International Journal of Systematic and Evolutionary Microbiology (2007), **57**, 941–946 **DOI** 10.1099/ijs.0.64778-0

Prevotella copri sp. nov. and *Prevotella stercorea* sp. nov., isolated from human faeces

Hidenori Hayashi,[1]† Kensaku Shibata,[1,2] Mitsuo Sakamoto,[1] Shinichi Tomita[2] and Yoshimi Benno[1]

[1]Microbe Division/Japan Collection of Microorganisms, RIKEN BioResource Center, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

[2]Department of Life Science, Faculty of Agriculture, Tamagawa University, 6-1-1 Tamagawa-Gakuen, Machida, Tokyo 194-8610, Japan

Correspondence
Hidenori Hayashi
h-hayashi@maebashi-it.ac.jp

Six strains (CB7^T, CB18, CB23, CB26, CB28 and CB35^T) were isolated from human faeces. Based on phylogenetic analysis, phenotypic characteristics, cellular fatty acid profiles and menaquinone profiles, these strains could be included within the genus *Prevotella* and made up two clusters. 16S rRNA gene sequence analysis indicated that five strains were most closely related to *Prevotella veroralis*, sharing about 92 % sequence similarity; the remaining strain was most closely related to *Prevotella shahii*, sharing about 90 % sequence similarity. All six strains were obligately anaerobic, non-pigmented, non-spore-forming, non-motile, Gram-negative rods. The cellular fatty acid compositions of the six strains differed significantly from those of other *Prevotella* species. Five strains (CB7^T, CB18, CB23, CB26 and CB28) contained dimethyl acetals and the major menaquinones of these strains were MK-11, MK-12 and MK-13. The major menaquinones of CB35^T were MK-12 and MK-13. Based on phenotypic and phylogenetic findings, two novel species, *Prevotella copri* sp. nov. and *Prevotella stercorea* sp. nov., are proposed, representing the two different strain clusters. The DNA G+C contents of strains CB7^T and CB35^T were 45.3 and 48.2 mol%, respectively. The type strains of *P. copri* and *P. stercorea* are CB7^T (=JCM 13464^T=DSM 18205^T) and CB35^T (=JCM 13469^T=DSM 18206^T), respectively.

The application of molecular biological techniques has enabled phylogenetic analysis of bacterial 16S rRNA genes in the human gut. In particular, phylogenetic analysis based on 16S rRNA genes has made it possible to clarify the dominant human faecal microbiota (Eckburg *et al.*, 2005; Hold *et al.*, 2002; Suau *et al.*, 1999; Wilson & Blitchington, 1996; Zoetendal *et al.*, 1998). A large number of species that have not yet been identified and characterized exists in the human gut. It has been reported that the human faecal microbiota could be analysed by 16S rRNA gene libraries and strictly anaerobic culture-based methods (Hayashi *et al.*, 2002a, b). Many novel operational taxonomic units (OTUs) and isolates that have not yet been characterized were detected and phylogenetic correlation between novel isolated strains and 16S rRNA sequences has been shown. Some novel OTUs and isolates were phylogenetically related to the genus *Prevotella*. These isolates were the predominant species in human faeces. Suau *et al.* (1999) and Eckburg *et al.* (2005) also detected some novel OTUs belonging to the genus *Prevotella* from 16S rRNA gene library data. Although *Prevotella* species have been isolated mainly from the oral cavity, these species also inhabit the human gut. Here, two novel species within the genus *Prevotella* isolated from human faeces are reported.

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 containing 100 % CO_2. Strains CB7^T, CB18, CB23, CB26, CB28 and CB35^T were isolated from faeces of a healthy Japanese male (52 years old) using medium 10 and the 'plate-in-bottle' method as described previously (Hayashi *et al.*, 2002a; Mitsuoka *et al.*, 1969). Briefly, after collecting samples, each 0.5 g faecal sample was immediately suspended in dilution buffer and 50 µl 10^8-diluted faecal sample was plated anaerobically on medium 10 by using the 'plate-in-bottle' filled with 100 % CO_2. Isolates were subcultured on EG agar. Bacteroides bile aesculin agar (Shah,

†Present address: Faculty of Engineering, Maebashi Institute of Technology, 460-1 Kamisatori, Maebashi, Gunma 371-0816, Japan.

