoflavonifractor*, *Cellulosibacter* and *Gemmiger*, as well as the provisional taxonomic groups '*Clostridium* cluster III' and '*Clostridium* cluster IV' (Collins et al., 1994), but excluding the genus *Oscillospira* which currently remains uncultured. The NCBI

59 Taxonomy database (http://www.ncbi.nlm.nih.gov/Taxonomy/) also proposes 21 genera within
60 *Ruminococcaceae* partially overlapping with those in the RDP with the addition of
61 *Caproiciproducens*, *Ercella*, *Mageeibacillus*, *Pseudobacteroides* and *Ruminiclostridium* (the latter
62 taxon supersedes *Clostridium* clusters III and IV as suggested by Yutin & Galperin [2013]), to the
63 exclusion of *Butyricicoccus*, *Cellulosibacter*, *Flavonifractor*, and *Pseudoflavonifractor*. These and
64 other inconsistencies between different databases point out the necessity of further taxonomic
65 improvements within the *Clostridia* class. It is pertinent to note that all bacterial names should be
66 validly published in the International Journal of Systematic and Evolutionary Microbiology as
67 required by the Bacteriological Code. It is only these names that have standing in the literature,
68 which is often not reflected in the names used in DNA databases.

69 In spite of a high degree of phenotypic divergence, most members of the family
70 *Ruminococcaceae* share a number of common features. The majority of genera within this family
71 comprise strictly-anaerobic bacteria with a Gram-positive type of cell wall, albeit many species
72 actually stain Gram-negative. Metabolism is chemoorganoheterotrophic fermentative with a variety
73 of organic acids and H_2 produced as end products (Rainey, 2009). Some species are capable of
74 anaerobic respiration by utilizing fumarate and sulphur as electron acceptors (van Gelder *et al*.,
75 2014). Morphologically the family is very diverse and includes species with rod-shaped (Zellner *et*
76 *al*., 1996; Duncan *et al*., 2002), coccoid (Sijpesteijn, 1948), and pleomorhic (Holmstrøm *et al*.,
77 2004) cells, the most notable being a giant (10–40 × 3–6 μm) filamentous septate yet uncultured
78 bacterium *Oscillospira guilliermondii* (Yanagita *et al*., 2003). Some species form spores whilst
79 others are motile by peritrichous flagella (Grech-Mora *et al*., 1996; Zellner *et al*., 1996). A number
80 of genera (e.g. *Ruminococcus*, *Faecalibacterium*, *Anaerotruncus*, *Fastidiosipila*, *Oscillospira*, and
81 *Subdoligranulum*) are associated with human and animal hosts and have been isolated from feces,
82 rumen and intestinal contents, and blood. Other representatives of the family have more diverse
83 isolation sources including wastewater sludge, anaerobic digesters and bioreactors (Rainey, 2009).

84 One member of the *Ruminococcaceae* family, *Faecalibacterium prausnitzii*, has attracted
85 special attention during the last decade due to its important role in the human gut. Placed in the
86 genus *Fusobacterium* in 1973 (Cato *et al*., 1974), this acetate-consuming and butyrate-producing,
87 extremely oxygen-sensitive, anaerobic organism was re-assigned two decades later to the
88 *Clostridium leptum* group (clostridial cluster IV according to Collins taxonomy). Finally, it was
89 renamed as *F. prausnitzii* in 2002 by Duncan *et al*. This bacterial species constitutes around 5% of
90 total bacterial loads in the fecal samples from healthy adults as determined by metagenomic
91 sequencing (Arumugam *et al*., 2011) and from 2% to 45% according to 16S library sequencing (our
92 unpublished data). Depletion of this organism from the fecal microbiota has been implicated in the
93 pathogenesis of inflammatory bowel disease (IBD; Sokol *et al*., 2009). Moreover, recent studies

94 have confirmed anti-inflammatory activity of *F. prausnitzii* live cultures, cell supernatant and
95 certain purified components in animal models of IBD (Quévrain et al., 2015; Rossi et al., 2015).
96 Interestingly, despite its extreme air sensitivity *F. prausnitzii* may actually benefit from low oxygen
97 concentrations by using it for NADH regeneration through an extracellular electron shuttle (Khan *et*
98 *al*., 2012).

99 During an ongoing culture-based study of human fecal microbiome, in healthy adults and
100 children, two strains of strictly anaerobic Gram stain negative bacteria were isolated that
101 presumably belonged to the family *Ruminococcaceae* but could not be classified to species level
102 using routine identification approaches. Preliminary analysis has shown that the strains designated
103 as 585-1^T and 668 were completely identical by their partial 16S rRNA gene sequences and were
104 moderately related to *F. prausnitzii*, *Subdoligranulum variabile*, *Gemmiger formicilis*, and
105 *Anaerofilum* species. The goal of the current study was to determine the taxonomic position of these
106 strains using polyphasic approach.

107 Strain 585-1^T was isolated from a stool sample of a 31-year-old healthy Russian male where it
108 was present at a concentration of ~ 1×10^8 c.f.u. g^{-1}. Strain 668 was isolated from the stool of a 5-
109 year-old healthy Russian male child at a concentration of ~ 4×10^8 c.f.u. g^{-1}. Fecal samples were
110 weighed, serially diluted with saline and spread over EG agar plates supplemented with 5% (v/v)
111 defibrinated sheep blood. EG medium base consisted of (per 100 ml): 0.24 g Lab-Lemco powder
112 (Oxoid), 1.0 g Proteose peptone No. 3 (BD-Difco), 0.5 g yeast extract (BD-Difco), 0.4 g Na_2HPO_4,
113 0.15 g glucose, 0.05 g soluble starch, 0.02 g L-cystine, 1.5 g agar, 0.05 g L-cysteine·HCl·H_2O.
114 Plates were incubated in an atmosphere of 85% N_2, 10% H_2, 5% CO_2 at 37°C for 72 h in anaerobic
115 jars (Schuett-Biotec). Well isolated colonies representative of each morphological type were
116 selected and streaked out several times to obtain pure cultures on EG-blood agar. Upon isolation,
117 strains 585-1^T and 668 were cultured anaerobically on EG broth, PYG broth (Thermo Fisher) or
118 MRS broth (Himedia) supplemented with 5 mg l^{-1} haemin. Cultures were incubated at 37°C for 48-
119 96 h. Strains were preserved by freeze-drying of bacterial suspensions frozen in 10% (w/v) sucrose,
120 1% (w/v) gelatin solution. Susceptibility of the strains to bile and NaCl was tested in EG broth
121 supplemented with 0-3% (w/v) of Oxgall (Sigma-Aldrich) and 0-8% (w/v) of NaCl. Media were
122 inoculated from fresh agar cultures and growth was examined visually after 48 hours. Physiological
123 properties and enzyme profiles were determined using Vitek 2 ANC, Rapid ID 32A, and API 20A
124 identification systems (bioMérieux) essentially according to manufacturer's instructions except for
125 substitution of API 20A standard incubation medium for glucose-free MRS broth. Carbohydrate
126 fermentation was studied by supplementing MRS broth with 2% (w/v) of every substrate tested
127 instead of D-glucose as well as with 0.01% (w/v) of bromocresol purple. Cultures were incubated
128 anaerobically for 72-96 h.

