Paraprevotella clara gen. nov., sp. nov. and *Paraprevotella xylaniphila* sp. nov., members of the family '*Prevotellaceae*' isolated from human faeces

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Two anaerobic, non-spore-forming, pleomorphic, Gram-negative rods, designated YIT 11840^T and YIT 11841^T, were isolated from human faeces. The organisms were catalase-negative, produced succinic and acetic acids as end products of glucose metabolism and had DNA G+C contents of approximately 48–49 mol%. Although the phenotypic characteristics of these two strains were very similar, analysis of their 16S rRNA gene sequences showed that they are only distantly related (93.8 %), indicating that they represent two different species. A comparative sequence analysis revealed that these two species are members of the family '*Prevotellaceae*' but are phylogenetically distant (<88 % sequence similarity) from the known genera belonging to this family, including *Prevotella*, *Hallela* and *Xylanibacter*. On the basis of the phylogenetic analysis and physiological tests, strains YIT 11840^T and YIT 11841^T represent two novel species of a new genus, for which the names *Paraprevotella clara* gen. nov., sp. nov. (type strain YIT 11840^T =JCM 14859^T =DSM 19731^T), the type species, and *Paraprevotella xylaniphila* sp. nov. (type strain YIT 11841^T =JCM 14860^T =DSM 19681^T) are proposed.

Comparative 16S rRNA gene sequence analysis has revealed that the large intestine in humans harbours up to 10^{14} bacteria, comprising more than 500 species (reviewed by Rajilić-Stojanović *et al.*, 2007). Most of these organisms are members of the phyla *Firmicutes* and *Bacteroidetes* (Eckburg *et al.*, 2005; Hayashi *et al.*, 2002; Wang *et al.*, 2003). Species belonging to the families *Bacteroidaceae*, '*Prevotellaceae*', '*Porphyromonadaceae*' and '*Rikenellaceae*' of the order '*Bacteroidales*' are common members of the human intestinal and oral microbiota, whereas more than 70 % of the human intestinal '*Bacteroidales*' phylotypes reported are detected only in cultivation-independent studies (Rajilić-Stojanović *et al.*, 2007). This observation can be attributed (at least to some extent) to the lack of appropriate cultivation techniques and to incomplete cultivation efforts.

To clarify the physiological characteristics and functions of the majority of the members of the human gastrointestinal microbiota, we have performed several intensive cultivation trials aimed at isolating so-called 'unculturable' or 'as-yet-uncultured' bacteria from the human gastrointestinal tract (Sakon *et al.*, 2008; Morotomi *et al.*, 2008; Nagai *et al.*, 2009). In this article, we report the isolation of two novel members of the family '*Prevotellaceae*' isolated from human faeces. Although we propose novel taxonomic units (genus and species) based on a single isolate, these isolates displayed >98 % 16S rRNA gene sequence similarity to some of the human intestinal uncultured clones reported by several groups in the USA and other countries, as described below, indicating that these bacteria are common members of the human intestinal microbiota.

Faecal samples were collected from two healthy Japanese males (subjects A and B; aged 54 and 38 years, respectively) and immediately transferred anaerobically. Initial processing and subsequent weighing and dilution of the specimens were carried out under strictly anaerobic conditions by using a modification of the Hungate technique (Holdeman *et al.*, 1977). Each sample was weighed and diluted with pre-reduced 0.1 M PBS (pH 7). Diluted samples were spread onto modified Gifu medium (GAM; Nissui Pharmaceutical) containing agar and supplemented with a mixture of antibiotics (described below) to isolate subdominant groups in the intestinal microbiota. Plates were incubated at 37 °C for 3 days in an anaerobic glove box (Coy Laboratory Products) containing $N_2/H_2/CO_2$ (88 : 7 : 5). The composition of the modified GAM agar was described in our previous report (Sakon *et al.*, 2008). Strain YIT 11840^T was isolated from a GAM agar plate supplemented with trimethoprim (32 μg ml^{-1}), streptomycin sulfate (16 μg ml^{-1}) and vancomycin hydrochloride (2 μg ml^{-1}) and then inoculated with a 10^{-6} serially diluted faecal sample from subject A. Strain YIT

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YIT 11840^T and YIT 11841^T are AB331896 and AB331897.

