1 ***Butyribacter intestini* gen. nov., sp. nov., a butyric acid-producing**
2 **bacterium of the family *Lachnospiraceae* isolated from the**
3 **human faeces, and reclassification of *Acetivibrio ethanolgignens***
4 **as *Acetanaerobacter ethanolgignens* gen. nov., comb. nov.**

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27 **Short title:** *Butyribacter intestini* gen. nov., sp. nov.

28 **Contents category:** New taxa - *Firmicutes* and related organisms

29

30 **Footnote:**

31 The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Butyribacter*
 32 *intestini* TF01-11^T is KT388745. The GenBank/EMBL/DDBJ accession numbers for the genome
 33 sequences of TF01-11^T and *Acetivibrio ethanoligignens* ATCC 33324^T are LLKB000000000 and
 34 LNAM000000000, respectively. The data that support the findings of this study have also been
 35 deposited into CNGB Sequence Archive (CNSA: <https://db.cngb.org/cnsa/>) of CNGBdb with
 36 accession number CNPhis0003380 and CNPhis0003389.

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41 Abstract

42 A novel, non-motile, Gram-stain-positive, non-spore-forming, obligate anaerobic
 43 bacterium, designated strain TF01-11^T, was isolated from human faeces. The isolate was
 44 characterized by phylogenetic and phenotypic properties, as well as by determination of its
 45 whole genome sequence. The growth temperature and pH ranges were 30–42 °C and 6.0–8.5,
 46 respectively. The end products of glucose fermentation were butyric acid and a small
 47 amount of acetic acid. The genome was estimated to be 3.61Mbp with G+C content of 36.79
 48 mol%. Genes related to biosynthesis of diaminopimelic acid, polar lipids, polyamines,
 49 teichoic and lipoteichoic acids were present. The predominant fatty acids were C_{16:0} (37.9 %),
 50 C_{14:0} (16.4 %), C_{13:0} OH/iso-C_{15:1} H (11.1 %) and C_{18:1} ω_{9c} (10.6 %). Phylogenetic analyses
 51 based on 16S rRNA gene sequences, the isolate was a member of family *Lachnospiraceae*,
 52 with the highest sequence similarity to the type strain of *Roseburia intestinalis* DSM 14610^T
 53 at 92.18 % followed by *Acetivibrio ethanolgignens* ATCC 33324^T at 91.99 %. The average
 54 nucleotide identity (ANI) calculated for the genomes between strain TF01-11^T and these
 55 closest relatives were 70.5 % and 68.1 %. Based on results of phenotypic characteristics and
 56 genotypic properties presented in this study, strain TF01-11^T represent a novel species in a
 57 new genus, for which the name *Butyribacter intestini* gen. nov., sp. nov. is proposed. The type
 58 strain of the type species is TF01-11^T (CGMCC 1.5203^T = CGMCC 10984^T = DSM 105140^T).
 59 In addition, *Acetivibrio ethanolgignens* is proposed to be reclassified as *Acetanaerobacter*
 60 *ethanolgignens* gen. nov., comb. nov.

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66 Introduction

67 The human intestine is colonized by a large number of microbial communities, which is 10
68 times higher than the total cells in the human body [1]. In total there are more than 4000 different
69 species in the gut (Almeida *et al.*, 2020). Most of the intestinal microbiota species are obligate
70 anaerobes [2]. These gut microbiota play a key role involving nutrition extraction [3], host
71 metabolic [4-6], prevention against pathogens [7] and immune regulation [8, 9]. Current evidence
72 also suggests that the gut microbiota can be considered an environmental factor in development of
73 disease, including obesity [10, 11], diabetes [11-13], inflammatory bowel disease [14, 15] and
74 colorectal cancer [16-18]. Member of the family *Lachnospiraceae* was one of the most abundant
75 groups within the *Firmicutes* [19, 20]. The production of butyric acid, a short-chain fatty acid,
76 links with *Lachnospiraceae* has potentially beneficial effects on the host [21-23]. Butyrate is
77 considered as one beneficial metabolites, which serves as the major energy source of intestinal
78 epithelial cells and has anti-inflammatory properties [24, 25].

