Eisenbergiella tayi gen. nov., sp. nov., isolated from human blood

Itay Amir,[1,2] Philippe Bouvet,[3] Christine Legeay,[3] Uri Gophna[2] and Abraham Weinberger[4,5]

Correspondence
Itay Amir
itayamir@gmail.com

[1]The Microbiology Laboratory, Ma'ayanei Hayeshua Medical Center, Bnei-Brak 51544, Israel

[2]Department of Molecular Microbiology and Biotechnology, George S. Wise Faculty of Life Sciences, Tel-Aviv University, Ramat-Aviv, Tel-Aviv 69978, Israel

[3]Institut Pasteur, Centre National de Référence des Bactéries Anaérobies et du Botulisme, 25-28 rue du Docteur Roux, F-75724 Paris cedex 15, France

[4]Department of Internal Medicine, Ma'ayanei Hayeshua Medical Center, Bnei-Brak 51544, Israel

[5]The Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, 69978, Israel

A catalase-positive, rod-shaped, non-proteolytic, non-motile, anaerobic bacterial strain, designated B086562^T, was isolated from a blood culture of an 84-year-old male patient in Israel. According to 16S rRNA gene sequence phylogeny, this strain has no known close relatives among recognized bacteria but should be placed within the family *Lachnospiraceae*. The most closely related recognized bacteria were from the '*Clostridium clostridioforme* group': *C. clostridioforme* (92.4 %) and *Clostridium bolteae* (92.3 %). The isolate produced butyrate, lactate, acetate and succinate as major metabolic end products. The major fatty acids were $C_{16:0}$ and $C_{18:1}$ *cis* 9 DMA and the DNA G+C content was 46.0 mol%. On the basis of the phenotypic properties and phylogenetic distinctiveness, the blood isolate represents a novel species of a new genus in the family *Lachnospiraceae*, for which the name *Eisenbergiella tayi* gen. nov., sp. nov. is proposed. The type strain of *Eisenbergiella tayi* is B086562^T (=LMG 27400^T=DSM 26961^T=ATCC BAA-2558^T).

Anaerobic bacteria form an important part of the human microbiota. While the majority of these organisms are commensals, many of them behave as opportunistic pathogens (Lobo *et al.*, 2013; Murphy & Frick, 2013). Culture-independent molecular techniques have revealed new insights into the bacterial diversity of human habitats, e.g. the oral cavity, gastrointestinal tract, skin and vagina (The Human Microbiome Project Consortium, 2012; Costello *et al.*, 2009; Dethlefsen *et al.*, 2007). Conversely, these methods do not provide definitive information regarding basic phenotypic characteristics, such as spore formation, biochemical profiles, cell wall fatty acids and metabolic end products of unseen bacterial phylotypes. Thus, isolation and characterization of one of these phylotypes provides an opportunity to describe a novel species. Here we report the characterization of a bacterial isolate from a blood culture of an 84-year-old patient, which was phylogenetically affiliated to several clones that were described in studies of the gastrointestinal tract (Ley *et al.*, 2006; Li *et al.*, 2012). The presence of similar clones in the human gastrointestinal tract suggests that the anaerobic bacteraemia of this patient might be caused by bacterial translocation, a phenomenon in which live bacteria or their products cross the intestinal barrier. According to their 16S rRNA gene sequence similarities, these hitherto uncultivated bacteria, together with strain B086562^T, belong to the family *Lachnospiraceae* within the phylum *Firmicutes*. *Eisenbergiella* gen. nov. is proposed to accommodate this group of anaerobes and *Eisenbergiella tayi* sp. nov. to accommodate B086562^T.

Phenotypic characterization of the strain was carried out using standard methods, as recommended in the Wadsworth manual (Jousimies-Somer *et al.*, 2012) using trypticase yeast extract haemin (TYH) broth for fermentation reactions. We also used commercially available tests such as the Vitek2 ANC card as well as the rapid ID 32A and API ZYM (bioMérieux), which were performed according to the manufacturer's instructions. Volatile and non-volatile end products of glucose metabolism were detected by gas–liquid chromatography (Carlier *et al.*, 2004; Holdeman *et al.*, 1977). The results of the phenotypic and biochemical characterization are presented in the species description and in Table 1.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain B086562T is KF814111.

