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Fournierella massiliensis gen. nov., sp. nov., a new human-associated member of the family *Ruminococcaceae*

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Abstract

An anaerobic bacterium, strain AT2^T, was isolated from the fresh stool sample of a healthy French man using the culturomics approach. The 16S rRNA gene sequence analysis showed that strain AT2^T had 95.2 % nucleotide sequence similarity with *Gemmiger formicilis* ATCC 27749^T, the phylogenetically closest species with standing in nomenclature. Cells are Gram-stain-negative, catalase- and oxidase-negative, obligately anaerobic, non-motile, non-spore-forming, rod-shaped, and the bacilli were mesothermophilic. The major fatty acids were $C_{16:0}$ (43.8 %) and $C_{18:1n9}$ (20 %). The DNA G+C content of the strain based on its genome sequence was 56.8 mol%. Based on the phenotypic, biochemical and phylogenetic analysis, we propose the creation of the genus *Fournierella* gen. nov., which contains strain AT2^T (=CSUR P2014^T=DSM 100451^T) as the type strain of the type species *Fournierella massiliensis* gen. nov., sp. nov.

Culturomics is a new approach for the characterization of living microbial diversity in any environmental or human sample [1]. With the development of new technologies such as high-throughput sequencing enabling public access to the complete genome sequences of many bacterial species, we proposed the inclusion of the complete genome sequence analysis in a polyphasic approach to describe new bacterial taxa [2]. This strategy, which we named taxono-genomics, combines phenotypic characteristics, notably the matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS spectrum and genomic properties [3, 4].

During an exploratory study of fresh stool by culturomics [1], an isolate was obtained and a new genus was proposed to accommodate this strain as a member of the family *Ruminococcaceae* [5]. At the time of writing, the family *Ruminococcaceae* contained 17 genera, including *Acetanaerobacterium*, *Acetivibrio*, *Anaerobacterium*, *Anaerofilum*, *Anaerotruncus*, *Ercella*, *Ethanoligenens*, *Faecalibacterium*, *Fastidiosipila*, *Gemmiger*, *Hydrogenoanaerobacterium*, *Oscillibacter*, *Oscillospira*, *Papillibacter*, *Ruminococcus*, *Sporobacter* and *Subdoligranulum* (www.bacterio.net/ruminococcaceae.html). Among members of the family, *Faecalibacterium prausnitzii* is one of the most abundant bacteria of the human gut. It is an extremely oxygen-sensitive bacterium that is difficult to cultivate, even in anaerobic conditions [6]. *F. prausnitzii* sustains growth in the presence of low partial pressure of oxygen, in presence of antioxidants [7], and showed mutualism with epithelial cells, possibly through mucin [8]. *F. prausnitzii* is one of the leading representatives of the human healthy mature anaerobic gut microbiota (HMAGM), suggesting the link between dietary antioxidants and maintenance of the HMAGM [9, 10]. It contributes to maintaining host–microbial homeostasis by secreting a microbial anti-inflammatory molecule that inhibits cellular NF-κB signalling and inflammation [11]. Changes in the abundance of *F. prausnitzii* have been linked to dysbiosis in several human disorders [12]. Here, we propose the main phenotypic, phylogenetic and genomic properties of strain AT2^T (=CSUR P2014^T =DSM 100451^T), that is close to but substantially differs from *Gemmiger formicilis*.

Strain AT2^T was isolated from a fresh stool sample collected from a healthy 28-year-old French man in January 2015. The stool sample was immediately stored at 4 °C after collection until being used for culture. The donor gave a written informed consent and the study was validated by the ethics committee of the Institut Federatif de Recherche

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Keywords: ; *Fournierella massiliensis*; ; taxonogenomics; ; culturomics; ; gut microbiota; ; human microbiome; ; anaerobic bacteria.
Abbreviations: dDDH, digital DNA-DNA hybridization; FAME, fatty acid methyl esters; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; ML, maximum-likelihood; SCFA, short-chain fatty acid.
The GenBank/EMBL/DDBJ accesion numbers for the genome sequence and 16S rRNA gene sequence of strain AT2^T are FAUK00000000 and LN846908, respectively.
Four supplementary figures and five supplementary tables are available with the online Supplementary Material.

