Dialister succinatiphilus sp. nov. and *Barnesiella intestinihominis* sp. nov., isolated from human faeces

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Two anaerobic, non-spore-forming, bacteria (YIT 11850^T and YIT 11860^T) that stained Gram-negative, were isolated from human faeces. Cells of strain YIT 11850^T were coccobacilli, asaccharolytic and largely unreactive, with only traces of lactate and propionate as metabolic end products; however, strain YIT 11850^T was able to decarboxylate succinate to propionate. The DNA G+C content of strain YIT 11850^T was 51.9 mol%. Following 16S rRNA gene sequence analysis, this strain was found to be most closely related to *Dialister propionicifaciens*, with 95.1 % sequence similarity between the two taxa. Biochemical data supported the affiliation of strain YIT 11850^T to the genus *Dialister*. Strain YIT 11850^T therefore represents a novel species for which the name *Dialister succinatiphilus* sp. nov. is proposed; the type strain is YIT 11850^T (=DSM 21274^T=JCM 15077^T). Cells of the other isolate, strain YIT 11860^T, were non-motile, rod-shaped, positive for aesculin hydrolysis, negative for indole production, produced succinic and acetic acids as end products of glucose metabolism and possessed a DNA G+C content of 45.5 mol%. On the basis of 16S rRNA gene sequence similarity values, this strain was shown to belong to the family 'Porphyromonadaceae' related to *Barnesiella viscericola* (96.0 %); similarity values with species within the family 'Porphyromonadaceae' with validly published names were less than 86 %. Biochemical data supported the affiliation of strain YIT 11860^T to the genus *Barnesiella*. Strain YIT 11860^T therefore represents a novel species for which the name *Barnesiella intestinihominis* sp. nov. is proposed; the type strain is YIT 11860^T (=DSM 21032^T=JCM 15079^T).

Many novel bacteria have been detected in the human gastrointestinal (GI) tract and faeces using culture-independent approaches based on small subunit (16S and 18S) rRNA diversity (for review see Rajilić-Stojanović *et al.*, 2007). The recent study of Eckburg *et al.* (2005) indicated that the proportion of reported cultivable bacteria was only 20 % for faecal and mucosal samples of three healthy individuals. To better understand the physiological characteristics and function of the majority of human GI microbiota, several intensive cultivation trials aimed at isolating so-called 'unculturable' or 'as-yet-uncultured' bacteria from the human GI tract have been performed (Sakon *et al.*, 2008). In this article, the isolation of two novel species from human faeces is reported. Although

novel taxonomic units (species) based on a single isolate are proposed, 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 C and M, 38 and 29 years old, respectively) and immediately transferred anaerobically. Each sample was weighed and diluted with pre-reduced 0.1 M PBS (0.145 M NaCl, 0.15 M sodium phosphate; pH 7) in an anaerobic glove box (Coy Laboratory Products) containing 88 % nitrogen, 7 % hydrogen and 5 % carbon dioxide. Then, each dilution was spread on modified Gifu anaerobic medium (GAM) agar (Nissui Pharmaceutical) and anaerobe basal agar (Oxoid). The composition of the modified GAM agar has been described in our previous report (Sakon *et al.*, 2008). Plates were incubated at 37 °C for 3 days in the same anaerobic cabinet. Strain YIT 11850^T was isolated from the GAM agar plate inoculated with a 10⁻⁸ serially diluted faecal sample from subject C. Strain

Abbreviation: GI, gastrointestinal.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YIT 11850^T and YIT 11860^T are AB370249 and AB370251, respectively.

16S rRNA gene sequence similarity values between *Dialister succinatiphilus* sp. nov. and other members of the genus *Dialister* are available with the online version of this paper.

