Bacteroides dorei sp. nov., isolated from human faeces

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Two Gram-negative, anaerobic, non-spore-forming rod-shaped organisms were isolated from human faeces. These isolates were tentatively identified as *Bacteroides* based on morphological and biochemical criteria and appeared closely related to *Bacteroides vulgatus* ATCC 8482^T. The 16S rRNA gene sequence analysis showed that the isolates were highly related to each other (99.5 %) and confirmed their placement in the genus *Bacteroides*. 16S rRNA gene sequence similarity values with close phylogenetic neighbours *Bacteroides vulgatus* ATCC 8482^T (96 %) and *Bacteroides massiliensis* CCUG 48901^T (93 %) preliminarily demonstrated that the organisms represented a novel species. The results of phenotypic, chemotaxonomic and 16S rRNA gene sequence analyses, and DNA–DNA homology values provided evidence that these two unknown isolates represent a single species and should be assigned to a novel species of the genus *Bacteroides*, as *Bacteroides dorei* sp. nov. The type strain is JCM 13471^T (= DSM 17855^T).

The genus *Bacteroides* are Gram-negative, non-spore-forming, non-motile, anaerobic rods, generally isolated from the gastrointestinal tract of humans and animals as members of the normal microflora (Smith *et al.*, 2005). *Bacteroides* species represent predominant organisms in the human colon (Benno *et al.*, 1989). They play a variety of roles as members of the indigenous flora that contribute to normal intestinal physiology and function. Several *Bacteroides* species, including the type species, *Bacteroides fragilis*, are important opportunistic pathogens and the most frequently isolated organisms from anaerobic infections (Finegold & George, 1989). Novel phylotypes of yet to be cultured *Bacteroides* species have been detected by 16S rRNA gene clone libraries of microbiota from the human intestine (Hayashi *et al.*, 2002; Suau *et al.*, 1999). Isolation and characterization of previously unknown *Bacteroides* species are important as they are related to human and animal health. Recently, *Bacteroides coprocola* (Kitahara *et al.*, 2005), *Bacteroides coprosuis* (Whitehead *et al.*, 2005), *Bacteroides fingoldii* (Bakir *et al.*, 2006b), *Bacteroides intestinalis* (Bakir *et al.*, 2006a) and *Bacteroides plebeius* (Kitahara *et al.*, 2005)

have been described. During the characterization of bacteria isolated from human faeces, two anaerobic bacteria were recovered on polyamine-deficient medium. The aim of this study was to determine the taxonomic position of the two isolates. The results of phenotypic, chemotaxonomic and 16S rRNA gene sequence analyses, and DNA–DNA homology values provided evidence that these two isolates represent a single species and that these isolates should be classified as a new species of the genus *Bacteroides*, for which the name *Bacteroides dorei* is proposed.

Isolates 175^T and 219 were recovered from the faeces collected from one healthy, Japanese, 23-year-old male. Polyamine-deficient medium (Noack *et al.*, 1998) with minor modification and a standard dilution plate method were used for isolation and enumeration of faecal bacteria as described previously (Bakir *et al.*, 2006a). AnaeroPack (Mitsubishi Gas) was used for creating anaerobic conditions and the plates were incubated at 37 °C for 72–120 h. Single colonies were picked and streaked out until single cultures were obtained on Eggerth Gagnon (EG) agar (Merck) medium supplemented with 5 % horse blood for 2 days at 37 °C in an anaerobic jar (Hirayama) filled with 100 % CO₂. Colony and cell morphology were observed by phase-contrast microscopy (Nikon) with an oil immersion objective lens and by Gram-staining after 48 h of culture on EG agar medium supplemented with 5 % horse blood at 37 °C. The two isolates originating from human faeces, 175^T and 219, were anaerobic, non-spore-forming, non-motile,

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Bacteroides dorei* 175^T is AB242142.

Tables showing biochemical properties, fatty acid composition, DNA base composition and DNA–DNA hybridization values of *Bacteroides dorei* sp. nov. and closely related *Bacteroides* species, and figures showing maximum-parsimony and UPGMA trees are available as supplementary material in IJSEM Online.

