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Draft Genome and Description of *Eisenbergiella massiliensis* Strain AT11^T: A New Species Isolated from Human Feces After Bariatric Surgery

Amadou H. Togo¹ · Awa Diop² · Matthieu Million¹ · Marie Maraninchi³ · Jean-Christophe Lagier¹ · Catherine Robert² · Fabrizio Di Pinto¹ · Didier Raoult¹ · Pierre-Edouard Fournier² · Fadi Bittar^{1,4}

Abstract

A novel strain of a Gram-stain negative, non-motile, non-spore forming rod-shaped, obligate anaerobic bacterium, designated AT11^T, was isolated from a stool sample of a morbidly obese woman living in Marseille, France. This bacterium was characterized using biochemical, chemotaxonomic, and phylogenetic methods. The 16S rRNA gene sequence analysis showed that strain AT11^T had a 97.8% nucleotide sequence similarity with *Eisenbergiella tayi* strain B086562^T, the closest species with standing in nomenclature. The major cellular fatty acids of the novel isolate were C_{16:0} followed by saturated or unsaturated C₁₈ fatty acids (C_{18:1n9}, C_{18:1n5} and C_{18:0}). The draft genome of strain AT11^T is 7,114,554 bp long with 48% G+C content. 6176 genes were predicted, including 6114 protein-coding genes and 62 were RNAs (with 2 5S rRNA genes, two 16S rRNA genes, two 23S rRNA genes, and 56 tRNA genes). The digital DNA–DNA hybridization (dDDH) relatedness between the new isolate and *E. tayi* strain B086562^T was 23.1% ± 2.2. Based on the phenotypic, chemotaxonomic, genomic, and phylogenetic characteristics, *Eisenbergiella massiliensis* sp. nov., is proposed. The type strain is AT11^T (=DSM 100838^T = CSUR P2478^T).

Introduction

The number of people suffering from obesity has increased in recent decades [25]. It has been well established that the gut microbiota contributes to the development of human metabolic disorders such as obesity [18, 24]. Bariatric surgery is the most effective treatment for morbid obesity. It

induces a sustainable weight loss, improves complications related to obesity, and increases the diversity of the gut flora [14, 34].

We conducted a study comparing the gut microbiota from obese patients before and after bariatric surgery using a new microbial high-throughput culture approach known as culturomics [16]. This new approach makes it possible to isolate and describe the living microbial diversity of any environmental and clinical sample. Using culturomics, we isolated a new anaerobic bacterium, strain AT11^T, from a stool sample harvested following bariatric surgery. The discovery of this bacterium has been previously reported as a new species announcement without a thorough description [31].

Herein, strain AT11^T was analyzed by a polyphasic approach in order to describe it as a new bacterial taxon. This combines phenotypic characteristics, the matrix laser desorption ionization-time of flight mass spectrometry (MALDI-TO MS) spectrum, and genomic properties known as taxono-genomics [27].

Here, we propose a classification and a set of phenotypic, chemical, and chemotaxonomic characteristics of a new bacterial species: strain AT11^T, which belongs to

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the genus *Eisenbergiella* [1], together with the description of the complete genome sequencing, annotation, and genomic comparison. To date, this genus includes only one species *Eisenbergiella tayi*, the type strain B086562^T (= LMG 27400^T = DSM 26961^T = ATCC BAA-2558^T) as reported in List of Prokaryotic Names with Standing in Nomenclature (<http://www.bacterio.net/ruminococcus.html>).

Materials and Methods

Ethics and Sample Collection

Once informed consent had been obtained, stool samples were collected before and after surgery. These samples were obtained from a 56-year-old obese French woman following bariatric surgery on April 27, 2011. All samples were stored at -80°C before culturing. The study and the assent procedure were approved by the local ethics committee of IFR 48, under ascent number 09-022, 2010.

Isolation and Identification of the Strain

Strain AT11^T was first grown on July 22, 2015. One gram of stool was pre-incubated in BD BACTECTM Lytic/10 Anaerobic/F Culture Vials media culture bottles (Becton, Dickenson and Company, Le Pont de Claix, France) enriched with 4 ml of filtered rumen juice and 4 ml of sheep blood. The pre-incubated product was cultured on 5% sheep blood-enriched Columbia agar (bioMérieux, Marcy l'Etoile, France) as described elsewhere [32]. This strain was isolated 21 days after pre-incubation. The resulting colonies were then identified using MALDI-TOF mass spectrometry (Bruker Daltonics, Leipzig, Germany) as previously described [29]. When the spectra of a bacterium are not identified by MALDI-TOF MS screening, 16S rRNA gene amplification and sequencing is performed.