129 Analysis of short-chain fatty acids (SCFA) was performed in 168 h PYG broth culture
130 supernatant using HPLC as described before (Shkoporov *et al*., 2015). Alcohols were analyzed
131 using Pye-Unicam 304 gas chromatograph equipped with 2 m x 2 mm glass column packed with
132 Porapak QS matrix (Fluka) in isocratic mode with column, injector, and detector temperatures of
133 100, 120, and 170°C, respectively. Cellular fatty acids and menaquinone profiles were analyzed in
134 the late exponential phase cultures in MRS broth. Long-chain fatty acids were separated and
135 detected using gas chromatography − mass spectrometry (GC-MS) according to Zhilina *et al*.
136 (2012). Respiratory quinones were detected following the procedure of Collins (1985). For
137 peptidoglycan amino acid analysis cell walls were prepared as described by Schleifer & Kandler,
138 1972. The cell wall preparations were hydrolysed with 6 M HCl at 105°C for 6 h (Schuman, 2011).
139 Quantitative amino acid analysis was performed with an LC 600 amino acid analyser (Biotronic).
140 Ultrathin sections (50-60 nm thick) of culture pellets were prepared as described by Duda *et*
141 *al*. (2009) and examined in a JEOL JEM-1200EX transmission electron microscope with
142 accelerating voltage of 80 kV.
143 Genomic DNA was extracted and sequenced on Roche/454 GS Junior as described before
144 (Shkoporov *et al*., 2015). Reads were assembled using Newbler v 2.7 into 108 contigs for strain
145 585-1^{T} (N50 = 101,736 bp) with a combined length of 4,111,078 bp and 20.0x coverage and 108
146 contigs for strain 668 (N50 = 114,065 bp) resulting in a combined length of 3,951,525 bp and 17.9x
147 coverage. Draft genome sequences of strains 585-1^{T} and 668 were deposited in GenBank
148 Nucleotide database under the accession numbers JXXK01000000 and LMUA01000000,
149 respectively. Annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline
150 (Angiuoli *et al*., 2008) with metabolic pathways constructed using the KEGG automated annotation
151 server (Moriya *et al*., 2007) followed by manual curation.
152 The complete 16S rRNA sequences of strains 585-1^{T} and 668 (KM098109 and KM098110,
153 respectively) and of the type strains from the family *Ruminococcaceae* were aligned using
154 MUSCLE (Edgar 2004).). All positions containing gaps and missing data were eliminated.
155 Phylogenetic inference was performed using the neighbor-joining (NJ) approach in MEGA 6
156 (Tamura *et al*., 2013) with evolutionary distances calculated using Tamura-Nei substitution model
157 (Tamura & Nei, 1993). The robustness of the tree topology was evaluated by a bootstrapping with
158 1000 re-samplings (Felsenstein, 1985). Furthermore, in order to check the validity of the NJ tree,
159 maximum likelihood phylogeny was inferred by RAxML (Stamatakis, 2014) using the GTR model
160 with gamma distributed rate heterogeneity and 1000 rapid bootstrap re-samplings. Additionally,
161 phylogeny of strains 585-1^{T} and 668 was inferred by using core proteome sequences across 23
162 representative strains of the family *Ruminococcaceae*. To select conserved orthologous proteins
163 encoded by publicly available *Ruminococcaceae* genomes we performed clustering of translated

164 genomic ORFs using OrthoMCL (Li *et al*. 2003) with an e-value cut-off 1E-5, percent identity cut-
165 off 40% and MCL inflation index I = 1.1. As a result a core proteome of 204 conserved protein
166 families with a single representative encoded by every *Ruminococcaceae* genome from the set was
167 obtained. Amino acid sequences were concatenated and aligned using MUSCLE excluding all gaps.
168 Phylogenetic inference was carried out using Neighbor-Joining in MEGA 6 with the JTT
169 substitution model (Jones *et al*., 1992) and 1000 bootstrap re-samplings. For each reconstructed
170 phylogeny *Clostridium perfringens* ATCC 13124^T was selected as an outgroup.

171 Average nucleotide identity (ANI) was calculated using 'Blast+'-based algorithm on
172 JSpeciesWS server (Richter et al., 2015).

173 The novel strains described in this study were obligately anaerobic, non-sporeforming, non-
174 motile, Gram-negative staining rods. Cells collected from 96 h EG blood agar plates were
175 1.6±0.3×0.4±0.1 μm in size and occurred singly and in pairs. Minute coccoid cells attached or
176 budding from the poles of rods were seen in some light and electron micrographs. Transmission
177 electron micrographs of ultrathin sections of strain $585\text{-}1^T$ revealed highly heterogenous cytoplasm
178 with circular electron dense inclusion bodies (114±13 nm in diameter) and lamellar structures
179 visible in some cells (Fig. 1). Cell envelope organization was elaborate with several electron dense
180 and transparent layers visible, somewhat resembling a Gram-negative trilaminar cell envelope.
181 However, KOH test was negative for both strains. Similar elements were previously seen in the cell
182 envelope of *Gemmiger formicilis* (Gossling & Moore, 1975; Salanitro *et al*., 1976), a member of the
183 family *Ruminococcaceae* moderately related to strains $585\text{-}1^T$ and 668 by 16S rRNA gene
184 sequence. Other related members of the family, *A. agile* and *F. prausnitzii* were shown, however, to
185 have a typical Gram-positive cell wall architecture with thin murein layer, despite their variable
186 staining in Gram method (Zellner *et al*., 1996; Rossi *et al*., 2015). In addition, thin microcapsule
187 was visible on the surface of strain $585\text{-}1^T$. Flagella, pili, and other types of surface appendages
188 were not detected in negatively stained preparations of strain $585\text{-}1^T$.

189 After 96 h of anaerobic growth on EG blood agar colonies reached 0.15-0.4 mm in diameter
190 and were non-haemolytic, colorless, circular, flat, dry, with entire margins and rough surface.
191 Supplementation of EG, MRS, and PYG broth media with 0.5% (w/v) maltose and 5 mg l^{-1} haemin
192 strongly stimulated growth of both strains which otherwise was poor even after prolonged
193 incubation. The best overall growth support of the strains was obtained with MRS broth
194 supplemented with maltose and haemin. The strains were not only resistant to 3% (w/v) of Oxgall
195 in EG broth, but also demonstrated enhanced growth in the form of threadlike, ropy and mucous
196 sediment. Both strains were resistant to up to 1% (w/v) NaCl in MRS broth. Using both MRS and
197 EG broth growth was observed at 37°C but not at 32° or 42°C. Aesculine and starch were

198 hydrolized by both strains. Indole, hydrogen sulphide, catalase, urease, and gelatinase were not
199 produced. Oxidase, nitrate reductase, and alkaline phosphatase reactions were also negative.
200 In Rapid ID 32A and Vitek 2 ANC identification panels, based on the use of chromogenic
201 enzyme substrates, both strains demonstrated positive reactions for a number of glycosyl
202 hydrolases, inlcuding α- and β-galactidases, α- and β-glucosidases, β-glucuronidase, α-
203 mannosidase, β-N-acetyl-glucosaminidase, but not for α-arabinosidase, α-L-fucosidase, β-D-
204 fucosidase, and β-mannosidase (Tables S1, S2). By contrast, all carbohydrate fermentation reactions
205 included in these panels were negative. All chromogenic arylamidase (exopeptidase) tests included
206 in the Rapid ID 32A and Vitek 2 ANC panels were negative for both strains, indicating that these
207 microorganisms specialize in the utilization of carbohydrate rather than protein substrates as carbon
208 source. Both strains were negative for most of the carbohydrate fermentation reactions in API 20A
209 tests. Acid production was detected from maltose, salicin, and weakly from D-glucose and L-
210 rhamnose (Table S3). In conventional carbohydrate fermentation tests acid production was detected
211 from maltose, salicin, D-galactose, L-rhamnose, weakly from D-mannose, melibiose and D-sorbitol.
212 Variable results were obtained with sucrose (Table S4). Growth on MRS without carbohydrates was
213 very poor.
214 In disk-diffusion experiments strain 585-1^T was resistant to amikacin, ampicillin,
215 azithromycin, cephalothin, clindamycin, levofloxacin, linezolid, and penicillin G, but sensitive to
216 amoxyclav and vancomycin (Table S5).
217 When grown in PYG broth with 0.5% (w/v) glucose strains 585-1^T/668 produced 22.2/24.4
218 mM of D-lactate and 9.7/9.4 of mM succinate. In addition, strain 668 produced 7.2 mM of acetate.
219 Supplementation of PYG with 0.5% (w/v) maltose led to increased production of succinic acid
220 (17.2 mM) and formation of formic (11.0 mM) and acetic (6.9 mM) acids by strain 585-1^T. By
221 contrast, growth of strains 585-1^T/668 on MRS broth supplemented with 0.5% (w/v) maltose
222 resulted in a wide range fermentation end products including 8.5/8.1 mM of formate, 13.1/10.1 mM
223 of acetate, 20.1/19.1 mM of D-lactate, 15.9/12.2 mM of succinate, 9.6/9.3 mM of propionate and
224 2.8/3.4 mM of butyrate. The overall composition of metabolic end-products in strains 585-1^T and
225 668 resembles those of other members of the family *Ruminococcaceae* isolated from human and
226 animal feces. The striking predominance of lactic acid among the end-products relates this group of
227 strains with the genus *Anaerofilum* (Zellner *et al*., 1996). However, unlike *Anaerofilum*, the novel
228 strains produced D-lactate isomer.
229 Cellular fatty acids (CFA) in strain 585-1^T were mostly composed of monounsaturated
230 species: $C_{18:1}ω9$ (31.4-31.9%), $C_{18:1}ω9$ aldehyde (20.6-21.0%), $C_{16:1}ω7$-cis (5.3-6.0%). Palmitic
231 acid (C16:0, 6.1-6.6%) and other saturated acids were present in minor amounts (Table S6).
232 According to literature, cell membranes of the closest related genera are composed mostly of