11841^T was isolated from a GAM agar plate supplemented with trimethoprim (32 μg ml^{-1}), streptomycin sulfate (16 μg ml^{-1}), vancomycin hydrochloride (0.5 μg ml^{-1}) and erythromycin (0.25 μg ml^{-1}) and then inoculated with a 10^{-6} serially diluted faecal sample from subject B. All of these antibiotics were obtained from Sigma-Aldrich. Single colonies were picked and streaked on the modified GAM agar until single cultures were obtained.

The end products of bacterial metabolism of glucose and xylan, in pre-reduced peptone/yeast extract medium (Holdeman *et al.*, 1977) supplemented with 1 % glucose or 1 % birch-wood xylan (Sigma), respectively, were analysed using HPLC according to a previously described procedure (Chonan *et al.*, 1995). Cellular morphology was determined after Gram-staining of 3-day plates. Bile resistance was tested by growing the bacteria on GAM agar plates supplemented with 2 % Bacto oxgall (Difco). Biochemical characteristics were determined using the API Rapid ID 32A, API ZYM and API 20A systems (bioMérieux) according to the manufacturer's instructions. Fatty acid methyl esters were obtained from lyophilized cells by saponification, methylation and extraction, using the method of Miller (1982) but with minor modifications (Kuykendall *et al.*, 1988). Fatty acid methyl esters were determined with a Shimadzu gas chromatograph (model GC-14A) and a Shimadzu chromatograph-data processor (model C-R5A). Fatty acid methyl ester peaks were identified with a bacterial acid methyl ester mix (Supelco) by using retention-time comparisons against standard compounds. Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987) and were analysed by means of an HPLC-atmospheric pressure chemical ionization-MS/MS system (API3200; Applied Biosystems) with an L-column ODS (2.1 × 150 mm; Chemicals Evaluation and Research Institute, Japan), using the modified method of Katsuta *et al.* (2005). The G+C content was determined by hydrolysing the DNA enzymically and quantifying the nucleosides by means of HPLC according to the method of Ezaki *et al.* (1990).

Closely related sequences were retrieved from the DNA Data Bank of Japan by using the program FASTA (Pearson & Lipman, 1985). The sequences were aligned and used to produce an unrooted phylogenetic tree with the neighbour-joining method (Saitou & Nei, 1987), using CLUSTAL_X (version 1.83) (Thompson *et al.*, 1997). The stability of the groups was estimated by means of bootstrap analysis (1000 replications) in CLUSTAL_X. The trees were visualized by using the program TreeView (version 1.6.6) (Page, 1996). The minimum-evolution method (1000 bootstrap replicates) in MEGA4 (Tamura *et al.*, 2007) and the maximum-likelihood method from the PHYLIP program package (Felsenstein, 1993) were used to confirm the phylogenetic placement of the aligned sequences. 16S rRNA gene sequence similarities between YIT 11840^T and YIT 11841^T and between these strains and the type strains of all described species within the family '*Prevotellaceae*' were calculated using FASTA (Pearson & Lipman, 1985) and EzTaxon (Chun *et al.*, 2007).