A supplementary figure is available with the online version of this paper.

Table 1. Characteristics useful to differentiating *Eisenbergiella tayi* strain B086562^T from the type strain of other closely related species

Strains: 1, *Eisenbergiella tayi* B086562^T; 2, *Clostridium clostridioforme* ATCC 25537^T (Mohan *et al.*, 2006); 3, *Clostridium bolteae* ATCC BAA-613^T (Song *et al.*, 2003); 4, *Clostridium hathewayi* CCUG 43506^T (Steer *et al.*, 2001); 5, *Clostridium lavalense* CCUG 54291^T (Domingo *et al.*, 2009); 6, *Clostridium citroniae* ATCC BAA-1317^T (Warren *et al.*, 2006); 7, *Clostridium aldenense* ATCC BAA-1318^T (Warren *et al.*, 2006); 8, *Clostridium xylanolyticum* ATCC 49623^T (Rogers & Baeker, 1991); 9, *Moryella indoligenes* AIP 220.04^T (Carlier *et al.*, 2007). +, Positive; −, negative; w, weak reaction; v, variable; ND, no data.

Characteristic	1	2	3	4	5	6	7	8	9
Acid from:									
Arabinose	−	+	+	+	w,v	v	v	−	−
Lactose	−	+	−	+	+	−	+	−	−
Melezitose	−	−	+	+	−	−	−	+	−
Raffinose	−	+	+	+	v	−	+	+	−
Rhamnose	−	+	+	+	−	+	−	+	−
Ribose	−	+	−	+	w,v	ND	ND	−	v
Salicin	−	−	−	+	+	−	w	+	−
Sorbitol	−	−	+	+	w,v	−	−	−	−
Xylose	−	+	+	+	w,v	+	+	+	−
Indole production	−	−	−	−	+	+	+	−	+
Aesculin hydrolysis	+	+	−	+	−	−	v	ND	−
End products of glucose metabolism*	B,l,a,s	A,L,E	A,L	A,E	A,l,s	ND	ND	F,L,A	A,B,l
DNA G+C content	46	ND	50.5	50.7-50.9	ND	ND	ND	40	50.2
Catalase	+	−	−	−	−	ND	ND	−	−
N-Acetyl-β-glucosaminidase (β NAG)	+	−	−	+	ND	−	−	ND	ND
α-Arabinosidase (αARA)	+	−	−	+	ND	−	+	ND	ND
Alkaline phosphatase (PAL)	−	+	−	−	ND	+	+	ND	ND
α-Galactosidase (αGAL)	+	+	+	+	−	−	+	ND	ND
β-Galactosidase (βGAL)	+	+	−	+	+	−	+	ND	ND
α-Glucosidase (αGLU)	+	+	−	ND	−	−	−	ND	ND

*A, Acetate; B, butyrate; E, ethanol; F, formate; L, lactate; S, succinate. Minor end products are indicated by lower-case letters.

For fatty acid composition, cells were grown for 48 h at 35 °C on brain heart infusion agar plates supplemented with 5 % (v/v) horse blood (BHIBLA) in anaerobic conditions. Inoculation, cell harvesting, extraction and analysis were performed according to the recommendation of the commercial identification system MIDI (Microbial identification System), except that cells were harvested from the whole plate and from two plates to obtain sufficient concentration of fatty acids in the extract. The whole-cell fatty acid composition was analysed by gas chromatography using this system by the BCCM/LMG Bacteria Collection Identification Service (Laboratory of Microbiology, Ghent University, Ghent, Belgium). The major fatty acids were $C_{16:0}$ and $C_{18:1}$ *cis* 9 dimethyl acetal (DMA) (>19 %) (Table 2). The fatty acid profile of B086562^T did not match any of the presently known profiles in the MIDI database (BHIBLA 3.8). However, the saturated fatty acid $C_{16:0}$ is the major component in other close relatives of strain B086562^T such as *Clostridium bolteae* ATCC BAA-613^T (31.8 %), *Clostridium lavalense* CCUG 54291^T (30.6 %) and *Clostridium citroniae* ATCC BAA-1317^T (31.5 %) [Data for reference strains were taken from the fatty acids database of the culture collection, University of Göteborg (CCUG): http://www.ccug.se].

