Members of the genus *Bacteroides* are a predominant component of human faecal microbiota. Although many species were originally included in this group, the taxonomy of the genus *Bacteroides* has undergone significant changes in the last decade (Jousimies-Somer & Summanen, 2002). Consequently, some species that were not included in *Bacteroides sensu stricto* (Shah & Collins, 1989) have been transferred to novel genera, such as *Alistipes* (Rautio et al., 2003), *Dialister* (Moore & Moore, 1994), *Dichelobacter* (Dewhirst et al., 1990) and *Tannerella* (Sakamoto et al., 2002). More recently, the misclassified species *Bacteroides distasonis* (Eggerth & Gagnon, 1933), *Bacteroides goldsteinii* (Song et al., 2005) and *Bacteroides merdae* (Johnson et al., 1986) were ultimately reclassified as *Parabacteroides distasonis*, *Parabacteroides goldsteinii* and *Parabacteroides merdae* (Sakamoto & Benno, 2006). In the process of collecting *P. merdae* JCM 13405 (Sakamoto & Benno, 2006), we found a *P. merdae*-like strain isolated from human faeces that was not identified as *P. merdae* by a PCR technique using species-specific primers (Liu et al., 2003). The present study was designed to determine the taxonomic status of this strain.

The strains used in the present study were maintained for 2 days at 37°C under CO2 on Eggerth Gagnon (EG) agar (Merck) supplemented with 5% (v/v) horse blood. Strain M-165T was isolated from human faeces. Bacteroides bile- aesculin agar (Shah, 1992) was used to check whether the growth of the isolate was inhibited on this medium. A PCR technique involving species-specific primers (Liu et al., 2003) was used to identify the *P. merdae*-like strain, as described previously (Sakamoto & Benno, 2006). Physiological reactions were determined with an API 20A anaerobe test kit (in duplicate) as recommended by the manufacturer (bioMérieux). The metabolic end products were prepared as described by Holdeman et al. (1977) and were analysed as described previously (Sakamoto et al., 2005). Fatty acid methyl esters were obtained from wet cells (approx. 40 mg) by saponification, methylation and extraction using the method of Miller (1982) with minor modifications (Kuykendall et al., 1988). Cellular fatty acid profiles were determined by using the MIDI microbial identification system (Microbial ID). Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987) and were analysed as described previously (Sakamoto et al., 2002). Biochemical reactions were determined with the Rapid ID 32A anaerobe identification kit (in duplicate) as recommended by the manufacturer (bioMérieux). Chromosomal DNA was isolated by using methods described previously (Marmur, 1961; Saito & Miura, 1963), but with some modifications. The DNA G+C

---

**Parabacteroides johnsonii** sp. nov., isolated from human faeces

Mitsuo Sakamoto, Maki Kitahara and Yoshimi Benno

Microbe Division/Japan Collection of Microorganisms, RIKEN BioResource Center, Wako, Saitama 351-0198, Japan

A bacterial strain isolated from human faeces, M-165T, was characterized in terms of its phenotypic and biochemical features, cellular fatty acid profile, menaquinone profile and phylogenetic position (based on 16S rRNA gene sequence analysis). A 16S rRNA gene sequence analysis showed that the isolate was a member of the genus *Parabacteroides*. Strain M-165T was closely related to *Parabacteroides merdae* strains, showing 98% sequence similarity. The strain was obligately anaerobic, non-pigmented, non-spore-forming, non-motile, Gram-negative, rod-shaped and was able to grow on media containing 20% bile. Although the phenotypic characteristics of the strain M-165T were similar to those of *P. merdae*, the isolate could be differentiated from *P. merdae* by means of API 20A tests for L-arabinose and L-rhamnose fermentation. DNA–DNA hybridization experiments revealed the genomic distinctiveness of the novel strain with respect to *P. merdae* JCM 9497T (≤60% DNA–DNA relatedness). The DNA G+C content of the strain is 47.6 mol%. On the basis of these data, strain M-165T represents a novel species of the genus *Parabacteroides*, for which the name *Parabacteroides johnsonii* sp. nov. is proposed. The type strain is M-165T (= JCM 13406T = DSM 18315T).