79 In this study, we report on the taxonomic characterization of a new butyrate-producing bacterial
80 strain, TF01-11^T, which was isolated from the faeces of a 17-year-old Chinese female. On the
81 basis of the phenotypic, chemotaxonomic, genotypic and phylogenetic data, strain TF01-11^T
82 represents a novel genus in the family *Lachnospiraceae*, with the proposed name, *Butyribacter*
83 *intestini* gen. nov., sp. nov. Furthermore, we suggest reclassification of *Acetivibrio ethanolgignens*
84 to *Acetanaerobacter ethanolgignens* gen. nov., comb. nov.

85

86 Material and methods

87 *Sample collection and bacteria isolation*

88 The fresh faeces sample was transferred immediately to anaerobic box (Bactron Anaerobic
89 Chamber, Bactron[®]-2, shellab, USA) and suspended in 0.1 M PBS (pH 7.0) after collected from a
90 a 17-year-old Chinese female living in Shenzhen. The faeces sample was tenfold diluted and
91 spread-plated onto peptone-yeast extract-glucose (PYG) plates and incubated under anaerobic
92 condition (contained 90 % nitrogen, 5 % hydrogen and 5 % carbon dioxide, by vol.) at 37 °C for 3
93 days. Single colonies were picked and streaked onto PYG agar until a pure culture was obtained
94 according the method previously [26]. The strain was maintained in glycerol suspension (20 %,
95 v/v) at -80 °C and preserved by lyophilization at 4 °C.

96 *Phenotypical characterization*

97 Cellular morphology of strain TF01-11^T was examined using phase contrast microscopy
98 (Olympus BX51, Japan) by using cells grown in prereduced anaerobically sterilized PYG broth at
99 37 °C for 24 h. The Gram reaction and spore formation were performed by staining using Gram
100 stain kit (Solarbio) and spore stain kit (Solarbio) according to the manufacturer's instructions.
101 Motility of cells grown in PYG broth was examined by phase-contrast microscopy. All growth
102 experiments, described below, were evaluated using the PYG medium in two replicates (1 %, v/v,
103 inoculum) and recorded by measuring the OD₆₀₀ of the cultures after 24, 48 h and 7 d,
104 respectively. The temperature range for growth at 4, 10, 15, 20, 25, 30, 37, 42, 45 and 50 °C and
105 the pH range for growth was assessed at pH 3.0–10.0 (at interval of 0.5 pH units). Salt tolerance
106 was determined in PYG broth containing 0–6.0 % (w/v) NaCl (at 1.0 % intervals). Biochemical
107 reactions and carbon source utilization were investigated by using the API ZYM, API 20A and
108 API 50CH tests (bioMérieux) according to the manufacturer's instructions. Short-chain fatty
109 acids (SCFA) produced from fermentation in PYG medium was measured by gas chromatograph
110 (GC-2014C, Shimadzu) using capillary columns packed with porapak HP-INNOWax (Cross-
111 Linked PEG, 30 m × 0.25 mm × 0.25 µm) and detected with a flame-ionization detector. Column
112 temperature was 220 °C, N₂ was used as the carrier gas in all analyses.

113 *Chemotaxonomic analyses*

Cells grown for 48 h at 37 °C on PYG agar plates were used for the whole-cell fatty acid and peptidoglycan analyses. Cellular fatty acids (CFA) of strain TF01-11^T and related species were extracted, methylated and analysed by GC as described previously (Chen & Dong, 2004). The analysed of peptidoglycan structure was carried as described previously [27].

Phylogenetic analysis based on 16S rRNA gene

The genomic DNA was extracted from cells grown in PYG broth for 24 h at 37°C and purified using the method described by Drancourt *et al.* [28]. The 16S rRNA gene sequence was amplified by PCR and sequenced as described previously [27]. The sequence obtained was compared with entries in EzBioCloud server [29]. Phylogenetic analysis was performed by using software package MEGA 7 [30]. Sequences of TF01-11^T and related type species were aligned and used to construct a phylogenetic tree by the neighbour-joining method [31] and maximum likelihood method using CLUSTAL W [32]. In each case, bootstrap values were calculated based on 1000 replications.

