Bacteroides faecis sp. nov., isolated from human faeces

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Two anaerobic, Gram-negative, non-motile and non-spore-forming bacterial strains, designated MAJ27^T and MAJ26, were isolated from human faeces. Both isolates grew optimally at 37 °C, were oxidase- and catalase-negative, were sensitive to bile and produced acid from fermentation of several substrates, including glucose. A study based on 16S rRNA gene sequences showed that both isolates were closely related to type strains of species of the genus *Bacteroides*. Comparisons of the isolates with *Bacteroides thetaiotaomicron* VPI 5482^T and *Bacteroides finegoldii* JCM 13345^T showed high levels of 16S rRNA gene sequence similarity (98.6–98.7 and 96.9–97.0 %, respectively), but low levels of DNA–DNA relatedness (⩽22 %). The DNA G+C content (42.7 ± 1 mol%) and the major fatty acid (anteiso-$C_{15:0}$, 39.3–42.5 %) supported the assignment of the isolates to the genus *Bacteroides*. Based on phenotypic, chemotaxonomic, genotypic and phylogenetic studies, we propose that strains MAJ27^T and MAJ26 be classified as representing a novel species, *Bacteroides faecis* sp. nov. The type strain is MAJ27^T (=KCTC 5823^T=JCM 16478^T).

Since the completion of the Human Genome Project, the contribution of symbiotic human gastrointestinal tract microbiota to normal physiology and predisposition to disease has been the focus of many studies (Turnbaugh *et al.*, 2007). The phylum *Bacteroidetes* constitutes the dominant gastrointestinal microbiota, followed by the phylum *Firmicutes* (Eckburg *et al.*, 2005; Gill *et al.*, 2006; Palmer *et al.*, 2007; Wang *et al.*, 2005). Recently, it was reported that changes in the relative abundance of these phyla are associated with obesity (Turnbaugh *et al.*, 2006). The phylum *Bacteroidetes* is composed of five major subgroups that constitute the *Cytophaga–Flavobacter–Bacteroides* (CFB) group and includes the genus *Bacteroides* (Gherna & Woese, 1992). The phylum *Bacteroidetes* seems to account for about 23 % of human intestinal microbiota (Eckburg *et al.*, 2005; Frank *et al.*, 2007; Hattori & Taylor, 2009; Ley *et al.*, 2005) and the genus *Bacteroides* seems to account for up to 20 % (Matsuki *et al.*, 2004; Rigottier-Gois *et al.*, 2003).

Bacteria belonging to the genus *Bacteroides* are Gram-negative, non-spore-forming, non-motile, anaerobic rods and are generally isolated from the gastrointestinal tract environment (Smith *et al.*, 2006). Some species of the genus *Bacteroides*, including *Bacteroides thetaiotaomicron*, are known to be decomposers in the colon by fermenting carbohydrates and catabolizing polysaccharides (hemicellulose and xylan) (Falony *et al.*, 2009; Flint, 2006; Salyers, 1995; Van der Meulen *et al.*, 2006). Novel strains of members of the genus *Bacteroides* from human faeces and intestinal organs have been identified using culture-independent techniques based on 16S rRNA gene sequence analysis (Dore *et al.*, 1998; Eckburg *et al.*, 2005; Gill *et al.*, 2006; Hayashi *et al.*, 2003; Li *et al.*, 2009; Palmer *et al.*, 2007) and several novel species have recently been identified and characterized (Bakir *et al.*, 2006a, b, c; Chassard *et al.*, 2008; Hayashi *et al.*, 2007; Kitahara *et al.*, 2005; Robert *et al.*, 2007; Song *et al.*, 2004).

In this study, two strains, designated MAJ27^T and MAJ26, were isolated from faeces of a healthy 26-year-old male during a Korean study on the diversity of cultivable intestinal microbiota. The strains were isolated on supplemented brain heart diffusion medium (BHIS) containing 50 mg kanamycin l^{-1} (Sigma) after 2 days at 37 °C in an anaerobic chamber (Bactron II; Shel Lab) containing $N_2/H_2/CO_2$ (90 : 5 : 5) (Bacic & Smith, 2008; Hecht, 2006; Smith *et al.*, 2006). The isolates were subcultured on Eggerth–Gagnon (EG) medium supplemented with 5 % horse blood under anaerobic conditions

Abbreviation: CFB, *Cytophaga–Flavobacter–Bacteroides*.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains MAJ27^T and MAJ26 are GQ496624 and GQ496623, respectively.

