Parasutterella secunda sp. nov., isolated from human faeces and proposal of Sutterellaceae fam. nov. in the order Burkholderiales

Masami Morotomi, Fumiko Nagai and Yohei Watanabe

A novel, strictly anaerobic, non-sporing, Gram-negative coccobacillus bacterium, designated strain YIT 12071 T, was isolated from human faeces. Biochemically, this strain was largely unreactive and asaccharolytic. Growth of this strain in peptone-yeast-extract broth was weak, producing no visible turbidity, and no short-chain fatty acids were detected as an end product of metabolism. Following 16S rRNA gene sequence analysis, strain YIT 12071 T was found to be most closely related to Parasutterella excrementihominis (90 % sequence similarity) and phylogenetically distinct from other known genera belonging to the order Burkholderiales. Biochemical data supported the affiliation of this strain with the genus Parasutterella. Strain YIT 12071 T, therefore, represents a novel species of the genus Parasutterella, for which the name Parasutterella secunda sp. nov. is proposed. The type strain is YIT 12071 T (=DSM 22575 T =JCM 16078 T). On the basis of 16S rRNA gene sequence analysis, species of the genera Sutterella and Parasutterella form a distinct and deep evolutionary lineage of descent in the order Burkholderiales. This lineage could not be associated with any of the four known families of the order Burkholderiales. The distinct phylogenetic position and the unusual combination of chemotaxonomic characteristics shared by these genera, such as the predominant quinones and cellular fatty acid compositions, suggest that they constitute a novel family in the order Burkholderiales, for which the name Sutterellaceae fam. nov. is proposed to accommodate the genera Sutterella and Parasutterella.

Abbreviations: APCI, atmospheric pressure chemical ionization; ECL, equivalent chain-length; FAMEs, fatty acid methyl esters; ML, maximum-likelihood; MP, maximum-parsimony.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIT 12071 T is AB491209.

One supplementary table is available with the online version of this paper.

The human intestinal microbiota is a complex ecosystem containing hundreds of microbial species, a substantial proportion of which have not yet been cultured. Recent molecular ecological studies based on rRNA gene sequences have revealed that members of nine bacterial phyla were found to inhabit the human gastrointestinal tract, two of which, Firmicutes and Bacteroidetes, are dominant (see review Rajilic-Stojanovic et al., 2007). The phylum Proteobacteria is usually secondary in numbers of bacteria present and members of the classes Alpha-, Beta-, Gamma-, Delta- and Epsilonproteobacteria have also been identified by using molecular techniques (Eckburg et al., 2005). Species of Proteobacteria such as Escherichia coli, Klebsiella pneumoniae and Enterobacter cloacae as well as some species of Proteus and Citrobacter have been identified, using conventional culture techniques, as members of the human intestinal microbiota (Finegold et al., 1974; Holdeman et al., 1976; Moore & Holdeman, 1974) and are all species belonging to the class Gammaproteobacteria. The species Sutterella wadsworthensis (Wexler et al., 1996; Engberg et al., 2000), Sutterella parvurubra (Sakon et al., 2008) and Parasutterella excrementihominis (Nagai et al., 2009) are also present but belong to the order Burkholderiales in the class Betaproteobacteria and were isolated during the course of 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, 2009, 2010; Nagai et al., 2009, 2010a, b; Watanabe et al., 2010).

At the time of writing, the order Burkholderiales contains the families Burkholderiaceae, Oxalobacteraceae, Alcaligenaceae and Comamonadaceae (Garrity et al., 2005) and the genera Sutterella and Parasutterella have been placed in the family Alcaligenaceae (Busse & Auling, 2005a; Wexler et al., 1996, 2005; Nagai et al., 2009; http://www.bacterio.cict.fr). In this study, a novel strain was isolated from human faeces, which represents a novel species of the genus Parasutterella. In addition, due to both the distinct phylogenetic positions and
biological and biochemical differences of the genera *Sutterella* and *Parasutterella* from known genera in the family *Alcaligenaceae*, we propose that a novel family, with the name *Sutterellaceae* fam. nov., be instated to accommodate these two genera.

