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

Yakult Central Institute for Microbiological Research, 1796 Yaho, Kunitachi, 186-8650 Tokyo, Japan

Correspondence
Masami Morotomi
masami-morotomi@yakult.co.jp

A novel, strictly anaerobic, non-spore-forming, Gram-reaction-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*.

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 Rajilić-Stojanović *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 parvirubra* (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

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.

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 12071^T 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 14016^T and *Sutterella stercoricanis* DSM 17807^T, purchased from the DSMZ, Germany, and *P. excrementihominis* YIT 11859^T (Nagai *et al.*, 2009) and *Sutterella parvirubra* YIT 11816^T (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 described previously (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 (1982) 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

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

Taxa: 1, strain YIT 12071^T; 2, *Parasutterella excrementihominis* YIT 11859^T; 3, *Sutterella parvirubra* YIT 11816^T; 4, *Sutterella wadsworthensis* DSM 14016^T; 5, *Sutterella stercoricanis* DSM 17807^T; 6, *Achromobacter xylosoxidans* (*n*=10; data from Coenye *et al.*, 2003; Busse & Auling, 2005c); 7, *Advenella incenata* (*n*=8; Coenye *et al.*, 2005); 8, *Alcaligenes faecalis* DSM 30033^T (Lipski *et al.*, 1992; Busse & Auling, 2005b); 9, *Bordetella pertussis* (*n*=12; Vancanneyt *et al.*, 1995; von Wintzingerode *et al.*, 2001; Sanden & Weyant, 2005); 10, *Brackiella oedipodis* LMG 19451^T (Willems *et al.*, 2002); 11, *Castellaniella defragrans* DSM 12141^T (Kämpfer *et al.*, 2006); 12, *Derxia gummosa* IAM 13946^T (Xie & Yokota, 2004); 13, *Kerstersia gyiorum* (*n*=6; Coenye *et al.*, 2003); 14, *Oligella urethralis* (*n*=8; Rossau *et al.*, 1987); 15, *Pelistega europaea* (*n*=13; Vandamme *et al.*, 1998); 16, *Pigmentiphaga kullae* K24^T (Blümel *et al.*, 2001); 17, *Pusillimonas noertemannii* BN9^T (Stolz *et al.*, 2005); 18, *Taylorella equigenitalis* LMG 6222^T (Vandamme *et al.*, 1998; Bleumink-Pluym *et al.* 2005); 19, *Tetrathiobacter kashmirensis* WT001^T (Ghosh *et al.*, 2005). +, Positive; −, negative; ND, no data available; SF, summed feature.

Characteristic	*Sutterellaceae*					*Alcaligenaceae*													
	1*	2*	3*	4*	5*	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Aerobic growth	−	−	−	−	−	+	+	+	+	+	−	+	+	+	+	+	+	−	+
Oxidase	−	−	−	−	−	+	+	+	+	+	ND	+	−	+	+	+	+	+	+
Catalase	−	−	−	−	−	+	+	+	ND	+	ND	−	+	+	+	+	ND	+	+
Major fatty acids†	$C_{18:1}\omega9c$, $C_{16:0}$, $C_{14:0}$	$C_{18:1}\omega9c$	$C_{18:1}\omega9c$, $C_{16:0}$ SF10	$C_{18:1}\omega9c$, $C_{16:0}$	$C_{18:1}\omega9c$, $C_{16:0}$, $C_{16:1}\omega7c$	$C_{16:0}$, $C_{17:0}$ cyclo, SF2	$C_{18:1}\omega7c$, SF3, $C_{16:0}$, SF2	$C_{16:0}$, $C_{17:0}$ cyclo, $C_{14:0}$ 3-OH	$C_{16:1}\omega7c$, $C_{16:0}$	$C_{18:1}\omega7c$, $C_{16:0}$, $C_{19:0}$ cyclo $\omega8c$	$C_{16:0}$, SF7	$C_{18:1}\omega7c$, $C_{16:1}\omega7c$, $C_{16:0}$	$C_{16:0}$, $C_{17:0}$ cyclo, SF2, $C_{18:1}\omega7c$	$C_{18:1}\omega7c$, $C_{16:0}$	SF7, SF2‡, $C_{16:1}\omega7c$, $C_{16:0}$	$C_{16:0}$, $C_{17:0}$ cyclo, $C_{19:0}$ cyclo $\omega8c$	$C_{17:0}$ cyclo, $C_{19:1}$ cyclo $\omega8c$, $C_{16:0}$	SF7, SF2‡, $C_{16:0}$	$C_{18:1}\omega7c$, SF3, $C_{16:0}$, SF2
Major quinone	MK-5, MMK-5	MK-6, MMK-6	MMK-5	MK-5, MMK-5	MK-5, MMK-5	Q-8	ND	Q-8	Q-8	ND	Q-8	Q-8	ND	ND	ND	Q-8	Q-8	ND	ND

*Data from this study.