Whole Genome analysis

The draft whole-genome sequence of strain TF01-11^T was performed by using a paired-end strategy with the platform Illumina HiSeq 2000 at BGI-Shenzhen (Shenzhen, China). The paired-end library had an mean insert length of 500 bp. Paired-end de novo genome assembly was performed with SOAPdenovo 2 package [33]. The genome sequence of strain TF01-11^T was compared with available genome sequences of representatives of the family *Lachnospiraceae*. All genome sequences were obtained from the GenBank sequence database. Individual coding sequences were annotated using the Rapid Annotation Subsystem Technology (RAST) server [34], freely available at (<http://rast.nmpdr.org>). DNA base content (mol% G+C) was calculated from the whole genome sequence. The average nucleotide identity (ANI) values were calculated for strain TF01-11^T and the most closely related species *R. intestinalis* DSM 14610^T, *A. ethanoligignens* ATCC 33324^T, *Lachnospira multipara* DSM 3073^T and *Coprococcus eutactus* ATCC 27759^T.

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Results and discussion

16S rRNA gene sequencing and phylogenetic analyses

The almost complete 16S rRNA gene sequence of strain TF01-11^T of 1,400 bp was determined. Comparative sequence analysis of strain TF01-11^T and validly published names using the EzBioCloud server revealed that the most similar sequences were those of the members of family *Lachnospiraceae* of the phylum *Firmicutes*. The 16S rRNA gene sequence similarity of strain TF01-11^T and the closest relatives, *Roseburia intestinalis* DSM 14610^T [35] and *Acetivibrio ethanolgignens* ATCC 33324^T [36], were 92.18 % and 91.99 % (Table 3), respectively, which was below the ‘lower cut-off window’ of 95 % for differentiation of a new genus [37, 38]. Furthermore, the phylogenetic analysis showed that strain TF01-11^T, together with the closest relative *A. ethanolgignens* ATCC 33324^T, formed a separate branch within the family *Lachnospiraceae* (Fig. 1 and Supplementary Fig. S1). Additionally, the type species of the genus *Acetivibrio*, *Acetivibrio cellulolyticus*, is phylogenetically classified in the family *Ruminococcaceae* according to the taxonomy list in LPSN (<https://lpsn.dsmz.de/genus/acetivibrio>) and shared a low 16S rRNA gene sequence similarity (85.2 %) with *A. ethanolgignens* ATCC 33324^T. It is obvious that strain *A. ethanolgignens* ATCC 33324^T does not cluster together with *Acetivibrio cellulolyticus* in the family *Ruminococcaceae*, but more closely group in the family *Lachnospiraceae*. This result was also confirmed by the maximum-likelihood method (Supplementary Fig. S1), suggesting that *A. ethanolgignens* ATCC 33324^T should be reclassified as a new genus of the family *Lachnospiraceae*.

Whole genome sequencing and G+C content

Sequencing of the genome produced an annotated genome size of approximately 3.61 Mbp. The G+C content of DNA was 36.79 mol% as calculated from the whole-genome sequence (Table 2). The ANI between strain TF01-11^T and *R. intestinalis* DSM 14610^T, *A. ethanolgignens* ATCC 33324^T, *L. multipara* DSM 3073^T and *C. eutactus* ATCC 27759^T had a maximum value of 70.5 % (Table 3).

For RAST annotation with genome of strain TF01-11^T, there were 11 genes associated with diaminopimelic acid synthesis, 7 genes associated with metabolism of polyamines, 12 genes associated with teichoic and lipoteichoic acids and 20 genes associated with metabolism of polar lipids, respectively, present in the genome (Table 4 and Table S2). No predicted gene sequences with recognizable similarity to those responsible for respiratory lipoquinones, mycolic acids or lipopolysaccharides.

Phenotypic and chemotaxonomic characteristics

Strain TF01-11^T was non-motile, Gram-stain-positive and non-spore-forming. No growth occurred under aerobic conditions. Cells were approximately 0.5–1.0 µm in width and 2.0–8.0 µm in length and occurring singly or in chains. Colonies on PYG agar plates were approximately 2.0 mm in diameter, grayish white, opaque, flat, smooth, dull and irregular with rhizoid margins after 48 h incubation at 37 °C under anaerobic conditions. The temperature for growth range from 30 °C to 42 °C (optimum 37 °C). The pH growth range was pH 6.0–8.5 (optimum pH 7.0). The NaCl tolerance range was 0 %–2.0 % (w/v). The major SCFAs produced in PYG broth were butyric and acetic acids. The main physiological and biochemical properties of strain TF01-11^T are given in Table 1 in comparison with closely related genera within family *Lachnospiraceae*. The predominant cellular fatty acid (>10 %) of strain TF01-11^T were C_{16:0} (37.9 %), C_{14:0} (16.4 %), C_{13:0} OH/iso-C_{15:1} H (11.1 %) and C_{18:1} ω_{9c} (10.6 %) (Table S1, available in the online Supplementary Material), The whole-cell hydrolysate of strain TF01-11^T contained meso-diaminopimelic acid (m-DAP).