Abbreviation: OTU, operational taxonomic unit.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *P. copri* CB7^T and *P. stercorea* CB35^T are AB064923 and AB244774, respectively.

API 20A test results, cellular fatty acid compositions, menaquinone compositions and API ZYM and API An-Ident test results of *P. copri* sp. nov., *P. stercorea* sp. nov. and related species are available as supplementary material in IJSEM Online.

Downloaded from www.microbiologyresearch.org by
IP: 93.91.26.97
On: Tue, 13 Oct 2015 08:53:18

1992) was used to check whether growth of the isolates was inhibited on this medium.

Physiological, biochemical and enzyme activity tests were performed by inoculation of API 20A, API ZYM and API An-Ident (bioMérieux) test strips according to the manufacturer's instructions followed by incubation at 37 °C in anaerobic jars. The isolates were cultured in PYG broth for analysis of metabolic end products (Sakamoto *et al.*, 2004, 2005a). The metabolic end products were prepared as described by Holdeman *et al.* (1977) and analysed as described previously (Sakamoto *et al.*, 2004, 2005a). Cellular fatty acid profiles were determined using the MIDI Microbial Identification System (Microbial ID). Saponification, methylation, extraction and determination of cellular fatty acid profiles were conducted as described previously (Sakamoto *et al.*, 2002). Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987) and analysed as described previously (Sakamoto *et al.*, 2004, 2005a). Genomic DNA was extracted from cells harvested from Gifu anaerobic medium (Nissui). Cells were suspended in buffer A (10 mM Tris/HCl and 10 mM EDTA, pH 8.0) containing Triton X-100 (final concentration, 1.5 %) and centrifuged at 19 000 ***g*** for 5 min. Cells were then resuspended in buffer A containing proteinase K (final concentration, 2 mg ml^{-1}). After incubation at 37 °C for 10 min, Triton X-100 was added to a final concentration of 1.5 % and the mixture was centrifuged at 19 000 ***g*** for 5 min. The following operations were carried out as described previously (Sakka *et al.*, 1989). The G+C content was determined by using the HPLC method (Kitahara *et al.*, 2001; Tamaoka & Komagata, 1984). The 16S rRNA gene was analysed as described previously (Hayashi *et al.*, 2002a). Sequence data were aligned with the CLUSTAL W package (Thompson *et al.*, 1994) and corrected by manual inspection. Nucleotide substitution rates (K_{nuc} values) were calculated and phylogenetic trees were constructed 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 CB7^T, CB18, CB23, CB26 and CB28 were obligately anaerobic, non-spore-forming, non-motile, Gram-negative rods. Cells of these five strains on EG agar were 0.83 × 0.91–0.99 μm in size and occurred singly; colonies were 0.3–2.0 mm in diameter, milk-white, circular, entire, slightly convex and smooth on EG agar plates. Cells of strain CB35^T were obligately anaerobic, non-spore-forming, non-motile, Gram-negative rods. Cells of strain CB35^T on EG agar were 0.25–0.42 × 1.08–1.25 μm in size and occurred singly; colonies were 0.2–1.8 mm in diameter, translucent, circular, entire, slightly convex and smooth on EG agar plates. Physiological and biochemical properties of the six novel strains, *Prevotella melaninogenica* JCM 6325^T, *Prevotella loescheii* JCM 8530^T, *Prevotella marshii* JCM 13450^T, *Prevotella oralis* JCM 12251^T, *Prevotella salivae* JCM 12084^T, *Prevotella shahii* JCM 12083^T and *Prevotella veroralis* JCM 6290^T were determined by API 20A. Five strains (CB7^T, CB18, CB23, CB26 and CB28) could be differentiated from *P. melaninogenica* JCM 6325^T, *Prevotella oulorum* NCTC 11871^T, *P. salivae* JCM 12084^T and *P. veroralis* JCM 6290^T by D-mannose and L-rhamnose fermentation (see Supplementary Table S1 available in IJSEM Online). Strain CB35^T could be differentiated from *P. loescheii* JCM 8530^T by gelatin digestion and aesculin hydrolysis, from *P. marshii* JCM 13450^T by lactose and sucrose fermentation and gelatin digestion, from *P. oralis* JCM 12251^T by aesculin hydrolysis and D-cellobiose and lactose fermentation and from *P. shahii* JCM 12083^T by gelatin digestion.