233 saturated CFA, e.g.: $C_{14:0}$, $C_{16:0}$ and $C_{16:0}$, $C_{18:0}$, predominate in *Faecalibacterium* and
234 *Subdoligranulum*, respectively (Jantzen & Hofstad, 1981; Holmstrøm *et al*., 2004), while iso-$C_{16:0}$,
235 iso-$C_{12:0}$, anteiso-$C_{17:0}$ are found in *Ethanoligenens* (Xing *et al*., 2006). Species of *Ruminococcus*
236 are quite diverse in their CFA composition, but $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$,
237 and anteiso-$C_{19:0}$ are the most common (Minato *et al*., 1988). Other genera of *Ruminococcaceae*
238 that are closely related to strains 585-1^T and 668 have not been characterized yet in terms of CFA
239 composition. Respiratory quinones were not detected in whole cell extracts from strains 585-1^T and
240 668. Amino acid composition of cell wall acid hydrolysates of the two strains was roughly identical
241 and contained glycine (31.85- 33.99 nM), glutamic acid (31.46-33.75 nM), lysine (23.77-28.23
242 nM), alanine (23.36-28.87 nM), and aspartic acid (21.92-25.2 nM). The exact structure of of peptide
243 moiety has to be determined. However, such amino acid composition is consistent with
244 peptidoglycan type A4α (cross-linkage by L-Lys-D-Asp) according to Schleifer & Kandler (1972)
245 nomenclature or type A11.31 according to Schumann (2011). Similarly to *Anaerofilum* the lactyl
246 group of muramic acid is likely to be esterified with glycine.

247 The 4.11 Mbp draft genomic assembly of strain 585-1^T had an overall G+C content of 56.5
248 mol%, a total of 3,802 protein-coding genes, 51 tRNA, a set of rRNA genes and 1 CRISPR array.
249 The combined length of strain 668 draft assembly was slightly smaller, 3.95 Mbp, with a G+C
250 content of 56.6 mol% , encoding a total of 3,556 genes, 50 tRNA, a set of rRNA genes and 1
251 CRISPR array. Central carbon metabolism genes in both strains include a full complement for
252 Embden–Meyerhof–Parnas and pentose phosphate pathways, an almost complete set of genes for
253 Entner–Doudoroff pathway (excluding glucose-6-phosphate 1-dehydrogenase), a pyruvate
254 ferredoxin oxidoreductase gene, and genes required for first carbon oxidation in citrate cycle.
255 Therefore, the metabolic capabilities of the new strains presumably differ from those of *F.*
256 *prausnitzii* and *Ruminococcus bromii* (which lack identifiable transaldolase gene) and
257 *Ethanoligenens harbinense* (which lacks Entner–Doudoroff pathway genes, but possesses an almost
258 complete TCA cycle). Unlike *F. prausnitzii* the novel strains do not have any recognizable genes for
259 butyrate production from acetate including, acetyl-CoA acetyltransferase (thiolase), 3-hydroxyacyl-
260 (β-hydroxybutyryl-) CoA dehydrogenase, 3-hydroxybutyryl-CoA dehydratase (crotonase), butyryl-
261 CoA dehydrogenase, and butyryl-CoA:acetate CoA-transferase (Louis & Flint, 2009). No butyrate
262 kinase genes were detected as well. Furthermore, unlike *F. prausnitzii* the new strains lack succinate
263 dehydrogenase genes, but possess genes coding for lactate dehydrogenase, and aldehyde
264 dehydrogenase. The presence of alcohol dehydrogenase genes can enable the new strains to produce
265 ethanol, which, however, could not be detected in broth cultures under conditions used in this study.

266 The repertoire of glycan degradation enzymes encoded by genomes of strains 585-1^T and 668
267 include α- and β-galactidases, hexosaminidases, α- and β-mannosidases, β-glucuronidase

268 glucosylceramidase, α-L-fucosidase, α-amylase (only in 585-1^{T}) and sialidase (only in 668).
269 Analysis of amino acid biosynthesis genes suggest that the strains are able to synthesize from
270 common precursors most amino acids with the exception of tryptophan, tyrosine, alanine, arginine,
271 and lysine. The strains appeared to be auxotrophic for most vitamins and enzyme cofactors,
272 however an almost complete pathway of anaerobic transformation of uroporphyrinogen III into
273 cobamide coenzyme (Roper *et al*., 2000) was found in both genomes.
274 A number of ABC-transporters were identified in the genomes of the new strains, which
275 include predicted transport systems for spermidine/putrescine, raffinose/melibiose, methyl-
276 galactoside, phosphate, phosphonate, branched-chain amino acid, oligopeptide, iron complex, zinc
277 (only in 585-1^{T}), cobalt, nickel, and biotin. The genomes of strains 585-1^{T} and 668 encode proteins
278 required for RecF homologous recombination pathway. In addition, strain 585-1^{T}, but not 668,
279 possess several genes coding for β-lactamases, which correlates well with resistance of the former
280 strain to penicillin G, ampicillin, and cephalothin.
281 The ecological distribution of the new bacterium across the human population and across
282 various sites in human body has to be established. However, a brief search in NCBI 'nr' database
283 revealed that numerous 16S rRNA gene sequences from uncultured bacteria with ≥97% identity to
284 strain 585-1^{T} are present in 16S rRNA gene datasets from a number of studies, including a study of
285 a Chinese family fecal microbiota (Li *et al*., 2008), study of ileal microbiota in patients with Crohn's
286 disease (Li *et al*., 2012), and a study of gut microbiota in obese subjects (Ley *et al*., 2006). We also
287 conducted a blastn search against an inhouse dataset of 16S rRNA gene sequences (V1-V3 region),
288 which was generated using 454 platform from fecal samples of 19 healthy human subjects including
289 the two subjects from whom strains 585-1^{T} and 668 were originally isolated. This search revealed
290 the presence of sequences with ≥97% identity in 7 samples (36.8%) with relative abundance
291 ranging from 0.01% to 0.4% of the total number of sequences.
292 Alignment of complete 16S rRNA genes from 585-1^{T} and 668 showed that they were 99.9%
293 identical. The strains also had 91.7/91.6% identity to *Anaerofilum pentosovorans* Fae^{T}, 91.6/91.5%
294 to *A. agile* F^{T}, 91.3/91.2% to *G. formicilis* ATCC 27749^{T}, 90.1% to *S. variabile* BI 114^{T}, and
295 88.9/88.8% identity to *F. prausnitzii* ATCC 27768^{T}. A search in NCBI 'wgs' database using 585-1^{T}
296 16S rRNA gene sequence revealed several highly identical (>99%) genes on gut shotgun
297 metagenomic contigs (LBCJ01000006, LBCI01000027), as well as a 16S rRNA gene from
298 unpublished draft assembly of human gut isolate '*Ruminococcaceae* bacterium cv2'
299 (NZ_CYPT01000000). This 4.26 Mbp genomic sequence with a G+C content of 56.6 mol% has
300 been included in the phylogenetic analysis described herein.
301 To establish the taxonomic positions of strains 585-1^{T} and 668 within the family
302 *Ruminococcaceae* two different approaches of phylogenetic analyses were carried out. One was

303 based on 16S rRNA gene sequences from the type strains of family *Ruminococcaceae* and was
304 conducted using both neighbor-joining and maximum likelihood inference methods (Fig. 2). The
305 other was performed on a concatenated alignment of 204 conserved orthologous proteins (Table S7)
306 encoded by the currently available complete and draft genomic sequences from the family
307 *Ruminococcaceae* (Fig. 3) using the NJ approach. Both approaches reliably placed strains 585-1^T,
308 668 and cv2 inside the family *Ruminococcaceae* According to 16S rRNA phylogeny, the three
309 strains formed a separate branch which was located as a sister clade to the
310 *Gemmiger*/*Subdoligranulum*/*Faecalibacterium* clade. These two clades along with the genus
311 *Anaerofilum* clade together were a part of a larger phylogenetic cluster, which also included *R.*
312 *bromii*, [*Clostridium*] *leptum*, [*Clostridium*] *sporosphaeroides* and which corresponded to clostridial
313 cluster IV in Collins's taxonomy. The conserved proteins tree had a similar topology and placed the
314 three strains as a separate branch again as the sister clade to the *Subdoligranulum*/*Faecalibacterium*
315 clade. As an additional phylogenetic measure ANI was calculated after pairwise blastn all-versus-all
316 searches between 585-1^T, 668, cv2, *F. prausnitzii* A2-165^T and *S. variabile* DSM 15176^T genomes.
317 The ANI between the strains 585-1^T, 668, cv2 ranged from 97.4 to 98.1%. *F. prausnitzii* A2-165^T
318 had 69.3-69.8% ANI to the new strains and 72.6% to *S. variabile* DSM 15176^T. *S. variabile* DSM
319 15176^T in turn displayed 68.8-69.5% identity to strains 585-1^T, 668, cv2 and 72.8% to *F. prausnitzii*
320 A2-165T.