Taxonomic conclusions

Base on genomic, phylogeny, phenotypic and chemotaxonomic characteristics of strain TF01-11^T presented above, we propose that this strain isolated from the faeces represents a novel species of a new genus distinct from other currently known species of family *Lachnospiraceae*, for which the name *Butyribacter intestini* gen. nov., sp. nov. is proposed. In addition, we propose reclassifying *Acetivibrio ethanolignens* ATCC 33324^T within a new genus in the family *Lachnospiraceae*, *Acetanaerobacter ethanolignens* gen. nov., comb. nov.

193

194 **Description of *Butyribacter* gen. nov.**

195 *Butyribacter* (Bu.ty.ri.bac'ter. N.L. n. *acidum butyricum* butyric acid; N.L. masc. n. *bacter* a rod;
196 N.L. masc. n. *Butyribacter* a butyric acid-producing rod).

197 Gram-stain-negative, non-motile, non-spore-forming rods, about 2.0–8.0 µm long and 0.5–1.0 µm
198 wide, occurring singly or in chains. Obligately anaerobic. Optimum growth temperature is
199 approximately 37 °C. Butyric and acetic acids are the major metabolic end products in PYG broth.
200 Meso-diaminopimelic acid is present in the hydrolysate of the peptidoglycan. The main fatty acids
201 are C_{16:0}, C_{14:0}, C_{13:0} OH/iso-C_{15:1} H and C_{18:1} ω_{9c}. The genome size is circa 3.6 Mbp. The genus is
202 affiliated to the family *Lachnospiraceae*. The type species is *Butyribacter intestini*.

203

204 **Description of *Butyribacter intestini* sp. nov.**

205 *Butyribacter intestini* (in.tes'ti.ni. L. gen. n. *intestini* of the gut, referring to the ecosystem of origin
206 of the bacterium).

207 Cell morphology is the same as described for the genus. Colonies are approximately 2.0 mm in
208 diameter, grayish white, opaque, flat, smooth, dull and irregular with rhizoid margins after 48 h at
209 37 °C. Growth occurs between 30 and 42 °C (optimum 37 °C) and at pH 6.0–8.5 (optimum pH
210 7.0–7.5). Positive result in tests for acid phosphatase (weak reaction) and α-glucosidase activities,
211 but negative for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine
212 arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-
213 phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-
214 glucosaminidase, α-mannosidase and β-fucosidase. Acid is produced from glucose, ribose (weak
215 positive), galactose, fructose, methyl-D-glucopyranoside, cellobiose, maltose, melibiose, sucrose,
216 starch, turanose, but not from xylose, adonitol, salicin, methyl-β-D-xylopyranoside, arabinose,
217 glycerol, sorbose, dulcitol, melezitose, inositol, raffinose, mannitol, sorbitol, rhamnose, methyl-α-