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 members of the genus *Bacteroides* (Shah & Collins, 1983). The major cellular fatty acids of strains CB7^T, CB18, CB23, CB26 and CB28 were $C_{16:0}$, $C_{18:1}\omega9c$ and anteiso-$C_{15:0}$. In addition, these strains contained dimethyl acetals. Recently, *Prevotella multisaccharivorax* has been reported to possess dimethyl acetals (Sakamoto *et al.*, 2005b). The major cellular fatty acids of strain CB35^T were anteiso-$C_{15:0}$, iso-$C_{15:0}$ and $C_{18:1}\omega9c$ (see Supplementary Table S2 available in IJSEM Online).

The major menaquinones of strains CB7^T, CB18, CB23, CB26 and CB28 were MK-11, MK-12 and MK-13 (see Supplementary Table S3 available in IJSEM Online), whereas the major menaquinones of strain CB35^T were MK-12 and MK-13. The major menaquinones of other *Prevotella* species are generally MK-10 and MK-11 (Sakamoto *et al.*, 2005a).

The API ZYM and API An-Ident systems have been reported to be useful in the identification of oral and non-oral Gram-negative bacteria (Laughon *et al.*, 1982; Slots, 1981; Tanner *et al.*, 1985). The biochemical characteristics of *Prevotella* species determined using these two systems are given in Supplementary Table S4 (available in IJSEM Online). Based on the results of API ZYM and API An-Ident, the six strains were divided into two groups, with one group consisting of five strains (CB7^T, CB18, CB23, CB26 and CB28) and one strain (CB35^T) in the other.

The 16S rRNA gene sequences determined in this study were about 1500 bp long and phylogenetic analysis was based on about 1435 aligned homologous nucleotides (*Escherichia coli* positions 34–1482). The phylogenetic tree clearly indicated that the isolates were related to strains within the genus *Prevotella* (Fig. 1). Five strains (CB7^T, CB18, CB23, CB26 and CB28) formed a single cluster and a distinct line of descent; their sequence similarity to each other was 99.1–99.5 %. The 16S rRNA gene of CB7^T showed highest sequence similarity to that of *P. veroralis* JCM 6290^T (92 %). CB35^T formed a single cluster and a distinct line of descent. The 16S rRNA gene of CB35^T showed highest sequence similarity to that of *P. shahii* JCM 12083^T (90 %). These results indicated that strains CB7^T and CB35^T could

Downloaded from www.microbiologyresearch.org by
IP: 93.91.26.97
On: Tue, 13 Oct 2015 08:53:18

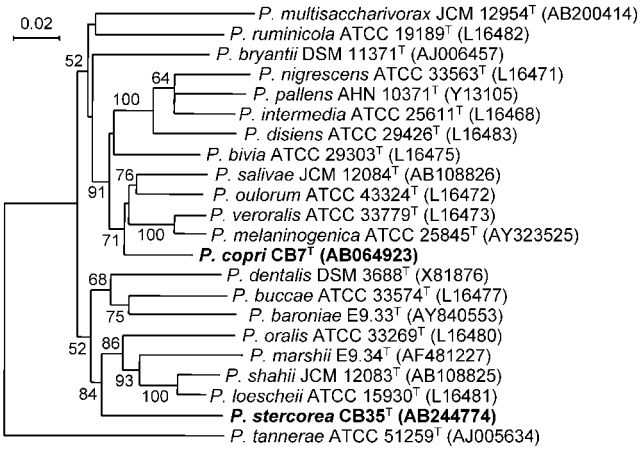

Fig. 1. Phylogenetic tree showing the relationship between strain CB7^T (*Prevotella copri* sp. nov.), strain CB35^T (*Prevotella stercorea* sp. nov.) and related *Prevotella* species. The tree was constructed by the neighbour-joining method based on 16S rRNA gene sequences. Numbers at nodes indicate bootstrap values greater than 50 %. Bar, 0.02 substitutions per nucleotide position.

represent novel species, since 16S rRNA gene sequence similarity to the most closely related species was <97 % (Stackebrandt & Goebel, 1994). The DNA G+C contents of strains CB7^T, CB18, CB23, CB26 and CB28 were 44.2–45.9 mol%; that of strain CB35^T was 48.2 mol%.