Gram-negative rods. Typical cells were 1.6–4.2 µm by 0.8–1.2 µm. Colonies on EG agar plates after 48 h incubation at 37 °C under 100 % CO₂ gas were circular, whitish, raised and convex, and attained a diameter of 2.0 mm. The isolates were incubated under various oxygen conditions (aerobic, microaerophilic, anaerobic) and at different temperatures (25–40 °C) to examine bacterial growth. Strains grew at temperatures from 25 to 40 °C with an optimum of 37 °C. The isolates were grown on Gifu anaerobic medium (GAM; Nissui) agar supplemented with 2 % bacto-oxgall (Difco), which is equivalent to 20 % bile for testing bile resistance. The two isolates were observed to be resistant to 20 % bile. The isolates were stab-inoculated into tubes containing semisolid GAM agar (0.5 %) for motility testing, and were incubated at 37 °C for up to 72 h before a negative result was recorded (McClung & Lindberg, 1957).

The results of phenotypic tests are listed in the species description. Biochemical tests were performed using API 20 A and API rapid ID 32 A strips (bioMérieux), according to the manufacturer's instructions, and incubated at 37 °C as described by Fenner *et al.* (2005) and Kitahara *et al.* (2005). Biochemical tests that showed differences from other recognized species of the genus *Bacteroides* are listed in Table 1. Biochemical test results of the isolates and the close neighbours *Bacteroides vulgatus* and *Bacteroides massiliensis* are listed in Supplementary Table S1 (available in IJSEM Online). The two isolates showed identical biochemical profiles. They showed the following differential biochemical test results with *B. vulgatus*, the most closely related species: β-glucosidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase, histidine arylamidase and serine arylamidase (Supplementary Table S1, available in IJSEM Online). Biochemical properties of the isolates and the close neighbour *B. massiliensis* are listed in Table 1 and in supplementary Table S1. The isolates demonstrated different biochemical reactions with *B. massiliensis* for aesculin, arabinose, rhamnose, xylose, sorbitol, 6-phospho-β-galactosidase, β-glucosidase, α-arabinosidase, β-glucuronidase, phenylalanine arylamidase, tyrosine arylamidase, glycine arylamidase, histidine arylamidase and serine arylamidase.

A loopful of well grown cells was harvested for fatty acid methyl ester (FAME) analysis. Saponification, methylation, extraction and determination of cellular fatty acid profiles were conducted as described by Sakamoto *et al.* (2002). Fatty acid profiles of the isolates and the closest relative *B. vulgatus* are provided in Supplementary Table S2 (available in IJSEM Online). The major fatty acids of the isolates and *B. vulgatus* were anteiso-C_{15:0}, iso-C_{17:0} 3-OH and C_{18:1ω9c}. The major cellular fatty acid content of the isolates supports their affiliation as members of the genus *Bacteroides*, particularly anteiso-C_{15:0} (Miyagawa *et al.*, 1979).

The 16S rRNA genes of the two isolates were amplified by PCR and sequenced to determine their phylogenetic affiliation. The genes were amplified by PCR (Biometra) with universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTACCTGTTACGACTT-3'). PCR

Table 1. Biochemical characteristics for distinguishing *Bacteroides dorei* sp. nov. from some members of the genus *Bacteroides*

Taxa are listed as: 1, *Bacteroides dorei* 175^T (this study); 2, *Bacteroides massiliensis* JCM 13223^T (this study); 3, *Bacteroides caccae* (Jousimies-Somer *et al.*, 2003); 4, *Bacteroides coprocola* (Kitahara *et al.*, 2005); 5, *Bacteroides eggerthii* (Jousimies-Somer *et al.*, 2003); 6, *Bacteroides finegoldii* (Bakir *et al.*, 2006b); 7, *Bacteroides fragilis* (Jousimies-Somer *et al.*, 2003); 8, *Bacteroides helcogenes* (Bakir *et al.*, 2006a); 9, *Bacteroides intestinalis* (Bakir *et al.*, 2006a); 10, *Bacteroides nordii* (Song *et al.*, 2004); 11, *Bacteroides plebeius* (Kitahara *et al.*, 2005); 12, *Bacteroides ovatus* JCM 5824^T (Jousimies-Somer *et al.*, 2003); 13, *Bacteroides salyersiae* (Song *et al.*, 2004); 14, *Bacteroides stercoris* (Jousimies-Somer *et al.*, 2003). Characteristics are scored as: +, positive reaction; –, negative reaction; v, variable reaction. *B. vulgatus* JCM 5827^T did not show any difference in the biochemical properties listed in this table to *B. dorei* JCM 13471^T.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Indole (L-tryptophan)	–	–	–	–	+	–	–	–	+	+	–	+	+	+
Aesculin	–	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Arabinose	+	–	+	–	+	+	–	–	+	–	+	+	+	–
D-Cellobiose	–	–	+	+	–	+	+	+	+	+	+	+	+	–
L-Rhamnose	+	–	+	+	+	+	–	–	+	+	+	+	+	+
Salicin	–	–	+	–	+	–	+	–	–	–	–	–	–	–
D-Sucrose	+	+	+	+	–	+	+	+	+	+	+	+	+	+
D-Trehalose	–	–	+	–	–	–	–	–	–	–	–	+	–	–
D-Xylose	+	–	+	+	+	+	+	+	+	+	+	+	+	+
α-Fucosidase	+	+	+	+	–	–	+	+	+	–	+	+	–	v