218 D-mannopyranoside, *N*-acetyl-glucosamine, amygdalin, arbutin, trehalose, inulin, glycogen,
 219 xylitol, gentiobiose, lyxose, tagatose, fucose, arabinol, gluconate, 2-ketogluconate and 5-
 220 ketogluconate. Indole is not produced. Gelatin is liquefied. Aesculin is not hydrolysed. There
 221 were 11 genes/proteins responsible for biosynthesis of DAP, including 4-hydroxy-
 222 tetrahydrodipicolinate reductase (EC 1.17.1.8) (1 gene), 4-hydroxy-tetrahydrodipicolinate
 223 synthase (EC 4.3.3.7) (1 gene), aspartate-semialdehyde dehydrogenase (EC 1.2.1.11) (1 gene),
 224 aspartokinase (EC 2.7.2.4) (1 gene), diaminopimelate decarboxylase (EC 4.1.1.20) (1 gene),
 225 diaminopimelate epimerase (EC 5.1.1.7) (1 gene), L,L-diaminopimelate aminotransferase (EC
 226 2.6.1.83) (1 gene), *N*-acetyl-L, L-diaminopimelate deacetylase (EC 3.5.1.47) (2 genes), UDP-*N*-
 227 acetylmuramoylalanyl-D-glutamate--2,6-diaminopimelate ligase (EC 6.3.2.13) (1 gene), and UDP-
 228 *N*-acetylmuramoylalanyl-D-glutamyl-2,6-diaminopimelate--D-alanyl-D-alanine ligase (EC 6.3.2.10)
 229 (1 gene), 7 genes/proteins responsible for biosynthesis of polyamines, including 5'-
 230 methylthioadenosine nucleosidase (EC 3.2.2.16) @ *S*-adenosylhomocysteine nucleosidase (EC
 231 3.2.2.9) (1 gene), ABC transporter, periplasmic spermidine putrescine-binding protein PotD (TC
 232 3.A.1.11.1) (1 gene), arginine decarboxylase (EC 4.1.1.19) / lysine decarboxylase (EC 4.1.1.18) (1
 233 gene), carbamate kinase (EC 2.7.2.2) (1 gene), putrescine transport ATP-binding protein PotA
 234 (TC 3.A.1.11.1) (1 gene), spermidine Putrescine ABC transporter permease component PotB (TC
 235 3.A.1.11.1) (1 gene), and spermidine putrescine ABC transporter permease component potC
 236 (TC..3.A.1.11.1) (1 gene), 12 genes/protein responsible for biosynthesis of teichoic and
 237 lipoteichoic acids, including 2-*C*-methyl-D-erythritol 4-phosphate cytidyltransferase (EC
 238 2.7.7.60) (2 genes), CDP-glycerol:poly(glycerophosphate) glycerophosphotransferase (EC
 239 2.7.8.12) (2 genes), membrane protein involved in the export of O-antigen, teichoic acid
 240 lipoteichoic acids (1 gene), minor teichoic acid biosynthesis protein GgaB (1 gene), *N*-
 241 acetylmannosaminyltransferase (EC 2.4.1.187) (1 gene), teichoic acid export ATP-binding protein
 242 TagH (EC 3.6.3.40) (3 genes), teichoic acid glycosylation protein (1 gene), and teichoic acid
 243 translocation permease protein TagG (1 gene), and 20 genes/protein responsible for biosynthesis
 244 of polar lipids, including 1-acyl-sn-glycerol-3-phosphate acyltransferase (EC 2.3.1.51) (2 genes),
 245 acyl carrier protein (2 genes), acyl-phosphate:glycerol-3-phosphate O-acyltransferase PlsY (1
 246 gene), alcohol dehydrogenase (EC 1.1.1.1) (1 gene), acetaldehyde dehydrogenase (EC 1.2.1.10) (1

gene), aldehyde dehydrogenase (EC 1.2.1.3) (1 gene), cardiolipin synthetase (EC 2.7.8.-) (1 gene),
 CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase (EC 2.7.8.5) (1 gene), CDP-
 diacylglycerol--serine O-phosphatidyltransferase (EC 2.7.8.8) (1 gene), dihydroxyacetone kinase
 family protein (1 gene), glycerate kinase (EC 2.7.1.31) (1 gene), glycerol-3-phosphate
 dehydrogenase (EC 1.1.5.3) (1 gene), glycerol-3-phosphate dehydrogenase [NAD(P)⁺] (EC
 1.1.1.94) (1 gene), phosphate:acyl-ACP acyltransferase PlsX (1 gene), phosphatidate
 cytidyltransferase (EC 2.7.7.41) (1 gene), phosphatidylglycerophosphatase B (EC 3.1.3.27) (1
 gene), and phosphatidylserine decarboxylase (EC 4.1.1.65) (1 gene). There are no genes
 responsible for biosynthesis of respiratory lipoquinones, mycolic acids or lipopolysaccharides.

The type strain is TF01-11^T (CGMCC 1.5203^T = CGMCC 10984^T = DSM 105140^T), isolated from
 the faeces obtained from a 17-year-old Chinese female. The DNA G+C content of the type strain
 is 36.79 mol%.

