Bacteroides finegoldii sp. nov., isolated from human faeces

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Two strains, 199^T and 176, of Gram-negative, anaerobic, rod-shaped, non-spore-forming bacteria were isolated during studies of microbiota in human faeces using polyamine-deficient medium. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the isolated strains belonged to the genus *Bacteroides*. The G + C content (42.4–43.0 mol%) and major fatty acid composition (anteiso-C_{15:0}, 31.8–36.2%) supported the affiliation of the two strains to the genus *Bacteroides*. 16S rRNA gene sequence similarities with their closest neighbours, *Bacteroides thetaiotaomicron* and *Bacteroides ovatus*, ranged from 94.6 to 96.5%. DNA–DNA hybridization and phenotypic analysis showed that the two strains belonged to the same species (DNA–DNA relatedness of 89%) and were distinct from their close relatives, *B. thetaiotaomicron* and *B. ovatus* (<23% DNA–DNA relatedness). On the basis of these data, a novel species of the genus *Bacteroides*, *Bacteroides finegoldii* sp. nov., is proposed, comprising the designated type strain 199^T (=JCM 13345^T=DSM 17565^T) and strain 176 (=JCM 13346).

Bacteroides species are predominant among the cultivable microbiota in human faeces (Benno *et al.*, 1989; Finegold *et al.*, 1983). In addition to cultivable species, novel phylogenetic types of *Bacteroides* that have not yet been cultivated have been detected by using 16S rRNA gene clone libraries of microbiota of the human intestine (Hayashi *et al.*, 2002; Suau *et al.*, 1999). Recovery of micro-organisms from any sample depends primarily on the isolation media used. Inclusion or exclusion of media ingredients could affect the recovery of micro-organisms. In general, polyamines (putrescine, spermidine and spermine) are ubiquitous and necessary for cell growth (Noack *et al.*, 1998). Micro-organisms cannot grow without polyamines. This growth inhibition can be overcome by simply adding polyamines to the culture medium (Macrae *et al.*, 1998). Bacteria synthesize polyamines by decarboxylation of the amino acids

ornithine, arginine and lysine (Macfarlane & Macfarlane, 1997). Members of the genus *Bacteroides* have been identified as spermidine producers (Noack *et al.*, 1998). The use of polyamine-deficient media facilitates the isolation of polyamine-producing bacteria (Noack *et al.*, 2000). *Bacteroides intestinalis* was isolated recently using polyamine-deficient medium (Bakir *et al.*, 2006). We isolated the strains described in this study from human faeces.

Two strains, 199^T and 176, were isolated from the faeces of a healthy, 23-year-old Japanese male. Polyamine-deficient medium (Noack *et al.*, 1998), with minor modifications, and a standard dilution plate method were used for isolation, as described previously (Bakir *et al.*, 2006). An AnaeroPack (Mitsubishi Gas) was used for creating anaerobic conditions and the incubation period used was 72–120 h at 37 °C. The strains were subcultured on Eggerth–Gagnon (EG) agar (Merck) supplemented with 5% horse blood for 2 days at 37 °C in an anaerobic jar (Hirayama) filled with 100% CO₂. Colony and cell morphologies were observed by using phase-contrast microscopy (Nikon) with an oil-immersion objective lens and by Gram-staining cultures after growth for 48 h on EG agar supplemented with 5% horse blood at 37 °C in an anaerobic jar. To examine bacterial growth, the isolates were incubated under various oxygen conditions (aerobic, microaerophilic, anaerobic) and at various temperatures (20, 25, 30, 37 and 40 °C). The strains were grown on Gifu anaerobic medium (GAM) agar (Nissui) supplemented with

Published online ahead of print on 16 December 2005 as DOI 10.1099/ijs.0.64084-0.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 199^T is AB222699.

API 20A and API rapid ID 32 A test results, cellular fatty acid compositions, DNA base composition and levels of DNA–DNA hybridization of strains 199^T and 176 and related strains and a maximum-parsimony phylogenetic tree showing the positions of strains 199^T and 176 among representative members of the genera *Prevotella*, *Bacteroides*, *Porphyromonas* and *Tannerella* are available as supplementary material in IJSEM Online.