321 Strains 585-1^T and 668 differ from phylogenetically related genera within the family
322 *Ruminococcaceae* by cell morphology, physiological culture properties, enzymatic activity,
323 spectrum of metabolic end-products, CFA composition, and genome characteristics. Based on the
324 phenotypic and genotypic properties of strains 585-1^T and 668 it is concluded that they represent a
325 new species in a new genus within the family *Ruminococcaceae*, for which the name
326 *Ruthenibacterium lactatiformans* gen. nov., sp. nov is proposed. The main chemotaxonomic
327 characteristics of the new taxon in comparison with some of the related genera are given in Table 1.
328

329 **Description of *Ruthenibacterium* gen. nov.**

330 *Ruthenibacterium* (Ru.the.ni.bac.te'ri.um. M.L. fem. n. *Ruthenia* medieval Latin name of
331 Russia; Gr. dim. n. *bakterion* a small rod; N.L. neut. n. *Ruthenibacterium* a rod-shaped bacterium
332 isolated in Russia).

333 Cells are Gram-negative, rod-shaped, obligately anaerobic, non-sporeforming, non-motile,
334 1.6±0.3×0.4±0.1 μm in size and occur singly and in pairs. Metabolism is chemoorganoheterotrophic
335 fermentative. Optimal growth temperature is 37°C. D-lactate and succinate are the major end-
336 products of fermentation. Predominant cellular fatty acids are $C1_{8:1}\omega9$, $C1_{8:1}\omega9a$, $C_{16:0}$, and $C_{16:1}\omega7$-
337 cis. The peptidoglycan contains glycine, glutamic acid, lysine, alanine, and aspartic acid.

338 Menaquinones are not produced. Member of the family *Ruminococcaceae*. The type species is
339 *Ruthenibacterium lactatiformans*.
340
341 **Description of *Ruthenibacterium lactatiformans* sp. nov.**
342 *Ruthenibacterium lactatiformans* (lac.ta.ti.for'mans. L. part. perf. pass. masc. *lactatus* fed
343 with milk, lactate; L. part. adj. *formans* forming; N.L. part. adj. *lactatiformans* lactate forming).
344 Exhibits the following characteristics in addition to those given in the description of the
345 genus. Growth on EG blood agar are visible after 72-96 h of anaerobic incubation at 37°C.
346 Colonies are 0.15-0.4 mm in diameter, non-haemolytic, colorless, circular, entire, flat, dry, and with
347 rough surface. Colonies are positive for aesculin and starch hydrolysis and tolerant to bile. In broth
348 cultures growth is stimulated by 0.5% (w/v) maltose, 5 mg l^{-1} haemin, and 2-3% (w/v) of Oxgall.
349 Indole, catalase, and urease are not produced. Gelatin is not digested. Acid is produced from D-
350 glucose, D-galactose, maltose, salicin, L-rhamnose but not from L-arabinose, adonitol, lactose, D-
351 mannitol, D-raffinose, and D-trehalose. In chromogenic substrates tests positive reactions are
352 obtained for α- and β-galactidases, α- and β-glucosidases, β-glucuronidase, β-N-acetyl-
353 glucosaminidase, but not for α-arabinosidase and α-fucosidase. The DNA G+C content is 56.4-56.6
354 mol%.
355 The type strain of the species, isolated from human feces, is 585-1^T (= DSM 100348^T, =
356 VKM B-2901^T)
357
358
359
360
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364
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490

491 **Table 1. Differential characteristics of strain 585-1^T and 668 in comparison with some related genera.** Data for other genera within the family
492 *Ruminococcaceae* are taken from Cato *et al*. (1974), Duncan *et al*. (2002), Jantzen & Hofstad (1981), Holmstrøm *et al*. (2004), Gossling & Moore
493 (1975), Zellner *et al*. (1996), Xing *et al*. (2006), Wozny *et al*. (1977), Minato *et al*. (1988) and Rainey (2009). NA, data not available; V, variable; W,
494 weak; a/b/f/ib/iv/l/p/s/v/e/bdl;, fermentation end products (acetic, butyric, fumaric, isobutyric, isovaleric, lactic, propionic, succinic and valeric acids,
495 ethanol, and 2,3-butanediol, respectively; capital and small letters indicate major and minor products, respectively).
496

	Strains 585-1 and 668	***Faecalibacterium***	***Subdoligranulum***	***Gemmiger***	***Anaerofilum***	***Ethanoligenens***	***Anaerotruncus***	***Ruminococcus***
Isolation source	Human feces	Human and animal feces	Human feces	Human feces and chicken ceca	Anaerobic sewage sludge	Anaerobic sewage sludge	Human feces and blood	Rumen, large bowel, or cecum of many animals and humans
Cell shape	Straight rods (occur singly and in pairs	Variable length straight rods (occur singly)	Coccoid and pleomorphic	Spherical to drop-like (often in pairs and short chains)	Thin straight rods (single and in pairs)	Rods and filaments	Thin rods	Cocci and coccobacilli (often in pairs and chains)
Cell size, µm	1.6±0.3×0.4±0.1	0.5–0.8 × 2.0–14.0	0.6–2.5	1–2.3 × 0.5–1.2	3.0–6.0 × 0.3–0.6	0.4–0.8 × 1.5–8.0	2–5 × 0.5	0.3–1.5 × 0.7–1.8
Gram stain result	-	-	-	-	+	+	+	V
Spores	-	-	-	-	-	-	+	-
Motility	-	-	-	-	+	+	-	-
Growth temperature, °C	37	37–45	37–45	37-45	18–44	20–44	36–40	37–42
Aesculin hydrolysis	+	+	+	+	V	+	-	V
Starch hydrolysis	+	V	-	V	-	W	-	V
Indole production	-	-	-	-	NA	+	+	-
Urease	-	-	-	-	NA	+	-	+
Optimal growth medium	EG, MRS	M2GSC, YCFA, Wilkins-Chalgrene broth (Oxoid)	M2GSC, Fastidious Anaerobe Broth (Oxoid)	E medium*	DSMZ medium 119	PYG broth	Brucella blood agar (Anaerobe Systems)	Rumen fluid agar
Major end products	L, S, a (PYG)	B, L, F, s†, p† (PYG-RF)	B, L, a, s	F, B, l, a (PYG)	L, A, E, F, bdl, CO_2	A, E, H_2, CO_2	A, B (PYG)	A, F, S, L, e, H_2 (PYG)
Major cellular fatty acids	$C_{18:1}\omega9$, $C_{18:1}\omega9a$, $C_{16:0}$, $C_{16:1}\omega7$-cis	$C_{14:0}$, $C_{16:0}$	$C_{16:0}$, $C_{18:0}$, $C_{18:1}\omega9$-cis	NA	NA	iso-$C_{16:0}$, iso-$C_{12:0}$, anteiso-$C_{17:0}$	NA	$C_{14:0}$, $C_{15:0}$, $C_{16:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$, ai-$C_{17:0}$ ai-$C_{19:0}$
G+C content, mol%	56.4-56.6‡	47-67 (56.2-57.7‡)	52.2 (57.9‡)	59	54-55	47.8-49.0 (55.6‡)	53-54 (54.2‡)	37-47 (41.1-53.4‡)
Genome size, Mb	3.9-4.3§	2.9-3.3	3.25	NA	NA	3.0	3.7	2.2-4.6

497
498 * Holdeman & Moore, 1973
499 † trace amounts
‡ based on draft and complete genome assemblies
§ includes a closely related genome of strain '*Ruminococcaceae* bacterium cv2' (NZ_CYPT01000000)
500