The physiological characteristics of strains YIT 11840^T and YIT 11841^T were very similar, except for the production of acid from maltose (YIT 11840^T, weakly positive; YIT 11841^T, negative) and from D-xylose (YIT 11840^T, positive; YIT 11841^T, weakly positive). The cells were Gram-negative, obligately anaerobic, non-motile, pleomorphic coccobacilli (YIT 11840^T, 0.4–1.3 × 0.7–2.2 μm; YIT 11841^T, 0.4–1.5 × 0.9–2.2 μm) that occurred singly, in pairs and in short chains. After 3 days of anaerobic incubation on GAM agar, colonies were 1.5–4.0 mm (YIT 11840^T) or 0.3–1.0 mm (YIT 11841^T) in diameter, entire, shiny and translucent with irregular or round margins. Growth of these strains in peptone/yeast extract/glucose broth was weak; small amounts of succinate and acetate were detected as end products of metabolism. However, growth of these strains was stimulated by xylan: large amounts of succinate (YIT 11840^T, 6.7 mM; YIT 11841^T, 18.3 mM) and acetate (YIT 11840^T, 7.0 mM; YIT 11841^T, 13.2 mM) were detected after incubation in peptone/yeast extract/xylan broth. Biochemical characteristics determined using the API Rapid ID 32A, API ZYM and API 20A systems are described in Table 1.

The chemotaxonomic characteristics of strains YIT 11840^T and YIT 11841^T were very similar. The major cellular fatty acids were anteiso-$C_{15:0}$ (33.0 and 21.8 %, respectively, in strains YIT 11840^T and YIT 11841^T), iso-$C_{15:0}$ (17.0 and 17.3 %) and $C_{18:1}\omega9c$ (20.8 and 38.0 %). The following other fatty acids were detected: $C_{14:0}$ (5.4 and 2.6 %), $C_{15:0}$ (8.5 and 2.4 %), $C_{16:0}$ (2.2 and 2.4 %), $C_{18:2}\omega9,12c$ (1.1 and 1.2 %), $C_{18:1}\omega9t$ (0.8 and 1.8 %) and unknown fatty acids (11.3 and 12.5 %; peaks with equivalent chain-lengths of 13.6 and 17.3 in both strains and 16.3 in YIT 11840^T or 22.2 in YIT 11841^T). For both strains, the major respiratory quinones were MK-10(H_0) and MK-11(H_0) (Table 1). Although the predominant menaquinones of strains YIT 11840^T and YIT 11841^T were similar to those of *Prevotella melaninogenica* and *Prevotella veroralis*, the overall pattern of the cellular fatty acid content and the other biological and biochemical characteristics differed from those of phylogenetically related, phenotypically similar *Prevotella* species of human oral and intestinal origin (Table 1).

Approximately 1500 bp of the 16S rRNA genes of YIT 11840^T and YIT 11841^T were sequenced. Although YIT 11840^T and YIT 11841^T had very similar phenotypic characteristics, comparison of their partial 16S rRNA gene sequences revealed that these strains were only distantly related (93.8 % sequence similarity) and therefore (on the basis of the phylogenetic species concept) represented two different species. In database searches, the highest levels of similarity were found with respect to *Prevotella* species (YIT 11840^T, 88.5–84.9 %; YIT 11841^T, 88.6–84.6 %). A phylogenetic analysis of these and other related sequences was performed: it confirmed that strains YIT 11840^T and YIT 11841^T were phylogenetically most closely associated with members of the '*Prevotellaceae*' (species belonging to the genera *Prevotella*, *Hallela* and *Xylanibacter*) but formed a separate cluster (Fig. 1). In contrast, the 16S rRNA gene sequences most similar to those of YIT 11840^T (98.9–

Table 1. Differential characteristics of strains YIT 11840^T and YIT 11841^T and strains of phylogenetically related, phenotypically similar *Prevotella* species of human oral and intestinal origin