Phylogenetic Analysis

The 16S rRNA gene amplification PCR and sequencing were performed using GeneAmp PCR System 2720 thermal cyclers (Applied Bio systems, Bedford, MA, USA) and ABI Prism 3130xl Genetic Analyzer capillary sequencer (Applied Bio systems), respectively, as described by Drancourt et al. [6]. The CodonCode Aligner was used to correct sequences and BLASTn searches were performed on the NCBI (National Centre for Biotechnology Information) web server at <http://blast.ncbi.nlm.nih.gov/gate1.inist.fr/Blast>

.cgi for the taxonomic assignation. Pairwise sequence similarities were calculated using the method recommended by Meier-Kolthoff et al. [23] and as described previously [33]. Sequences were aligned using ClustalW with default parameters and phylogenies were inferred using the GGDC web server available at <http://ggdc.dsmz.de/> using the DSMZ phylogenomics pipeline.

Phenotypic, Biochemical, and Chemotaxonomic Characterization

Different growth temperatures (room temperature, 28, 37, 45, and 55 °C) were tested on sheep blood-enriched Columbia agar (bioMérieux) under anaerobic conditions using GENbag anaer system (bioMérieux), microaerophilic conditions using GENbag microaer system (bioMérieux), and aerobic conditions, with or without 5% CO₂.

Phenotypic and biochemical characteristics were performed as described elsewhere [32]. In addition to the three API gallery systems (API® ZYM, API® 20A, and API® 50 CH) usually used in our laboratory, API® Rapid ID 32A gallery system was added and the tests were done according to the manufacturer's instructions (bioMérieux).

E test strips for Amikacin 0.016–256 µg/ml, Vancomycin 0.016–256 µg/ml, Imipenem 0.002–32 µg/ml, Ceftriaxone 0.016–256 µg/ml, Rifampicin 0.002–32 µg/ml, Benzyl penicillin 0.002–32 µg/ml, Amoxicillin 0.016–256 µg/ml, Minocycline 0.016–256 µg/ml, Teicoplanin 0.016–256 µg/ml, Erythromycin 0.016–256 µg/ml, and Daptomycin 0.016–256 µg/ml (bioMérieux) were used for the antimicrobial agent susceptibility of strain AT11^T as recommended by EUCAST [4, 22]. Breakpoint tables for the interpretation of MICs and inhibition zone diameters, version 7.1, 2017, were used to interpret the results: these are available at <http://www.eucast.org>.

Cellular fatty acid methyl ester (FAME) analysis of this was then performed using gas chromatography/mass spectrometry (GC/MS) as described by Dione et al. [5].

Genome Sequencing and Assembling

The genomic DNA of strain AT11^T was sequenced and assembled as described in previous studies [33]. It was quantified by a Qubit assay using the high sensitivity kit (Life Technologies, Carlsbad, CA, USA) to 107.7 ng/µl and mechanically sheared with a circular shear to small fragments with an optimal length of 1401 bp using the Covaris device S2 in T6 tubes (Covaris, Woburn, MA, USA). A High Sensitivity Bioanalyzer LabChip (Agilent Technologies Inc, Santa Clara, CA, USA) was used to visualize the library profile and the final concentration library was measured at 34.4 nmol/l. The libraries were then normalized

Results and Discussion

Phylogenetic Analysis

The spectrum generated from strain AT11^T spots did not match those of Bruker and our in-house database (Supplementary Fig. 1) available at <http://www.mediterranean-infection.com/article.php?laref=933&titre=c-d-e>. This new strain exhibited 97.76% nucleotide sequence similarity with *Eisenbergiella tayi*, the closest species with standing in nomenclature according to the 16S rDNA sequence analysis. Figure 1 presents the neighbor-joining phylogenetic tree (Fig. 1) based on 16S rRNA gene sequences and shows the relationships between strain AT11^T and some related taxa. This sequence of the strain was deposited in EMBL-EBI under accession number LN881600.