The biochemical characteristics and fatty acid compositions of strains MAJ27^T and MAJ26 and their closest phylogenetic neighbours are available with the online version of this paper.

and stored as a suspension in 10 % skimmed milk (BBL) with 10 % glycerol at −80 °C. Reference strains *B. thetaiotaomicron* VPI 5482^T and *Bacteroides finegoldii* 199^T were obtained from DSMZ and KCTC, respectively, and maintained under the same conditions.

For phenotypic, chemotaxonomic and phylogenetic characterization, strains were cultivated on EG medium at 37 °C and pH 7.6–7.8 for 2 or 3 days, unless otherwise stated. Growth of the isolates at 12, 15, 25, 30, 37, 43 and 47 °C and under aerobic conditions was observed on PYG medium (DSMZ 104). To determine bile resistance, the isolates were cultivated on PYG medium supplemented with 0.1–0.4 % (w/v) bile salts (Sigma). Gram staining was performed with a Gram staining kit (bioMérieux). Spore staining was determined with malachite green dye. Cell morphology and Gram and spore staining were observed using phase-contrast microscopy (Eclipse 50*i*; Nikon). Catalase and oxidase activities were investigated with 3 % (v/v) hydrogen peroxide solution and 1 % (w/v) *p*-tetramethyl phenylenediamine solution (bioMérieux), respectively. The motility of the isolates was determined by stabbing the centre of a column of PYG medium containing 0.4 % agar. Other phenotypic characteristics were determined using API 20A and Rapid ID 32A (bioMérieux), according to the manufacturer's instructions (Bakir *et al.*, 2006a). The results of the biochemical analyses are given in Table 1, Supplementary Table S1 (available in IJSEM Online) and the species description.

Fatty acids of the isolates and *B. thetaiotaomicron* VPI 5482^T were obtained from cells by saponification, methylation and extraction according to the Sherlock Microbial Identification System (MIDI, 1999). Fatty acid compositions were determined using GC (Hewlett Packard 6890) and analysed using Microbial Identification software (Sasser, 1990). The predominant fatty acids of strains MAJ27^T and MAJ26 were similar to those of *B. thetaiotaomicron* VPI 5482^T (see Supplementary Table S2 in IJSEM Online). Strains MAJ27^T and MAJ26 contained anteiso-$C_{15:0}$ (42.5 and 39.3 %, respectively) and iso-$C_{17:0}$ 3-OH (14.8 and 14.2 %, respectively). Within the CFB group, large amounts of branched fatty acids are found (Brondz *et al.*, 1991; Paster *et al.*, 1994) and branched 15-carbon non-hydroxy and 17-carbon 3-hydroxy acids are the predominant fatty acids for the genus *Bacteroides* (Mayberry *et al.*, 1982).

For 16S rRNA gene sequence analysis, the genes of the isolates were amplified by colony PCR using four bacteria-specific primers (8F, 968F, 518R and 1492R; Baker *et al.*, 2003). The PCR products were purified using a QIAquick PCR Purification kit (Qiagen) and sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems), according to the manufacturers' instructions. The reaction mixtures were analysed using an automated system (PRISM 3730XL DNA Analyzer; Applied Biosystems). The partial 16S rRNA gene sequences were assembled using DNASTAR in the SeqMan software package and compared with other sequences in the GenBank database. The isolates were found to be closely related to strains of species belonging to the genus *Bacteroides*. The 16S rRNA gene sequence similarity between strain MAJ27^T and strain MAJ26 was 99.9 %. The 16S rRNA gene sequence similarities between strains MAJ27^T and MAJ26 with *B. thetaiotaomicron* VPI 5482^T were 98.8 and 98.7%, respectively, and with *B. finegoldii* JCM 13345^T were 97.0 and 96.9 %, respectively. The sequences from the isolates were aligned with 16S rRNA gene sequences of the genus *Bacteroides* from GenBank using the multiple sequence alignment program CLUSTAL_X (1.83) (Thompson *et al.*, 1997). The trimmed alignment was converted to MEGA

Table 1. Comparative characteristics of *Bacteroides faecis* sp. nov. with closely related type strains of species of the genus *Bacteroides*

Taxa: 1, *Bacteroides faecis* sp. nov. (*n*=3); 2, *B. thetaiotaomicron* VPI 5482^T; 3, *B. finegoldii* 199^T; 4, *B. ovatus* ATCC 8483^T; 5, *B. xylanisolvens* XB1A^T; 6, *B. caccae* ATCC 43185^T; 7, *B. nordii* WAL 11050^T; 8, *B. salyersiae* WAL 10018^T. Data were taken from this study, Bakir *et al.* (2006a), Chassard *et al.* (2008), Johnson *et al.* (1986) and Song *et al.* (2004). All strains were positive for acid production from D-xylose, cellobiose and L-rhamnose and hydrolysis of aesculin. +, Positive; W, weakly positive; −$^+$, most strains negative; −, negative.