Matsuki *et al.* (2004) analysed human gut microbiota (46 healthy adults) by real-time PCR using a *Prevotella*-specific primer. *Prevotella* was found in 21 of 46 subjects (46 %), with the $\log_{10}$ of the number of cells per g wet weight being 9.7 ± 0.8. Some OTUs that belong to the genus *Prevotella* were also detected in 16S rRNA gene libraries (Eckburg *et al.*, 2005; Hayashi *et al.*, 2002a; Suau *et al.*, 1999). These results suggest that members of the genus *Prevotella* are common in the human gut. The novel *Prevotella* species described here (represented by CB7^T and CB35^T) were detected at high frequency using anaerobic culture-based methods (Hayashi *et al.*, 2002a). These isolates represent two species that are important constituents of human gut microbiota.

On the basis of the results presented in this study, these strains could be classified as representatives of two novel species of the genus *Prevotella*. The names *Prevotella copri* sp. nov. and *Prevotella stercorea* sp. nov. are proposed for the five strains (CB7^T, CB18, CB23, CB26 and CB28) and for strain CB35^T, respectively. Differential characteristics of *P. copri* sp. nov., *P. stercorea* sp. nov. and some related *Prevotella* species are shown in Table 1.

Description of *Prevotella copri* sp. nov.

Prevotella copri (cop′ri. N.L. gen. n. *copri* from Gr. gen. n. *kopron* of/from faeces).

Cells are Gram-negative rods that are anaerobic and non-spore-forming. Colonies on EG agar plates after 48 h incubation at 37 °C under 100 % CO_2 are white, circular and convex. Optimum temperature for growth is 37 °C. Growth is inhibited on Bacteroides bile aesculin agar. Indole-negative and aesculin is hydrolysed. No activity is detected for urease and gelatin is not hydrolysed. Acid is produced from glucose, lactose, sucrose, maltose, raffinose, salicin, xylose, L-arabinose, cellobiose and L-rhamnose. Positive reactions are obtained using API ZYM for alkaline phosphatase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase and β-glucosidase; negative reactions are obtained for lipase (C4), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, β-gluconidase, *N*-acetyl-β-D-glucosaminidase, α-mannosidase and α-fucosidase. Positive reactions are obtained using An-Ident for α-glucosidase, α-arabinofuranosidase, β-gluconidase, alkaline phosphatase, α-galactosidase, indoxylacetate hydrolase and arginine and alanine aminopeptidases; negative reactions are obtained for indole, *N*-acetyl-β-D-glucosaminidase, α-L-fucosidase, β-galactosidase, pyroglutamic acid arylamidase, leucine, proline, tyrosine, arginine, histidine, phenylalanine and glycine aminopeptidases and catalase. The major metabolic end products are succinic and acetic acids. Major fatty acids are $C_{16:0}$ (12–13 %), $C_{18:1}\omega9c$ (13–18 %) and anteiso-$C_{15:0}$ (20–27 %). The principal respiratory quinones are menaquinones MK-10 (8–10 %), MK-11 (23–26 %), MK-12 (43–45 %) and MK-13 (15–19 %). Minor menaquinones are MK-8 (0–1 %) and MK-9 (1–4 %).

The type strain is CB7^T (=JCM 13464^T=DSM 18205^T), isolated from human faeces. Strains CB18 (=JCM 13465), CB23 (=JCM 13466), CB26 (=JCM 13467) and CB28 (=JCM 13468) are included in this species. The DNA G+C contents of the five known strains are 44.2–45.9 mol%.

Description of *Prevotella stercorea* sp. nov.

Prevotella stercorea (ster.co′re.a. L. fem. adj. *stercorea* pertaining to faeces).

Cells are Gram-negative rods that are anaerobic and non-spore-forming. Colonies on EG agar plates after 48 h incubation at 37 °C under 100 % CO_2 gas are translucent, circular, entire, slightly convex and smooth. Optimum temperature for growth is 37 °C. Growth is inhibited on Bacteroides bile aesculin agar. Negative for indole, does not hydrolyse aesculin or gelatin and no urease activity is detected. Acid is produced from glucose, lactose, sucrose, maltose, mannose and raffinose. Positive reactions are obtained using API ZYM for alkaline phosphatase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase and α-L-fucosidase; negative reactions are obtained for esterase (C4), esterase lipase (C8), lipase (C4), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, β-gluconidase and β-glucosidase. Positive reactions are obtained using An-Ident for *N*-acetyl-β-D-glucosaminidase,