Phenotypic and Biochemical Characterization

Strain AT11^T is strictly anaerobic, its growth temperature was between 28 and 45 °C, and optimal growth was observed at 37 °C. Colonies appeared light gray in color and exhibited an irregular form with a diameter between 0.5 and 1.5 mm after 72 h of culture on Columbia agar with 5% sheep blood (bioMérieux). No growth was observed above 5 g/l (10–100 g/l) salt on Schaedler agar with 5% sheep blood (bioMérieux). Cells were Gram-negative, non-motile, non-spore-forming, catalase positive, and rod shaped, measuring 1–3 µm in length and 0.4–0.5 µm wide using electron microscopy (Supplementary Fig. 2). The negativity of Gram staining was confirmed by the positive KOH test, but the strain had a positive Gram structure in electron microscopy. The characteristics of strain AT11^T, according to API® gallery systems (50 CH, 20A, Zym and Rapid ID 32A), along with those of the closest species, *Eisenbergiella tayi* strain B086562^T, are listed in Supplementary Table 1 and the differences between these two species are presented in Table 1.

Hexadecanoic acid was the most abundant fatty acid (63%), followed by saturated and unsaturated C₁₈ fatty acids representing approximately (33%) of total relative abundance. The fatty acid profiles of strain AT11^T and the closest strain *E. tayi* B086562^T are shown in Table 2.

Antimicrobial agent susceptibility was tested according to the EUCAST recommendations leading to the following MIC results: 32, 0.5, 0.125, 1.6, 0.064, 0.38, 0.5, 0.5, and 0.125 µg/ml, respectively, for Amikacin, Vancomycin, Imipenem, Ceftriaxone, Rifampicin, Benzyl penicillin, Amoxicillin, Minocycline, and Teicoplanin.

Table 1 Differential characteristic of strain AT11^T with *Eisenbergiella tayi* B086562^T

Properties	<i>Eisenbergiella massiliensis</i> AT11 ^T	<i>Eisenbergiella tayi</i> B086562 ^{Ta}
Indole production	V	–
Arabinose	+	–
Arbutin	+	–
Cellulose	+	–
Dulcitol	+	–
Gelatin	+	–
Glucose	+	–
Lactose	+	–
Maltose	V	–
Mannitol	V	–
Mannose	+	–
Raffinose	+	–
Rhamnose	+	–
Saccharose	+	–
Salicin	+	–
Sorbose	+	–
Tagatose	+	–
Trehalose	+	–
Trypsin	+	–
Xylose	V	–
Potassium 5-cetogluconate	+	–
Acid phosphatase	+	–
Alkaline phosphatase	V	+
Arginine hydrolase	+	–
Esterase	+	–
Esterase lipase	+	–
Naphthol-AS-BI-phosphohydrolase	+	–
Tyrosine arylamidase	–	+
α-Arabinosidase	–	+
α-Fructosidase	+	–
β-Glucuronidase	+	–
Isolated from	Human feces	Blood

+ Positive, – negative

v Variable

^aData for *E. tayi* were obtained from Amir et al. [25]

Genome Properties

The genome deposited in EMBL-EBI under accession number OEZA00000000 (Fig. 2) is 7,114,554 bp long with 48% GC content. It is composed of 19 contigs consisting of 17 scaffolds. Of the 6176 predicted genes, 6114 were protein-coding genes and 62 were RNAs (two 5S rRNA genes, two 16S rRNA genes, two 23S rRNA genes, 56 tRNA genes). A total of 4321 genes (70.67%) were assigned a putative

Table 2 Cellular fatty acid composition (%) of strain AT11^T compared to its closest neighbor *Eisenbergiella tayi* strain B086562^T