For determination of the DNA G+C content, genomic DNA of strain B086562^T was prepared according to a modified version of the procedure of Gevers *et al.*, 2001. The G+C content of the DNA sample was determined in three independent analyses using HPLC (Mesbah *et al.*, 1989) by the BCCM/LMG Bacteria Collection Identification Service. The DNA G+C content of strain B086562^T was 46.0 mol%.

For electron microscopy of negative staining, bacteria grown on trypticase soya agar (TSA) + 5 % (v/v) defibrinated sheep blood (DSB) (Novamed; 72 h, 37 °C) were suspended in saline, fixed to a carbon-coated grid, and stained with 2 % (w/v) uranyl acetate. For electron microscopy, ultrathin sections of cells were prepared as described elsewhere (Carlier *et al.*, 2004) and examined using a JEOL 1010 transmission electron microscope at 80 Kv.

Colonies appeared on TSA + 5 % (v/v) DSB after 48–72 h of incubation. They were irregular with rhizoid margins, flat, smooth, dull, opaque, approximately 0.17–0.6 mm in

Table 2. Fatty acid compositions (%) of *Eisenbergiella tayi* B086562^T

Fatty Acid	%
C16 : 0	31.8
C18 : 1 *cis* 9 DMA	19.7
C18 : 1 *cis* 9	10.4
C18 : 0	9.0
C17 : 2	8.1
C14 : 0	6.3
C16 : 0 DMA	3.5
C14 : 0 DMA	2.6
C18 : 1 *trans*-11*	2.3
C13 : 1 *cis* 12	1.5
C16 : 0 2-OH	1.1
C16 : 0 ALDE	1.1
C18 : 2 *cis* 9, 12	0.9
C18 : 1 *cis*11 DMA	0.8
C15 : 0	0.6
anteiso-C15 : 0	0.4

*The exact position of the double bond cannot be determined with the type of GC analysis used.

diameter (up to 0.8 mm after 7 days), non-pigmented and non-haemolytic (Fig. 1a). Colonies differed on *Brucella* blood agar and appeared smaller and more regularly circular. Cells were thin elongated rods, sometimes with tapered ends, (approximately 3.4–7.3 μm × 0.4–0.7 μm), and usually occurred singly or in pairs. Bacteria were non-motile, Gram-stain-negative (Fig. 1b, c), but appeared to be structurally similar to Gram-stain-positive cells (Fig. 1d–g). This characteristic has also been reported in members of the RIC group (*Clostridium ramosum*, *Clostridium innocuum* and *C. clostridioforme*). The strain was negative when it was subjected to the KOH string test (Gregersen, 1978). No flagella were observed. Spore formation was not observed with either Gram staining or Schaeffer–Fulton staining.

The isolate was strictly anaerobic; however, the strain survived exposure to air for more than 12 h. The isolate was susceptible to potency discs containing 5 μg vancomycin and 5 μg metronidazole and was resistant to 1 mg kanamycin and 10 μg colistin. The isolate was found to be resistant to bile, although no growth was observed on *Bacteroides* Bile Aesculin agar. Catalase reaction was positive. Urease production was negative when the strain was tested using the urea–indole test (Bio-Rad). Indole was not produced and nitrates were not reduced. Gelatin was not liquefied and casein was not hydrolysed (the strain grew on skimmed milk agar after 5 days without proteolysis). The strain was negative for lecithinase and lipase activity. Growth occurred in the temperature range 15–45 °C (optimum at 30–37 °C), with no growth observed at 4 or 55 °C. Starch was not hydrolysed. Acid was produced from aesculin and aesculin was hydrolysed. Acid was not produced from glucose, lactose, arabinose, cellobiose, fructose, galactose, glycerol, inositol, maltose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, ribose, sucrose, salicin, sorbitol, starch, trehalose or xylose. Using Vitek2 ANC cards, positive reactions with the strain were obtained for Ellman, 5-bromo-4-chloro-3-indoxyl-β-glucoside, urease, β-galactopyranosidase indoxyl, α-arabinosidase, 5-bromo-4-chloro-3-indoxyl-α-galactoside, β-mannosidase, aesculin hydrolysis, β-D-fucosidase, 5-bromo-4-chloro-3-indoxyl-α-mannoside, α-L-fucosidase and α-L-arabinofuranoside, with all other tests negative. Although the presence of enzymes related to utilization of sugars seemed inconsistent with its asaccharolytic characteristics, this phenomenon has also been documented for other organisms such as *Tannerella forsythensis* (Sakamoto *et al.*, 2002; Tanner *et al.*, 1986). The metabolic end products in trypticase yeast extract haemin broth with glucose (TGYH) were butyric acid (13 mmol l^{-1}), lactic acid (9 mmol l^{-1}), acetic acid (5 mmol l^{-1}) and succinic acid (1 mmol l^{-1}). Butyric acid is the major energy source for colonocytes and is involved in the maintenance of colonic mucosal health (Hamer *et al.*, 2008). The fact that butyric acid is the main fermentation end product of this bacterium suggests that this organism might have a beneficial effect in the human gastrointestinal tract.