Faecal samples were collected from three healthy Japanese males (subjects H, O and K; aged 56, 38 and 28, respectively) and were immediately transferred to an anaerobic glove box (Coy Laboratory Products), containing 88% nitrogen, 7% hydrogen and 5% carbon dioxide, where each sample was weighed and diluted with pre-reduced 0.1 M PBS (pH 7). Each dilution was then spread on modified Gifu anaerobic medium (GAM; Nissui Pharmaceutical) containing 1.5% (w/v) agar that was supplemented with one of seven antibiotics, at three different concentrations, in an attempt to isolate subdominant groups of the intestinal microbiota. The composition of the modified GAM agar was described previously by Sakon et al. (2008). The inoculated plates were incubated at 37°C for 3 days in an anaerobic glove box. Strain YIT 12071T was isolated from GAM agar plates supplemented with oxacillin (4 μg ml⁻¹; Sigma) and inoculated with a 10⁻⁶ serially diluted faecal sample from subject O. Single colonies were picked and streaked on the modified GAM agar until pure cultures were obtained. *S. wadsworthensis* DSM 14016T and *Sutterella stercoricanis* DSM 17807T, purchased from the DSMZ, Germany, and *P. excrementihominis* YIT 11859T (Nagai et al., 2009) and *Sutterella parvibirua* YIT 11816T (Sakon et al., 2008), isolated previously from human faeces, were used as reference strains.

The end-products of bacterial metabolism in pre-reduced peptone-yeast extract (PY) medium (Holdeman et al., 1977) and PY medium supplemented with 1% glucose (PYG), lactate or succinate were analysed by HPLC as previously described (Chonan et al., 1995). Cell morphology was determined by examining 4-day-old modified GAM agar cultures using phase-contrast light microscopy. Biochemical characteristics were tested in duplicate using the API Rapid ID32A, API ZYM and API 20A systems (bioMérieux), according to the manufacturer’s instructions. Oxidase activity was determined with Oxidase test strips (Eiken Chemical). Catalase activity was determined by the production of bubbles in a 3% hydrogen peroxide solution.

Cellular fatty acid methyl esters (FAMEs) were obtained from cells grown on modified GAM agar by saponification, methylation and extraction using the method of Miller (1988) with minor modifications (Kuykendall et al., 1988). FAMEs were determined by using the MIDI system with MOORE5 of the MIS Standard Libraries. Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987) and were analyzed by using an HPLC-atmospheric pressure chemical ionization (APCI)-MS/MS system (API 3200, Applied Biosystems) with an L-column ODS (2.1 × 150 mm, Chemicals Evaluation and Research.

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Table 1. Differential characteristics of strain YIT 12071T and members of related genera

Institute), and an HPLC-APCI-MS system (Micromass ZQ equipped with 2996 photodiode array detector; Waters) with a Cadenza CD-C18 column (3.0 × 150 mm; Imtakt), following the modified method of Katsuta et al. (2005). 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 GenBank/EMBL/DDBJ by using the FASTA program (Lipman & Pearson, 1985). Sequences were aligned and used to produce an unrooted phylogenetic tree according to 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). Trees were visualized by using the TreeView program (version 1.6.6) (Page, 1996). Maximum-parsimony (MP) and maximum-likelihood (ML) methods were used to confirm the phylogenetic placement of the aligned sequences. MP analysis was performed using the software package MEGA4 (Tamura et al., 2007). The ML tree was constructed via the PHYML program (Guindon & Gascuel, 2003) using Kimura's two-parameter nucleotide substitution model (Kimura, 1980). The input file was prepared via the SEQBOOT program in the PHYLIP software package (Felsenstein, 2004).

