Bacteroides coprophilus sp. nov., isolated from human faeces

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Three Gram-negative, anaerobic, rod-shaped bacteria (strains CB40, CB41 and CB42^T) were isolated from human faeces. Based on phylogenetic analysis and specific phenotypic characteristics, these strains were included in the genus *Bacteroides*, and 16S rRNA gene sequence analysis indicated that these strains represented a novel species. The strains were most closely related to the type strains of *Bacteroides barnesiae* and *Bacteroides salanitronis*, with sequence similarities of 93.4 and 89.8 %, respectively. The G + C content of strain CB42^T is 44.7 mol%. Major fatty acids were anteiso-C_{15:0}, C_{16:0}, iso-C_{17:0} 3-OH and C_{18:1}ω9c. On the basis of the data presented, a novel *Bacteroides* species, *Bacteroides coprophilus* sp. nov., is proposed, with CB42^T (=JCM 13818^T=DSM 18228^T) as the type strain.

The culture-independent approach based on 16S rRNA gene sequence analysis has made it possible to clarify the dominant microbiota of the human gut (Eckburg *et al.*, 2005; Suau *et al.*, 1999; Wilson & Blichington, 1996; Zoetendal *et al.*, 1998). We have previously reported that the human faecal microbiota can be analysed based on 16S rRNA gene libraries and strictly anaerobic culture-based methodologies (Hayashi *et al.*, 2002a, b). We have detected many potentially novel species that have not yet been characterized and have demonstrated correlations between novel isolates and 16S rRNA gene sequences. Among the isolates that we have studied are some that have been shown to be representatives of novel species of the genus *Bacteroides* (Hayashi *et al.*, 2002a, b). Some potential novel species belonging to the genus *Bacteroides* have also been observed from 16S rRNA gene sequence libraries (Eckburg *et al.*, 2005; Hayashi *et al.*, 2002a; Suau *et al.*, 1999). *Bacteroides* is one of the predominant genera in the human gut microbiota (Benno *et al.*, 1989; Finegold *et al.*, 1983; Hayashi *et al.*, 2002a, b). Recently, five novel species belonging to the genus *Bacteroides* (*Bacteroides coprocola*, *B. dorei*, *B. finegoldii*, *B.*

intestinalis and *B. plebius*) that were isolated from human faeces have been described (Bakir *et al.*, 2006a, b, c; Kitahara *et al.*, 2005). The 16S rRNA gene sequences of these species corresponded to undescribed sequences detected in 16S rRNA gene sequence libraries derived from human faecal samples (Eckburg *et al.*, 2005; Hayashi *et al.*, 2002a). Here we describe a novel species belonging to the genus *Bacteroides* that was isolated from human faeces by using a strictly anaerobic culture-based method.

The strains used in the present study were maintained on Eggerth Gagnon (EG) agar (Merck) supplemented with 5 % (v/v) horse blood for 2 days at 37 °C, in an atmosphere of 100 % CO₂. Strains CB40, CB41 and CB42^T were isolated from the faeces of a healthy Japanese male (52 years old) by using medium 10 and the 'plate in bottle' method as described by Hayashi *et al.* (2002a) and Mitsuoka *et al.* (1969). Briefly, after collecting samples, each 0.5 g faecal sample was immediately suspended in dilution buffer, and 50 µl of 10⁸-diluted faecal samples were plated anaerobically on medium 10 by using the 'plate in bottle' filled with 100 % CO₂. Bile resistance was tested by growing the bacteria on GAM (Nissui) agar plates supplemented with 2 % Bacto oxgall (Difco). Physiological, biochemical and enzyme activity tests were performed by inoculation of API 20A and Rapid ID 32A (bioMérieux) test strips according to the manufacturer's instructions and incubation at 37 °C in an anaerobic jar. The isolates were cultured in PYG broth for analysis of metabolic end products (Sakamoto *et al.*, 2004, 2005). The metabolic end products were prepared as described by Holdeman *et al.* (1977) and analysed as described by Sakamoto *et al.* (2004, 2005). Cellular fatty acid

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains CB40, CB41 and CB42^T are AB064919, AB260025 and AB260026.