501
502 **Figure legends.**
503
504 **Figure 1**. Transmission electron micrographs of ultrathin sections of strain 585-1^T cells showing overall cell morphology (a), spherical inclusion
505 bodies (b), laminate structures (c), and cell wall organization (d). C, microcapsule; CW, multilayer cell wall; IB, inclusion bodies; IM, intracytoplasmic
506 membrane structures; LB, lamellar bodies; MC, minute cells; N, nucleoid.
507
508 **Figure 2**. Neighbor-joining phylogenetic tree of 16S rRNA gene sequences. Evolutionary distances were computed using the Tamura-Nei
509 substitution matrix. The scale bar represents 0.02 substitutions per nucleotide position. Accession number in Genbank database is given next to a strain
510 name. Node labels represent bootstrap confidence levels obtained using Neighbor-joining/Maximum likelihood methods. Only the nodes with both
511 bootstrap levels higher than 70% are labeled.
512
513 **Figure 3.** Neighbor-joining phylogenetic tree of concatenated sequences of 204 conserved proteins. Evolutionary distances were computed using
514 the JTT matrix. The scale bar represents 0.1 substitutions per amino acid position. Accession numbers in Genbank database is given next to a strain
515 name. Node labels represent bootstrap confidence levels obtained using neighbor-joining method.

Figure 1

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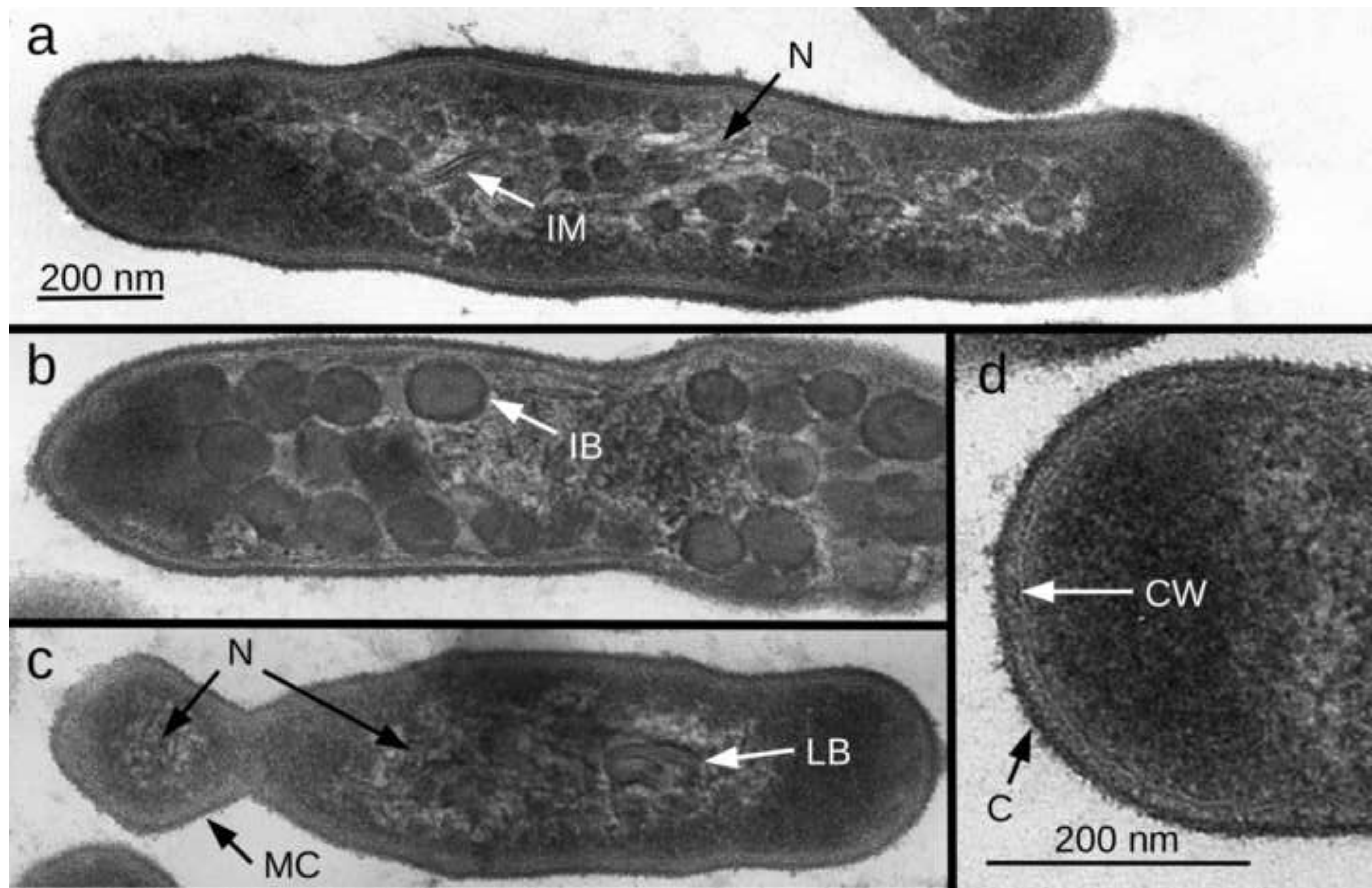

Figure 2

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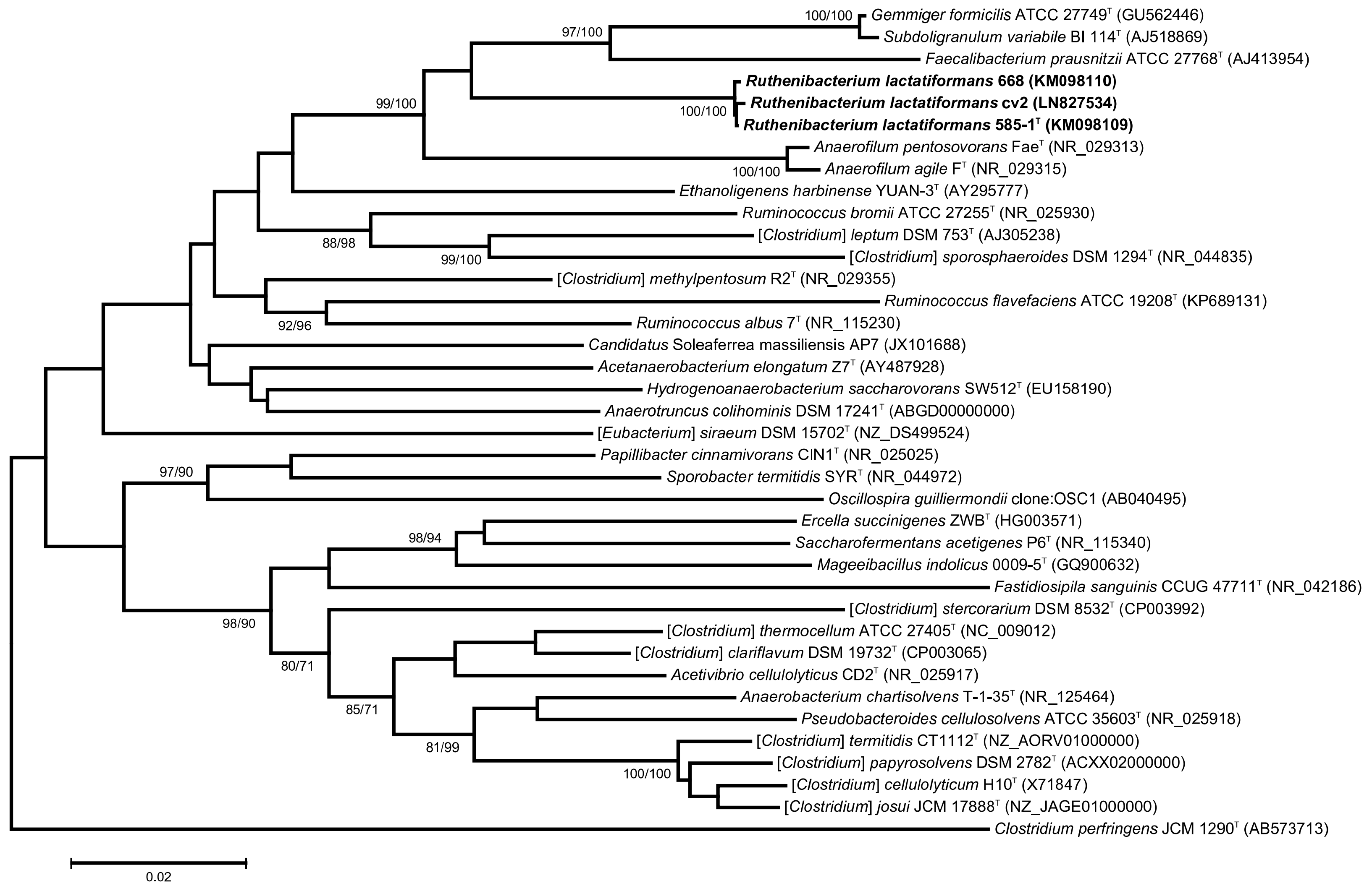