---

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain M-165T is AB261128.

The cellular fatty acid compositions of strain M-165T and closely related species are available in a supplementary table in IJSEM Online.

---

**Correspondence**

Mitsuo Sakamoto
sakamoto@jcm.riken.jp

---

International Journal of Systematic and Evolutionary Microbiology (2007), 57, 293–296

DOI 10.1099/ijs.0.64588-0
content was determined by using the HPLC method of Tamaoka & Komagata (1984). The elution solvent was a mixture of 0.02 M NH₄H₂PO₄ and acetonitrile (20:1, v/v). DNA–DNA hybridization experiments were carried out in microplate wells as described by Ezaki et al. (1989). Hybridization was performed at 44°C for 16 h. The 16S rRNA gene sequence was analysed as described previously (Sakamoto et al., 2002). Related sequences were aligned with the CLUSTAL W program (Thompson et al., 1994) and corrected by manual inspection. Nucleotide substitution rates (K_{sub} values) were calculated (Kimura, 1980) after gaps and unknown bases had been eliminated. The phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987). A bootstrap resampling analysis (Felsenstein, 1985) was performed to estimate the confidence of the tree topologies.

Strain M-165T was obligately anaerobic, non-pigmented, non-spore-forming, non-motile, Gram-negative and rod-shaped; it was not identified as *P. merdae* by the PCR. Growth of the isolate was not inhibited on medium containing 20% bile. Cells on EG agar were 0.8 μm by 1.7–2.5 μm in size and occurred singly. On EG agar plates, colonies were 1–2 mm in diameter, grey to off-white in colour, circular, entire, slightly convex and smooth. The phenotypic characteristics are given in the species description. The isolate could be differentiated from *P. merdae* by the ability of the former to ferment L-arabinose and L-rhamnose in the API 20A panel. Catalase production was a key characteristic for differentiating the isolate (and *P. distasonis*) from *P. merdae*. Biochemical characteristics of the isolate determined using the Rapid ID 32A kit were similar to those of *P. merdae* (Sakamoto & Benno, 2006); only pyroglutamic acid arylamidase activity was different from *P. merdae*.

The major cellular fatty acids of the isolate were anteiso-15:0 and iso-17:0 3-OH (32 and 21%, respectively). The amount of 18:1o9c (9%) present was slightly lower than that found in *Parabacteroides* species (14–16%), while the amount of 15:0 (5.5%) present was slightly higher than that found in *Parabacteroides* species (1.3–3.1%) (see Supplementary Table S1 available in IJSEM Online).

The major menaquinones of the isolate were MK-9 (54%) and MK-10 (39%). A small amount of MK-8 (5%) was also present. These data are in agreement with the description of the genus *Parabacteroides* (Sakamoto & Benno, 2006). The menaquinone compositions of strain M-165T and *P. merdae* JCM 9497T were almost the same.

Approximately 1500 bases of the 16S rRNA gene sequence were determined for the isolate. For the phylogenetic analysis, 1340 bp (positions 61–1375; *Escherichia coli* numbering system) sequences of each strain were used. The 16S rRNA gene sequence analysis showed that strain M-165T represented a species within the genus *Parabacteroides* (Fig. 1), being closely related to *P. merdae* (similarities of 97.9 and 98% for JCM 9497T and JCM 13405, respectively).

The DNA G+C content of strain M-165T was 47.6 mol%. This value is almost the same as those for the reference strains (Table 1). The levels of DNA–DNA relatedness observed serve to distinguish strain M-165T from *P. merdae* JCM 9497T and JCM 13405 (relatively high DNA–DNA relatedness, ≤60% ; Table 1).