2 % bacto-oxgall (Difco) to test for bile resistance. Strains were inoculated by stabbing into tubes containing semisolid GAM agar (0.5 %) to test for motility, and were incubated at 37 °C for up to 72 h before a negative reaction was recorded (McClung & Lindberg, 1957). Results of phenotypic analyses are listed in the species description. Other physiological, biochemical and enzymic activity tests were performed by using API 20A and API rapid ID 32 A kits (bioMérieux), according to the manufacturer's instructions, with incubation at 37 °C in an anaerobic jar. Results from chemotaxonomic analyses that were useful in distinguishing the two novel strains from some other recognized *Bacteroides* species are summarized in Table 1. The biochemical test results from API 20A and API rapid ID 32 A for the two novel strains and the closely related species *Bacteroides thetaiotaomicron* and *Bacteroides ovatus* are listed in Supplementary Table S1 in IJSEM Online. Strains 199^T and 176 had identical biochemical profiles. The two strains could be differentiated from their closest relative, *B. thetaiotaomicron* JCM 5827^T, by utilization of trehalose, indole production, acid production from salicin and activities of α -fucosidase, arginine arylamidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase, glycine arylamidase, histidine arylamidase and serine arylamidase. Biochemical characteristics of strains 199^T and 176 that differed from those of their close relative *B. ovatus* JCM 5824^T included indole production, acid production from trehalose and activities of α -fucosidase, glutamyl glutamic acid arylamidase and leucine arylamidase.

A loopful of well-grown cells was harvested for fatty acid methyl ester analysis. Saponification, methylation,

extraction and determination of cellular fatty acid profiles were performed as described previously (Sakamoto *et al.*, 2002). The results are given in Supplementary Table S2 in IJSEM Online. No significant differences in fatty acid profiles were found for strains 199^T and 176 and *B. thetaiotaomicron* JCM 5827^T, except that *B. ovatus* JCM 5824^T contained <1 % C_{16:0}. The major cellular fatty acid of strains 199^T and 176 was anteiso-C_{15:0} (36.2 and 31.8 %, respectively), in agreement with data for the genus *Bacteroides* as described by Miyagawa *et al.* (1979). The closely related strains *B. thetaiotaomicron* JCM 5827^T and *B. ovatus* JCM 5824^T also contained anteiso-C_{15:0} (35.4 and 38.9 %, respectively) as the major cellular fatty acid.

Almost complete (1485 and 1488 bases) 16S rRNA gene sequences of strains 199^T and 176 were amplified by PCR (Biometra) using the universal primers 27F (5'-AGAGTTT-GATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGT-TACGACTT-3'). The products were purified by using a Montage PCR₉₆ filter plate (Millipore) and sequenced directly using the dideoxynucleotide chain-termination method with a DNA sequencer (ABI PRISM 3100; Applied Biosystems/Hitachi) and a BigDye Terminator version 3.1 cycle sequencing RR-100 kit (Applied Biosystems), according to the manufacturer's instructions. Strains closely related to the novel strains were determined by retrieval of closely related 16S rRNA gene sequences from DDBJ, EMBL and GenBank. The sequences were aligned using CLUSTAL_X (version 1.81) (Thompson *et al.*, 1997). Alignment gaps and ambiguous bases were removed prior to phylogenetic analysis by using MacClade (version 4.03) (Maddison & Maddison, 2002). A phylogenetic tree was

Table 1. Biochemical characteristics that differentiate *Bacteroides finegoldii* sp. nov. from some other members of the genus *Bacteroides*