For DNA extraction, cells were grown anaerobically for 72 h using Wheaton serum bottles containing 60 ml brain heart infusion broth (Merck) supplemented with 5.7 mM cysteine to scavenge traces of oxygen and 0.0001 % (w/v) resazurin as a redox indicator (Masalha *et al.*, 2001). Cells were then centrifuged for 10 min at 1727 ***g*** and DNA was extracted using a Wizard Genomic DNA purification kit (Promega) according to the manufacturer's instructions. PCR-mediated amplification of the 16S rRNA gene was performed using the universal primers: 8f 5′-CACGGATCCAGACTTTGATYM-TGGCTCAG-3′ and 1512r 5′-GTGAAGCTTACGGYT-AGCTTGTTACGACTT-3′ (Felske *et al.*, 1997) using 1U Phusion *Taq* polymerase (Finnzyme) in a thermal cycling program of 98 °C for 3 min, 35 cycles at 98 °C for 10 s, 62 °C for 35 s and 72 °C for 35 min and finally 72 °C for 7 min. PCR products were purified with a Gel/PCR DNA fragments Extraction kit (GeneAid) using the PCR cleanup protocol. The purified PCR product was then sequenced using the AB 3500xl Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions. Sequencing was performed using 8f, 534r (5′-ATTACCGCGGCTG-CTGG-3′), 968f (5′-AACGCGAAGAACCTTAC-3′) and 1512r primers. Phylogenetic neighbours were initially identified using the BLAST program against the database of cultured and uncultured bacteria (Altschul *et al.*, 1997). High quality sequences with the highest scores were then selected along with sequences of representative clones for alignment using the MAFFT server (Katoh *et al.*, 2002) (http://mafft.cbrc.jp/alignment/server/). Poorly aligned regions were manually removed, and the phylogenetic tree was reconstructed using the maximum-likelihood method (Felsenstein, 1981) with 1000 bootstrap resamplings using

