Bacteroides plebeius sp. nov. and *Bacteroides coprocola* sp. nov., isolated from human faeces

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Nine strains of Gram-negative, anaerobic rod were isolated from human faeces. Based on phylogenetic analysis and specific phenotypic characteristics, these strains were included within the *Bacteroides* cluster and were divided into two clusters. Strains from the two clusters showed 16S rRNA gene sequence similarities of 90.4 and 92.7% to the nearest recognized species, *Bacteroides vulgatus*. The strains also formed two clusters exhibiting a 16S rRNA gene sequence divergence of approximately 6%. DNA–DNA hybridization studies confirmed that the two novel strain clusters were distinct from each other. Based on the phenotypic and phylogenetic findings, two novel species, *Bacteroides plebeius* sp. nov. and *Bacteroides coprocola* sp. nov., are proposed, each representing one of the two strain clusters. The DNA G+C content of the type strains were 43.9 mol% for *B. plebeius* (M12^T=JCM 12973^T=DSM 17135^T) and 42.4 mol% for *B. coprocola* (M16^T=JCM 12979^T=DSM 17136^T).

Recently, culture-independent approaches using a 16S rRNA gene sequence clone library have demonstrated that approximately 75% of the clones from human faecal samples represented novel phylotypes, indicating that our knowledge of the predominant members is very limited (Hayashi *et al.*, 2002a, b; Suau *et al.*, 1999). *Bacteroides* is one of the predominant genera in human faecal microbiota revealed by culture methods (Benno *et al.*, 1989; Finegold *et al.*, 1983) and 16S rRNA gene sequence clone libraries have shown that many novel phylotypes in the *Bacteroides* cluster were retrieved from human faecal samples (Hayashi *et al.*, 2002a, b; Suau *et al.*, 1999). Therefore, it is important for our understanding of the composition of human faeces to isolate and identify novel bacterial strains included in the genus *Bacteroides*. Here, we report two novel species within the genus *Bacteroides* isolated from human faeces.

Nine strains (M12^T, M14, M35, M122, M131, M132, M16^T, M11 and M156) were isolated from faeces of three healthy

Japanese men and women (22 years old, female; 29, male; 31, male) via growth on medium 10 and using the 'plate-in-bottle' method (Hayashi *et al.*, 2002a; Mitsuoka *et al.*, 1969). Briefly, after collecting faecal samples, each (0.5 g) was suspended immediately in dilution buffer and samples (50 µl) of faeces diluted to 10⁻⁸ were plated anaerobically on medium 10 by using the 'plate in bottle' filled with 100% CO₂. Isolates were subcultured on Eggerth Gagnon (EG; Merck) agar plates supplemented with 5% horse blood for 2 days at 37 °C in an anaerobic jar (Hirayama Manufacturing Corp.) filled with 100% CO₂.

Bile resistance was tested by growing the bacteria on GAM (Nissui) agar plates supplemented with 2% bacto-oxgall (Difco). Other physiological, biochemical and enzymic-activity tests were performed by inoculation using the API 20A and API Rapid ID 32A systems (bioMérieux) according to the manufacturer's instructions and incubation at 37 °C in an anaerobic jar. Nearly complete (1500 bases) 16S rRNA gene sequences of the strains investigated were amplified by PCR with universal primers 27F (5'-dAGAGTTTGATCC-TGGCTCAG-3') and 1492R (5'-dGGTTACCTTGTTACG-ACTT-3') using a Biometra Thermocycler Tgradient. PCR products were purified by using an Ultraclean PCR Clean-up kit (MO BIO) and were sequenced by using a BigDye Terminator cycle sequencing kit and ABI PRISM 3100 Genetic Analyser (both Applied Biosystems). The closest recognized relatives of the isolates were determined by performing database searches, and sequences of closely related

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Bacteroides plebeius* M12^T and *Bacteroides coprocola* M16^T are AB200217 and AB200224, respectively.