Fatty acid	Name	Strain AT11	<i>E. tayi</i> ^a
C _{16:0}	Hexadecanoic acid	62.7	45.4
C _{18:1n9}	9-Octadecenoic acid	10.3	14.8
C _{18:1n5}	13-Octadecenoic acid	9.2	ND
C _{18:0}	Octadecanoic acid	7.8	12.8
C _{18:2n6}	9,12-Octadecadienoic acid	4.4	1.3
C _{18:1n7}	11-Octadecenoic acid	1.6	3.2
C _{17:0}	Heptadecanoic acid	1.5	ND
C _{15:0}	Pentadecanoic acid	< 1	< 1
C _{14:0}	Tetradecanoic acid	< 1	6.3
C _{16:1n7}	9-Hexadecenoic acid	< 1	ND
C _{20:4n6}	5,8,11,14-Eicosatetraenoic acid	< 1	ND
9,10-Methylene-C _{16:0}	2-Hexyl-cyclopropanoic acid	< 1	ND
Iso-C _{16:0}	14-Methyl-pentadecanoic acid	< 1	ND
Iso-C _{15:0}	13-Methyl-tetradecanoic acid	< 1	ND
C16: 0 2-OH	2-Hydroxyhexadecanoic acid	ND	1.6
C _{17:2}	Heptadecadienoic acid	ND	11.6
C _{13:1 cis 12}	12-Tridecanoic acid	ND	2.1
Anteiso-C _{15:0}	12-Methyl-tetradecanoic acid	ND	< 1

ND Not detected

^aData for *E. tayi* were obtained from Amir et al. [25]

function by COGs or by NR BLAST. 130 genes were identified as ORFans (2.13%). The remaining 1525 genes (24.94%) were annotated as hypothetical proteins. Two genes associated with Vancomycin (Vancomycin B-type resistance protein, VanW) resistance and 20 genes associated with beta-lactamase resistance were found using the RAST web server [26]. The remaining 1525 genes (24.94%) were annotated as hypothetical proteins.

Genome Comparison

The draft genome sequence of strain AT11^T (7.11 MB) is larger in size than those of *C. bolteae*, *B. producta*, *C. clostridioforme*, *Eubacterium ruminantium*, and *A. hadrus* (6.38, 6.09, 5.46, 2.84, and 2.77 MB, respectively) but almost equal to that of *E. tayi* (7.15). Its G+C (48%) content is lower than that of *C. clostridioforme* and *C. bolteae* (49 and 49.6%, respectively), but higher than that of *E. tayi*, *B. producta*, *Eubacterium ruminantium*, and *A. hadrus* (46.3, 45.7, 37.2, and 37.2, respectively). Its gene content (6114) is higher than that of *C. bolteae*, *B. producta*, *C. clostridioforme*, *A. hadrus*, and *Eubacterium ruminantium* (5892, 5666, 5376, 2716, and 2533, respectively) but lower than that of *E. tayi* at 6156. The distribution of genes into COG categories was not entirely similar in all compared genomes (Fig. 3). The average genomic identity of orthologous gene sequences (AGIOS)

values ranged from 61.7% between *C. bolteae* and *Eubacterium ruminantium* to 90.8% between *C. bolteae* and *C. clostridioforme* among compared species without strain AT11^T (Supplementary Table 2). When strain AT11^T was included in the comparison, these values ranged from 62.2% with *Eubacterium ruminantium* to 78.4% with *E. tayi* (Supplementary Table 2). The dDDH values for strain AT11^T ranged from 19.5% with *A. hadrus* to 34.4% with *C. clostridioforme* (Supplementary Table 3) with a probability of error of $\pm 2\%$. These values are very low and below the cutoff of 70%, thus also confirming that this strain is a new species.

Based on the phenotypic, chemotaxonomic, genomic, and phylogenetic characteristics, a novel bacterium isolated from the stool sample of a morbidly obese French woman, under the name *Eisenbergiella massiliensis* sp. nov., is proposed. The type strain is AT11^T = DSM 100838^T = CSUR P2478^T.

Description of *Eisenbergiella massiliensis* sp. nov

Eisenbergiella massiliensis (*mas.si.li.en'sis*. L. fem. adj. massiliensis, of Massilia, the Latin name for Marseille). It is a strictly anaerobic bacterium which grows at a mesothermal temperature of 37 °C. The colonies grown on Columbia agar with 5% enriched sheep blood are light gray, non-hemolytic, and irregular with a diameter of 0.5 mm.