IFR48 under agreement number 09–022. To isolate the novel strain, 1 g stool sample was injected in an anaerobic blood culture bottle (BACTEC Lytic/10 Anaerobic/F Culture Vials) supplemented with 4 ml filter-sterilized rumen fluid and 5 % sheep blood, and then incubated at 37 °C. After incubation for 3 days, 100 µl culture suspension was collected, plated on 5 % sheep blood-enriched Columbia agar (BioMérieux) and incubated at 37 °C in an anaerobic atmosphere for 48 h. Emerging colonies were subcultured individually for purification using the same conditions, and identified by MALDI-TOF MS as described by Seng *et al.* [13]. Isolates were re-streaked three times and purity was confirmed by direct examination and MALDI-TOF MS analysis. Purity was confirmed when all 12 spots from 12 different colonies yielded 12 perfectly matching MALDI-TOF MS spectra. When the strain was not identified by MALDI-TOF MS, its 16S rRNA gene sequence was assessed as previously reported [14] using the fD1-rP2 primers, a GeneAmp PCR System 2720 thermal cycler (Applied Biosystems) and an ABI Prism 3130-XL capillary sequencer (Applied Biosciences).

For taxonomic assignment, the Chromas Pro 1.34 software (Technelysium Pty.) was used to correct sequences. Pairwise sequence similarities were calculated using the method recommended by Meier-Kolthoff [15] for the 16S rRNA gene sequence, available via the GGDC web server [16] and at http://ggdc.dsmz.de/. Phylogenies were inferred by the GGDC web server using the DSMZ phylogenomics pipeline [17] adapted to single genes. A multiple sequence alignment was created with MUSCLE [18]. A maximum-likelihood (ML) tree was inferred from the alignment with RAxML [19]. Rapid bootstrapping in conjunction with the autoMRE boot stopping criterion [20] and subsequent search for the best tree was used. The sequences were checked for a compositional bias using the X^2 test as implemented in PAUP* [21].

Different growth temperatures (25, 28, 37, 45 and 55 °C) were tested. Growth of the strain was tested under anaerobic and microaerophilic conditions using GENbag Anaer and GENbag Microaer systems, respectively (BioMérieux), and under aerobic conditions, with or without 5 % CO_2. Transmission electron microscopy of the strain, using a Tecnai G20 (FEI company) at an operating voltage of 60kV, was performed after negative staining. The pH for growth of the strain was tested at a range from pH 6 to 8.5. Tolerance to NaCl was tested using a range of 5–100 g l^{-1} NaCl on Schaedler agar with 5 % sheep blood (BioMérieux) in an anaerobic atmosphere. Gram staining was performed and observed using a Leica DM 2500 photonic microscope (Leica Microsystems) with a ×100 oil immersion lens. In addition to Gram staining, the KOH test was carried out to confirm the cell-wall type according to the procedures described elsewhere [22, 23]. Staining was also performed under anaerobic conditions as differences can occur with exposure to oxygen [24].

Motility of the bacterium was assessed using a Leica DM 1000 photonic microscope (Leica Microsystems) at ×100 magnification. A thermic shock at 80 °C for 20 min on fresh colonies of the strain was carried out in order to test sporulation. The viability of cells was checked by subculturing them on the same media before heating, while the motility of strain $AT2^T$ was tested observing fresh colonies using a DM1000 photonic microscope (Leica Microsystems) with a ×100 oil-immersion objective lens. Catalase (BioMérieux) activity was determined in 3 % hydrogen peroxide solution, and oxidase activity was assessed using an oxidase reagent (Becton Dickinson). Biochemical properties of the strain were investigated using API ZYM, 20A and 50CH strips (BioMérieux) according to the manufacturer's instructions. The antibiotic susceptibility of strain $AT2^T$ was tested using the disc diffusion method [25]; the results are shown in the supplementary data. Cellular fatty acid methyl ester (FAME) analysis was performed by GC/MS. Two samples were prepared with approximately 5 mg bacterial biomass per tube harvested from several culture plates. FAMEs were prepared as described by Sasser [26]. GC/MS analyses were carried out as described before [27]. Briefly, FAMEs were separated using an Elite 5 MS column and monitored by mass spectrometry (Clarus 500 - SQ 8 s; Perkin Elmer). Spectral database search was performed using MS Search 2.0 operated with the Standard Reference Database 1A (NIST) and the FAMEs mass spectral database (Wiley).