Tables detailing results using the API 20A and Rapid ID 32A systems, cellular fatty acid contents, and DNA base composition and levels of DNA–DNA relatedness among the new and related strains are available as supplementary material in IJSEM Online.

species were retrieved from DDBJ, EMBL and GenBank. Phylogenetic analysis was performed with CLUSTAL X (version 1.83) (Thompson *et al.*, 1997) and a phylogenetic tree was constructed according to the neighbour-joining method (Saitou & Nei, 1987). Topology of the tree was evaluated by a bootstrap analysis with 1000 replicates using the CLUSTAL X software. The virtually complete 16S rRNA gene sequences of *Bacteroides helcogenes* JCM 6297^T (Benno *et al.*, 1983), *Bacteroides pyogenes* JCM 6294^T (Benno *et al.*, 1983) and *Bacteroides tectus* JCM 10003^T (Love *et al.*, 1986) were also determined because their 16S rRNA gene sequences have not been deposited in GenBank/EMBL/DDBJ. For DNA–DNA hybridization experiments, bacterial DNA of the isolated strains and of *B. helcogenes* JCM 6297^T and *Bacteroides vulgatus* JCM 5826^T was extracted from cells harvested from EGF broth (Kitahara *et al.*, 2001) after growth for 12 h at 37 °C, as described previously, and purified by using the methods of Saito & Miura (1963). Levels of DNA–DNA hybridization were determined by using the method of Ezaki *et al.* (1989) using photobiotin and microplates. The DNA G + C content was determined as described previously (Kitahara *et al.*, 2001).

The nine isolates investigated were obligately anaerobic, non-spore-forming, non-motile, Gram-negative short rods or rods.

Physiological and biochemical properties of the nine strains, *B. helcogenes* 6427^T and *B. vulgatus* JCM 5826^T were determined by using the API 20A and Rapid ID 32A systems. Based on results using the API 20A system, the nine strains were divided into two groups, with one group consisting of six strains (M12^T, M14, M35, M122, M131, M132) and the other group consisting of three strains (M12^T, M14, M35) (see Supplementary Table S1 in IJSEM Online). Based on the Rapid ID 32A results, the cluster of six strains could be further divided into two subgroups, comprising strains M12^T, M14 and M35, and strains M122, M131 and M132 (see Supplementary Table S2 in IJSEM Online). Strains M16^T, M11 and M156 showed the same results using the Rapid ID 32A system.

The cellular fatty acid profiles of *Bacteroides* species have been determined (Shah & Collins, 1980) and used to provide a classification for the genus (Shah & Collins, 1983). The major cellular fatty acids of the nine strains investigated here

Fig. 1. Phylogenetic tree showing the relationship between *Bacteroides plebeius* sp. nov., *Bacteroides coprocola* sp. nov. and related *Bacteroides* species. The tree was constructed by the neighbour-joining method based on 16S rRNA gene sequences. The numbers at nodes indicate bootstrap values greater than 50%. Bar, 1% sequence divergence.

included anteiso-branched C_{15:0} (11.0–25.8 % of total) and significant amounts of C_{16:0} 3-OH (9.8–21.8 %), C_{16:0} (7.5–22.5 %), iso-C_{17:0} 3-OH (2.6–18.2 %) and C_{18:0}ω9c (9.6–13.6 %) (see Supplementary Table S3 in IJSEM Online).

Approximately 1500 bases of the 16S rRNA gene sequences were determined for the isolated strains, and also for *B. helcogenes* JCM 6427^T, *B. pyogenes* JCM 6294^T and *B. tectus* JCM 10003^T (the phylogenetic relationships of these three species with other species of the genus *Bacteroides* were not clear in previous studies). The phylogenetic tree thus constructed indicated that the isolates were related to strains in the genus *Bacteroides* (Fig. 1). Six strains (M12^T, M14, M35, M122, M131 and M132) formed a single cluster and a distinct line of descent, with 16S rRNA gene sequence similarities of between 98.3 and 100 %. Highest sequence similarity to M12^T was found with M16^T (94.0 %), *Bacteroides massiliensis*, a recently described species (Fenner *et al.*, 2005) (90.9 %), and *B. vulgatus* (90.4 %). Strains M16^T, M11 and M156 also formed a single cluster and a distinct line of descent, with levels of sequence similarity among the three strains of between 98.9 and 99.9 %. Highest sequence similarity to M16^T was found with M12^T (94.0 %), *B. massiliensis* (91.2 %) and *B. vulgatus* (92.7 %). These results indicated that strains M12^T and M16^T each represent a novel species, as the level of 16S rRNA gene sequence similarity to the closest related species was <97 % (Stackebrandt & Goebel, 1994). *B. pyogenes* JCM 6294^T and *B. tectus* JCM