† Fatty acids constituting >10 % of the total are shown in descending order. Summed feature 2 comprises $C_{14:0}$ 3-OH, iso-$C_{16:1}$ I, unknown fatty acid of ECL 10.928 and/or $C_{12:0}$ aldehyde. Summed feature 3 comprises $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH. Summed feature 7 comprises $C_{17:1}\omega9c$ and/or unknown fatty acid of ECL 16.760. Summed feature 10 comprises $C_{18:1}\omega7c$ and/or unknown fatty acid of ECL 17.834. Summed feature 12 comprises iso-$C_{19:0}$ and/or unknown fatty acid of ECL 18.622.

‡Referred to as SF3 in Vandamme *et al.* (1998).

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 12071^T were Gram-reaction-negative, obligately anaerobic, non-motile coccobacilli, 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 12071^T 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 12071^T 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 12071^T and type strains of the genera *Parasutterella* and *Sutterella*

Taxa: 1, strain YIT 12071^T; 2, *Parasutterella excrementihominis* YIT 11859^T; 3, *Sutterella parvirubra* YIT 11816^T; 4, *Sutterella wadsworthensis* DSM 14016^T; 5, *Sutterella stercoricanis* DSM 17807^T. Values are percentages of total fatty acids; only those fatty acids that make up >1 % of the total are shown. Data were obtained in this study.

Fatty acid	1	2	3	4	5
Saturated straight-chain					
$C_{12:0}$	2.82				
$C_{14:0}$	10.78	5.96	4.25	8.54	2.25
$C_{15:0}$			1.36	1.01	1.10
$C_{16:0}$	14.09	8.66	21.13	15.68	23.19
$C_{18:0}$	2.82	1.46	3.25	1.04	1.38
Unsaturated straight-chain					
$C_{16:1}\omega7c$	1.29		6.13	1.69	23.10
$C_{18:2}\omega6,9c$			1.27		
$C_{18:1}\omega9c$	39.95	68.10	43.36	56.37	31.77
Hydroxy acids					
$C_{16:0}$ 3-OH		6.26			
Dimethyl acetal (DMA)					
$C_{16:0}$	1.63				
$C_{18:1}\omega9c$	6.95				
Summed features*					
1		2.37			
2	4.17		2.43	2.07	2.81
5	3.68		2.19	3.79	1.86
7	1.05				
10	6.60	4.43	12.98	5.06	7.78
12	2.57			1.11	

*Summed feature composition is as follows: 1, $C_{13:1}\omega12c$ and/or $C_{14:0}$ aldehyde; 2, $C_{12:0}$ 3-OH and/or $C_{13:0}$ DMA; 5, $C_{15:0}$ DMA and/or $C_{14:0}$ 3-OH; 7, $C_{17:1}\omega9c$ and/or unknown fatty acid of ECL 16.760; 10, $C_{18:1}\omega7c$ and/or unknown fatty acid of ECL 17.834; 12, iso-$C_{19:0}$ 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 12071^T 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 $C_{18:1}\omega9c$ (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 $C_{18:1}\omega9c$, 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 12071^T 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 11589^T, 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 12071^T was sequenced. Database searches revealed a high similarity between strain YIT 12071^T and *P. excrementihominis* (90.0 %). Phylogenetic analyses of these and other related sequences were performed and confirmed that strain YIT 12071^T was phylogenetically most closely associated with, but formed a separate cluster from, *P. excrementihominis* YIT 11859^T (Fig. 1). Despite this close association, the 16S rRNA gene sequence of strain YIT 12071^T 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 12071^T, 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 12071^T was 48.2 mol%, similar to that of *P. excrementihominis* (49.8 %).

Based on the phylogenetic, phenotypic and chemotaxonomic evidence, strain YIT 12071^T 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