Characteristic	1	2	3	4	5	6	7	8
Isolation source (human)	Faeces	Faeces	Faeces	Faeces	Faeces	Faeces	Intestinal specimens	Faeces
Gelatin hydrolysis	−	−	−	−	−	W	+	+
Indole formation	+	+	−	+	−	−	+	+
Acid production from:								
L-Arabinose	+	+	+	+	+	+	−	+
Salicin	−	−	+	+	+	−$^+$	−	−
Trehalose	−	+	−	+	+	+	−	−
Enzyme activity								
α-Fucosidase	+	+	−	+	+	+	−	−
Arginine arylamidase	−	−	+	−	−	+	−	−
DNA G+C content (mol%)	42.7	42	43	ND	42.8	40	41.4	42.0

format for phylogenetic analyses. Phylogenetic consensus trees were constructed using the neighbour-joining and maximum-parsimony methods with MEGA4 (Tamura *et al.*, 2007) and evaluated using 1000 bootstrap replicates (Kluge & Farris, 1969; Saitou & Nei, 1987). The phylogenetic analysis positioned the isolates within the *Bacteroides* group and also demonstrated that the isolates were closely related to *B. thetaiotaomicron* VPI 5482^T and *B. finegoldii* JCM 13345^T (Fig. 1).

Genomic DNA of the isolates and the reference strains was extracted using a G-spin Genomic DNA Extraction kit (Intron Biotechnology). DNA–DNA hybridization was performed using the fluorometric method described by Ezaki *et al.* (1989) with modifications (Hirayama *et al.*, 1996). As reported by Wayne *et al.* (1987), strains with DNA–DNA relatedness >70 % are generally considered as belonging to the same species. DNA–DNA relatedness between strain MAJ27^T and strain MAJ26 was 97 %,