For the analysis of short-chain fatty acid (SCFA) production, a Wilkins-Chalgren-Anaerobe broth supplemented with cysteine and DTT (reduced WCA broth described previously by Kläring *et al.* and Pfeiffer *et al.* [28, 29]) and prepared using strictly anaerobic techniques (100 % N_2) was used. Samples were collected at 24, 48 and 72 h after inoculation. Acetic, propanoic, butanoic, isobutanoic, pentanoic, hexanoic and heptanoic acids were purchased from Sigma Aldrich. A stock solution was prepared in water/methanol (50 %, v/v) at 0.1 M for each SCFA and then stored at –20 °C. Calibration standards were freshly prepared in water: 0.05, 0.5, 1, 5 and 10 mM. Culture samples, prepared in duplicate, were centrifuged 5 min at 13 000 r.p.m. and the supernatants were collected. All solutions were adjusted to pH 2–3 with HCl before injection. SCFAs were measured with a Clarus 500 chromatography system connected to a SQ8s mass spectrometer (Perkin Elmer). Analysis was performed with an Elite FFAP column (30 m, 0.25 mm id, 0.25 µm film thickness) such as detailed previously [30]. Injection volume was 0.5 µl (split less, 200 °C). Helium was supplied at 1 ml min^{-1} as the carrier gas. Compounds were separated according to a linear temperature gradient from 100 to 200 °C at 8 °C min^{-1}. Selected ion recording mass spectrometry SCFA analysis by GC/MS was performed using the following masses: 43 m/z for isobutanoic acid; 60 m/z for acetic, butanoic, pentanoic, hexanoic and heptanoic acids; 74 m/z for propanoic acid. The transfer line and the electron impact source were set at 200 °C. Quadratic calibration curves were automatically fitted with an acceptable coefficient of determination above 0.999 and deviation below 20 % (Turbo mass 6.1, Perkin Elmer). SCFA

quantities in samples were presented after subtraction of the quantities found in the blank samples.

Genomic DNA (gDNA) of the strain was sequenced using a MiSeq sequencer (Illumina) with the mate-pair strategy. The gDNA was barcoded in order to be mixed with 11 other projects with the Nextera Mate Pair sample prep kit [31]. The gDNA was quantified by a Qubit assay with the high sensitivity kit (Life technologies) to 72.2 ng μl^{-1}. The mate-pair library was prepared with 1.5 µg genomic DNA using the Nextera mate-pair Illumina guide. The gDNA sample was simultaneously fragmented and tagged with a mate-pair junction adapter. The fragmentation pattern was validated on an Agilent 2100 BioAnalyzer (Agilent Technologies) with a DNA 7500 labchip. The DNA fragments ranged in size from 1.5 kb up to 11 kb with a maximum at 6.7 kb. No size selection was performed and 412 ng of tagmented fragments were circularized. The circularized DNA was mechanically sheared to small fragments with a maximum at 1033 bp on the Covaris device S2 in T6 tubes (Covaris). The library profile was visualized on a High Sensitivity Bioanalyzer LabChip (Agilent Technologies) and the final concentration library was measured at 24.1 nmol l^{-1}. The libraries were normalized at 4 nM and pooled. After a denaturation step and dilution, the pool of libraries was loaded onto the reagent cartridge and then onto the instrument along with the flow cell. Automated cluster generation and sequencing run were performed in a single 2×251 bp run.

MALDI-TOF MS failed to identify strain AT2^T at the genus and species levels. Therefore, its spectrum was added to our database to improve its content. For phylogenetic analysis, the input nucleotide matrix comprised 11 operational taxonomic units and 1572 characters, 405 of which were variable and 267 of which were parsimony-informative. The base-frequency check indicated no compositional bias (P=0.99, α=0.05). ML analysis under the GTR+Gamma model yielded a highest log likelihood of −5973.03, whereas the estimated alpha parameter was of 0.17. The ML bootstrapping converged after 100 replicates; the average support was of 87.75 %.

The 16S rRNA gene sequencing showed that the strain AT2^T exhibited 95.2 % nucleotide sequence similarity with *Gemmiger formicilis*, the phylogenetically closest species with standing in nomenclature [32]. The resulting phylogenetic tree highlighting the position of strain AT2^T with the phylogenetically closest species with a validly published name is shown in Fig. 1 (see also Figs S1 and S2, available in the online Supplementary Material), and strain AT2^T was thus classified in the family *Ruminococcaceae* [5]. Differences in MALDI-TOF MS spectra between strain AT2^T and other closely related species with available spectrum are presented in Fig. S3.