Figure 3

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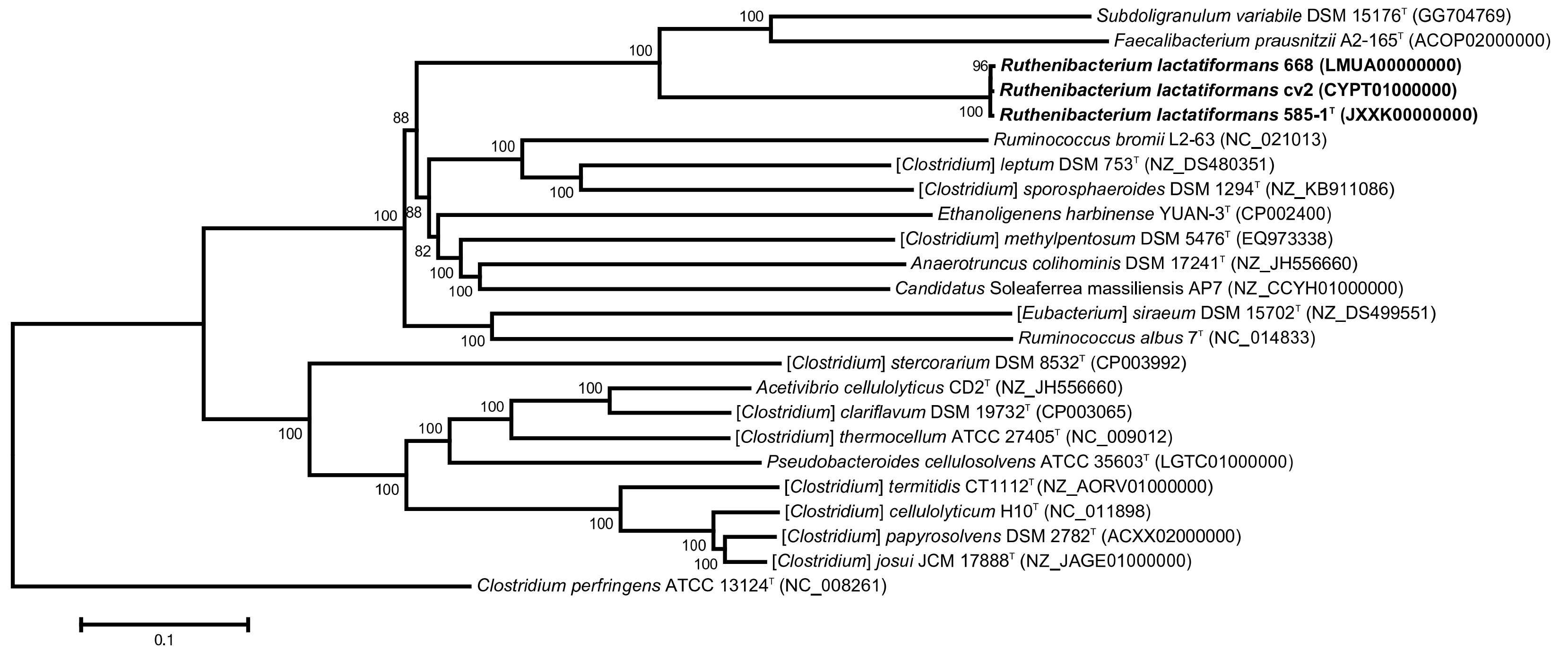

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Supplementary data

***Ruthenibacterium lactatiformans* gen. nov., sp. nov., a new anaerobic, lactate-producing member of the family *Ruminococcaceae* isolated from human feces**

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Table S1. Biochemical profiles of strains 585-1^{T} and 668 obtained using the Vitek 2 ANC identification panel.

	Biochemical test	Code	Strains	
			585-1	668
4	D-Galactose	dGal	-	-
5	Leucine arylamidase	LeuA	-	-
6	ELLMAN	ELLM	+	+
7	Phenylalanine Arylamidase	PheA	-	-
8	L-Proline arylamidase	ProA	-	-
10	L-Pyrrolydonyl arylamidase	PyrA	-	-
11	D-Cellobiose	dCEL	-	-
13	Tyrosine arylamidase	TyrA	-	-
15	Ala-Phe-Pro arylamidase	APPA	-	-
18	D-Glucose	dGLU	-	-
20	D-Mannose	dMNE	-	-
22	D-Maltose	dMAL	-	-
28	Sucrose	SAC	-	-
30	Arbutine	ARB	-	-
33	N-Acetyl-glucosamine	NAG	-	-
34	5-Bromo-4-chloro-3-indoxyl-β-glucoside	BGLUi	+	+
36	Urease	URE	-	-
37	5-Bromo-4-chloro-3-indoxyl-β-glucuronide	BGURi	+	+
39	5-Bromo-4-chloro-3-indoxyl-β-galactopyranoside	BGALi	+	+
41	α-Arabinosidase	AARA	+	+
42	5-Bromo-4-chloro-3-indoxyl-α-galactoside	AGALi	+	+
43	β-Mannosidase	BMAN	-	-
44	Arginine GP	ARG	-	-
45	Pyruvate	PVATE	-	-
51	Maltotriose	MTE	-	-
53	Aesculine, hydrolysis	ESC	+	+
54	β-D-Fucosidase	BdFUC	-	-
55	5-Bromo-4-chloro-3-indoxyl-β-N-acetyl-glucosamide	BNAGi	+	+
56	5-Bromo-4-chloro-3-indoxyl-α-mannoside	AMANi	+	+
57	α-L-Fucosidase	AIFUC	-	-
59	Phosphatase	PHOS	-	-
60	L-arabinose	IARA	-	-
61	D-Ribose 2	dRIB2	-	-
62	Phenylphosphonate	OPS	-	-
63	α-L-Arabinosidase	AARAF	-	-
64	L-Xylose	dXYL	-	-

Table S2. Biochemical profiles of strains 585-1^{T} and 668 obtained using the Rapid ID 32 A panel.

Biochemical test	Code	Substrate	Strains	
			585-1	668
Urease	URE	Urea	-	-
Arginine dihydrolase	ADH	L-arginine	-	-
α-galactosidase	αGAL	4-nitrophenyl-αD-galactopyranoside-	+	+
β–galactosidase	βGAL	4-nitrophenyl-βD-galactopyranoside	+	+
β–galacostidase 6 phophate	βGP	4-nitrophenyl-βD-galactopyranoside-6-phosphate-2CHA	-	-
α-glucosidase	αGLU	4-nitrophenyl-αD-glucopyranoside	+	+
β-glucosidase	βGLU	4-nitrophenyl-βD-glucopyranoside	+	+
α-arabinosidase	αARA	4-nitrophenyl- αL-arabinofuropyranoside	-	-
β-glucuronidase	βGUR	4-nitrophenyl-βD-glucuronide	+	+
β-N-acetyl glucosaminidase	βNAG	4-nitrophenyl-N-acetyl-β-D-glucosaminide	+	+
Mannose fermentation	MNE	D-mannose	-	-
Raffinose fermentation	RAF	D-raffinose	-	-
glutamate decarboxylase	GDC	Glutamic acid	-	-
α-fucosidase	αFUC	4-nitrophenyl- αL-fucopyranoside	-	-
nitrate reductase	NIT	Potassium nitrate	-	-
Indole production	IND	L-tryptophane	-	-
alkaline phosphatase	PAL	2-naphtyl-phosphate	-	-
Arginine arylamidase	ArgA	L-arginine- β-naphtylamide	-	-
Proline arylamidase	ProA	L-proline- β-naphtylamide	-	-
leucyl glycine arylamidase	LGA	L-leucyl-L-glycine - β-naphtylamide	-	-
phenylalanine arylamidase	PheA	L- phenylalanine - β-naphtylamide	-	-
leucine arylamidase	LeuA	L-leucine- β-naphtylamide	-	-
Pyroglutamic acid arylamidase	PyrA	Pyroglutamic acide - β-naphtylamide	-	-
Tyrosine arylamidase	TyrA	L-tyrosine- β-naphtylamide	-	-
Alanine arylamidase	AlaA	L-alanyl-L-alanine- β-naphtylamide	-	-
Glycine arylamidase	GlyA	L-glycine- β-naphtylamide	-	-
Histidine arylamidase	HysA	L-histidine- β-naphtylamide	-	-
Glutamyl Glutamic acid arylamidase	GGA	L-glutamyl-L-glutamic acide-- β-naphtylamide	-	-
Serine arylamidase	SerA	L-serine- β-naphtylamide	-	-

Table S3. Biochemical characteristics of strains 585-1[T] and 668 determined using API 20 A identification system with MRS medium (readings after 72 h incubation at 37°C).

