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Parabacteroides johnsonii sp. nov., isolated from human faeces

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A bacterial strain isolated from human faeces, M-165^T, 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-165^T 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-165^T 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 9497^T ($\leqslant$60 % DNA–DNA relatedness). The DNA G+C content of the strain is 47.6 mol%. On the basis of these data, strain M-165^T represents a novel species of the genus *Parabacteroides*, for which the name *Parabacteroides johnsonii* sp. nov. is proposed. The type strain is M-165^T (=JCM 13406^T=DSM 18315^T).

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 CO_2 on Eggerth Gagnon (EG) agar (Merck) supplemented with 5 % (v/v) horse blood. Strain M-165^T 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

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

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

content was determined by using the HPLC method of Tamaoka & Komagata (1984). The elution solvent was a mixture of 0.02 M $NH_4H_2PO_4$ 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_{nuc} 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-165^{T} 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 : 1ω9*c* (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-165^{T} and *P. merdae* JCM 9497^{T} 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-165^{T} represented a species within the genus *Parabacteroides* (Fig. 1), being closely related to *P. merdae* (similarities of 97.9 and 98 % for JCM 9497^{T} and JCM 13405, respectively).

The DNA G + C content of strain M-165^{T} 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-165^{T} genomically from *P. merdae* JCM 9497^{T} and JCM 13405 (relatively high DNA–DNA relatedness, $\leqslant$60 %; Table 1).

On the basis of the above-mentioned findings and the 16S rRNA gene sequence analysis, strain M-165^{T} represents a novel species of the genus *Parabacteroides*, for which the name *Parabacteroides johnsonii* sp. nov. is proposed. The

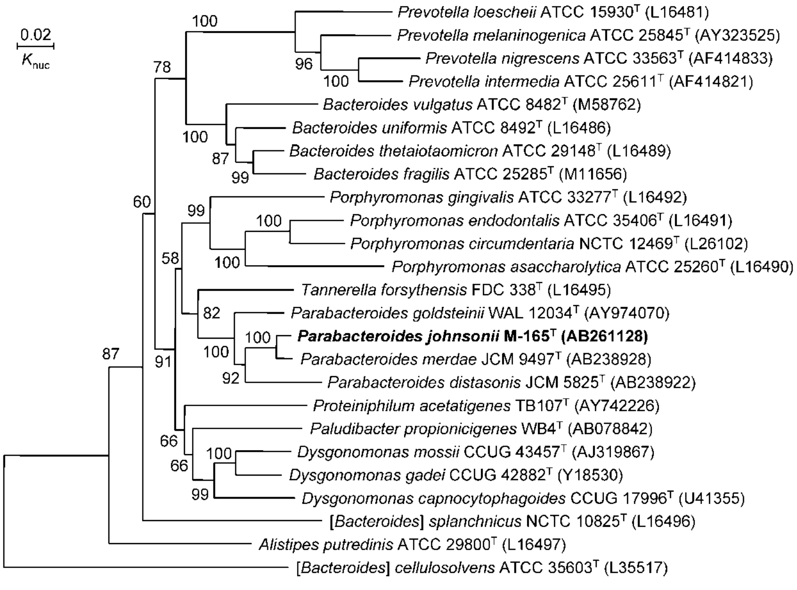

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between strain M-165^{T} and related species. Accession numbers are shown in parentheses. Numbers at nodes indicate bootstrap percentages from 1000 replicates. Bar, 0.02 substitutions per nucleotide position.

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

Strain	G+C content (mol%)	DNA–DNA relatedness (%) with:				
		1	2	3	4	5
1. Strain M-165^T	47.6	100	60	59	6	11
2. *P. merdae* JCM 9497^T	47.2	58	100	90	7	4
3. *P. merdae* JCM 13405	47.5	60	91	100	8	4
4. *P. distasonis* JCM 5825^T	47.1	13	15	16	100	9
5. *P. goldsteinii* JCM 13446^T	46.5	12	8	8	4	100

Table 2. Differential characteristics of strain M-165^T (*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.

Characteristic	Strain M-165^T	*P. merdae*	*P. distasonis*	*P. goldsteinii*
Catalase production	+	−	+	V
Acid production from:				
Salicin	−	−	+	−
L-Arabinose	+	−	−	−
D-Cellobiose	−	−	+	+
D-Melezitose	−	−	+	−
L-Rhamnose	+	−	+	+
Enzyme activities:				
β-Glucosidase	−	−	+	+
β-Glucuronidase	+	+	−	+
Pyroglutamic acid arylamidase	−	+	−	−

differential characteristics of *P. johnsonii* sp. nov. and other *Parabacteroides* species are shown in Table 2.

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-xylose 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, leucyl glycine arylamidase, phenylalanine 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, 6-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^T (=JCM 13406^T=DSM 18315^T), was isolated from human faeces.

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