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 MAJ27T 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 5482T and Bacteroides finegoldii JCM 13345T 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-C15:0, 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 MAJ27T and MAJ26 be classified as representing a novel species, Bacteroides faecis sp. nov. The type strain is MAJ27T (=KCTC 5823T=JCM 16478T).

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-spor-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 MAJ27T 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 (BHI) containing 50 mg kanamycin l−1 (Sigma) after 2 days at 37 °C in an anaerobic chamber (Bactron II; Shel Lab) containing N2/H2/CO2 (90 : 5 : 5) (Bacic & Smith, 2008; Bae et al., 2003) after 2 days at 37 °C in an anaerobic chamber (Bactron II; Shel Lab) containing N2/H2/CO2 (90 : 5 : 5) (Bacic & Smith, 2008; Hecht, 2006; Smith et al., 2006). The isolates were subcultured on Eggерт–Gagnon (EG) medium supplemented with 5 % horse blood under anaerobic conditions.
and stored as a suspension in 10% skimmed milk (BBL) with 10% glycerol at -80 °C. Reference strains B. thetaiotaomicron VPI 5482T and Bacteroides finegoldii 199T 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 anaerobic 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 50i; Nikon).

Catalase and oxidase activities were investigated with tetramethyl phenylenediamine solution (bioMérieux), according to the manufacturer’s instructions. Other phenotypic characteristics were determined using API 20A and Rapid ID 32A (bioMérieux) or with 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 MAJ27T and strain MAJ26 was 99.9%. The 16S rRNA gene sequence similarities between strains MAJ27T and MAJ26 with B. thetaiotaomicron VPI 5482T were 98.8 and 98.7%, respectively, and with B. finegoldii JCM 13345T 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

<table>
<thead>
<tr>
<th>Characteristic</th>
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<tr>
<td>Isolation source (human)</td>
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<td>Acid production from:</td>
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<td>D-Arabinose</td>
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<td>Salicin</td>
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<td>Enzyme activity</td>
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<td>DNA G+C content (mol%)</td>
<td>42.7</td>
<td>42</td>
<td>43</td>
<td>ND</td>
<td>42.8</td>
<td>40</td>
<td>41.4</td>
<td>42.0</td>
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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 MAJ27T and strain MAJ26 was 99.9%. The 16S rRNA gene sequence similarities between strains MAJ27T and MAJ26 with B. thetaiotaomicron VPI 5482T were 98.8 and 98.7%, respectively, and with B. finegoldii JCM 13345T 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

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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<sup>T</sup> and <i>B. thetaiotaomicron</i> VPI 5482<sup>T</sup> was 22% and between strain MAJ27<sup>T</sup> and <i>B. finegoldii</i> 199<sup>T</sup> was 21%. This indicated that the isolates belonged to a single novel species of the genus <i>Bacteroides</i>. 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 <i>Escherichia coli</i> 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 <i>Bacteroides</i> (40–48 mol%; Shah, 1992).

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

**Description of <i>Bacteroides faecis</i> sp. nov.**

<i>Bacteroides faecis</i> (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 0.1–1.5 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 β-glucuronidase, urease, arginine dihydro-α-galactosidase, α-fucosidase, β-glucuronidase, α- and β-glucosidases, <i>N</i>-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 D-sorbitol or trehalose. With API Rapid ID 32A, positive for arabinosidase, β-D-galactosidase, α-D-glucosidase, malto- and manno- and raffinose, L-rhamnose, sucrose and D-xylose, but not from glycerol, mannitol, melezitose, salicin, D-sorbitol or trehalose. With API Rapid ID 32A, positive for x-fucosidase, α- and β-galactosidases, x- and β-glucosidases, <i>N</i>-acetyl-β-glucosaminidase, glutamic acid decarboxylase, alkaline phosphatase, leucyl glycine arylamidase, alanine arylamidase, glutamyl glutamic acid arylamidase, production of indole and fermentation of <i>D</i>-mannose and raffinose, but negative for β-galactosidase 6-phosphate, α-arabinosidase, β-glucuronidase, urease, arginine dihydro-lase, 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<sub>15:0</sub>, iso-C<sub>17:0</sub> 3-OH and C<sub>16:0</sub> 3-OH. The DNA G+C content of the type strain is 42.7 ± 1 mol%.

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

**Acknowledgements**

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**References**