YIT 11860^T was isolated from an anaerobe basal agar plate inoculated with a 10⁻⁶ serially diluted faecal sample from subject M. Single colonies were picked and streaked out until single cultures were obtained on modified GAM agar. The end products of bacterial metabolism of glucose, lactate or succinate in pre-reduced peptone-yeast extract (PY) medium (Holdeman *et al.*, 1977) supplemented with glucose (PYG medium), lactate or succinate, respectively, were analysed by HPLC according to a previously described procedure (Chonan *et al.*, 1995). Cellular morphology was recorded after Gram-staining of 3 day plate or 1 day broth cultures. Biochemical characteristics were determined using the API Rapid ID 32A, API ZYM and API 20A systems (bioMérieux) according to the manufacturer's instructions. The DNA G+C content was determined by hydrolysing the DNA enzymically and quantifying the nucleosides by HPLC according to the method of Ezaki *et al.* (1990). Closely related sequences were retrieved from the DDBJ (DNA DataBank of Japan) using the FASTA program (Lipman & Pearson, 1985). Sequences were aligned and used to produce an unrooted phylogenetic tree by the neighbour-joining method (Saitou & Nei, 1987) using CLUSTAL_X (version 1.83) (Thompson *et al.*, 1997). The stability of the groupings was estimated by bootstrap analysis (1000 replications) in CLUSTAL_X. Trees were visualized by using the TREEVIEW program (version 1.6.6) (Page, 1996). The minimal 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.

Cells of YIT 11850^T stained Gram-negative and were obligately anaerobic, non-motile coccobacilli (0.4–

0.9 × 0.8–2.0 µm), occurring singly, in pairs and in short chains. Colonies after 3 days anaerobic incubation on ATCC medium 1257 (ETSA medium) were translucent, entire, circular, convex and pin-point. They were asaccharolytic in API test systems. Although the growth of YIT 11850^T in PYG broth produced no visible turbidity, trace amounts of lactate and propionate were detected as end products of metabolism. In the API Rapid ID 32A and API ZYM test systems, strain YIT 11850^T was very unreactive; apart from activities for esterase C4, naphthol-AS-BI-phosphohydrolase, acid phosphatase and weak alkaline phosphatase, all other tests were negative. Addition of succinate, but not lactate, enhanced the growth of YIT 11850^T and subsequent HPLC analysis revealed that this strain produced a large amount of propionate from PY medium supplemented with succinate. Strain YIT 11850^T was able to decarboxylate succinate to propionate, similar to *Dialister propionificiens* (Jumas-Bilak *et al.*, 2005) and members of the genus *Veillonella*. Enzymic profiles obtained with Rapid ID 32A highlighted the phenotypic differences within the genus *Dialister*, which are summarized in Table 1.

Approximately 1500 bp of the 16S rRNA gene of strain YIT 11850^T was sequenced and database searches revealed highest sequence relatedness to that of the type strain of *D. propionificiens* (95.1 % sequence similarity; see supplementary Table S1 in IJSEM Online). Phylogenetic analysis of these and other related sequences was performed and data confirmed that strain YIT 11850^T was phylogenetically most closely associated with *D. propionificiens* and *Dialister invisus* (Fig. 1). Currently, only four species of the genus *Dialister* are known: *Dialister pneumosintes*, formerly classified as *Bacteroides pneumosintes* and origin-

Table 1. Major characteristics of strain YIT 11850^T and phylogenetically related species of the genus *Dialister*

Taxa: 1, strain YIT 11850^T; 2, *Dialister propionificiens* CCUG 49291^T; 3, *Dialister invisus* CCUG 47026^T; 4, *Dialister microaerophilus* CCUG 48837^T; 5, *Dialister pneumosintes* ATCC 33048^T. Data for strain YIT 11850^T are from this study. Data for other species are from Jumas-Bilak *et al.* (2005). Rapid ID 32A API codes for these type strains are 0000400000, 0000000000, 0000000000, 2000013305 and 0000012401, respectively. +, Positive; –, negative; NA, no data available.

Characteristic	1	2	3	4	5
Isolation source	Human faeces	Human clinical samples	Human oral cavity	Human clinical samples	Human nasopharyngeal secretions
DNA G+C content (mol%)	51.9	NA	45	36.3	35
Rapid ID 32A API:					
Alanine arylamidase	–	–	–	+	–
Alkaline phosphatase	+	–	–	–	–
Arginine arylamidase	–	–	–	+	+
Arginine dihydrolase	–	–	–	+	–
Glycine arylamidase	–	–	–	–	+
Histidine arylamidase	–	–	–	+	+
Leucine arylamidase	–	–	–	+	+
Phenylalanine arylamidase	–	–	–	+	–
Serine arylamidase	–	–	–	+	–
Tyrosine arylamidase	–	–	–	+	–

