Parabacteroides gordonii sp. nov., isolated from human blood cultures

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Three bacterial strains that had been isolated from human blood cultures, MS-1^T, MS-2 and MS-3, were characterized for their phenotypic and biochemical features, cellular fatty acid profiles, menaquinone profiles and phylogenetic positions based on 16S rRNA gene sequence analysis. 16S rRNA gene sequence analysis showed that the isolates were members of the genus *Parabacteroides*. These isolates were most closely related to *Parabacteroides goldsteinii* JCM 13446^T, with 95.9% 16S rRNA gene sequence similarity. The levels of sequence similarity among the three strains were 99.7–100%. The isolates were obligately anaerobic, non-pigmented, non-spore-forming, non-motile, Gram-negative and rod-shaped. The strains grew on media containing 20% bile. These strains could be differentiated from *P. goldsteinii* by their ability to ferment L-arabinose and inability to ferment cellobiose, L-rhamnose and trehalose or to hydrolyse aesculin. The major menaquinone of the isolates was MK-10. Based on these data, we propose a novel *Parabacteroides* species, *Parabacteroides gordonii* sp. nov. The type strain is MS-1^T (=JCM 15724^T =CCUG 57478^T).

The genus *Parabacteroides* was proposed by Sakamoto & Benno (2006) for three 'outmembers' of the genus *Bacteroides*, *Bacteroides distasonis* (Eggerth & Gagnon, 1933), *B. goldsteinii* (Song *et al.*, 2005) and *B. merdae* (Johnson *et al.*, 1986). Sakamoto *et al.* (2007) subsequently proposed a fourth species of the genus, *Parabacteroides johnsonii*. These species are phylogenetically distinct from species of the genus *Bacteroides* and are related to *Tannerella forsythia* (Sakamoto *et al.*, 2002). *Parabacteroides* species are isolated mainly from human faeces and clinical specimens, such as peritoneal fluids, appendix tissues and intra-abdominal abscesses (Song *et al.*, 2005). The present study was designed to determine the taxonomic status of three strains of Gram-negative, obligately anaerobic rods that had been isolated from human blood cultures.

Strains MS-1^T, MS-2 and MS-3 were isolated from blood cultures (days 1, 6 and 9) from a 73-year-old Japanese male

presenting with a 1-week history of fever and diarrhoea. An abdominal computed tomography scan showed a large liver with central necrosis and hepatocellular carcinoma. Due to the patient's unstable status, a liver biopsy and treatment for the presumed carcinoma were deferred. Sputum, urine and stool cultures did not contain the same isolates. Strains MS-1^T, MS-2 and MS-3 were maintained on Eggerth Gagnon (EG) agar (Merck) supplemented with 5% (v/v) horse blood for 2 days at 37 °C in an atmosphere containing 100% CO₂. For this study, *Parabacteroides goldsteinii* JCM 13446^T was obtained and subjected to some of the phenotypic and biochemical tests.

Bacteroides bile aesculin agar (Shah, 1992) was used to check whether bile inhibited growth of the isolates. Physiological and biochemical reactions were determined in duplicate with the API 20A anaerobe test kit and the Rapid ID 32A anaerobe identification kit, respectively (bioMérieux), according to the manufacturer's instructions. The metabolic end products were prepared as described by Holdeman *et al.* (1977) and were analysed as described previously by Sakamoto *et al.* (2005). Fatty acid methyl esters (FAMES) were obtained from about

Abbreviation: FAME, fatty acid methyl ester.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains MS-1^T, MS-2 and MS-3 are AB470343–AB470345, respectively.

40 mg wet cells by saponification, methylation and extraction using minor modifications (Kuykendall *et al.*, 1988) of the method of Miller (1982). Cellular fatty acid profiles were determined with the Microbial Identification System (Microbial ID; MIDI). Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987) and were analysed as described previously by Sakamoto *et al.* (2002).