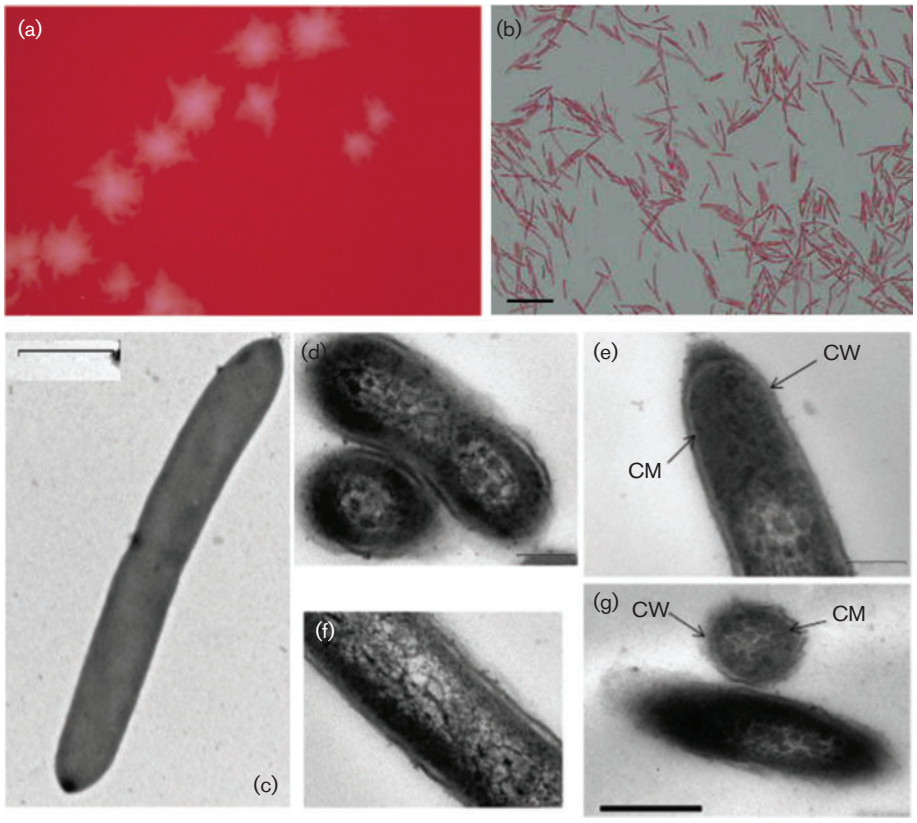

Fig. 1. (a) Colony morphology of strain B086562^T on TSA + 5 % (v/v) DSB. (b) Gram-stain of cells of isolate B086562^T grown in brain heart infusion broth, bar, 5 μm. (c) Transmission electron micrograph of B086562^T – general morphology after negative staining, bar, 1 μm. (d–g) Ultrathin sections showing the Gram-positive cell wall (CW) and the cytoplasmic membrane (CM) in cells of B086562^T. Bars, 200 nm (d–f) and 0.5 μm (g).

the PhyML web-server (Guindon & Gascuel, 2003). Evolutionary distances were calculated according to the algorithm of Kimura's two-parameter model (Kimura, 1980). Visualization of the phylogenetic tree was carried out using N-J plot software (Perrière & Gouy, 1996).

Comparative sequence analysis revealed that the DNA sequence of B086562^T was closely related to '*Clostridiaceae* bacterium MS3' (HF95290), which was isolated from human stools, having 99.2 % similarity. These 16S rRNA gene sequences grouped together and formed a separate clade (Fig. 2). Furthermore, the 16S rRNA gene sequence of B086562^T showed high sequence similarity to NLAE-zl- sequences such as NLAE-zl-H61 (with a maximum similarity value of 99.7 %) and other human faecal isolates (Cherie J. Ziemer, unpublished; GenBank nos JX006307, JX006308, JX006403). Within this lineage is placed a clone associated with human ileum ELU0096-T30 S-NI_000255 (described by Li *et al.*, 2012), and another clone HAW-RM37-2-B-1600d-E, associated with human gut (described by Ley *et al.*, 2006) is branched off (Fig. S1, available in the online Supplementary Material). The nearest phylogenetic neighbour to B086562^T was *C. clostridioforme* ATCC 25537^T with a sequence similarity of 91.7 %, which was below the 'lower cut-off window' of 95 % for differentiation of a new genus (Tindall *et al.*, 2010; Yarza *et al.*, 2008). We analysed the 16S rRNA gene sequences that were obtained using the Sanger sequencing method in the database of Li and coworkers (Li *et al.*, 2012). We found that 102 out of 81644 sequences showed more than 97 % sequence similarity with the 16S rRNA gene sequence of B086562^T. All of these were from normal ileal samples.

The phylogenetic analysis also showed that the unknown anaerobic isolates fitted into the clostridial rRNA cluster XIVa, which consists of a phenotypically heterogeneous collection of organisms (Collins *et al.*, 1994). From their 16S rRNA sequences, other strains are closely related to B086562^T, such as AIP 541.12 isolated from a blood culture of a 63-year-old man (P. Bouvet, unpublished data) and strain *Lachnospiraceae* 3_1_57FAA_CT1 (ACTP020000) from a transverse colon biopsy of a female Crohn's disease patient (Fig. S1). These strains share as much as 99.3 % similarity (1381 alignable positions over 1390 bases) with strain B086562^T. Interestingly, variations among 16S rRNA sequences in the genome were noted for strain AIP 541.12 (98.5 % similarity between the two sequences) (P.