Strains: 1, YIT 11840^T; 2, YIT 11841^T; 3, *P. copri* (data for five strains, including the type strain); 4, *P. stercorea* JCM 13469^T; 5, *P. loescheii* JCM 8530^T; 6, *P. marshii* JCM 13450^T; 7, *P. melaninogenica* JCM 6325^T; 8, *P. oralis* JCM 12251^T; 9, *P. salivae* JCM 12084^T; 10, *P. shahii* JCM 12083^T; 11, *P. veroralis* JCM 6290^T. Data for reference strains were obtained from Hayashi *et al.* (2007) unless indicated otherwise. +, Positive; −, negative; V, variable; W, weak; ai-, anteiso-branched; i-, iso-branched; ND, no data. All strains are positive for acid production from D-glucose, but negative for acid production from D-mannitol, glycerol, D-sorbitol and trehalose. All strains are positive for acid phosphatase, negative for esterase (C4), lipase (C4), leucine, valine and cystine arylamidases, trypsin and β-glucuronidase activities. All strains are negative for indole formation and for urease and catalase activities.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Aesculin hydrolysis	−	−	+	−	+	−	−	+	+	−	+
Gelatin hydrolysis	−	−	−	−	+	+	+	−	−	−	−
Acid production from:											
L-Arabinose	+	+	+	−	−	−	−	−	+	−	−
Cellobiose	−	−	+	−	−	−	−	+	+	−	+
Lactose	−	−	+	+	+	−	+	+	+	+	+
Maltose	W	−	+	+	+	+	+	+	+	+	+
D-Mannose	+	+	−	+	+	+	+	+	+	+	+
Melezitose	−	−	V	−	−	−	−	−	−	−	−
Raffinose	−	−	+	+	+	+	+	+	+	+	+
L-Rhamnose	−	−	+	−	−	−	−	−	−	−	−
Salicin	−	−	+	−	−	−	−	−	+	−	−
Sucrose	−	−	+	+	+	−	+	+	+	+	+
D-Xylose	+	W	+	−	−	−	−	−	+	−	−
Enzyme activities											
N-Acetyl-β-D-glucosaminidase	−	−	−	+	+	−	+	+	+	+	+
Chymotrypsin	−	−	W	−	−	W	−	W	−	−	−
Esterase lipase (C8)	−	−	W	−	−	−	−	−	−	−	−
α-Fucosidase	−	−	−	+	+	−	+	+	+	+	+
α-Galactosidase	+	+	+	+	+	−	+	+	W	W	W
β-Galactosidase	+	+	−	−	+	−	+	W	+	W	+
α-Glucosidase	−	−	+	+	+	+	+	+	+	+	+
β-Glucosidase	W	W	+	−	V	−	−	W	+	−	−
α-Mannosidase	−	−	−	W	−	−	−	W	−	−	−
Major cellular fatty acids	ai-$C_{15:0}$, $C_{18:1}\omega9c$, i-$C_{15:0}$	$C_{18:1}\omega9c$, ai-$C_{15:0}$, i-$C_{15:0}$	$C_{16:0}$, $C_{18:1}\omega9c$, ai-$C_{15:0}$	$C_{18:1}\omega9c$, i-$C_{15:0}$, ai-$C_{15:0}$	$C_{16:0}$, $C_{18:1}\omega9c$, ai-$C_{15:0}$	$C_{18:1}\omega9c$, ai-$C_{15:0}$	$C_{18:1}\omega9c$, ai-$C_{15:0}$	$C_{16:0}$, $C_{18:1}\omega9c$, $C_{16:0}$ 3-OH, ai-$C_{15:0}$	$C_{18:1}\omega9c$, i-$C_{17:0}$ 3-OH, ai-$C_{15:0}$	$C_{13:0}$, $C_{16:0}$, $C_{18:1}\omega9c$, $C_{16:0}$ 3-OH	$C_{18:1}\omega9c$, ai-$C_{15:0}$
Predominant menaquinone(s)	10, 11	10, 11	11, 12, 13	12, 13	10	11, 12	10, 11	13	11, 12	10, 11, 12	10, 11
DNA G+C content (mol%)	48.1	49.0	44.2–45.9	48.2	46.9	51[a]*	41.1[b]	43.1[c]	41.3	44.3	42.1[c]