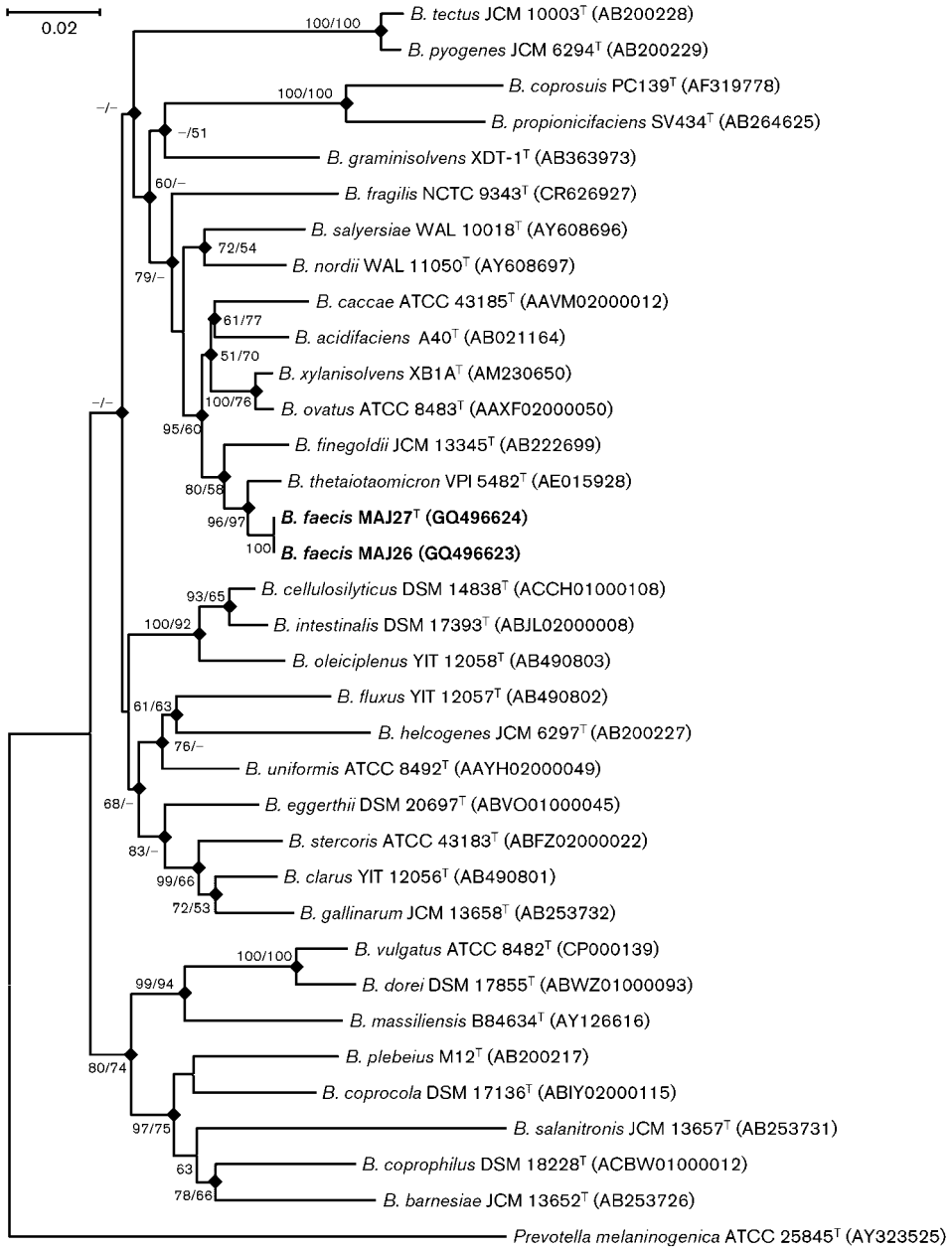

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic position of strains MAJ27^T and MAJ26. Filled diamonds indicate that the corresponding nodes were also recovered in trees generated with the maximum-parsimony method. Bootstrap values (>50 %) based on 1000 replicates for the neighbour-joining and maximum-parsimony methods, respectively, are shown at branch nodes. Bar, 0.02 substitutions per site.

between strain MAJ27^T and *B. thetaiotaomicron* VPI 5482^T was 22 % and between strain MAJ27^T and *B. finegoldii* 199^T was 21 %. This indicated that the isolates belonged to a single novel species of the genus *Bacteroides*. The DNA G+C content of the isolates was determined using a fluorometric method with SYBR Green I and real-time PCR (Gonzalez & Saiz-Jimenez, 2002). The genomic DNA of *Escherichia coli* K-12 was used as the calibration reference (Gonzalez & Saiz-Jimenez, 2002). The DNA G+C content of the isolates was 42.7±1 mol%, which fell within the limits of the range reported for the genus *Bacteroides* (40–48 mol%; Shah, 1992).

On the basis of phenotypic, chemotaxonomic, genotypic and phylogenetic studies, we propose that strain MAJ27^T and strain MAJ26 be classified as representing a novel species of the genus *Bacteroides*, for which the name *Bacteroides faecis* sp. nov. is proposed.

Description of *Bacteroides faecis* sp. nov.

Bacteroides faecis (fa.e′cis. L. gen. n. *faecis* of dregs, of faeces, referring to faecal origin).

Anaerobic, Gram-negative, non-motile and non-spore-forming rods, 1.5–2.0 μm in length and 1.0 μm in width, generally observed singly. After cultivation on PYG medium at 37 °C for 4 days, colonies are pale yellow, circular, glistening and convex with a buttery texture and 1.0–1.5 mm in diameter. Grows at 25–43 °C (optimum 37 °C). Oxidase-, catalase- and urease-negative and indole-positive. Sensitive to bile. Hydrolyses aesculin, but not gelatin. With API 20A, produces acid from L-arabinose, cellobiose, D-glucose, lactose, maltose, D-mannose, raffinose, L-rhamnose, sucrose and D-xylose, but not from glycerol, D-mannitol, melezitose, salicin, D-sorbitol or trehalose. With API Rapid ID 32A, positive for α-fucosidase, α- and β-galactosidases, α- and β-glucosidases, *N*-acetyl-β-glucosaminidase, glutamic acid decarboxylase, alkaline phosphatase, leucyl glycine arylamidase, alanine arylamidase, glutamyl glutamic acid arylamidase, production of indole and fermentation of D-mannose and raffinose, but negative for β-galactosidase 6-phosphate, α-arabinosidase, β-glucuronidase, urease, arginine dihydrolase, arginine arylamidase, proline arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, glycine arylamidase, histidine arylamidase, serine arylamidase and reduction of nitrate. The major fatty acids are anteiso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and $C_{16:0}$ 3-OH. The DNA G+C content of the type strain is 42.7±1 mol%.

The type strain, MAJ27^T (=KCTC 5823^T=JCM 16478^T), was isolated from human faeces. Strain MAJ26 (=KCTC 5822=JCM 16477) is a reference strain.

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