Fig. 1. Phylogenetic tree showing the positions of *Dialister succinatiphilus* YIT 11850^T and other species belonging to the family Veillonaceae based on 16S rRNA gene sequence similarity data. The tree was rooted with *Escherichia coli* and constructed by using the neighbour-joining method; bootstrap values from 1000 replications are given at the nodes. GenBank/EMBL/DDBJ accession numbers are shown in parentheses. Bar, 0.1 substitutions per nucleotide position.

ally isolated from nasopharyngeal secretions of patients with influenza (Willems & Collins, 1995); *D. invisus*, isolated from the human oral cavity (Downes *et al.*, 2003); and *Dialister microaerophilus* and *D. propionificiens*, isolated from human clinical samples (Jumas-Bilak *et al.*, 2005). Although there is no evidence that species of the genus *Dialister* have been isolated from human faeces as common members of human indigenous microbiota, many uncultured human intestinal bacterial clones with 16S rRNA gene sequences that are highly similar to that of strain YIT 11850^T have been deposited in the DDBJ (Ley *et al.*, 2006; Gill *et al.*, 2006) (Fig. 1). The DNA G + C content of strain YIT 11850^T was 51.9 mol%, whereas that of the recognized species of the genus *Dialister* is 35–46 mol% (Downes *et al.*, 2003). It is evident from the results of the taxonomic study that the Gram-negative staining, coccobacillus strain YIT 11850^T recovered from human faeces represents a hitherto unknown species.

Cells of YIT 11860^T stained Gram-negative and were obligately anaerobic, non-motile, pleomorphic rods (0.4–1.0 × 1.3–13.0 µm) that hydrolysed aesculin but were negative for indole production. Colonies after 3 days anaerobic incubation on anaerobe basal agar were 1–3 mm in diameter, translucent to pale orange–yellow in colour with irregular margins and a low convex shape. Analysis of metabolic end products by HPLC from PYG broth revealed succinic and acetic acids. Results based on the API Rapid ID 32A, 20A and API ZYM systems are summarized in Table 2. Many of the characteristics were similar to those of *Barnesiella viscericola* (Sakamoto *et al.*, 2007).

An almost-complete 16S rRNA gene sequence of strain YIT 11860^T was determined (1484 bp). The most similar 16S rRNA gene sequences (98.6–99.9% similarity) were derived from studies of uncultured colonic bacteria (Ley

et al., 2006; Eckburg *et al.*, 2005; Mai *et al.*, 2006) (Fig. 2). The cultured bacterium most closely related to strain YIT 11860^T was *B. viscericola* (96.0% similarity). *B. viscericola*, isolated from chicken caecum, is currently the sole recognized species of the genus *Barnesiella*. A tree showing the phylogenetic relationships of the unknown bacterium is depicted in Fig. 2 and reveals that strain YIT 11860^T is related to *B. viscericola*. The DNA G + C content of strain YIT 11860^T was 45.5 mol%.

In this report, the isolation of two novel species from human faeces is described. Their phenotypic criteria corresponded to earlier studies of the genera *Dialister* and *Barnesiella*, respectively. Based on their phylogenetic distinctiveness, it is considered that strain YIT 11850^T represents a novel species in the genus *Dialister* and, on the basis of the presented findings, it is proposed that the unknown species from human faeces be assigned as a novel

Table 2. Major characteristics that differentiate strain YIT 11860^T from the phylogenetically related bacterium *Barnesiella viscericola*

Data for YIT 11860^T are from this study. Data for *B. viscericola* JCM 13660^T are from Sakamoto *et al.* (2007).

Characteristic	YIT 11860 ^T	<i>B. viscericola</i>
Isolation source	Human faeces	Chicken caecum
DNA G + C content (mol%)	45.5	52.0
Acid production from:		
Lactose	+	–
Sucrose	–	+
Rapid ID 32A API:		
β-Glucuronidase	+	–
Fermentation of mannose	+	–

Fig. 2. Phylogenetic tree showing the position of strain YIT 11860^T among selected clones or strains belonging to the family *Porphyromonadaceae*. The tree was rooted with *Escherichia coli* and constructed by using the neighbour-joining method; bootstrap values from 1000 replications are given at the nodes. GenBank/EMBL/DBJ accession numbers are shown in parentheses. Bar, 0.1 substitutions per nucleotide position.

species, *Dialister succinatiphilus* sp. nov. Likewise, on the basis of the presented findings, it is proposed that strain YIT 11860^T be assigned to the genus *Barnesiella* as a novel species, *Barnesiella intestinihominis* sp. nov.