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

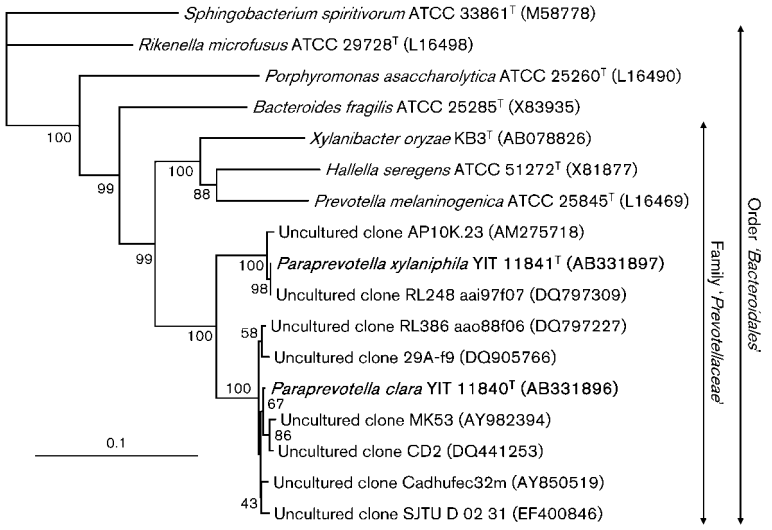

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strains YIT 11840^T and YIT 11841^T and species belonging to the family '*Prevotellaceae*'. GenBank/EMBL/DDBJ accession numbers are shown in parentheses. Values shown at branch points are bootstrap percentages (based on 1000 replications). The tree was rooted with *Sphingobacterium spiritivorum* ATCC 33861^T. Bar, 10 % sequence divergence.

98.6 %) and YIT 11841^T (99.9–99.5 %) were derived from studies of uncultured human faecal or colonic bacteria (Eckburg *et al.*, 2005; Gophna *et al.*, 2006; Mai *et al.*, 2006; Ley *et al.*, 2006; Li *et al.*, 2008; Kassinen *et al.*, 2007) (Fig. 1). These data indicate that these bacteria are common members of the human intestinal microbiota.

Species belonging to the genus *Prevotella* are common members of the human indigenous intestinal and oral microbiota (for reviews, see Rajilić-Stojanović *et al.*, 2007; Sakamoto *et al.*, 2005). As described above, both the phylogenetic and phenotypic characteristics of strains YIT 11840^T and YIT 11841^T were similar to those of *Prevotella* species (Willems & Collins, 1995). Characteristics that serve to differentiate strains YIT 11840^T and YIT 11841^T from *Prevotella* type strains of human oral and intestinal origin are shown in Table 1. On the basis of their phylogenetic distinctiveness, therefore, strains 11840^T and 11841^T represent two novel species in a novel genus of the family '*Prevotellaceae*', for which we propose the names *Paraprevotella clara* gen. nov., sp. nov. and *Paraprevotella xylaniphila* sp. nov., respectively.

Description of *Paraprevotella* gen. nov.

Paraprevotella (Pa.ra.pre.vo.tel′la. Gr. prep. *para* beside, next to; N.L. fem. n. *Prevotella* name of a bacterial genus; N.L. fem. n. *Paraprevotella* a genus similar to *Prevotella*).

Cells are Gram-negative, non-spore-forming and non-motile. Strictly anaerobic. Utilize various sugars and produce succinic and acetic acids as major fermentation end products. The major cellular fatty acids are iso-$C_{15:0}$, anteiso $C_{15:0}$ and $C_{18:1}\omega9c$. The major respiratory quinones are MK-10(H_0) and MK-11(H_0). The type species is *Paraprevotella clara*.

Description of *Paraprevotella clara* sp. nov.