Colonies of strain AT2^T obtained on 5 % sheep blood-enriched Columbia agar (BioMérieux) were translucent with a diameter of 0.3 to 1 mm. Growth of the strain was observed in anaerobic and microaerophilic atmospheres at 28, 37 and 45 °C but optimal growth was observed in an anaerobic atmosphere at 37 °C after incubation for 48 h. No growth was obtained at 55 °C or in an aerobic atmosphere. Cells of strain AT2^T were Gram-stain-negative (confirmed by the KOH test and under anaerobic conditions), rod-shaped, measured 0.5 µm in diameter and 2 µm in length (Fig. 2), were non-motile, non-spore-forming and without catalase and oxidase activities. The strain grew at a pH ranging from 6 to 8.5, with optimal growth at pH 7.0 to 7.3. No growth was observed on Schaedler agar enriched with 5 % sheep blood at 10 g l^{-1} NaCl. The main characteristics of strain AT2^T compared to the closest species are shown in Table 1. The classification and general features of strain AT2^T are summarized in Table S1. Analysis of the total cellular fatty acid composition demonstrated that the major fatty acid was the saturated acid $C_{16:0}$ (43.8 %) followed by the unsaturated acid $C_{18:1n9}$ (20 %). Values represent the GC area percentage from total identified fatty acid methyl esters only (aldehydes, dimethyl acetates and unidentified 'summed features' described previously were not included). Cellular fatty acid profiles of strain AT2^T compared with other closely related species are summarized in Table 2.

Strain AT2^T produced SCFAs after 24, 48 and 72 h of culture in reduced WCA broth. After 72 h, the production of acetic acid was predominant (>10 mM), higher than butanoic (6.0±0.3 mM), isobutanoic (2.4±0.1 mM), propanoic (0.6±0.1 mM), pentanoic (0.1±0.1 mM), isopentanoic and isohexanoic acids (the last two were not quantified). Hexanoic and heptanoic acids were not detected.

The draft genome of strain AT2^T was deposited in EMBL-EBI under accession number FAUK00000000 (Fig. S4), it is 3 829 842 bp long with a G+C content of 56.8 %. It is composed of 19 scaffolds (27 contigs). Of the 3632 predicted genes, 3553 were protein-coding genes, and 79 were RNAs (one 16S rRNA, four 23S rRNA, six 5S rRNA, 68 tRNAs). A total of 2514 genes (70.7 %) were assigned a putative function by COGs or NR BLAST comparison. A total of 298 genes (8.4 %) were identified as ORFans. Using ARG-ANNOT [33], two genes (0.06 %) associated with resistance were detected and seven genes (0.20 %) associated to PKS or NRPS [34] were discovered through genome analysis. Using PHAST and RAST, 1799 genes (50.6 %) were associated to mobilome elements. The remaining genes (616) were annotated as hypothetical proteins (Table S2). The distribution of genes into COGs functional categories is shown in Table S3. Considering closest species with available genome, the digital DNA–DNA hybridization (dDDH) values ranged from 23.4 % with *Subdoligranulum variabile* BI 114^T to 36.6 % with *F. prausnitzii* ATCC 27768^T (Table S4).

Compared with the closest phylogenetic species (Fig. 1), the phylogenetic distance between strain AT2^T and its closest neighbour was superior to the distance between *G. formicilis* X2-56^T and *S. variabile* BI 114^T, between *G. formicilis* X2-56^T and *F. prausnitzii* ATCC 27768^T and between *S. variabile* BI 114^T and *F. prausnitzii* ATCC 27768^T. Phylogenomics analysis was not possible given the unavailability of the