10003^T were related closely to '*Bacteroides denticanum*' (J. M. Hardham, K. W. King, K. Dreier, C. Strietzel, C. Sfintescu & R. T. Evans, unpublished data).

Based on the results of physiological, biochemical, cellular fatty acid and phylogenetic analyses, six strains (M12^T, M35, M122, M131, M16^T and M156) were selected for determination of the DNA base composition and DNA–DNA hybridization experiments, together with reference strains *B. helcogenes* JCM 6427^T and *B. vulgatus* JCM 5826^T. Probe DNA from strains M12^T, M35, M122 and M131 showed DNA–DNA hybridization values of between 71 and 112 % with each other, and that from strains M16^T and M156 showed values of 73 and 76 %. Strain M12^T showed relatively low levels of DNA–DNA relatedness to strain M16^T (18 %), *B. helcogenes* JCM 6427^T (2 %) and *B. vulgatus* JCM 5826^T (3 %). Strain M16^T also showed relatively low levels of DNA–DNA relatedness to strain M12^T (5 %), *B. helcogenes* JCM 6427^T (2 %) and *B. vulgatus* JCM 5826^T (1 %). The DNA G + C contents of strains M12^T, M35, M122 and M131 ranged from 42.4 to 43.9 mol%. The DNA G + C contents of strains M16^T and M156 were 42.4 and 41.1 %, respectively. On the basis of the results presented, M12^T and M16^T are classified as the type strains of two novel species of the genus *Bacteroides*, for which the names *Bacteroides plebeius* sp. nov. and *Bacteroides coprocola* sp. nov., respectively, are proposed. Differential characteristics of the novel species and some related *Bacteroides* species are shown in Table 1.

Table 1. Differential characteristics of *Bacteroides plebeius* sp. nov., *Bacteroides coprocola* sp. nov. and related *Bacteroides* species

+, Positive; –, negative; v, variable.

Characteristic	<i>B. plebeius</i>	<i>B. coprocola</i>	<i>B. helcogenes</i>	<i>B. massiliensis</i> *	<i>B. vulgatus</i>
Aesculin hydrolysis	+	+	+	–	–
Acid produced from:					
Salicin	–	+	+	–	–
L-Arabinose	+	–	–	–	+
D-Cellobiose	+	+	+	–	–
L-Rhamnose	+	+	–	–	+
Enzymic reactions:					
β-Glucuronidase	+	–	+	–	+
Arginine arylamidase	v	–	–	–	+
Phenylalanine arylamidase	v	–	–	–	–
Leucine arylamidase	v	–	–	+	+
Glycine arylamidase	+	–	–	–	+
Histidine arylamidase	v	–	–	–	–
Major cellular fatty acid(s)	Anteiso-C _{15:0} , iso-C _{17:0} 3-OH, C _{16:0} 3-OH, C _{18:1} ω9c, C _{16:0}	Anteiso-C _{15:0} , iso-C _{17:0} 3-OH, C _{16:0} 3-OH, C _{18:1} ω9c, C _{16:0}	Anteiso-C _{15:0} , iso-C _{17:0} 3-OH	Anteiso-C _{15:0}	Anteiso-C _{15:0} , iso-C _{17:0} 3-OH, C _{15:0}
DNA G + C content (mol%)	42.4–43.9	41.1–42.4	45.0	49.0	42.3

*Data were taken from Fenner *et al.* (2005).

Description of *Bacteroides plebeius* sp. nov.

Bacteroides plebeius (ple.bei'us. L. masc. adj. *plebeius* common, of low class.