259

260 **Description of *Acetanaerobacter* gen. nov.**

261 *Acetanaerobacter* (A.cet.an.ae.ro. bac'ter. L. n. *acetum* vinegar; Gr. pref. *an* not; Gr. masc. n. *aer*
 262 air; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Acetanaerobacter* vinegar-producing anaerobic rod).

263 Cells are nonsporeforming, motile, Gram-stain-negative rods, obligately anaerobic, which do not
 264 grow under microaerophilic or aerobic conditions. Growth occurs between 30 and 42 °C
 265 (optimum 37 °C) and at pH 7.0–9.0 (optimum pH 7.0). Acetic and lactate acids are the major
 266 metabolic end products in PYG broth. Major fatty acids are C_{14:0}, C_{16:0} and C_{18:1} ω_{9c}. The
 267 genome size is circa 3.7 Mbp. The genomic DNA G+C content of the type species is 41.0 mol%.
 268 The type species is *Acetanaerobacter ethanoligignens*.

269

270 **Description of *Acetanaerobacter ethanoligignens* comb. nov.**

271 The description of the species is given by Robinson & Ritchie (1881) and determined from this
 272 study. Cells are about 0.5–0.9 µm long and 1.5–2.5 µm wide, occurring singly, in pairs, and often
 273 in short chains. Colonies are yellowish-white, circular, convex, smooth, translucent, and 0.5 to 1.5
 274 mm in diameter on PYG plate after 2–3 days of cultivation. The fermentation products is acetic
 275 acid, ethanol, hydrogen, and carbon dioxide. Positive result in tests for leucine arylamidase
 276 activity, but negative for acid phosphatase, α -glucosidase activities, alkaline phosphatase, esterase
 277 (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -
 278 chymotrypsin, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, β -
 279 glucuronidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and β -fucosidase. Acid
 280 is produced from glucose, mannose, lactose, maltose, salicin, mannose, cellobiose (weak positive),
 281 raffinose (weak positive), fructose, galactose, mannitol, pyruvate and starch (weak positive), but
 282 not from xylose, arabinose, glycerol, melezitose, sorbitol, rhamnose, trehalose, adonitol, lactate,
 283 raffinose, ribose and sucrose. Indole is not produced. Gelatin is liquefied. Aesculin is not
 284 hydrolysed.

285 The type strain is 77-6^T (= ATCC 33324^T = DSM 3005^T)

286

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418 **Figure legends**

419 **Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequence of strain TF01-11^T
 420 and related type species of the family *Lachnospiraceae* and *Ruminococcaceae*. Bootstrap values (>
 421 90 %) based on 1000 replications are shown at branch nodes. *Clostridium butyricum* is used as an
 422 out-group. Bar, 20 % nucleotide sequence divergence.

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437 **Table 1.** Differential phenotypic features among TF01-11^T and the type species of
438 phylogenetically closely related members of family *Lachnospiraceae*.

439 Strains: 1, TF01-11^T; 2, *R. intestinalis* DSM 14610^T; 3, *A. ethanoligignens* ATCC 33324^T; 4, *L.*
440 *multipara* DSM 3073^T. Data were from Duncan *et al.* (2006), Robinson & Ritchie (1981), Bryant
441 (1986) and this study. +, Positive; w, weakly positive reaction; –, negative; ND, no data available;
442 SCFA, short-chain fatty acid.

Characteristic	1	2	3	4
Isolation source	Human faeces	Human faeces	Pigs colons	Cattle rumen
Cell size (µm)	0.5–1.0×2.0–8.0	0.5×1.5–3	0.5–0.9×1.5–2.5	0.4–0.6×2.0–4.0
Gram-stain	Positive	Variable	Negative	Weakly positive
Motility/flagella	–	+ / Multiple	+ / Fascicle	+ / Monotrichous
Major SCFA(s) produced	a, b, l	b, f, l	a, l	a, f, l
Growth:				
Temperature range (°C)	30–42	25–42	30–42	25–50
pH range	6.0–8.5	6.0–8.0	7.0–9.0	6.0–9.0
Salt tolerance (%)	2	2	1	2
Aesculin hydrolysis	–	+	–	+
Gelatin hydrolysis	+	–	+	–
Acid from:				
Cellobiose	+	+	–	+
D-Arabinose	–	+	–	–
D-Galactose	+	ND	+	–