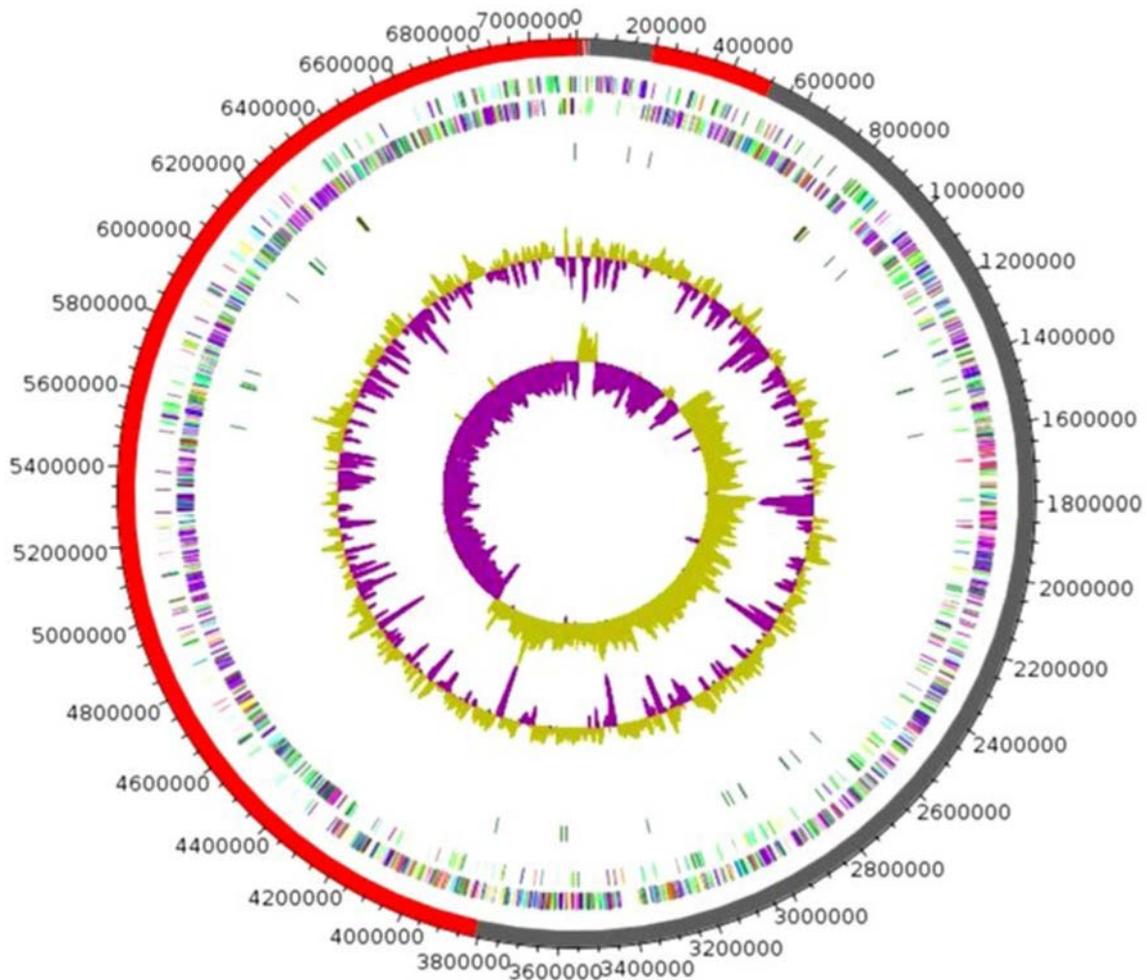


Fig. 2 Graphical circular map of the genome of strain AT11^T: From outside to the center: Contigs (red/gray), Clusters of Orthologous Groups (COGs) category of genes on the forward strand (three circles), G+C content. (Color figure online)

cles), genes on forward strand (blue circle), genes on the reverse strand (red circle), COGs category on the reverse strand (three circles), G+C content. (Color figure online)

Cells exhibit a negative Gram-stain, are non-spore-forming, non-motile, catalase positive, and rod-shaped bacilli, with a size of 0.4/2 μm . Using the API Gallery systems (API® ZYM API® 50CH API® 20A and API® rapid ID 32A) in anaerobic condition, positive reactions were observed for acid phosphatase, alkaline phosphatase, esterase, esterase lipase, naphthol-AS-BI-phosphohydrolase, *N*-acetyl- β -glucosaminidase, α -arabinosidase, α -fucosidase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, β -glucuronidase, 6-phosphate- β -galactosidase, arbutin, D-cellobiose, D-glucose, D-lactose, D-lyxose, D-maltose, D-mannose, D-raffinose, D-saccharose, D-tagatose, D-trehalose, dulcitol,

D-xylose, L-arabinose, L-rhamnose, L-sorbose, potassium 5-cetogluconate, and salicin. Urease and indole are not produced, gelatin was not liquefied and nitrate was not reduced, although esculin was hydrolyzed. The major cellular fatty acids detected were C_{16:0} (62.7%) and C_{18:1n9} (10.3%). Its genome, consisting of one chromosome, is 7,114,554 bp in length with 48% of G+C content. The type strain AT11^T = CSUR P2478^T = DSM 100838^T was isolated from the stool sample of a French morbidly obese woman following bariatric surgery.