Downloaded from www.microbiologyresearch.org by
IP: 93.91.26.97
On: Tue, 13 Oct 2015 08:53:18

Table 1. Differential characteristics of *Prevotella copri* sp. nov., *Prevotella stercorea* sp. nov. and related *Prevotella* type strains

Strains: 1, *P. copri* sp. nov. ($n=5$); 2, *P. stercorea* sp. nov. CB35^T; 3, *P. loescheii* JCM 8530^T; 4, *P. marshii* JCM 13450^T; 5, *P. melaninogenica* JCM 6325^T; 6, *P. oralis* JCM 12251^T; 7, *P. oulorum* NCTC 11871^T; 8, *P. salivae* JCM 12084^T; 9, *P. shahii* JCM 12083^T; 10, *P. veroralis* JCM 6290^T. +, Positive; −, negative; V, variable; W, weak; ND, no data. Data were obtained in this study unless indicated otherwise with the exception of *P. oulorum* NCTC 11871^T (data from Shah *et al.*, 1985).

Characteristic	1	2	3	4	5	6	7	8	9	10
Aesculin hydrolysis	+	−	+	−	−	+	+	+	−	+
Acid production from:										
Lactose	+	+	+	−	+	+	+	+	+	+
Sucrose	+	+	+	−	+	+	+	+	+	+
Salicin	+	−	−	−	−	−	−	+	−	−
D-Xylose	+	−	−	−	−	−	−	+	−	−
L-Arabinose	+	−	−	−	−	−	−	+	−	−
Gelatin	−	−	+	+	+	−	−	−	+	−
D-Cellobiose	+	−	−	−	−	+	−	+	−	+
D-Mannose	−	+	+	+	+	+	+	+	+	+
D-Melezitose	V	−	−	−	−	−	−	−	−	−
L-Rhamnose	+	−	−	−	−	−	−	−	−	−
Enzyme activities:										
Esterase lipase (C8)	W	−	−	−	−	−	ND	−	−	−
Chymotrypsin	W	−	−	W	−	W	ND	−	−	−
β-Glucosidase	+	−	V	−	−	W	ND	+	−	−
α-Mannosidase	−	W	−	−	−	W	ND	−	−	−
N-Acetyl-β-D-glucosaminidase	−	+	+	−	+	+	ND	+	+	+
α-Arabinofuranosidase	+	−	−	−	−	−	ND	+	−	−
α-L-Fucosidase	−	+	+	−	+	+	ND	+	+	+
α-Galactosidase	+	+	+	−	+	+	ND	W	W	W
β-Galactosidase	−	−	+	−	+	W	ND	+	W	+
Pyroglutamic acid arylamidase	−	−	−	+	−	−	ND	−	−	−
Arginine aminopeptidase	−	−	+	−	+	−	ND	−	−	−
Histidine aminopeptidase	−	−	−	−	+	−	ND	−	−	−
Major cellular fatty acids	$C_{16:0}$, $C_{18:1}\omega9c$, anteiso-$C_{15:0}$	$C_{18:1}\omega9c$, iso-$C_{15:0}$, anteiso-$C_{15:0}$	$C_{16:0}$, $C_{18:1}\omega9c$, anteiso-$C_{15:0}$	$C_{18:1}\omega9c$, anteiso-$C_{15:0}$	$C_{18:1}\omega9c$, anteiso-$C_{15:0}$	$C_{16:0}$, $C_{18:1}\omega9c$, $C_{16:0}$ 3-OH, anteiso-$C_{15:0}$	iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{17:0}$	$C_{18:1}\omega9c$, iso-$C_{17:0}$ 3-OH, anteiso-$C_{15:0}$	$C_{13:0}$, $C_{16:0}$, $C_{18:1}\omega9c$, $C_{16:0}$ 3-OH	$C_{18:1}\omega9c$, anteiso-$C_{15:0}$
Predominant menaquinone(s)	11, 12, 13	12, 13	10[a*]	11, 12	10, 11[a]	13	10, 11	11, 12[a]	10, 11, 12[a]	10, 11[a]
DNA G+C content (mol%)	44.2–45.9	48.2	46.9	51[b]	41.1[a]	43.1[c]	45.1	41.3	44.3	42.1[c]

*Data from other studies as follows: *a*, Sakamoto *et al.* (2004); *b*, Downes *et al.* (2005); *c*, Watabe *et al.* (1983).