Chromosomal DNA was isolated using described methods (Marmur, 1961; Saito & Miura, 1963) with some modifications. The DNA base composition was determined with the HPLC method of Tamaoka & Komagata (1984). The elution solvent was a mixture of 0.02 M $\text{NH}_4\text{H}_2\text{PO}_4$ and acetonitrile (20:1, v/v). The 16S rRNA gene sequence was analysed as described previously (Sakamoto *et al.*, 2002). Related sequences were aligned with the CLUSTAL X version 2.0 program (Larkin *et al.*, 2007) and the alignment was corrected manually. After gaps and unknown bases were eliminated, nucleotide substitution rates (K_{nuc} values) were calculated (Kimura, 1980). The phylogenetic tree was constructed with the neighbour-joining method (Saitou & Nei, 1987). A bootstrap resampling analysis (Felsenstein, 1985) was performed to evaluate the confidence limits of tree topologies.

Cells of strains MS-1^T, MS-2 and MS-3 were non-pigmented, non-spore-forming and non-motile. The growth of the isolates 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. Colonies on EG agar after 48 h of incubation at 37 °C under anaerobic conditions were 1–2 mm in diameter, grey to off-white-grey, circular, entire, slightly convex and smooth. Phenotypic characteristics are given in the species description and in Table 1. These strains could be differentiated from *Parabacteroides goldsteinii* JCM 13446^T by their ability to ferment L-arabinose and inability to ferment cellobiose, L-rhamnose and trehalose or to hydrolyse aesculin with the API 20A kit. Catalase activity was not detected with the API 20A kit but it was detected on EG agar. Using the Rapid ID 32A kit, these strains could be differentiated from *P. goldsteinii* JCM 13446^T by the presence of α-arabinosidase activity and the lack of β-glucosidase, phenylalanine arylamidase, tyrosine arylamidase, glycine arylamidase, histidine arylamidase, glutamyl glutamic acid arylamidase and serine arylamidase activities.

The major cellular fatty acid of the isolates was anteiso-15:0 (42–47 %) (Table 2). The proportion of anteiso-15:0 was larger than those recorded for other *Parabacteroides* species (25–32 %), while the proportion of iso-17:0 3-OH (2–5 %) was significantly smaller than for other *Parabacteroides* species (21–26 %) (Sakamoto & Benno, 2006; Sakamoto *et al.*, 2007). The major menaquinones of two isolates (MS-1^T and MS-2) were MK-9 (10 and 9 %), MK-10 (72 and 71 %) and MK-11 (13 and 14 %), respectively. Minor amounts of MK-8 (1 and 2 %) were also present. The menaquinone compositions of the

Table 1. Differential characteristics of *Parabacteroides gordonii* sp. nov. and other *Parabacteroides* species

Taxa: 1, *Parabacteroides gordonii* sp. nov. (n=3); 2, *P. goldsteinii* JCM 13446^T (data from this study and Song *et al.*, 2005); 3, *P. distasonis*; 4, *P. johnsonii*; 5, *P. merdae* [data in columns 3–5 from Sakamoto & Benno (2006) and Sakamoto *et al.* (2007)]. +, Positive; w, weakly positive; v, variable; –, negative.

Characteristic	1	2	3	4	5
Catalase production	v	v	+	+	–
Aesculin hydrolysis	–	+	+	+	+
Acid produced from:					
Salicin	–	–	+	–	–
L-Arabinose	+	–	–	+	–
Cellobiose	–	w	+	–	–
Melezitose	–	–	+	–	–
L-Rhamnose	–	+	+	+	–
Trehalose	–	+	+	+	+
Enzyme activities					
α-Arabinosidase	+	–	+	+	+
β-Glucosidase	–	+	+	–	–
β-Glucuronidase	–	–*	–	+	+
Glutamic acid decarboxylase	–	–	+	+	+
Glutamyl glutamic acid arylamidase	–	+	+	+	+
Glycine arylamidase	–	+	+	+	+
Histidine arylamidase	–	+	+	+	+
Phenylalanine arylamidase	–	+	+	+	+
Pyroglutamic acid arylamidase	–	–	–	–	+
Serine arylamidase	–	+	+	+	+
Tyrosine arylamidase	–	+	+	+	+

*Reported as positive by Song *et al.* (2005) using Rosco diagnostic tablets.

isolates were somewhat different from those of other *Parabacteroides* species (Sakamoto & Benno, 2006; Sakamoto *et al.*, 2007). For *P. goldsteinii* JCM 13446^T, the major menaquinones are MK-9 (24 %) and MK-10 (67 %) and the minor menaquinones are MK-7 (<1 %), MK-8 (3 %) and MK-11 (4 %). The DNA G+C contents of strains MS-1^T, MS-2 and MS-3 were 44.6, 45.2 and 42.3 mol%, respectively. These values were slightly lower than those recorded for other *Parabacteroides* species (46.5–47.6 mol%) (Sakamoto *et al.*, 2007).