D-Lactose	–	–	+	–
D-Maltose	+	–	+	–
D-Mannitol	–	–	+	–
D-Mannose	–	–	+	–
Salicin	–	–	+	–
Starch	+	+	w	–
Sucrose	+	+	–	+
Enzyme activity (API ZYM)				
Esterase (C4)	–	+	–	–
Esterase lipase (C8)	–	w	–	–
Leucine arylamidase	–	–	+	w
Acid phosphatase	w	–	–	+
β -Galactosidase	–	+	–	–
β -Glucuronidase	–	w	–	–
α -Glucosidase	+	–	–	–
β -Glucosidase	–	w	–	–

‡ a, Acetate; b, butyrate; f, formate; l, lactate;

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445 **Table 2.** Genome size and DNA G+C content of Strain TF01-11^T compared with three closely
446 related isolates of the family *Lachnospiraceae*. The data of *R. intestinalis* DSM 14610^T, *A.*
447 *ethanolgignens* ATCC 33324^T, *C. eutactus* ATCC 27759^T and *L. multipara* DSM 3073^T were
448 from NCBI.

Strain	Accession number	Genome size (×10 ⁶ bp)	G+C content (mol%)
Strain TF01-11 ^T	LLKB00000000	3.61	36.79
<i>R. intestinalis</i> DSM 14610 ^T	ABYJ00000000	4.38	42.6
<i>A. ethanolgignens</i> ATCC 33324 ^T	LNAM00000000	3.66	41.0
<i>C. eutactus</i> ATCC 27759 ^T	ABEY00000000	3.10	43.1
<i>L. multipara</i> DSM 3073 ^T	AUJG00000000	2.87	35.3

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Table 3. Levels of 16S rRNA gene sequence similarity and ANI values (in percentages) based on BLAST for strains TF01-11^T and the most closely related members of the family *Lachnospiraceae*.

Strains: TF01-11^T; 2, *R. intestinalis* DSM 14610^T; 3, *A. ethanolgignens* ATCC 33324^T; 4, *C. eutactus* ATCC 27759^T; 5, *L. multipara* DSM 3073^T.

Strain	Accession no.	1	2	3	4	5
16S rRNA gene sequence similarity (%)						
TF01-11 ^T	KT388745	100				
<i>R. intestinalis</i> DSM 14610 ^T	AJ312385	92.18	100			
<i>A. ethanolgignens</i> ATCC 33324 ^T	FR749897	91.99	93.22	100		
<i>C. eutactus</i> ATCC 27759 ^T	NR044049	89.84	91.54	91.08	100	
<i>L. multipara</i> DSM 3073 ^T	FR733699	90.60	92.34	91.48	92.43	100
ANI values (%)						
TF01-11 ^T	LLKB00000000	100				
<i>R. intestinalis</i> DSM 14610 ^T	ABYJ00000000	70.5	100			
<i>A. ethanolgignens</i> ATCC 33324 ^T	LNAM00000000	68.1	68.3	100		
<i>C. eutactus</i> ATCC 27759 ^T	ABEY00000000	69.3	69.5	68.9	100	
<i>L. multipara</i> DSM 3073 ^T	AUJG00000000	66.9	66.3	66.2	66.7	100

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Table 4. Number of genes identified in biosynthetic pathway from whole genome sequences of strain TF01-11^T and related organisms identified by RAST.

Taxa: 1, TF01-11^T; 2, *R. intestinalis* DSM 14610^T; 3, *A. ethanolgignens* ATCC 33324^T; 4, *C. eutactus* ATCC 27759^T; 5, *L. multipara* DSM 3073^T; 6, *Anaerostipes caccae* DSM 14662^T. Data are for type strains. Numbers of genes identified for benzoquinones (ubiquinones, rhodoquinones, plastoquinones), naphthoquinones (menaquinones, demethylmenaquinones, monomethylmenaquinones, menathioquinones), lipopolysaccharides and mycolic acids were zero for all taxa studied.

Genes responsible for biosynthesis	1	2	3	4	5	6
Diaminopimelic acid	11	12	10	13	12	13
Polar lipids	20	26	19	18	18	39
Polyamines	7	17	12	7	13	9
Teichoic and lipoteichoic acids	12	13	8	2	3	10