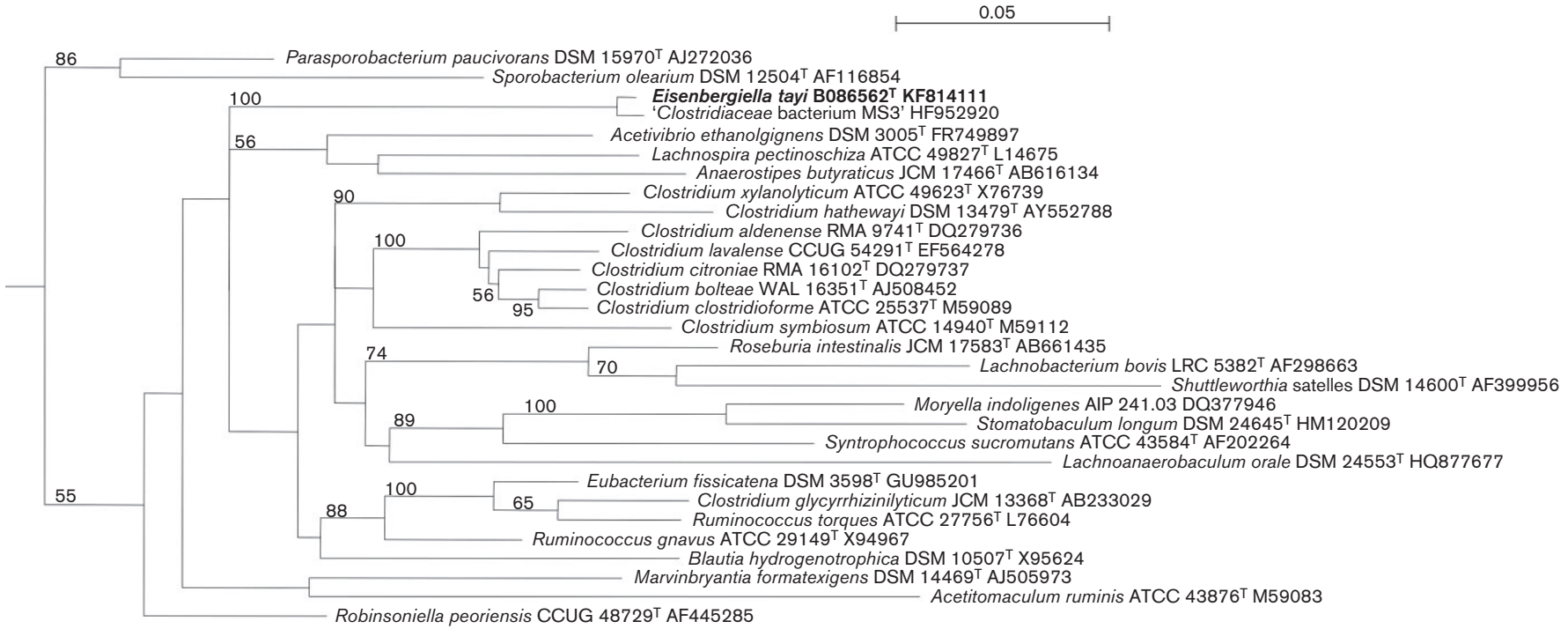

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequence comparisons over 1340 aligned bases indicating the relationships between the clinical isolate B086562^T and related species within the class *Clostridia*. The sequence of *Peptostreptococcus anaerobius* JCM 1470 (AB640688) was used as an outgroup (not shown). Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are shown at branch points. Bar, 5 % sequence divergence.

Bouvet, unpublished data) and for strain *Lachnospiraceae* 3_1_57FAA_CT1 (ACTP020000) (98.2–98.9 % similarities between the three sequences available). Interoperon sequence variation (between two or more copies of the 16S rRNA gene) within a single strain is found in both *Archaea* and *Bacteria* and has been previously described in the anaerobic genera *Prevotella* and *Veillonella* (Alauzet *et al.*, 2010; Marchandin *et al.*, 2003) as well as in other organisms (Ludwig *et al.*, 2011). There is a 4.2 % overall probability of encountering a species with significant intragenomic diversity among its 16S rRNA genes (Pei *et al.*, 2010; Větrovský & Baldrian, 2013). For practical purposes it is more convenient to use a strain that has a single rRNA sequence rather than isolates that harbour internal variation in their 16S rRNA gene sequences. This is especially true for strains of clinical relevance, as in this case, where taxonomic results should not be ambiguous for the end user. This approach also has the advantage of simplifying the assignment of related species in the future. This phenomenon was not observed in strain B086562^T.