On the basis of the above-mentioned findings and the 16S rRNA gene sequence analysis, strain M-165T represents a novel species of the genus *Parabacteroides*, for which the name *Parabacteroides johnsonii* sp. nov. is proposed.
differential characteristics of *P. johnsonii* sp. nov. and other *Parabacteroides* species are shown in Table 2.

**Table 1.** DNA G+C contents and levels of DNA–DNA relatedness

<table>
<thead>
<tr>
<th>Strain</th>
<th>G+C content (mol%)</th>
<th>DNA–DNA relatedness (%) with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1. Strain M-165&lt;sup&gt;T&lt;/sup&gt;</td>
<td>47.6</td>
<td>100</td>
</tr>
<tr>
<td>2. <em>P. merdae</em> JCM 9497&lt;sup&gt;T&lt;/sup&gt;</td>
<td>47.2</td>
<td>58</td>
</tr>
<tr>
<td>3. <em>P. merdae</em> JCM 13405</td>
<td>47.5</td>
<td>60</td>
</tr>
<tr>
<td>4. <em>P. distasonis</em> JCM 5825&lt;sup&gt;T&lt;/sup&gt;</td>
<td>47.1</td>
<td>13</td>
</tr>
<tr>
<td>5. <em>P. goldsteinii</em> JCM 13446&lt;sup&gt;T&lt;/sup&gt;</td>
<td>46.5</td>
<td>12</td>
</tr>
</tbody>
</table>

**Table 2.** Differential characteristics of strain M-165<sup>T</sup> (*Parabacteroides johnsonii* sp. nov.) and other *Parabacteroides* species

+ , Positive; − , negative; V, variable. Data for *P. goldsteinii* were taken from Song et al. (2005) and this study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain M-165&lt;sup&gt;T&lt;/sup&gt;</th>
<th><em>P. merdae</em></th>
<th><em>P. distasonis</em></th>
<th><em>P. goldsteinii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase production</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>V</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salicin</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>D-Cellobiose</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Melezitose</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Enzyme activities:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Pyroglutamic acid arylamidase</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

**Description of Parabacteroides johnsonii** sp. nov.

*Parabacteroides johnsonii* (john.so’ni.i. N.L. gen. n. johnsonii of Johnson, named after the American molecular taxonomist John L. Johnson, who was the first to describe *P. merdae*).

Cells are obligately anaerobic, non-spore-forming, non-motile, Gram-negative rods (0.8 × 1.7–2.5 μm). On EG agar plates, colonies are 1–2 mm in diameter, grey to off-white in colour, circular, entire, slightly convex and smooth. Indole and urease are not produced. Catalase is produced. Aesculin is hydrolysed. Gelatin is not digested. Grows on medium containing 20% bile. Acid is produced from L-arabinose, glucose, lactose, maltose, D-mannose, D-raffinose, L-rhamnose, sucrose, D-trehalose and D-xylene but not from D-cellobiose, glycerol, D-mannitol, D-melezitose, salicin or D-sorbitol. Positive Rapid ID 32A reactions are obtained for α-galactosidase, β-galactosidase, α-glucosidase, α-arabinosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, glutamic acid decarboxylase, alkaline phosphatase, arginine arylamidase, leucine arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, glutamyl glutamic acid arylamidase and serine arylamidase. Mannose and raffinose are fermented. All of the other tests (for urease, arginine dihydrolase, β-phospho-β-galactosidase, β-glucosidase, α-fucosidase, nitrate reduction, indole production, proline arylamidase and pyroglutamic acid arylamidase) are negative. The major end products from PYG broth cultures (1% peptone, 1% yeast extract, 1% glucose) are succinic and acetic acids; small amounts of isovaleric acid and propionic acid are also produced. The major cellular fatty acids are anteiso-15 : 0 and iso-17 : 0 3-OH. The predominant respiratory quinones are menaquinones MK-9 and MK-10. The DNA G+C content of the type strain is 47.6 mol%.

The type strain, M-165<sup>T</sup> ( = JCM 13406<sup>T</sup> = DSM 18315<sup>T</sup>), was isolated from human faeces.