Taxa: 1, *B. finegoldii* sp. nov.; 2, *B. thetaiotaomicron* JCM 5827^T; 3, *B. ovatus* JCM 5824^T (data in columns 1–3 from this study); 4, *Bacteroides caccae* (Jousimies-Somer *et al.*, 2003); 5, *Bacteroides coprocola* (Kitahara *et al.*, 2005); 6, *Bacteroides eggerthii* (Jousimies-Somer *et al.*, 2003); 7, *Bacteroides fragilis* (Jousimies-Somer *et al.*, 2003); 8, *Bacteroides helcogenes* (Bakir *et al.*, 2006); 9, *Bacteroides intestinalis* (Bakir *et al.*, 2006); 10, *Bacteroides massiliensis* (Fenner *et al.*, 2005); 11, *Bacteroides nordii* (Song *et al.*, 2004); 12, *Bacteroides plebeius* (Kitahara *et al.*, 2005); 13, *Bacteroides salyersiae* (Song *et al.*, 2004); 14, *Bacteroides stercoris* (Jousimies-Somer *et al.*, 2003); 15, *Bacteroides uniformis* (Bakir *et al.*, 2006); 16, *Bacteroides vulgatus* (Jousimies-Somer *et al.*, 2003). Characteristics are scored as: +, positive reaction; –, negative reaction; v, variable reaction; +⁺, most strains positive; –⁺, most strains negative.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Indole production (L-tryptophan)	–	+	+	–	–	+	–	–	+	–	+	–	+	+	+	–
Aesculin hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	– ⁺
Acid production from:																
L-Arabinose	+	+	+	+	–	+	–	–	+	–	–	+	+	– ⁺	+	+
D-Cellobiose	+	+	+	+	+	– ⁺	+	+	+	–	+	+	+	– ⁺	+	–
L-Rhamnose	+	+	+	+	+	– ⁺	–	–	+	–	+	+	+	+	–	+
Salicin	+	–	+	– ⁺	+	–	–	+	–	–	–	–	–	– ⁺	+	–
D-Sucrose	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
D-Trehalose	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
D-Xylose	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
α -Fucosidase activity	–	+	+	+	+	–	+	+	+	+	–	+	–	v	+	+

constructed based on the neighbour-joining method (Saitou & Nei, 1987) using PAUP version 4.0b10 (Swofford, 2000). Distance matrices were calculated by using Kimura's 2-parameter distances for neighbour-joining analysis (Gascuel, 1997). Parsimony analysis was carried out with maximum-parsimony implemented in PAUP version 4.0b10. Maximum-parsimony trees were obtained by using 100 random addition, heuristic search replicates and the tree bisection-reconnection branch-swapping option (Dauga, 2002). The topologies of the phylogenetic trees were evaluated by using the bootstrap resampling method of Felsenstein (1985), with 1000 replicates. Analysis of the 16S rRNA gene sequences revealed that strains 199^T and 176 were members of the genus *Bacteroides*. The phylogenetic position of the two strains among *Bacteroides* species and representative members of the genera *Prevotella*, *Bacteroides*, *Porphyromonas* and *Tannerella* are shown in Fig. 1 and Supplementary Fig. S1 in IJSEM Online, respectively. The phylogenetic trees clearly showed that strains 199^T and 176 formed a single cluster and had a distinct line of descent with *B. thetaiotaomicron* ATCC 29148^T. Close phylogenetic relatives of strains 199^T and 176 were *B. thetaiotaomicron* ATCC 29148^T and *B. ovatus* NCTC 11153^T. Sequence similarity values were calculated using the program GENETYX (MAC version 11.2.0; Software Development) (Yumoto *et al.*, 2005). 16S rRNA gene sequence similarities of strain 199^T with *B. thetaiotaomicron* ATCC 29148^T and *B. ovatus* NCTC 11153^T were 95.9 and 94.6 %, respectively. Strain 176 showed 96.5 and 95.2 % sequence similarity with *B. thetaiotaomicron* ATCC 29148^T and *B. ovatus* NCTC 11153^T, respectively. The 16S rRNA

gene sequence similarity between strains 199^T and 176 was 99.2 %.

DNA was extracted from cells harvested from EGF broth [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 7.6] as described previously (Kitahara *et al.*, 2001) after incubation for 12 h at 37 °C, and purified by using the methods of Saito & Miura (1963). Cells were disrupted with lysozyme (Wako Pure Chemical Industries) and SDS, extracted with phenol, and the DNA was recovered using alcohol precipitation and suspended in TE buffer (1 mM EDTA, 10 mM Tris/HCl, pH 8.0). The DNA was treated with RNase A, RNase T₁ (both from Sigma) and proteinase K (Wako Pure Chemical Industries), and extracted with phenol and phenol/chloroform/isoamyl alcohol (25:24:1, by vol.). The DNA was recovered by alcohol precipitation and dissolved in TE buffer. The DNA concentration was measured by using a UV-spectrophotometer (Shimadzu, UV-2100). DNA base compositions were determined by using HPLC (Tamaoka & Komagata, 1984) after enzymic digestion of the DNA to deoxyribonucleosides. An equimolar mixture of four deoxyribonucleotides from a Yamasa G + C kit (Yamasa Shoyu) was used as a quantitative standard. The G + C contents of strains 199^T, 176 and the closely related strains *B. thetaiotaomicron* JCM 5827^T and *B. ovatus* JCM 5824^T were 42, 43, 43 and 42 mol%, respectively (Supplementary Table S3 in IJSEM Online). The G + C contents of strains 199^T and 176 supported the affiliation of the novel strains with members of