Downloaded from www.microbiologyresearch.org by
IP: 93.91.26.97
On: Tue, 13 Oct 2015 08:53:18

α-glucosidase, α-L-fucosidase, alkaline phosphatase, α-galactosidase, indoxylacetate hydrolase and alanine aminopeptidase; negative reactions are obtained for indole, α-arabinofuranosidase, β-gluconidase, β-galactosidase, pyroglutamic acid arylamidase, arginine, leucine, proline, tyrosine, histidine, phenylalanine and glycine aminopeptidases and catalase. The major metabolic end products are succinic and acetic acids; small amounts of iso-valeric acid are also produced. Major fatty acids are $C_{18:1}\omega 9c$ (15 %), iso-$C_{15:0}$ (24 %) and anteiso-$C_{15:0}$ (26 %). The principal respiratory quinones are menaquinones MK-12 (26 %) and MK-13 (50 %); minor menaquinones are MK-9 (1 %), MK-10 (5 %) and MK-11 (7 %).

The type strain is CB35^T (=JCM 13469^T=DSM 18206^T), isolated from human faeces. The DNA G+C content of the type strain is 48.2 mol%.

Acknowledgements

We are grateful to Professor Dr H. G. Trüper, University of Bonn, Germany, for his suggestions regarding nomenclature. We thank Dr S. Karita, Mie University, Japan, for help in DNA extraction techniques.

References

Downes, J., Sutcliffe, I., Tanner, A. C. R. & Wade, W. G. (2005). *Prevotella marshii* sp. nov. and *Prevotella baroniae* sp. nov., isolated from the human oral cavity. *Int J Syst Evol Microbiol* **55**, 1551–1555.

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 on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

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.

Hold, G. L., Pryde, S. E., Russell, V. J., Furrie, E. & Flint, H. J. (2002). Assessment of microbial diversity in human colonic samples by 16S rDNA sequence analysis. *FEMS Microbiol Ecol* **39**, 33–39.

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 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 7α-dehydroxylating activity. *Int J Syst Evol Microbiol* **51**, 39–44.

Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.

Laughon, B. E., Syed, S. A. & Loesche, W. J. (1982). API ZYM system for identification of *Bacteroides* spp., *Capnocytophaga* spp., and spirochetes of oral origin. *J Clin Microbiol* **15**, 97–102.

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. (2005a). *Prevotella multiformis* sp. nov., isolated from human subgingival plaque. *Int J Syst Evol Microbiol* **55**, 815–819.

Sakamoto, M., Umeda, M., Ishikawa, I. & Benno, Y. (2005b). *Prevotella multisaccharivorax* sp. nov., isolated from human subgingival plaque. *Int J Syst Evol Microbiol* **55**, 1839–1843.

Sakka, K., Furuse, S. & Shimada, K. (1989). Cloning and expression in *Escherichia coli* of thermophilic *Clostridium* sp. F1 gene related to cellulose hydrolysis. *Agric Biol Chem* **53**, 905–910.

Shah, H. N. (1992). The genus *Bacteroides* and related taxa. In *The Prokaryotes*, 2nd edn, pp. 3593–3607. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.

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.

Shah, H. N., Collins, M. D., Watabe, J. & Mitsuoka, T. (1985). *Bacteroides oulorum* sp. nov., a nonpigmented saccharolytic species from the oral cavity. *Int J Syst Bacteriol* **35**, 193–197.

Slots, J. (1981). Enzymatic characterization of some oral and nonoral gram-negative bacteria with the API ZYM system. *J Clin Microbiol* **14**, 288–294.

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.

Tanner, A. C. R., Strzempko, M. N., Belsky, C. A. & McKinley, G. A. (1985). API ZYM and API An-Ident reactions of fastidious oral gram-negative species. *J Clin Microbiol* **22**, 333–335.

Downloaded from www.microbiologyresearch.org by
IP: 93.91.26.97
On: Tue, 13 Oct 2015 08:53:18

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.

Watabe, J., Benno, Y. & Mitsuoka, T. (1983). Taxonomic study of *Bacteroides oralis* and related organisms and proposal of *Bacteroides veroralis* sp. nov. *Int J Syst Bacteriol* **33**, 57–64.

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.

Downloaded from www.microbiologyresearch.org by
IP: 93.91.26.97
On: Tue, 13 Oct 2015 08:53:18