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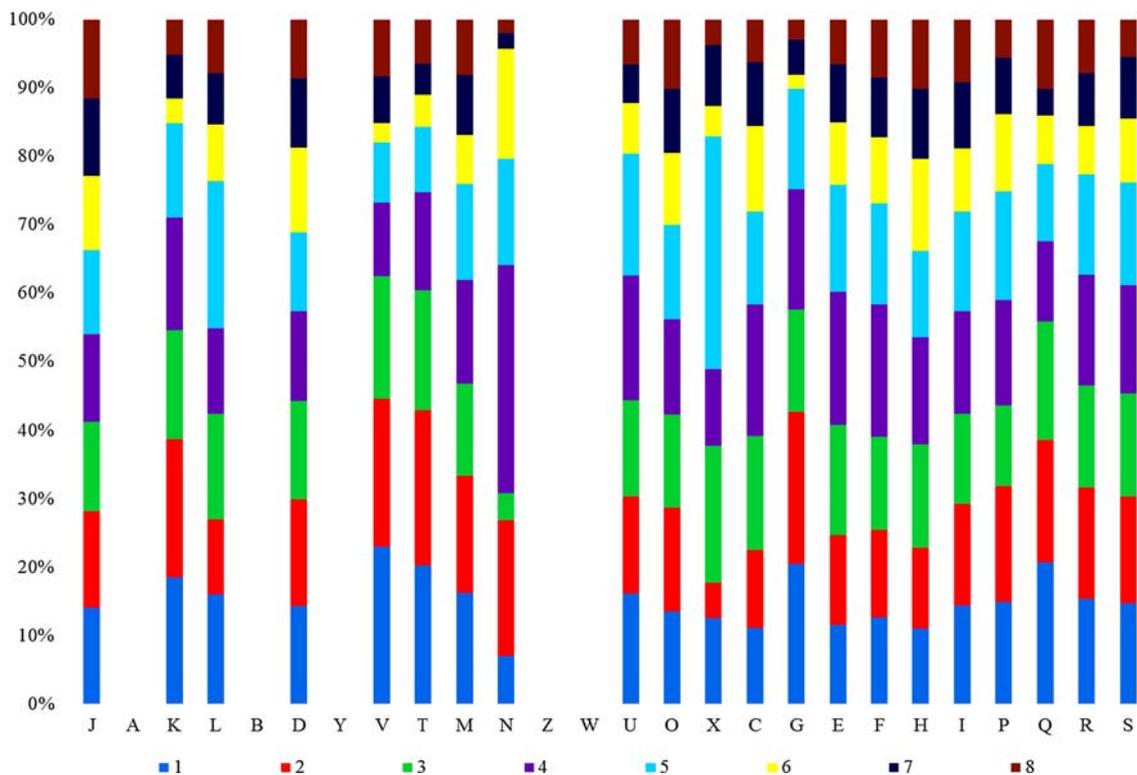


Fig. 3 Distribution of functional classes of predicted genes according to the Clusters of Orthologous Groups (COGs) of strain AT11^T with its closest species: 1, Strain AT11^T; 2, *Eisenbergiella tayi* strain DSM 26961^T; 3, *Blautia producta* strain ATCC 27340^T; 4, *Clostridium boltea* strain WAL 16351^T; 5, *Clostridium clostridioforme* strain

ATCC 25537^T; 6, *Parasporobacterium paucivorans* strain DSM 15970^T; 7, *Anaerostipes hadrus* strain ATCC 29173^T; and 8, *Eubacterium ruminantium* strain ATCC 17233^T. *Superscript T* Type strain, ATCC American Type Culture Collection, DSM Deutsche Sammlung von Mikroorganismen, WAL Wadsworth Anaerobe Laboratory

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Compliance with Ethical Standards

Conflict of interest The author declares that they have no conflicts of interest.

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