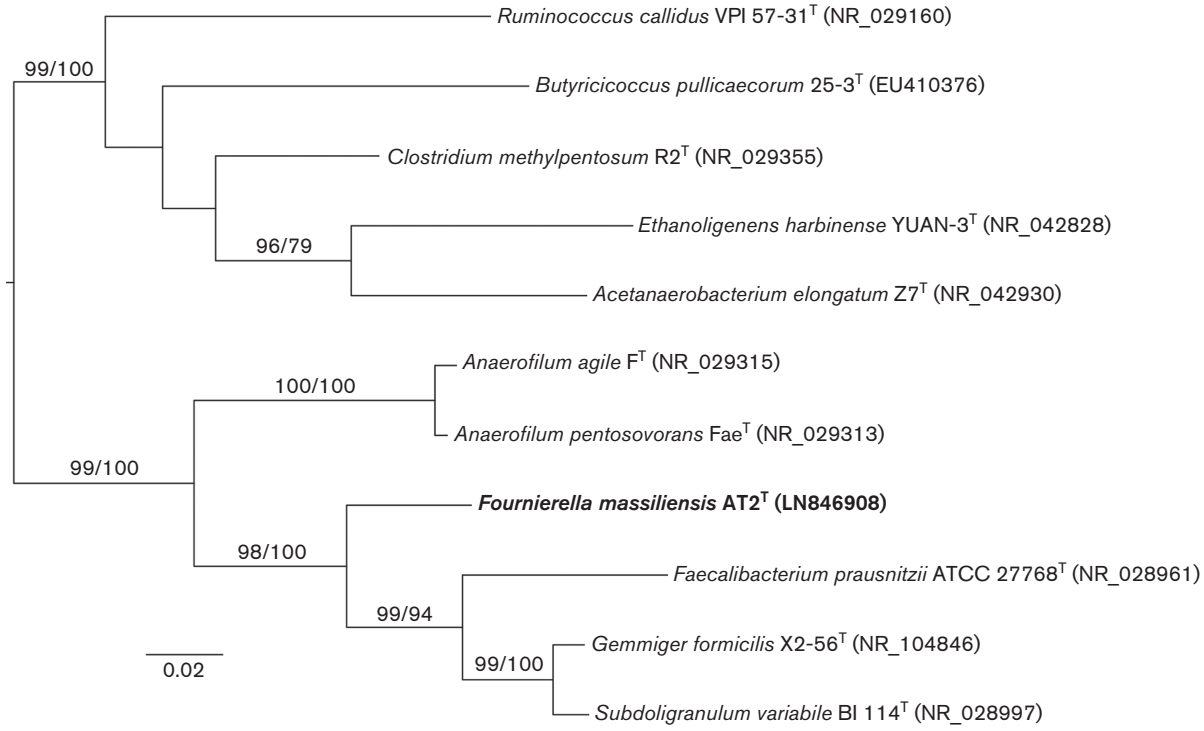

Fig. 1. ML tree inferred under the GTR+GAMMA model and rooted by midpoint-rooting. The branches are scaled in terms of the expected number of substitutions per site. umbers above the branches are support values when larger than 60 % from ML (left) and MP (right) bootstrapping.

closest species' genomes, but genomic comparisons (dDDH and AGIOS, reported in Tables S4 and S5, respectively) confirmed that the similarities between strain AT2^T and the closest species are in accordance with the proposition of a new genus in the family *Ruminococcaceae*. Moreover, the G+C% difference exceeded 1 % (−2.2 % compared with *G. formicilis* X2-56^T, +4.6 % compared with *S. variabile* BI 114^T). According to Qin *et al.* [35], a strain from a new genus will have less than 50 % pairwise percentage of conserved proteins with its closest phylogenetic neighbours. This percentage was 25.4 % (902/3553) with *F. prausnitzii* ATCC 27768^T and 29.4 % (1045/3553) with *S. variabile* BI 114^T, confirming strain AT2^T as a member of a new genus (Table S5). The genome of *G. formicilis* was not available but the 16S rRNA gene phylogenetic distance between strain AT2^T and *G. formicilis* X2-56^T was very similar to that of *F. prausnitzii* ATCC 27768^T (Fig. 1). The 16S rRNA gene similarity values further support the proposal of a novel genus. Indeed, Yarza *et al.* [36] reported a median sequence identity of 96.4 % (95 % confidence interval 96.2 to 96.55) to distinguish two genera. This confirms our strain as a new genus (95.2 % 16S rRNA gene sequence similarity with *G. formicilis* X2-56^T, its closest phylogenetic neighbour). Kim *et al.* [37] also confirmed a taxonomic coherence between genomic and 16S rRNA gene sequence similarity for taxonomic demarcation of prokaryotes.