products were purified by using a Montage PCR₉₆ filter plate (Millipore) and sequenced directly by the dideoxynucleotide chain-termination method using a DNA sequencer (ABI PRISM 3100; Applied Biosystems/Hitachi) with a BigDye Terminator, version 3.1 cycle sequencing RR-100 kit (Applied Biosystems), according to the manufacturer's instructions. 16S rRNA gene sequences of >1490 bp were obtained. Phylogenetic relatives of the isolates were determined by performing database searches, and sequences of related species were retrieved from DDBJ, EMBL and GenBank nucleotide sequence databases. Sequences were aligned using CLUSTAL X (version 1.81) (Thompson *et al.*, 1997). Alignment gaps and ambiguous bases were removed prior to phylogenetic analysis using MacClade (version 4.03) (Maddison & Maddison, 2002). Neighbour-joining and UPGMA phylogenetic trees were inferred using the software package MEGA version 3.1 (Kumar *et al.*, 2004), according to the Kimura two-parameter model. Parsimony analysis was carried out with maximum-parsimony implemented in the PAUP version 4.0b10 software package (Swofford, 2000). Maximum-parsimony trees were obtained by a heuristic search and by selecting the tree bisection/reconnection branch-swapping option (Dauga, 2002). The topology of phylogenetic trees was evaluated by the

bootstrap resampling method of Felsenstein (1985) with 1000 replicates. Sequence comparison against 16S rRNA gene sequences deposited in the DDBJ/EMBL/GenBank database and treeing analysis preliminarily revealed that the two isolates demonstrated a specific affinity with members of the genus *Bacteroides* (Fig. 1; Supplementary Figs A and B, available in IJSEM Online). The two isolates appeared to be genetically highly related to each other, displaying 99.5 % 16S rRNA gene sequence similarity (Stackebrandt & Goebel, 1994; Konstantinidis & Tiedje, 2005). The phylogenetic positions of isolates 175^T and 219 among recognized members of the genus *Bacteroides* are shown in Fig. 1. The phylogenetic tree indicated that the isolates represented a new subline within the genus *Bacteroides*. The isolates displayed phylogenetic affinity with *B. vulgatus* and the level 16S rRNA gene sequence divergence among them was 4 %. The branching of the isolates at the base of this group was supported by a bootstrap resampling value of 100 % (Fig. 1). Although there is no precise correlation between percentage 16S rRNA gene sequence divergence and species delineation, however, it is generally recognized that

divergence values of 3 % or more are significant (Stackebrandt & Goebel, 1994; Whitehead *et al.*, 2005).

DNA of the two isolates and of the closest relative, *B. vulgatus*, was extracted from cells cultivated in EGF broth as described by Kitahara *et al.* (2001) [2.4 g Lab-Lemco powder (Oxoid), 10 g Proteose Peptone No. 3 (Difco), 5 g yeast extract, 4 g Na₂HPO₄, 5 g glucose, 0.5 g soluble starch, 0.5 g L-cysteine.HCl.H₂O and 1 l distilled water; pH adjusted to 7.6] after 12 h at 37 °C and purified by the methods of Saito & Miura (1963). Lysozyme (Wako Pure Chemical Industries) and SDS were used for the disruption of cells, followed by phenol extraction. DNA was recovered with alcohol precipitation and suspended in TE (1 mM EDTA, 10 mM Tris/HCl, pH8.0) buffer. DNA was treated with RNase A and RNase T₁ (both from Sigma), and then extracted with phenol and phenol/chloroform/isoamyl alcohol (25:24:1). DNA was obtained by alcohol precipitation and dissolved in TE buffer. The concentration of DNA was measured by using a UV spectrophotometer (UV-2100; Shimadzu). DNA base compositions were determined