Tables detailing API 20A and Rapid ID 32A test results and the cellular fatty acid composition of strains of *B. coprophilus* sp. nov. and related species are available as supplementary material with the online version of this paper.

profiles were determined by using the MIDI microbial identification system (Microbial ID). Saponification, methylation, extraction and determination of cellular fatty acid profiles were conducted as described by Sakamoto *et al.* (2002). Genomic DNA was extracted from cells harvested from GAM broth as described by Sakka *et al.* (1989). Briefly, cells were suspended in buffer A (10 mM Tris/HCl and 10 mM EDTA, pH 8.0) containing lysozyme (1 mg ml⁻¹). After incubation at 37 °C for 5 min, 0.1 vols 10 % SDS was added, and the suspension was incubated at 60 °C for 30 min. The G + C content of the DNA was determined by using the HPLC method (Kitahara *et al.*, 2001; Tamaoka & Komagata, 1984). The 16S rRNA gene was analysed as described by Hayashi *et al.* (2002a). Sequence data were aligned with the CLUSTAL W (Thompson *et al.*, 1994) package and corrected by manual inspection. Nucleotide substitution rates (K_{nuc} values) were calculated and phylogenetic trees were constructed by using the neighbour-joining method (Kimura, 1980; Saitou & Nei, 1987). Bootstrap resampling analysis (Felsenstein, 1985) of 100 replicates was performed to estimate the confidence of tree topologies.

Cells of strains CB40, CB41 and CB42^T were obligately anaerobic, non-spore-forming, non-motile, Gram-negative short rods or rods. Cells on EG agar were 0.7–0.8 × 2.5–4.1 µm in size and occurred singly. Colonies were 0.5–1.2 mm in diameter, dark grey, translucent, lustrous, circular and slightly convex on EG agar plates. The three new strains could be differentiated from *Bacteroides barnesiae* JCM 13652^T and *Bacteroides salanitronis* JCM 13657^T based on fermentation of L-arabinose, D-cellobiose, L-rhamnose, salicin and D-xylose (see Supplementary Table S1 in IJSEM Online). Based on Rapid ID 32A test results, the new strains

could be differentiated from *B. barnesiae* JCM 13652^T and *B. salanitronis* JCM 13657^T based on activity data for α -arabinosidase, *N*-acetyl- β -glucosaminidase, α -fucosidase and arginine, glycine, glutamyl glutamic acid, histidine, leucine, phenylalanine, serine and tyrosine arylamidases (see Supplementary Table S2 in IJSEM Online).

The cellular fatty acid composition of *Bacteroides* species has been determined (Mayberry *et al.*, 1982; Miyagawa *et al.*, 1979; Shah & Collins, 1980) and used to provide a classification for the genus (Shah & Collins, 1983). The major cellular fatty acids of strains CB40, CB41 and CB42^T were anteiso-C_{15:0}, C_{16:0}, iso-C_{17:0} 3-OH and C_{18:1}ω9c (see Supplementary Table S3 in IJSEM Online).

The lengths of the 16S rRNA gene sequences of strains CB40, CB41 and CB42^T obtained in this study were about 1500 bases, and phylogenetic analysis was based on about 1472 aligned homologous nucleotides (*Escherichia coli* positions 8–1492). The phylogenetic tree clearly indicated that these strains were related to strains within the genus *Bacteroides* (Fig. 1). The three novel strains formed a single cluster and a distinct line of descent; their 16S rRNA gene sequences were identical. Highest sequence similarity to strain CB42^T was found with *B. barnesiae* JCM 13652^T (93.4 %), indicating that strain CB42^T could represent a novel species (similarity < 97 % to the closest related species; Stackebrandt & Goebel, 1994). The DNA G + C contents of strains CB40, CB41 and CB42^T were 44.2–45.2 mol%.

Matsuki *et al.* (2004) analysed the human gut microbiota by real-time PCR using a *Bacteroides fragilis* group-specific primer set. The *B. fragilis* group was detected from all subjects at a level of antilog₁₀ 9.9 ± 0.3 cells g⁻¹ (wet wt).

Fig. 1. Phylogenetic tree showing the relationship between strain CB42^T and related *Bacteroides* species. The tree was constructed by using the neighbour-joining method based on 16S rRNA gene sequences. The sequences of strains CB40 and CB41 were identical to that of strain CB42^T. Bootstrap values (from 100 replicates) of ≥ 50 are shown. The sequence of *Prevotella melaninogenica* ATCC 25845^T was used as the outgroup for rooting the tree. Bar, 0.05 substitutions per nucleotide position.

We detected isolates CB40, CB41 and CB42^T at high frequency [antilog₁₀ 10.4 cells g⁻¹ (wet wt)] by using a strictly anaerobic culture-based method (Hayashi *et al.*, 2002a). The 16S rRNA gene sequence of the isolates investigated herein has been detected previously from a 16S rRNA gene sequence library (Hayashi *et al.*, 2002a). These isolates may thus represent an important *Bacteroides* species occurring in human faeces.

On the basis of the results presented, strains CB40, CB41 and CB42^T are considered to represent a novel species of the genus *Bacteroides*, for which the name *Bacteroides coprophilus* sp. nov. is proposed. Differential characteristics of *B. coprophilus* and some related *Bacteroides* species are given in Table 1.

Description of *Bacteroides coprophilus* sp. nov.