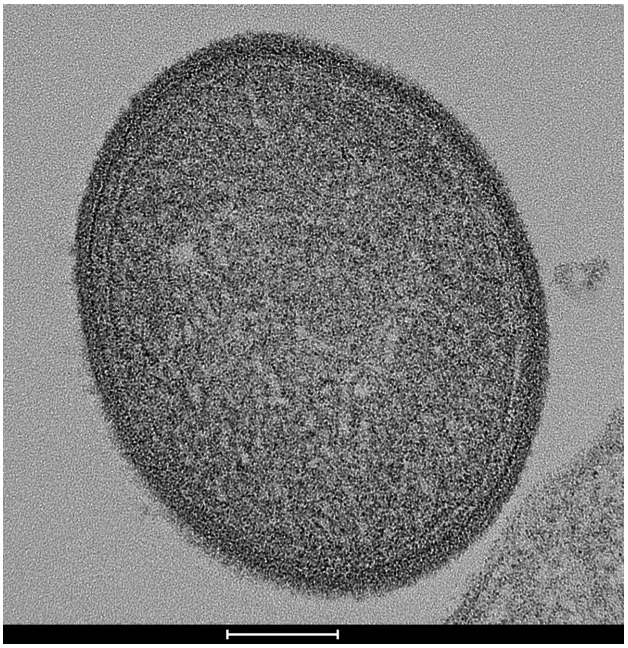

Fig. 2. Transmission electron micrograph of strain AT2^T, obtained using a Tecnai G20 (FEI company) at an operating voltage of 60kV. Bar, 100 nm.

By comparison with reference strains of other closely related species (Table 1), strain AT2^T differed in the combination of nitrate reductase activity (presence), use of L-arabinose (absence), and production of SCFA (acetic acid was the major SCFA produced, only a small amount of butyric acid was produced). Interestingly, strain AT2^T produced acetic acid while the reference strain of *F. prausnitzii* (ATCC 27768^T) consumed it. Moreover, MALDI-TOF analysis did not allow identifying previously known species. These phenotypic differences along with genomic and phylogenetic findings led us to propose that strain AT2^T (=CSUR P2014^T =DSM 100451^T) is the representative strain of a novel species of a new genus within the family *Ruminococcaceae* for which we propose the name *Fournierella massiliensis* gen. nov., sp. nov.

This bacterium was isolated from the faeces of a 28-year-old healthy French man living in Marseilles, France and may have a beneficial role in the gut through butyrate production. Butyrate is the preferred energy source for colonic epithelial cells and is thought to play an important role in maintaining colonic health in humans [38]. Moreover, the production of a significant amount of acetate promotes further the butyrate production in the gut since fifty percent of the butyrate-producing isolates are net acetate consumers during growth, probably because they employ the butyryl coenzyme A-acetyl coenzyme A1 transferase pathway for butyrate production [39].

DESCRIPTION OF *FOURNIERELLA* GEN. NOV.

Fournierella (Four.nier.el'la. N.L. fem. n. *Fournierella* named after the French clinical microbiologist Pierre-Edouard Fournier for his contribution to the taxono-genomic description of the bacteria).

Cells are Gram-negative-staining and the non-motile bacilli are 0.5 µm in diameter and 2 µm in length, and anaerobic. Optimal growth is observed at 37 °C and pH tolerance ranges from pH6–8.5. Cells do not produce catalase and oxidase.

The type species is *Fournierella massiliensis*.

DESCRIPTION OF *FOURNIERELLA MASSILIENSIS* SP. NOV.

Fournierella massiliensis (mas.si.li.en'sis. L. fem. adj. *massiliensis* of *Massilia*, the Latin name of Marseilles).

In addition to the characteristics given in the genus description, colonies grown on 5 % sheep blood-enriched Columbia agar are white with a diameter of 1 mm. Unable to produce indole. Using an API 50CH strip (BioMérieux), positive reactions are observed for glycerol, D-galactose, D-glucose, D-fructose, D-mannose, methyl α-D-glucopyranoside, aesculin ferric citrate, salicin, maltose, lactose, melibiose, sucrose, D-melezitose, rafinose, turanose and potassium 5-ketogluconate. Negative reactions are observed for erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, cellobiose, trehalose, inulin, starch, glycogen, xylitol, gentiobiose, D-xylose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate and potassium 2-ketogluconate.