**References**

Dewhirst, F. E., Paster, B. J., La Fontaine, S. & Rood, J. I. (1990). Transfer of *Kingella indologenes* (Snell and Lapage 1976) to the genus *Suttonella* gen. nov. as *Suttonella indologenes* comb. nov.; transfer of...
Bacteroides nodosus (Beveridge 1941) to the genus Dichelobacter gen.
nov., as Dichelobacter nodosus comb. nov.; and assignment of the
genera Cardiobacterium, Dichelobacter, and Suttonella to Cardio-
bacteriaceae fam. nov. in the gamma division of Proteobacteria on
the basis of 16S rRNA sequence comparisons. Int J Syst Bacteriol
40, 426–433.

Eggerth, A. H. & Gagnon, B. H. (1933). The Bacteroides of human

deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-
dilution wells as an alternative to membrane filter hybridization in
which radioisotopes are used to determine genetic relatedness among

approach using the bootstrap. Evolution 39, 783–791.

Institute and State University.

caccae sp. nov., Bacteroides merae sp. nov., and Bacteroides stercoris

Jousimies-Somer, H. & Summanen, P. (2002). Recent taxonomic
changes and terminology update of clinically significant anaerobic
gram-negative bacteria (excluding spirochetes). Clin Infect Dis 35
(Suppl. 1), S17–S21.

rates of base substitutions through comparative studies of nucleotide


Fatty acids, antibiotic resistance, and deoxyribonucleic acid
homology groups of Bradyrhizobium japonicum. Int J Syst Bacteriol
38, 358–361.

Liu, C., Song, Y., McTeague, M., Vu, A. W., Wexler, H. & Finegold,
fragilis group by multiplex PCR assays using group- and species-
specific primers. FEMS Microbiol Lett 222, 9–16.


of bacterial whole-cell fatty acid methyl esters, including hydroxy

gen. nov., sp. nov.; Catonella morbi gen. nov., sp. nov.; Hallella
serogens gen. nov., sp. nov.; Johnsonella ignava gen. nov., sp. nov.; and
Dialister pneumosintes gen. nov., comb. nov., nom. rev., anaerobic gram-negative bacilli from the human gingival crevice.

Rautio, M., Errola, E., Väisänen-Tunkelrott, M. L., Molitoris, D.,
Reclassification of Bacteroides putredinis (Weinberg et al. 1937) in
a new genus Alistipes gen. nov., as Alistipes putredinis comb. nov.,
and description of Alistipes finegoldii sp. nov., from human sources.
Syst Appl Microbiol 26, 182–188.

deoxyribonucleic acid by phenol treatment. Biochim Biophys Acta
72, 619–629.

method for reconstructing phylogenetic trees. Mol Biol Evol 4,
406–425.

distasonis, Bacteroides goldsteini and Bacteroides merdae as
Parabacteroides distasonis gen. nov., comb. nov., Parabacteroides
goldsteini comb. nov. and Parabacteroides merdae comb. nov. Int

Sakamoto, M., Suzuki, M., Umeda, M., Ishikawa, I. & Benno, Y.
(2002). Reclassification of Bacteroides forsythus (Tanner et al. 1986)
as Tannerella forsythensis corrig., gen. nov., comb. nov. Int J Syst Evol
Microbiol 52, 841–849.

Sakamoto, M., Huang, Y., Umeda, M., Ishikawa, I. & Benno, Y.

Prokaryotes, 2nd edn, pp. 3593–3607. Edited by A. Balows, H. G.
Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York:
Springer.

Bacteroides (Castellani and Chalmers) to Bacteroides fragilis and

Song, Y., Liu, C., Lee, J., Bolanos, M., Vaisanen, M. L. & Finegold,

composition by reversed-phase high-performance liquid chromato-

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W:
improving the sensitivity of progressive multiple sequence alignment
through sequence weighting, position-specific gap penalties and