Bacteroides coprophilus (cop.ro'phi.lus. Gr. n. *copros* dung, faeces; Gr. masc. adj. *philos* friendly to; N.L. masc. adj. *coprophilus* loving faeces).

Cells are anaerobic, non-spore-forming, Gram-negative short rods or rods. Colonies on EG agar plates after 48 h

incubation at 37 °C under 100 % CO₂ are circular, dark grey, translucent and convex. Grows in the presence of bile. Indole-negative. Aesculin is not hydrolysed. Nitrate is not reduced. No activity is detected for urease or gelatin. Acid is produced from D-glucose, lactose, maltose, D-mannose, D-raffinose and sucrose. Positive reactions are obtained using the API Rapid ID 32A system for *N*-acetyl- β -glucosaminidase, alkaline phosphatase, α -fucosidase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase and alanine, arginine, histidine, leucine and leucyl glycine arylamidases. Negative reactions are obtained for α -arabinosidase, arginine dihydrolase, β -galactosidase 6-phosphate, β -glucuronidase, glutamic acid decarboxylase, nitrate reductase, indole, proline arylamidase and pyroglutamic acid arylamidase. The major end products are succinic and acetic acids; small amounts of isovaleric acid, propionic acid and pyruvic acid are also produced. Major fatty acids are anteiso-C_{15:0} (12–16 %), C_{16:0} (9–12 %), iso-C_{17:0} 3-OH (17–19 %) and C_{18:1 ω 9c} (16–18 %). The DNA G+C content of the type strain is 44.7 mol%.

The type strain, CB42^T (= JCM 13818^T = DSM 18228^T), was isolated from human faeces. Strains CB40 (= JCM 13816) and CB41 (= JCM 13817) are included in this species.

Table 1. Differential characteristics of strain CB42^T and type strains of related *Bacteroides* species

Strains: 1, strain CB42^T (identical results were obtained for strains CB40 and CB41 unless indicated); 2, *B. barnesiae* JCM 13652^T; 3, *B. salanitronis* JCM 13657^T. Data for reference strains were taken from Lan *et al.* (2006). +, Positive; –, negative; w, weak.

Characteristic	1	2	3
Acid produced from:			
L-Arabinose	–	–	+
D-Cellobiose	w	+	+
L-Rhamnose	–	–	w
Salicin	–	+	w
D-Xylose	–	+	+
Enzyme activities			
<i>N</i> -Acetyl- β -glucosaminidase	+	+	–
α -Arabinosidase	–	+	+
Arginine arylamidase	+	–	–
α -Fucosidase	+	+	–
Glutamyl glutamic acid arylamidase	w	+	+
Glycine arylamidase	w	–	–
Histidine arylamidase	+	–	–
Leucine arylamidase	+	–	–
Phenylalanine arylamidase	w	–	–
Tyrosine arylamidase	w	–	–
Serine arylamidase	w	–	–
Major cellular fatty acids	anteiso-C _{15:0} , C _{16:0} , iso-C _{17:0} 3-OH, C _{18:1ω9c}	anteiso-C _{15:0} , iso-C _{15:0} , C _{16:0} , C _{16:0} 3-OH	anteiso-C _{15:0} , iso-C _{15:0} , iso-C _{17:0} 3-OH, C _{16:0} 3-OH
DNA G+C content (mol%)	44.2–45.2*	46.8	46.9

*Range for strains CB40, CB41 and CB42^T.