Approximately 1500 bases of the 16S rRNA gene sequence were determined for the isolates. For the phylogenetic analysis, 1340 bp (positions 61–1375; *Escherichia coli* numbering system) from each strain were used. 16S rRNA gene sequence analysis showed that strain MS-1^T was a member of the genus *Parabacteroides* (Fig. 1) and was closely related to *P. goldsteinii* JCM 13446^T, with 95.9 % 16S rRNA gene sequence similarity. According to Stackebrandt & Goebel (1994), strains showing 16S rRNA gene sequence similarity less than 97 % will not show DNA–DNA reassociation values greater than 60 % and will

Table 2. Cellular fatty acid compositions of *Parabacteroides gordonii* sp. nov. and closely related species

Taxa: 1, *Parabacteroides gordonii* sp. nov. ($n=3$); 2, *P. distasonis* ($n=6$); 3, *P. goldsteinii* JCM 13446^T; 4, *P. johnsonii* JCM 13406^T; 5, *P. merdae* ($n=2$). Data for reference taxa were taken from Sakamoto & Benno (2006) and Sakamoto *et al.* (2007). Values are percentages of total fatty acids. ECL, Equivalent chain-length; ALDE, aldehyde; DMA, dimethylacetal; tr, trace amount ($<1\%$); –, not detected/not reported.

Fatty acid	1	2	3	4	5
Saturated straight-chain					
12:0	tr	–	–	–	–
14:0	1.3 ± 0.1	1.3 ± 0.1	1.1	1.1	1.4 ± 0.1
15:0	1.8 ± 0.5	2.4 ± 0.4	2.0	5.5	1.4 ± 0.1
16:0	4.8 ± 0.2	5.3 ± 1.3	7.2	5.4	5.9 ± 1.6
17:0	–	–	–	tr	–
18:0	tr	1.1 ± 0.1	1.7	1.1	1.2 ± 0.2
Unsaturated straight-chain					
14:1 ω 5c	–	tr	–	tr	tr
16:1 ω 7c	1.5 ± 0.3	1.6 ± 0.1	1.9	1.0	1.6 ± 0.1
16:1 ω 7c DMA	tr	–	–	–	–
17:1 ω 8c	–	tr	tr	–	tr
18:2 ω 6,9c	1.2 ± 0.1	1.3 ± 0.1	2.2	2.4	1.3 ± 0.4
18:1 ω 9c	11.1 ± 1.9	14.8 ± 1.2	11.7	9.0	14.1 ± 0.6
Hydroxy					
iso-15:0 3-OH	–	tr	–	tr	tr
15:0 3-OH	–	tr	–	tr	–
iso-16:0 3-OH	–	tr	–	–	–
16:0 3-OH	1.1 ± 0.1	4.9 ± 0.6	3.6	3.0	5.7 ± 0.1
iso-17:0 3-OH	3.8 ± 1.6	24.7 ± 1.2	26.3	21.3	26.0 ± 0.1
anteiso-17:0 3-OH	1.7 ± 0.6	3.4 ± 0.4	7.9	3.1	4.2 ± 1.1
17:0 3-OH	–	1.2 ± 0.2	1.2	2.0	1.2
Aldehyde					
16:0 ALDE	1.1	–	–	–	–
Saturated branched-chain					
iso-13:0	tr	tr	–	tr	–
iso-14:0	–	tr	–	tr	–
iso-15:0	7.1 ± 0.4	7.2 ± 0.5	4.3	8.1	8.0 ± 0.3
anteiso-15:0	44.8 ± 2.7	28.0 ± 1.8	26.5	32.2	25.3 ± 0.3
anteiso-17:0	tr	tr	tr	tr	tr
Summed features*					
1	3.1 ± 0.6	–	–	–	–
3	12.2 ± 1.3	tr	–	1.4	tr
9	1.2 ± 0.1	–	–	–	–
10	1.5	1.5 ± 0.2	1.6	tr	1.7 ± 0.1
12	–	tr	–	tr	tr