Fig. 1. Phylogenetic positions of the isolated strains within the genus *Bacteroides*. The tree was constructed by using the neighbour-joining method based on 16S rRNA gene sequences. Bootstrap values >50 % based on 1000 replications are listed as percentages at branching points. Bar, 0.01 substitutions per site.

the genus *Bacteroides*, which have a G+C content of 40–48 mol% (Shah, 1992).

DNA–DNA relatedness was determined based on pairwise 16S rRNA gene sequence similarity values of strain 199^T with strain 176, *B. thetaiotaomicron* and *B. ovatus*. For the DNA–DNA hybridization experiments, DNA of the two isolates and *B. thetaiotaomicron* JCM 5827^T and *B. ovatus* JCM 5824^T was extracted from cells harvested from EGF broth (Kitahara *et al.*, 2001). DNA–DNA hybridization was performed by using the photobiotin-labelling method of Ezaki *et al.* (1989), using a microplate reader (Fluoroskan-Ascent; Labsystems). The hybridization temperature used was 42 °C. The DNA–DNA relatedness value between strains 199^T and 176 was 89 %. The two strains also had identical biochemical profiles and >97 % 16S rRNA gene sequence similarity. Therefore, they can be considered to represent a single species. Strain 199^T had DNA–DNA relatedness values of 20 and 23 % with its close neighbours *B. thetaiotaomicron* JCM 5827^T and *B. ovatus* JCM 5824^T, respectively (see Supplementary Table S3 in IJSEM Online). DNA–DNA hybridization values of <70 % with the closest *Bacteroides* species confirmed that strain 199^T represented a novel species (Stackebrandt & Goebel, 1994). That strain 199^T was distinct from other species of the genus *Bacteroides* with validly published names was also evident from the phenotypic analyses. On the basis of the results presented in this study, strain 199^T should be classified as the type strain of a novel species of the genus *Bacteroides*, for which we propose the name *Bacteroides finegoldii* sp. nov. An additional strain (176) is also included in this species.

Description of *Bacteroides finegoldii* sp. nov.

Bacteroides finegoldii (fi.ne.gol'di.i. L. gen. n. *finegoldii* of Finegold, in honour of Dr Sydney M. Finegold, a contemporary researcher in anaerobic bacteriology and an infectious diseases clinician).

Cells are strictly anaerobic, non-spore-forming, non-motile, Gram-negative rods, about 0.80 µm wide and 1.5–4.5 µm long, and occur singly. Surface colonies on EG blood agar plates after 2 days are 1–2 mm in diameter, circular, translucent–whitish, raised and convex. The optimum temperature for growth is about 37 °C. Grows in the presence of bile. Indole-negative but is able to hydrolyse aesculin. Nitrate is not reduced. No activity is detected for urease and gelatin is not hydrolysed. Acid is produced from glucose, lactose, sucrose, maltose, salicin, xylose, arabinose, cellobiose, mannose, raffinose and rhamnose, but not from mannitol, glycerol, melezitose, sorbitol or trehalose. Positive reactions are obtained using API rapid ID 32 A for α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, α-arabinosidase, N-acetyl-β-glucosaminidase, glutamic acid decarboxylase, alkaline phosphatase, leucyl glycine arylamidase, alanine arylamidase and glutamyl glutamic acid arylamidase. Negative reactions are obtained for arginine dihydrolase, 6-phospho-β-galactosidase, β-glucuronidase, α-fucosidase, 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} (31.8–36.2 %) and iso-C_{17:0} 3-OH (13.1–14.5 %). The DNA G+C content is 42.4–43.0 mol%.

The type strain is 199^T (=JCM 13345^T=DSM 17565^T), isolated from human faeces. Strain 176 (=JCM 13346) is included in this species.

Acknowledgements

We thank Dr Kuruto Hara, Kyodo Milk Industry Co. Ltd, Japan, for the preparation of polyamine-deficient medium.

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