Susceptibility testing was performed by using the disc diffusion method on Wilkins-Chalgren agar (Oxoid) according to the recommendations of the Antibiogram Committee of the French Society of Microbiology. We are aware that the agar diffusion method is not internationally recognized [Clinical and Laboratory Standards Institute (CLSI) M11 A-7] and disc diffusion breakpoint criteria for antimicrobial susceptibility testing of anaerobes have not yet been determined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). However, agar diffusion diameters that are high ($\geqslant$30 mm, i.e. susceptible strain) or low (6 mm, i.e. contact to the disc for a resistant strain) provide a realistic estimate of the strain's susceptibility to antibiotics. B086562^T is susceptible to penicillin G, ampicillin, amoxicillin, amoxicillin–clavulanic acid, ticarcillin, tetracycline, imipenem, cefalotin, cefotaxime, cefoxitin, rifampicin, vancomycin and metronidazole (all diameters $\geqslant$30 mm except cefotaxime 22 mm), and resistant to erythromycin and trimethoprim–sulfamethoxazole (all diameters=6 mm).

Description of *Eisenbergiella* gen. nov.

Eisenbergiella (Ei.sen.berg.i.el′la. N.L. fem. dim. n. *Eisenbergiella* named in memory of the Polish physician and bacteriologist Dr Filip Eisenberg (1876–1942) who perished during the Holocaust).

Cells are Gram-stain-negative, but appear Gram-positive structurally and do not react in the KOH test. They are catalase-positive non-spore-forming, non-motile, thin elongated rods, strictly anaerobic, which do not grow under microaerophilic or aerobic conditions. The predominant fatty acids are $C_{16:0}$ and $C_{18:1}$ *cis* 9 DMA. Butyric, lactic, acetic and succinic acids are the major metabolic end products in TGYH broth. Based on 16S rRNA gene sequence analysis, phylogenetically related to members of the family *Lachnospiraceae*. The type species is *Eisenbergiella tayi*.

Description of *Eisenbergiella tayi* sp. nov.

Eisenbergiella tayi (tay′i. N.L. gen. masc. n. *tayi* of Tay, named after Dr Waren Tay, who described Tay-Zachs disease).

Displays the following properties in addition to those given in the genus description. Cells are 3.4–7.3 μm long × 0.4–0.7 μm wide. On TSA + 5 % (v/v) DSB after 48–72 h of incubation, colonies are flat, opaque, irregular with rhizoid margins, non-pigmented and non-haemolytic. Cells are asaccharolytic (in TYH medium) and non-proteolytic. Aesculin is fermented and hydrolysed. Catalase-positive. Gelatin is not liquefied and casein is not hydrolysed. The fatty acid profile includes $C_{16:0}$, $C_{18:1}$ *cis* 9 DMA, $C_{18:1}$ *cis* 9, $C_{18:0}$, $C_{17:2}$ and $C_{14:0}$. Positive (in the Rapid ID 32A system) for α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, α-arabinosidase, *N*-acetyl-β-glucosaminidase, α-fucosidase and alkaline phosphatase, but negative for arginine dihydrolase, 6-phospho-β-galactosidase, β-glucuronidase, glutamic acid decarboxylase, reduction of nitrates, indole production, arginine arylamidase, proline arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, glutamyl glutamic acid arylamidase and serine arylamidase. Using the API ZYM system, acid phosphatase, esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase and *N*-acetyl-β-glucosaminidase are present. Leucine, valine and cysteine arylamidases, lipase (C14), trypsin, α-chymotrypsin and α-mannosidase activities are absent.

The type strain B086562^T (=LMG 27400^T=DSM 26961^T =ATCC BAA-2558^T) was isolated from a culture of blood from an 84-year-old-man. The DNA G + C content of the type strain is 46.0 mol%.

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