Cells cultivated on EG blood agar plates are strictly anaerobic, non-spore-forming, non-motile and Gram-negative. The short rods or rod-shaped cells are 0.8 µm in width and variable in length, generally in the range 1–5 µm. Colonies grown on EG blood agar plates are 1–3 mm in diameter, disc-shaped, greyish-white and translucent. Optimum temperature for growth is around 37 °C. All strains grow in the presence of bile. All strains produce acid from L-arabinose, D-cellobiose, glucose, lactose, maltose, D-mannose, D-raffinose, L-rhamnose, sucrose and D-xylose. None produce acid from glycerol, D-mannitol, D-melezitose, D-sorbitol or D-trehalose. Aesculin is hydrolysed. Gelatin is not hydrolysed. Indole is not produced. Catalase and urease are not produced. Using Rapid ID 32A, all strains are positive for α-galactosidase, β-galactosidase, β-galactosidase-6-phosphate, α-glucosidase, β-glucosidase, α-arabinosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, alkaline phosphatase, leucyl glycine arylamidase, alanine arylamidase, glycine arylamidase, glutamyl glutamic acid arylamidase, glutamic acid decarboxylase and α-fucosidase. All strains have positive reactions on urease, arginine dihydrolase, alkaline phosphatase, proline arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase and serine arylamidase. Arginine arylamidase, phenylalanine arylamidase, leucine arylamidase and histidine arylamidase are variable. Major fatty acids are anteiso-C_{15:0}, C_{16:0} 3-OH, C_{16:0}, iso-C_{17:0} 3-OH and C_{18:1}ω9c.

The DNA G+C content of the type strain is 43.9%. The type strain, M12^T (=JCM 12973^T=DSM 17135^T), was isolated from faeces of a healthy human. Five additional strains [M14 (=JCM 12974), M35 (=JCM 12975), M122 (=JCM 12976), M131 (=JCM 12977) and M132 (=JCM 12978)] are included in this species.

Description of *Bacteroides coprocola* sp. nov.

Bacteroides coprocola [cop.ro'co.la. Gr. n. *kopros* faeces; L. suffix *-cola* inhabitant of; N.L. masc. n. (in apposition) *coprocola* inhabitant of faeces].

Cells cultivated on EG blood agar plates are strictly anaerobic, non-spore-forming, non-motile and Gram-negative. The short rods or rod-shaped cells are 0.8 µm in width and variable in length, generally in the range 1–4 µm. Colonies on EG blood agar plates are 1.0 to approximately 3.0 mm in diameter, disc-shaped and greyish-white. Optimum temperature for growth is around 37 °C. All strains grow in the presence of bile. All strains produce acid from D-cellobiose, glucose, lactose, maltose, D-mannose, D-raffinose, L-rhamnose, salicin, sucrose and D-xylose. None produce acid from L-arabinose, glycerol, D-mannitol, D-melezitose, D-sorbitol or D-trehalose. Aesculin is hydrolysed. Gelatin is not hydrolysed. Indole is not produced. Catalase and urease are not produced. Using Rapid ID 32A, all strains have

positive reactions for α-galactosidase, β-galactosidase, β-galactosidase-6-phosphate, α-glucosidase, β-glucosidase, α-arabinosidase, N-acetyl-β-glucosaminidase, alkaline phosphatase, leucyl glycine arylamidase, alanine arylamidase, glutamyl glutamic acid arylamidase, glutamic acid decarboxylase and α-fucosidase. All strains have negative reactions on urease, arginine dihydrolase, β-glucuronidase, arginine arylamidase, proline arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, glycine arylamidase, histidine arylamidase and serine arylamidase. The major fatty acids are anteiso-C_{15:0}, C_{16:0} 3-OH, C_{16:0}, iso-C_{17:0} 3-OH and C_{18:1}ω9c.

The DNA G+C content of the type strain is 42.4%. The type strain, M16^T (=JCM 12979^T=DSM 17136^T), was isolated from faeces of a healthy human. Two additional strains [M11 (=JCM 12980) and M156 (=JCM 12981)] are included in this species.

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