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References

- Bakir, M. A., Kitahara, M., Sakamoto, M., Matsumoto, M. & Benno, Y. (2006a). *Bacteroides intestinalis* sp. nov., isolated from human faeces. *Int J Syst Evol Microbiol* **56**, 151–154.
- Bakir, M. A., Kitahara, M., Sakamoto, M., Matsumoto, M. & Benno, Y. (2006b). *Bacteroides finegoldii* sp. nov., isolated from human faeces. *Int J Syst Evol Microbiol* **56**, 931–935.
- Bakir, M. A., Sakamoto, M., Matsumoto, M., Kitahara, M. & Benno, Y. (2006c). *Bacteroides dorei* sp. nov., isolated from human faeces. *Int J Syst Evol Microbiol* **56**, 1639–1643.
- Benno, Y., Endo, K., Mizutani, T., Namba, Y., Komori, T. & Mitsuoka, T. (1989). Comparison of fecal microflora of elderly persons in rural and urban areas of Japan. *Appl Environ Microbiol* **55**, 1100–1105.
- Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S. R., Nelson, K. E. & Relman, D. A. (2005). Diversity of the human intestinal microbial flora. *Science* **308**, 1635–1638.
- Felsenstein, J. (1985). Confidence limits of phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Finegold, S. M., Sutter, V. L. & Mathisen, G. E. (1983). Normal indigenous flora. In *Human Intestinal Microflora in Health and Disease*, pp. 3–31. Edited by D. J. Hentges. New York: Academic Press.
- Hayashi, H., Sakamoto, M. & Benno, Y. (2002a). Phylogenetic analysis of the human gut microbiota using 16S rDNA clone libraries and strictly anaerobic culture-based methods. *Microbiol Immunol* **46**, 535–548.
- Hayashi, H., Sakamoto, M. & Benno, Y. (2002b). Fecal microbial diversity in a strict vegetarian as determined by molecular analysis and cultivation. *Microbiol Immunol* **46**, 819–831.
- Holdeman, L. V., Cato, E. P. & Moore, W. E. C. (1977). *Anaerobe Laboratory Manual*, 4th edn. Blacksburg, VA: Virginia Polytechnic Institute and State University.
- Kimura, M. (1980). A simple method for estimating the evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Kitahara, M., Takamine, F., Imamura, T. & Benno, Y. (2001). *Clostridium hiranonis* sp. nov., a human intestinal bacterium with bile acid 7 α -dehydroxylating activity. *Int J Syst Evol Microbiol* **51**, 39–44.
- Kitahara, M., Sakamoto, M., Ike, M., Sakata, S. & Benno, Y. (2005). *Bacteroides plebeius* sp. nov. and *Bacteroides coprocola* sp. nov., isolated from human faeces. *Int J Syst Evol Microbiol* **55**, 2143–2147.
- Lan, P. T. N., Sakamoto, M., Sakata, S. & Benno, Y. (2006). *Bacteroides barnesiae* sp. nov., *Bacteroides salanitronis* sp. nov. and *Bacteroides gallinarum* sp. nov., isolated from chicken caecum. *Int J Syst Evol Microbiol* **56**, 2853–2859.
- Matsuki, T., Watanabe, K., Fujimoto, J., Takada, T. & Tanaka, R. (2004). Use of 16S rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in human feces. *Appl Environ Microbiol* **70**, 7220–7228.
- Mayberry, W. R., Lambe, D. W., Jr & Ferguson, K. P. (1982). Identification of *Bacteroides* species by cellular fatty acid profiles. *Int J Syst Bacteriol* **32**, 21–27.
- Mitsuoka, T., Morishita, Y., Terada, A. & Yamamoto, S. (1969). A simple method (“plate-in-bottle method”) for the cultivation of fastidious anaerobes. *Jpn J Microbiol* **13**, 383–385.
- Miyagawa, E., Azuma, R. & Suto, T. (1979). Cellular fatty acid composition in gram-negative obligately anaerobic rods. *J Gen Appl Microbiol* **25**, 41–51.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sakamoto, M., Suzuki, M., Umeda, M., Ishikawa, I. & Benno, Y. (2002). Reclassification of *Bacteroides forsythus* (Tanner *et al.* 1986) as *Tannerella forsythensis* corrig., gen. nov., comb. nov. *Int J Syst Evol Microbiol* **52**, 841–849.
- Sakamoto, M., Suzuki, M., Huang, Y., Umeda, M., Ishikawa, I. & Benno, Y. (2004). *Prevotella shahii* sp. nov. and *Prevotella salivae* sp. nov., isolated from the human oral cavity. *Int J Syst Evol Microbiol* **54**, 877–883.
- Sakamoto, M., Huang, Y., Umeda, M., Ishikawa, I. & Benno, Y. (2005). *Prevotella multiformis* sp. nov., isolated from human subgingival plaque. *Int J Syst Evol Microbiol* **55**, 815–819.
- Sakka, K., Furuse, S. & Shimada, K. (1989). Cloning and expression in *Escherichia coli* of thermophilic *Clostridium* sp. F1 genes related to cellulose hydrolysis. *Agric Biol Chem* **53**, 905–910.
- Shah, H. N. & Collins, M. D. (1980). Fatty acid and isoprenoid quinone composition in the classification of *Bacteroides melaninogenicus* and related taxa. *J Appl Bacteriol* **48**, 75–87.
- Shah, H. N. & Collins, M. D. (1983). Genus *Bacteroides*. A chemotaxonomical perspective. *J Appl Bacteriol* **55**, 403–416.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Suau, A., Bonnet, R., Sutren, M., Godon, J.-J., Gibson, G. R., Collins, M. D. & Doré, J. (1999). Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. *Appl Environ Microbiol* **65**, 4799–4807.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Wilson, K. H. & Blitchington, R. B. (1996). Human colonic biota studied by ribosomal DNA sequence analysis. *Appl Environ Microbiol* **62**, 2273–2278.
- Zoetendal, E. G., Akkermans, A. D. & de Vos, W. M. (1998). Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. *Appl Environ Microbiol* **64**, 3854–3859.