Cells of strain YIT 12071T were Gram-reaction-negative, obligately anaerobic, non-motile cocccobacilli, 0.4–1.3 × 0.6–1.7 μm. After 4 days of anaerobic incubation at 37 °C on modified GAM agar, colonies were translucent to beige, circular, convex and pinpoint in size. Growth of strain YIT 12071T in PY broth was weak, producing no visible turbidity, and no short-chain fatty acids were detected as an end product of metabolism. Addition of glucose, lactate or succinate did not enhance growth or the production of short-chain fatty acids. The strain was asaccharolytic in API test systems. Tests for indole production, nitrate reduction, catalase and urease activities and aesculin and gelatin hydrolysis were negative. In the API Rapid ID 32A and API ZYM test systems, strain YIT 12071T was largely unreactive but was positive for arginine dihydrolase, esterase (C4), esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase activities. Alkaline phosphatase activity was positive in the API Rapid ID 32A system and weakly positive in the API ZYM test system. All other test results were negative. Other

Table 2. Fatty acid compositions of strain YIT 12071T and type strains of the genera Parasutterella and Sutterella

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*Summed feature composition is as follows: 1, C14:0 aldehyde; 2, C12:0 3-OH and/or C13:0 DMA; 5, C15:0 DMA and/or C14:0 3-OH; 7, C17:0ω9c and/or unknown fatty acid of ECL 16.760; 10, C18:1ω7c and/or unknown fatty acid of ECL 17.834; 12, iso-C19:0 and/or unknown fatty acid of ECL 18.622.
biochemical characteristics obtained by using the API Rapid ID32A and API ZYM test systems are included in the species description. The biological and biochemical characteristics that differentiate species of the genera Parasutterella and Sutterella from type species of genera in the family Alcaligenaceae are summarized in Table 1.

All strains of the genera Parasutterella and Sutterella tested were oxidase- and catalase-negative and no aerobic growth was observed. The opposite was found for type species of genera in the family Alcaligenaceae (Table 1).

Cellular fatty acid and isoprenoid quinone profiles of strain YIT 12071T and type strains of species of the genera Parasutterella and Sutterella are provided in Table 2 and Supplementary Table S1, respectively. All of these strains contained $C_{18:1 \alpha 9c}$ (32–68 %) and $C_{16:0}$ (9–23 %) as the predominant fatty acids. Some minor qualitative and quantitative differences in fatty acid content could be observed but the overall patterns were very similar among species of these genera. The fatty acid $C_{18:1 \alpha 9c}$, on the other hand, has not been reported to be a major component in species of the family Alcaligenaceae (Table 1). The major respiratory quinone of strain YIT 12071T was methylmenaquinone-5 (MMK-5; 91 %). Menaquinone-5 (MK-5; 9 %) was also detected (Supplementary Table S1). The major respiratory quinone of the other type strains of the genera Parasutterella and Sutterella was also MMK-5 except for P. excrementihominis YIT 11859T, in which MMK-6 was dominant (Supplementary Table S1). The typical fragmentation of a ubiquinone ring nucleus at mole peak $m/z$ 197 was not detected in all these strains, indicating ubiquinones are not present in strains of the genera Parasutterella and Sutterella. Contrary to this, species of the family Alcaligenaceae have, in general, been characterized by the presence of ubiquinone-8 (Q-8) as the major isoprenoid quinone (Table 1; Fletcher et al., 1987; Oyaizu-Masuchi & Komagata, 1988).

A 1485 bp region of the 16S rRNA gene of strain YIT 12071T was sequenced. Database searches revealed a high similarity between strain YIT 12071T and P. excrementihominis (90.0 %). Phylogenetic analyses of these and other related sequences were performed and confirmed that strain YIT 12071T was phylogenetically most closely associated with, but formed a separate cluster from, P. excrementihominis YIT 11859T (Fig. 1). Despite this close association, the 16S rRNA gene sequence of strain YIT 12071T shared highest sequence similarities (98.6–99.4 %) with uncultured intestinal bacteria derived from studies of swine, cows and turkeys [GenBank accession nos: AF371864 (Leser et al., 2002); DQ455891 (Scupham, 2007); EU009773 (Scupham et al., 2008); EU794169 (Patton et al., 2009)] (Fig. 1). In all phylogenetic trees, strain YIT 12071T, together with the recently observed uncultured clone sequences, formed a distinct monophyletic clade (99.9 % bootstrap support) within the order Burkholderiales (Fig. 1). This lineage could not be associated with any of the four known families in the order Burkholderiales. The DNA G+C content of strain YIT 12071T was 48.2 mol%, similar to that of P. excrementihominis (49.8 %).