Characteristic	***585***	***668***
Indole production	-	-
Urease activity	-	-
Acid production from D-glucose	weak	weak
Acid production from D-mannitol	-	-
Acid production from lactose	-	-
Acid production from sucrose	-	-
Acid production from maltose	+	+
Acid production from salicin	+	+
Acid production from D-xylose	-	-
Acid production from L-arabinose	-	-
Gelatin digestion	-	-
Aesculin hydrolysis	+	+
Acid production from glycerol	-	-
Acid production from D-cellobiose	-	-
Acid production from D-mannose	-	-
Acid production from D-melezitose	-	-
Acid production from D-raffinose	-	-
Acid production from D-sorbitol	-	-
Acid production from L-rhamnose	weak	weak
Acid production from D-trehalose	-	-
Catalase production	-	-

Table S4. Carbohydrate fermentation profiles of strains 585-1^{T} and 668 determined in MRS medium supplemented with 0.01% (w/v) bromocresol purple indicator (readings after 96 h incubation at 37°C).

Characteristic	***585***	***668***
Acid production from D-glucose	+	+
Acid production from D-mannitol	-	-
Acid production from lactose	-	-
Acid production from sucrose	+	weak
Acid production from maltose	+	+
Acid production from salicin	+	+
Acid production from D-galactose	+	+
Acid production from L-arabinose	-	-
Acid production from adonitol	-	-
Acid production from α-methyl-D-glucoside	-	-
Acid production from D-mannose	weak	weak
Acid production from melibiose	weak	weak
Acid production from D-raffinose	-	-
Acid production from D-sorbitol	weak	weak
Acid production from L-rhamnose	+	+
Acid production from D-trehalose	-	-
Acid production from amygdalin	-	-

Table S5. Antibiotic susceptibility profile of strain 585-1^T determined using disk-diffusion method on (growth inhibition zones were measured 72 h after inoculation on EG agar).

Antibiotic		Amount of substance in disk, µg	Diameter of growth inhibition zone, mm 585-1	**Sensitivity, Yes/No**
Amikacin	**Ak**	30	5	No
Amoxyclav	**Ac**	30	50	Yes
Ampicillin	**A**	2	5	No
Azithromycin	**At**	15	5	No
Cephalothin	**Ch**	30	5	No
Clindamycin	**Cd**	2	12	No
Levofloxacin	**Le**	5	5	No
Linezolid	**Lz**	30	20	No
Penicillin G	**P**	10 U	5	No
Vancomycin	**Va**	30	18	Yes

Table S6. Cellular fatty acids (CFA) analysis from 2 mg of washed and dried cells of strain 585-1.

CFA species*	Peak area (percentage of total), Experiment 1	Peak area (percentage of total), Experiment 2
14:1ω3	0.3%	0.7%
14:0	0.9%	2.2%
ai15	0.1%	0.4%
15:0	0.2%	0.3%
15:1ω6	0.1%	0.2%
i16	0.1%	0.1%
16:1ω9	0.2%	0.2%
16:1ω7c	**5.3%**	**6.1%**
16:1ω7t	0.2%	0.2%
16:1ω5	0.2%	0.1%
16:0	**6.1%**	**6.6%**
16:1ω7a	1.6%	1.4%
16a	3.0%	2.1%
i17	0.9%	0.8%
ai17	0.7%	2.9%
17:1ω8	0.8%	1.4%
17:0	0.7%	0.9%
i17a	0.1%	0.1%
ai17a	1.0%	0.7%
17:1ω8a	0.6%	0.5%
17a	0.1%	0.1%
18:2	0.2%	0.3%
18:1ω9	**31.9%**	**31.4%**
18:1ω7c	5.3%	4.8%
18:1ω7t	3.5%	3.0%
18:1ω5	0.4%	0.4%
18:0	2.3%	1.6%
18:1ω9a	**21.1%**	**20.6%**
18:1ω7ca	5.2%	3.8%
18:1ω7ta	1.0%	1.0%
18:1ω5a	0.3%	0.5%
18a	1.2%	0.9%
i19	0.4%	0.0%
19:0	0.1%	0.6%
i19a	0.1%	0.1%
ai19a	0.2%	0.1%
20:1ω9	0.4%	0.5%
10h18	3.1%	2.3%
20:0	0.2%	0.1%
	100.0%	100.0%

* c, *cis*; t, *trans*; i, *iso*; ai, *anteiso*; a, aldehyde; h, hydroxy

Table S7. List of 204 core proteins used for phylogenetic inference.

Locus tag in str	Protein annotation
TQ39_RS06915	peptidyl-tRNA hydrolase
TQ39_RS00675	RNA methyltransferase
TQ39_RS11970	ATP-dependent DNA helicase RecG
TQ39_RS07070	gamma-glutamyl-phosphate reductase
TQ39_RS06745	Holliday junction resolvase
TQ39_RS15555	thiamine pyrophosphokinase
TQ39_RS02850	RNA polymerase sigma factor RpoD
TQ39_RS10525	hypothetical protein
TQ39_RS05190	50S ribosomal protein L18
TQ39_RS05235	50S ribosomal protein L16
TQ39_RS13860	hypothetical protein
TQ39_RS00710	transcription antitermination protein NusB
TQ39_RS05200	30S ribosomal protein S8
TQ39_RS05765	mRNA interferase PemK
TQ39_RS10565	aminopeptidase
TQ39_RS05275	30S ribosomal protein S10
TQ39_RS02285	16S rRNA maturation RNase YbeY
TQ39_RS09960	non-canonical purine NTP pyrophosphatase
TQ39_RS04565	hypothetical protein
TQ39_RS06225	cysteine--tRNA ligase
TQ39_RS13430	phosphoglucosamine mutase
TQ39_RS01830	excinuclease ABC subunit B
TQ39_RS00625	queuine tRNA-ribosyltransferase
TQ39_RS05145	30S ribosomal protein S13
TQ39_RS10570	ribosomal protein S12 methylthiotransferase
TQ39_RS05280	RNA-binding protein
TQ39_RS10515	riboflavin biosynthesis protein RibF
TQ39_RS16585	ATPase
TQ39_RS05260	50S ribosomal protein L23
TQ39_RS05130	DNA-directed RNA polymerase subunit alpha
TQ39_RS05175	50S ribosomal protein L15
TQ39_RS09950	RNA-binding protein
TQ39_RS05240	30S ribosomal protein S3
TQ39_RS08300	chorismate synthase
TQ39_RS10005	50S ribosomal protein L35
TQ39_RS14235	50S ribosomal protein L27
TQ39_RS14210	transcriptional regulator
TQ39_RS14240	50S ribosomal protein L21
TQ39_RS10530	ribosome-binding factor A
TQ39_RS15615	hypothetical protein
TQ39_RS16435	hypothetical protein
TQ39_RS05725	cytidylate kinase
TQ39_RS00085	alanine--tRNA ligase
TQ39_RS10075	ferredoxin-NADP+ reductase subunit alpha
TQ39_RS04365	tRNA sulfurtransferase ThiI
TQ39_RS00745	stage III sporulation protein AC
TQ39_RS01765	hypothetical protein