Fig. 1. Neighbour-joining tree showing phylogenetic positions of isolates 175^T and 219 among recognized members of the genus *Bacteroides* based on 16S rRNA gene sequences available in the DDBJ/EMBL/GenBank databases. Scale bar represents 0.02 substitutions per nucleotide position. Bootstrap values (> 50 %) based on 1000 replications are listed as percentages at the branching points.

using HPLC (Tamaoka & Komagata, 1984) after enzymic digestion of DNA to deoxyribonucleosides. An equimolar mixture of four deoxyribonucleotides from the Yamasa GC kit (Yamasa Shoyu) was used as the quantitative standard. The DNA G + C contents of the isolates were determined as 43 mol%. The DNA G + C content of the nearest neighbour *B. vulgatus* was 41 mol% (Supplementary Table S3, available in IJSEM Online). The G + C content of the isolates also supports the affiliation of the isolates in the genus *Bacteroides*, which have G + C contents between 40 and 48 mol% (Shah, 1992).

DNA–DNA relatedness was determined based on close pairwise 16S rRNA gene sequence similarity values of strain 175^T with 219 and *B. vulgatus*. For DNA–DNA hybridization experiments, bacterial DNA was extracted from cells harvested from EGF broth (Kitahara *et al.*, 2001). DNA–DNA hybridization was performed by the photobiotin-labelling method of Ezaki *et al.* (1989) using a microplate reader (Spectroan FL-2575; Towa Scientific). The hybridization temperature was 42 °C. The DNA–DNA homology value between isolates 175^T and 219 was ≥73 %. The two isolates also showed identical biochemical profiles and a 99.5 % 16S rRNA gene sequence similarity value. Therefore, it was confirmed that they belonged to a single species. The level of DNA–DNA relatedness between strain 175^T and the closest neighbour, *B. vulgatus* was ≤46 % (Supplementary Table S3, available in IJSEM Online). DNA–DNA hybridization values of <70 % with the closest *Bacteroides* species confirmed the novelty of isolate 175^T (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Support for the distinctiveness of the isolate from other valid species of the genus *Bacteroides* was also very evident from phenotypic analyses. Based on the phenotypic and genotypic evidence presented, these two isolates should be assigned to the genus *Bacteroides* as a single species for which the name *Bacteroides dorei* sp. nov. is proposed

Description of *Bacteroides dorei* sp. nov.

Bacteroides dorei [do.re'i. N.L. gen. masc. n. *dorei* of Doré, in honour of the French microbiologist Joel Doré, in recognition of his many contributions to intestinal (gut) microbiology].

Cells are Gram-negative rods, anaerobic, non-motile and non-spore-forming. Typical cells are 1.6–4.2 µm by 0.8–1.2 µm and occur singly. Colonies on EG agar plates after 48 h incubation at 37 °C under 100 % CO₂ gas are circular, whitish, raised and convex, and attain a diameter of 2.0 mm. Optimum temperature for growth is 37 °C. Grows in the presence of bile. Indole-negative and aesculin is not hydrolysed. Nitrate is not reduced. No activity detected for urease and gelatin. Acid is produced from glucose, sucrose, xylose, rhamnose, lactose, maltose, arabinose, mannose and raffinose. Acid is not produced from cellobiose, salicin, trehalose, mannitol, glycerol, melezitose and sorbitol. Positive reactions obtained using API rapid ID 32 A for α-fucosidase, α-galactosidase, β-galactosidase, 6-phospho-β-galactosidase,

α-glucosidase, β-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. Negative reactions obtained for arginine dihydrolase, proline arylamidase and pyroglutamic acid arylamidase. Major fatty acids are anteiso-C_{15:0} (26–32 %), iso-C_{17:0} 3-OH (17–19 %) and C_{18:1ω9c} (9–12 %). DNA G + C content is 43 mol%. The type strain is 175^T (=JCM 13471^T=DSM 17855^T). Strain 219 (=JCM 13472) is included in this species.

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