Based on the phylogenetic, phenotypic and chemotaxonomic evidence, strain YIT 12071T represents a novel species of the genus Parasutterella, for which the name Parasutterella secunda sp. nov. is proposed.

At the time of writing, the order Burkholderiales contains four families, Burkholderiaceae, Oxalobacteraceae, Alcaligenaceae and Comamonadaceae (Garrity et al., 2005). Based on the distinct phylogenetic position of the genera Sutterella and

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**Fig. 1.** Phylogenetic tree showing the relationships between strain YIT 12071T, strains representing genera of the family Alcaligenaceae and related taxa in the order Burkholderiales based on 16S rRNA gene sequence analysis. The tree was rooted with Escherichia coli ATCC 11775T as an outgroup and was constructed by using the neighbour-joining method based on the comparison of sequences of ~1300 nt. Bootstrap values >50 % (based on 1000 replications) are shown at branch points. Similar tree topologies were obtained by using the MP and ML methods (data not shown). Filled circles indicate that the corresponding nodes were also recovered in trees generated with the MP and ML methods. GenBank accession numbers are shown in parentheses. Bar, 0.1 substitutions per nucleotide position.
Parasutterella within the order Burkholderiales and the differences observed in the biological and chemotaxonomic characterization, it is proposed that a novel family, Sutterellaceae fam. nov., should be created to accommodate these genera.

**Description of Sutterellaceae fam. nov.**

Sutterellaceae (Sut.te.rel.la’ceae. N.L. fem. n. Sutterella type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. Sutterellaceae the Sutterella family).

Cells are Gram-reaction-negative rods or coccobacilli and grow under anaerobic conditions or in a microaerophilic atmosphere. Negative for oxidase and catalase activities. The main isoprenoid quinone is MMK-5 or MMK-6. Phylogenetically, the family is a member of the class Betaproteobacteria of the order Burkholderiales. The type genus is Sutterella Wexler et al. 1996.

**Description of Parasutterella secunda sp. nov.**

*Parasutterella secunda* (se.cun’da. L. fem. adj. secunda next to the first, the second, referring to the second species of the genus *Parasutterella* to be described).

Cells are Gram-reaction-negative, non-motile, strictly anaerobic, asaccharolytic, non-spore-forming cocci to coccobacilli, 0.4–1.3 × 0.6–1.7 μm. Colonies are translucent to beige, circular, convex and pinpoint in size after 4 days of growth at 37 °C on modified GAM agar under anaerobic conditions. Negative for nitrate reduction, indole production, catalase, urease and oxidase activities and aesculin and gelatin hydrolysis. In the API test systems, tests are positive for alkaline phosphatase (weakly positive in the API ZYM system), arginine dihydrolase, esterase (C4), esterase lipase (C8) and naphthol-AS-BI phosphohydrolase activities. Negative for N-acetyl-β-glucosaminidase, acid phosphatase, alanine arylamidase, x-arabinosidase, arginine arylamidase, chymotrypsin, cystine arylamidase, x-fucosidase, x- and β-galactosidase, 6-phosphate-β-galactosidase, x- and β-glucosidase, β-glucuronidase, glutamic acid deacetylase, N-acetylglucosaminidase, aminopeptidase A, β-glucosidase and β-galactosidase activities. The major cellular fatty acids are 

The type strain, YIT 12071T (≡DSM 22575T ≡JCM 16078T), was isolated from human faeces. The DNA G+C content of the type strain is 48.2 mol%.

**Acknowledgements**

We are grateful to Dr Jean Euzéby of the École Nationale Vétérinaire in Toulouse for his suggestions regarding the etymology of the species epithet. We thank K. Harada, H. Sakon and K. Manabe for their advice and help with the quinone analysis. We also thank Dr Ryuichiro Tanaka and Dr Haruji Sawada for their understanding and encouragement throughout our research activities.

**References**