TQ39_RS12370	50S ribosomal protein L13
TQ39_RS16590	sigma-70 family RNA polymerase sigma factor
TQ39_RS14225	cysteine--tRNA ligase
TQ39_RS08165	hypothetical protein
TQ39_RS00670	hypothetical protein
TQ39_RS00090	histidine triad nucleotide-binding protein
TQ39_RS11410	ribosome biogenesis GTPase Der
TQ39_RS09185	membrane protein insertase
TQ39_RS10550	transcription termination factor NusA
TQ39_RS00595	DNA repair protein RadA
TQ39_RS07895	nucleoside triphosphate pyrophosphohydrolase
TQ39_RS15600	primosomal protein N'
TQ39_RS05620	RNA methyltransferase
TQ39_RS05165	adenylate kinase
TQ39_RS10805	adenylosuccinate lyase
TQ39_RS04610	DUF378 domain-containing protein
TQ39_RS10070	dihydropyrimidine dehydrogenase subunit A
TQ39_RS11560	argininosuccinate synthase
TQ39_RS13510	1-deoxy-D-xylulose-5-phosphate reductoisomerase
TQ39_RS10685	50S ribosomal protein L11
TQ39_RS09220	hypothetical protein
TQ39_RS02265	DNA mismatch repair protein MutS
TQ39_RS02365	50S rRNA methyltransferase
TQ39_RS02870	IMPACT family protein
TQ39_RS10830	phosphoribosylformylglycinamidine cyclo-ligase
TQ39_RS05790	phenylalanine--tRNA ligase subunit beta
TQ39_RS10510	30S ribosomal protein S15
TQ39_RS09275	16S rRNA methyltransferase
TQ39_RS05195	50S ribosomal protein L6
TQ39_RS05245	50S ribosomal protein L22
TQ39_RS07880	sporulation protein YabP
TQ39_RS13505	RIP metalloprotease RseP
TQ39_RS09270	kinase to dihydroxyacetone kinase
TQ39_RS09295	serine--tRNA ligase
TQ39_RS05215	50S ribosomal protein L24
TQ39_RS09350	ADP-ribose pyrophosphatase
TQ39_RS05700	tRNA-specific adenosine deaminase
TQ39_RS03410	triose-phosphate isomerase
TQ39_RS08545	RNA-binding protein
TQ39_RS10575	hypothetical protein
TQ39_RS15605	guanylate kinase
TQ39_RS10690	50S ribosomal protein L11
TQ39_RS10520	pseudouridine synthase
TQ39_RS08450	hypothetical protein
TQ39_RS08075	methionine--tRNA ligase
TQ39_RS13865	hypothetical protein
TQ39_RS14230	GTPase CgtA
TQ39_RS09250	tRNA modification GTPase
TQ39_RS01880	50S ribosomal protein L9
TQ39_RS04625	uracil phosphoribosyltransferase
TQ39_RS10375	nifR3 family TIM-barrel protein
TQ39_RS02210	elongation factor Ts
TQ39_RS10840	amidophosphoribosyltransferase

TQ39_RS02855	DNA primase
TQ39_RS06530	hypothetical protein
TQ39_RS09845	dimethyladenosine transferase
TQ39_RS00665	arginine repressor ArgR
TQ39_RS00740	stage III sporulation protein AD
TQ39_RS00630	aspartate-semialdehyde dehydrogenase
TQ39_RS02270	DNA repair protein RecO
TQ39_RS09190	ribonuclease P protein component
TQ39_RS15545	stage IV sporulation protein A
TQ39_RS02880	50S ribosomal protein L31
TQ39_RS16490	cell division protein FtsZ
TQ39_RS02295	hypothetical protein
TQ39_RS04700	hypothetical protein
TQ39_RS12375	30S ribosomal protein S9
TQ39_RS04400	formate--tetrahydrofolate ligase
TQ39_RS02275	GTPase Era
TQ39_RS09415	stage 0 sporulation protein
TQ39_RS09865	cell division protein FtsE
TQ39_RS08555	signal recognition particle protein
TQ39_RS02835	DNA-directed RNA polymerase subunit beta'
TQ39_RS13500	PolC-type DNA polymerase III
TQ39_RS13440	Holliday junction DNA helicase RuvB
TQ39_RS05230	50S ribosomal protein L29
TQ39_RS15665	hypothetical protein
TQ39_RS10410	N(6)-L-threonylcarbamoyladenine synthase TsaD
TQ39_RS05210	50S ribosomal protein L5
TQ39_RS10535	translation initiation factor IF-2
TQ39_RS00985	ATP-dependent DNA helicase PcrA
TQ39_RS11435	glucose-6-phosphate isomerase
TQ39_RS10715	hypothetical protein
TQ39_RS05270	50S ribosomal protein L3
TQ39_RS15925	YggS family pyridoxal phosphate enzyme
TQ39_RS02215	30S ribosomal protein S2
TQ39_RS00720	hypothetical protein
TQ39_RS05265	50S ribosomal protein L4
TQ39_RS13525	ribosome recycling factor
TQ39_RS00615	preprotein translocase subunit YajC
TQ39_RS06905	transcription-repair coupling factor
TQ39_RS08560	DNA-binding protein
TQ39_RS03480	adenylosuccinate synthetase
TQ39_RS01110	UDP-N-acetylenolpyruvoylglucosamine reductase
TQ39_RS12250	endonuclease III
TQ39_RS09900	elongation factor P
TQ39_RS01785	DNA mismatch repair protein MutL
TQ39_RS10670	50S ribosomal protein L7/L12
TQ39_RS10000	50S ribosomal protein L20
TQ39_RS10695	preprotein translocase subunit SecE
TQ39_RS10680	50S ribosomal protein L1
TQ39_RS09200	chromosomal replication initiator protein DnaA
TQ39_RS15645	sporulation protein YtfJ
TQ39_RS05180	50S ribosomal protein L30
TQ39_RS05155	translation initiation factor IF-1
TQ39_RS10845	N5-carboxyaminoimidazole ribonucleotide mutase

TQ39_RS00500	hypothetical protein
TQ39_RS04605	DUF378 domain-containing protein
TQ39_RS01165	NrdR family transcriptional regulator
TQ39_RS01190	isoleucine--tRNA ligase
TQ39_RS09995	hypothetical protein
TQ39_RS03760	glycine--tRNA ligase
TQ39_RS08550	30S ribosomal protein S16
TQ39_RS05140	30S ribosomal protein S11
TQ39_RS10555	ribosome assembly cofactor RimP
TQ39_RS08270	3-phosphoshikimate 1-carboxyvinyltransferase
TQ39_RS13520	UDP pyrophosphate synthase
TQ39_RS01770	hypothetical protein
TQ39_RS18975	GTP-binding protein YchF
TQ39_RS03400	phosphoglycerate kinase
TQ39_RS01125	HPr kinase
TQ39_RS13455	GTP-binding protein
TQ39_RS04585	AsnC family transcriptional regulator
TQ39_RS00700	exodeoxyribonuclease VII large subunit
TQ39_RS00620	queuine tRNA-ribosyltransferase
TQ39_RS09970	ribonuclease III
TQ39_RS06240	UDP-N-acetylmuramoylalanine--D-glutamate ligase
TQ39_RS08100	ribulose-phosphate 3-epimerase
TQ39_RS01725	hypothetical protein
TQ39_RS08005	branched chain amino acid aminotransferase
TQ39_RS08310	chorismate mutase
TQ39_RS05250	30S ribosomal protein S19
TQ39_RS01105	RNase adaptor protein RapZ
TQ39_RS05785	phenylalanine--tRNA ligase subunit alpha
TQ39_RS01870	hypothetical protein
TQ39_RS15670	peptidase M50
TQ39_RS00505	recombinase RecR
TQ39_RS09955	non-canonical purine NTP pyrophosphatase
TQ39_RS01865	hypoxanthine phosphoribosyltransferase
TQ39_RS02840	DNA-directed RNA polymerase subunit beta
TQ39_RS00230	NAD synthetase
TQ39_RS01650	GTP-binding protein
TQ39_RS00105	DNA polymerase III subunit delta
TQ39_RS15585	hypothetical protein
TQ39_RS10545	hypothetical protein
TQ39_RS02440	preprotein translocase subunit SecG
TQ39_RS05255	50S ribosomal protein L2
TQ39_RS01875	DNA helicase
TQ39_RS01100	hypothetical protein
TQ39_RS15560	ribosome small subunit-dependent GTPase A
TQ39_RS05125	50S ribosomal protein L17
TQ39_RS11175	RNA methyltransferase
TQ39_RS11220	30S ribosomal protein S20
TQ39_RS13530	UMP kinase
TQ39_RS01635	heat-shock protein Hsp33
TQ39_RS11190	50S ribosomal protein L11 methyltransferase
TQ39_RS15610	hypothetical protein