Paraprevotella clara (cla′ra. L. fem. adj. *clara* clear, bright, shining or brilliant, referring to the colony characteristics). Exhibits the following properties in addition to those given in the genus description. Cells are approximately 0.4–1.3 × 0.7–2.2 μm. After 3 days of anaerobic incubation on GAM agar, colonies are 1.5–4.0 mm in diameter, entire, shiny and translucent with irregular or round margins. Succinic and acetic acids are produced as end products of metabolism from peptone/yeast extract/glucose broth. Positive for acid production from L-arabinose, glucose, lactose, D-mannose, raffinose and D-xylose. Weakly positive for acid production from maltose. Cellobiose, glycerol, D-mannitol, melezitose, L-rhamnose, salicin, D-sorbitol, sucrose and trehalose are not utilized. In the API Rapid ID 32A and API ZYM test systems, positive results are obtained for acid phosphatase, alanine arylamidase, alkaline phosphatase, α-arabinosidase, α-galactosidase, β-galactosidase, glutamyl glutamic acid arylamidase and leucyl glycine arylamidase. Negative for *N*-acetyl-β-glucosaminidase, arginine arylamidase, arginine dihydrolase, chymotrypsin, cystine arylamidase, esterase lipase (C8), esterase (C4), α-fucosidase, 6-phospho-β-galactosidase, α-glucosidase, β-glucuronidase, glutamic acid decarboxylase, glycine arylamidase, histidine arylamidase, leucine arylamidase, lipase (C4), α-mannosidase, phenylalanine arylamidase, proline arylamidase, pyroglutamic acid arylamidase, serine arylamidase, trypsin, tyrosine arylamidase and valine arylamidase. Weakly positive for β-glucosidase and naphthol-AS-BI-phosphohydrolase. The DNA G+C content of the type strain is 48.1 mol%.

The type strain, YIT 11840^T (=JCM 14859^T =DSM 19731^T), was isolated from human faeces.

Description of *Paraprevotella xylaniphila* sp. nov.

Paraprevotella xylaniphila (xy.la.ni.phi′la. N.L. n. *xylanum* xylan; N.L. fem. adj. *phila* loving; N.L. fem. adj. *xylaniphila* xylan-loving).

Exhibits the following properties in addition to those given in the genus description. The biological and biochemical

characteristics of *P. xylaniphila* are very similar to those of *P. clara*, except that there are slight differences with regard to cell size, colony size and acid production from maltose (negative) and D-xylose (weakly positive). Cells are approximately 0.4–1.5 × 0.9–2.2 μm. After 3 days of anaerobic incubation on GAM agar, colonies are 0.3–1.0 mm in diameter, entire, shiny and translucent with irregular or round margins. Succinic and acetic acids are produced as end products of metabolism from peptone/yeast extract/glucose broth. Positive for acid production from L-arabinose, glucose, lactose, D-mannose and raffinose. Weakly positive for acid production from D-xylose. Cellobiose, glycerol, maltose, D-mannitol, melezitose, L-rhamnose, salicin, D-sorbitol, sucrose and trehalose are not utilized. In the API Rapid ID 32A and API ZYM test systems, cells are positive for acid phosphatase, alanine arylamidase, alkaline phosphatase, α-arabinosidase, α-galactosidase, β-galactosidase, glutamyl glutamic acid arylamidase and leucyl glycine arylamidase. Negative for *N*-acetyl-β-glucosaminidase, arginine arylamidase, arginine dihydrolase, chymotrypsin, cystine arylamidase, esterase lipase (C8), esterase (C4), α-fucosidase, 6-phospho-β-galactosidase, α-glucosidase, β-glucuronidase, glutamic acid decarboxylase, glycine arylamidase, histidine arylamidase, leucine arylamidase, lipase (C4), α-mannosidase, phenylalanine arylamidase, proline arylamidase, pyroglutamic acid arylamidase, serine arylamidase, trypsin, tyrosine arylamidase and valine arylamidase. Weakly positive for β-glucosidase and naphthol-AS-BI-phosphohydrolase. The DNA G+C content of the type strain is 49.0 mol%.

The type strain, YIT 11841^T (=JCM 14860^T =DSM 19681^T), was isolated from human faeces.

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