*Summed features represent groups of two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 1 consisted of 13:1 ω 1c and/or 14:0 ALDE. Summed feature 3 consisted of ECL 13.570 and/or iso-15:0 ALDE. Summed feature 9 consisted of iso-16:0 3-OH and/or ECL 17.157. Summed feature 10 consisted of one or more of ECL 17.834 and 18:1 ω 11c/ ω 9t/ ω 6t FAME. Summed feature 12 consisted of ECL 18.622 and/or iso-19:0.

thus represent different species. The levels of sequence similarity among the three strains were 99.7–100 %.

Based on the phenotypic and biochemical characteristics and the 16S rRNA gene sequence analysis, we propose that these three strains represent a novel *Parabacteroides* species, *Parabacteroides gordonii* sp. nov. *Parabacteroides* species have been isolated from human blood cultures as a small population (2 %) (Simmon *et al.*, 2008). The patient from

whom *Parabacteroides gordonii* sp. nov. was isolated died approximately 3 months after admission. It is not known whether infection with this species had a role in the death of the patient.

Description of *Parabacteroides gordonii* sp. nov.

Parabacteroides gordonii (gor.do'ni.i. N.L. masc. gen. n. *gordonii* of Gordon, named after the American biologist

Fig. 1. Phylogenetic tree showing the relationship between *Parabacteroides gordonii* sp. nov. MS-1^T and related species, based on 16S rRNA gene sequences and constructed with the neighbour-joining method. Percentages at nodes are levels of bootstrap support based on 1000 replicates. Bar, 0.02 substitutions per nucleotide position.

Jeffrey I. Gordon, in recognition of his many contributions to intestinal microbiology).

Cells are obligately anaerobic, non-spore-forming, non-motile, Gram-negative rods ($0.8 \times 1.7\text{--}2.5\ \mu\text{m}$). Colonies on EG agar after 48 h of incubation at $37\ ^\circ\text{C}$ under anaerobic conditions are 1–2 mm in diameter, grey to off-white-grey, circular, entire, slightly convex and smooth. Indole and urease are not produced. Catalase is variable (activity is not detected in the API 20A test but is detected on EG agar). Aesculin is not hydrolysed. Gelatin is not digested. Growth occurs on medium containing 20 % bile. Acid is produced from L-arabinose, glucose, lactose, maltose, D-mannose, raffinose, sucrose and D-xylose, but not from cellobiose, glycerol, D-mannitol, melezitose, L-rhamnose, salicin, D-sorbitol or trehalose. Positive reactions are obtained using Rapid ID 32A for α - and β -galactosidase, α -glucosidase, α -arabinosidase, N-acetyl- β -glucosaminidase, alkaline phosphatase, arginine arylamidase, leucyl glycine arylamidase, leucine arylamidase and alanine arylamidase. Mannose and raffinose are fermented. All of the other tests that were performed in this study (urease, arginine dihydrolase, 6-phospho- β -galactosidase, β -glucosidase, β -glucuronidase, glutamic acid decarboxylase, α -fucosidase, nitrate reduction, indole production, proline arylamidase, phenylalanine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, glycine arylamidase, histidine arylamidase, glutamyl glutamic acid arylamidase and serine arylamidase) gave negative results. The major end product in peptone-yeast extract-glucose broth (1 %, w/v, of each) is acetic acid; a small amount of succinic acid is also produced. The major cellular fatty acid is anteiso-15:0. The predominant respiratory quinone is menaquinone MK-10. The DNA G + C content of the type strain is 44.6 mol%.

The type strain is MS-1^T (=JCM 15724^T =CCUG 57478^T), isolated from human blood cultures. Two additional strains from the same source, MS-2 (=JCM 15725) and MS-3 (=JCM 15726), are included in this species.

Acknowledgements

We thank Dr M. A. Bakir for help with the 16S rRNA gene sequence analysis.

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