Table 1. Differential characteristics of strain AT2^T compared with other closely related species

Strains: 1, AT2^T (data from this study); 2, *Gemmiger formicilis* X2-56^T (=ATCC 27749^T) [40 – Salanitro *et al.*, 1976); 3, *Subdoligranulum variabile* BI 114^T (=DSM 15176^T) [41]; 4, *Faecalibacterium prausnitzii* ATCC 27768^T [6]; 5, *Anaerofilum pentosovorans* Fae^T (=DSM 7168^T) [42]; 6, *Anaerofilum agile* strain F^T (=DSM 4272^T) [42]. All strains were strict anaerobes. +, Positive; –, negative; NA, data not available; v, variable; w, weak.

	1	2	3	4	5	6
Cell diameter (µm)	0.5–2	0.3–1	0.6–2.5	0.5–0.9/2–14	0.2–0.6/3–6	0.2–0.6/3–6
Gram stain	–	v	–	–	v	v
Motility	–	–	–	–	v	+
Production of nitrate reductase	+	–	–	–	NA	NA
Utilization of:						
L-Arabinose	–	NA	NA	NA	+	+
D-Mannose	+	NA	+	NA	+	+
D-Mannitol	–	NA	–	NA	+	+
Maltose	+	+	+	w	+	+
Short chain fatty acid production						
Acetate	Major	Minor	Minor	Utilization	Major	Major
Formate	NA	Major	NA	Major	Major	Major
Lactate	NA	NA	Major	Major	Major	Major
Propionate	Minor	NA	NA	NA	–	–
Butyrate	Minor	Major	Major	Major	–	–
Isolation source	Human faeces	Human faeces	Human faeces	Human faeces	Industrial wastewater bioreactor	Sewage sludge

Table 2. Cellular fatty acid profile of strain AT2^T compared with other related species

Strains: 1, AT2^T (data from this study); 2, *Subdoligranulum variabile* BI 114^T [41]; 3, *Intestinimonas butyriciproducens* SRB-521–5-I^T [28]; 4, *Ethanoligenens harbinense* YUAN-3^T (=JCM 12961^T=CGMCC 1.5033^T) (Xing, *et al.*, 2004); 5, *Hydrogenoanaerobacterium saccharovorans* SW512^T (=AS 1.5070^T=JCM 14861^T) [43]; 6, *Acetanaerobacterium elongatum* Z7^T (=JCM 12359^T=AS 1.5012^T) [44]. Values are % of total fatty acids. Only cellular fatty acid >1 % in strain AT2^T are included. NA, Not available.

Fatty acid	1	2	3	4	5	6
$C_{14:0}$	11.8	6.2	67.4	NA	15. 6	NA
$C_{15:0}$	1.0	<1	NA	NA	NA	NA
$C_{16:0}$	43.8	33.0	3.9	4.98	29.1	NA
$C_{18:0}$	10.0	11.6	3.7	NA	NA	NA
$C_{18:1n9}$	20.3	38.5	5.7	NA	NA	NA
$C_{18:2n6}$	1.2	NA	<1	NA	NA	NA

Using an API 20A strip (BioMérieux), positive reactions are observed for D-glucose, lactose, sucrose, D-mannose, aesculin ferric citrate, salicin, maltose, D-melezitose, glycerol and raffinose but no reaction is obtained for urease, D-mannitol, D-xylose, gelatin, D-cellulose, D-sorbitol, L-arabinose, L-rhamnose and trehalose. Using an API ZYM strip (BioMérieux), exhibits esterase (C4), esterase lipase (C8), acid phosphatase, naphtol phosphohydrolase, α-galactosidase (melibase), α-glucosidase (maltase), β-glucosidase (cellulose), and *N*-acetyl-β-glucosaminidase (chitinase) activities. No alkaline phosphatase, lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, β-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase activities are observed. The major SCFA produced is acetic acid but butyric acid, isobutyric acid and propionic acid are also produced in small amounts. The predominant cellular fatty acids are the saturated acid $C_{16:0}$ and the unsaturated acid $C_{18:1n9}$.

The type strain is AT2^T (=CSUR P2014^T=DSM 100451^T), isolated from the faeces of a healthy 28-year-old French male. The DNA G+C content of the type strain is 56.8 % (genome sequence). The type strain is susceptible to amoxicillin, amoxicillin/clavulanic acid, cefalexin, ciprofloxacin, doxycycline, erythromycin, gentamicin, imipenem, metronidazole, nitrofurantoin, oxacillin, penicillin G, rifampicin, trimethoprim-sulfamethoxazole, tobramycin and vancomycin, but resistant to ceftriaxone.

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Conflicts of interest
The authors declare no conflicts of interest.

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