Emended description of the genus *Dialister* (ex Bergey *et al.* 1923) Moore and Moore 1994

The description is as emended by Downes *et al.* (2003) and Jumas-Bilak *et al.* (2005) with the following modification: the DNA G + C content ranges from 35 to 52 mol%.

Description of *Dialister succinatiphilus* sp. nov.

Dialister succinatiphilus (suc.ci.na.ti.phi'lus. N.L. n. *succinas* -atis succinate; Gr. adj. *philos* loving; N.L. masc. adj. *succinatiphilus* succinate-loving).

Cells are non-motile, non-spore-forming cocci to coccobacilli, approximately $0.4\text{--}0.9 \times 0.8\text{--}2.0\text{ }\mu\text{m}$, which stain Gram-negative. Colonies after 3 days anaerobic incubation on ATCC medium 1257 (ETSA medium) are translucent, entire, circular, convex and pin-point. Oxidase- and catalase-negative. Aesculin and gelatin are not hydrolysed and nitrate is not reduced. Indole is not produced. Asaccharolytic. Esterase C4, naphthol-AS-BI-phosphohydrolase, acid phosphatase and weak alkaline phosphatase activities may be detected. Using the commercially available API test systems, no activity is detected for alanine arylamidase, α -arabinosidase, arginine arylamidase, arginine dihydrolase, α -chymotrypsin, cystine arylamidase, esterase lipase C8, α -fucosidase, α -glucosidase, β -glucosidase, β -glucuronidase, α -galactosidase, β -galactosidase, 6-phospho- β -galactosidase, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, glycine arylamidase, histidine arylamidase, leucine arylamidase, leucyl glycine arylamidase, lipase C4, α -mannosidase, *N*-acetyl- β -gluco-

saminidase, proline arylamidase, phenylalanine arylamidase, phosphoamidase, pyroglutamic acid arylamidase, serine arylamidase, trypsin, valine arylamidase, urease or tyrosine arylamidase.

The type strain, YIT 11850^T (=DSM 21274^T=JCM 15077^T), was isolated from human faeces. The DNA G + C content of the type strain is 51.9 mol%.

Emended description of the genus *Barnesiella* Sakamoto *et al.* 2007

The description is as given by Sakamoto *et al.* (2007) with the following modification: the DNA G + C content is in the range 45–52 mol%.

Description of *Barnesiella intestinihominis* sp. nov.

Barnesiella intestinihominis (in.tes.ti.ni.ho'mi.nis. L. gen. n. *intestini* of the intestine; L. gen. n. *hominis* of a human being; N.L. gen. n. *intestinihominis* of the human intestine).

Cells stain Gram-negative and are obligately anaerobic, non-motile, pleomorphic rods ($0.4\text{--}1.0 \times 1.3\text{--}13.0\text{ }\mu\text{m}$) that hydrolyse aesculin, but are negative for indole production. Colonies after 3 days anaerobic incubation on anaerobe basal agar are 1–3 mm in diameter, translucent with irregular margins, pale orange–yellow in colour and low convex. Analysis of metabolic end products by HPLC from PYG broth reveals succinic and acetic acids. Catalase, urease and oxidase are not produced. Negative for nitrate reduction and gelatin hydrolysis. Acid is produced from glucose, lactose, maltose and D-mannose, but not from D-mannitol, sucrose, salicin, D-xylose, L-arabinose, glycerol, melezitose, raffinose, D-sorbitol, L-rhamnose or trehalose. Weak acid is produced from cellobiose. Using

the commercially available API test systems, activity is detected for *N*-acetyl- β -glucosaminidase, acid phosphatase, alanine arylamidase, alkaline phosphatase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, β -glucuronidase, glutamic acid decarboxylase, leucyl glycine arylamidase and naphthol-AS-BI-phosphohydrolase, but not for α -arabinosidase, arginine arylamidase, arginine dihydrolase, chymotrypsin, cystine arylamidase, α -fucosidase, 6-phospho- β -galactosidase, glutamyl glutamic acid arylamidase, 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. Weak activity is detected for esterase lipase C8 and esterase C4.

The type strain, YIT 11860^T (=DSM 21032^T=JCM 15079^T), was isolated from human faeces. The DNA G+C content of the type strain is 45.5 mol%.

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