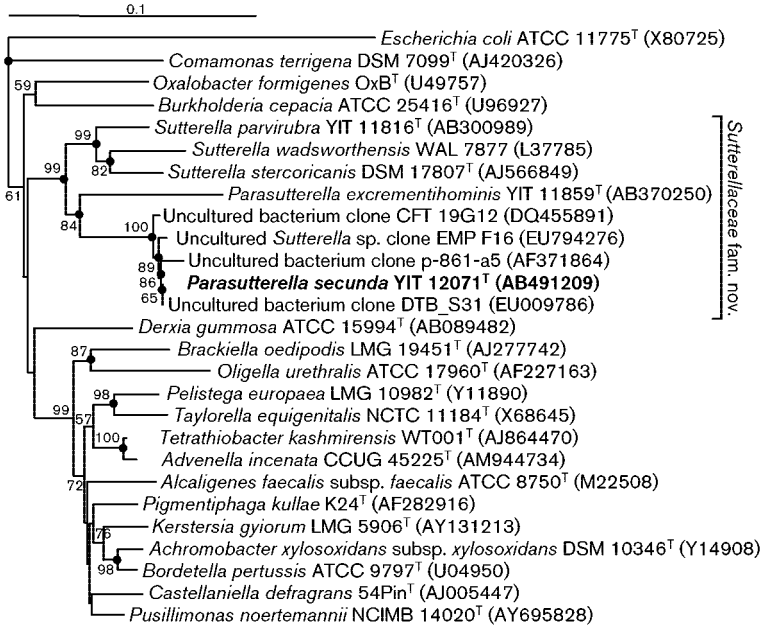

Fig. 1. Phylogenetic tree showing the relationships between strain YIT 12071^T, strains representing genera of the family *Alcaligenaceae* and related taxa in the order *Burkholderiales* based on 16S rRNA gene sequence analysis. The tree was rooted with *Escherichia coli* ATCC 11775^T as an outgroup 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 given 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′ce.ae. 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, α-arabinosidase, arginine arylamidase, chymotrypsin, cystine arylamidase, α-fucosidase, α- and β-galactosidase, 6-phosphate-β-galactosidase, α- and β-glucosidase, β-glucuronidase, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, glycine arylamidase, histidine arylamidase, leucine arylamidase, leucyl glycine arylamidase, lipase (C14), α-mannosidase, phenylalanine arylamidase, proline arylamidase, pyroglutamic acid arylamidase, serine arylamidase, trypsin, tyrosine arylamidase and valine arylamidase activities. The major cellular fatty acids are $C_{18:1}\omega9c$, $C_{16:0}$ and $C_{14:0}$. The major respiratory quinone is MMK-5 (H_0).

The type strain, YIT 12071^T (=DSM 22575^T =JCM 16078^T), 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

Bleumink-Pluym, N. M. C. & van der Zeijst, B. A. M. (2005). Genus IX. *Taylorella* Sugimoto, Isayama, Sakazaki and Kuramochi 1984, 503^{VP} (Effective publication: Sugimoto, Isayama, Sakazaki and Kuramochi 1983, 155). In *Bergy's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, The Proteobacteria, part C, The Alpha-, Beta-, Delta- and Epsilonproteobacteria, pp. 684–685. Edited by D. J. Brenner, N. P. Kreig, J. T. Staley & G. M. Garrity. New York: Springer.

Blümel, S., Mark, B., Busse, H.-J., Kämpfer, P. & Stolz, A. (2001). *Pigmentiphaga kullae* gen. nov., sp. nov., a novel member of the family *Alcaligenaceae* with the ability to decolorize azo dyes aerobically. *Int J Syst Evol Microbiol* **51**, 1867–1871.

Busse, H. & Auling, G. (2005a). Family *Alcaligenaceae* De Ley, Segers, Kersters, Mannheim and Lievens 1986, 412^{VP}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, The Proteobacteria, part C, The Alpha-, Beta-, Delta- and Epsilonproteobacteria, pp. 647–653. Edited by D. J. Brenner, N. P. Kreig, J. T. Staley & G. M. Garrity. New York: Springer.

Busse, H. J. & Auling, G. (2005b). Genus *Alcaligenes* Castellani and Chalmers 1919, 936^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, The Proteobacteria, part C, The Alpha-, Beta-, Delta- and Epsilonproteobacteria, pp. 653–658. Edited by D. J. Brenner, N. P. Kreig, J. T. Staley & G. M. Garrity. New York: Springer.

Busse, H. & Auling, G. (2005c). Genus *Achromobacter* Yabuuchi and Yano 1981, 477^{VP}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, The Proteobacteria, part C, The Alpha-, Beta-, Delta-, and Epsilonproteobacteria, pp. 658–662. Edited by D. J. Brenner, N. P. Kreig, J. T. Staley & G. M. Garrity. New York: Springer.

Chonan, O., Matsumoto, K. & Watanuki, M. (1995). Effect of galactooligosaccharides on calcium absorption and preventing bone loss in ovariectomized rats. *Biosci Biotechnol Biochem* **59**, 236–239.

Coenye, T., Vancanneyt, M., Cnockaert, M. C., Falsen, E., Swings, J. & Vandamme, P. (2003). *Kerstersia gyiorum* gen. nov., sp. nov., a novel *Alcaligenes faecalis*-like organism isolated from human clinical samples, and reclassification of *Alcaligenes denitrificans* Rüger and Tan 1983 as *Achromobacter denitrificans* comb. nov. *Int J Syst Evol Microbiol* **53**, 1825–1831.

Coenye, T., Vanlaere, E., Samyn, E., Falsen, E., Larsson, P. & Vandamme, P. (2005). *Advenella incenata* gen. nov., sp. nov., a novel member of the *Alcaligenaceae*, isolated from various clinical samples. *Int J Syst Evol Microbiol* **55**, 251–256.

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.

Engberg, J., On, S. L., Harrington, C. S. & Gerner-Smidt, P. (2000). Prevalence of *Campylobacter*, *Arcobacter*, *Helicobacter*, and *Sutterella* spp. in human fecal samples as estimated by a reevaluation of isolation methods for Campylobacters. *J Clin Microbiol* **38**, 286–291.

Ezaki, T., Saidi, S. M., Liu, S. L., Hashimoto, Y., Yamamoto, H. & Yabuuchi, E. (1990). Rapid procedure to determine the DNA base composition from small amounts of gram-positive bacteria. *FEMS Microbiol Lett* **67**, 127–130.

Felsenstein, J. (2004). PHYLIP (phylogeny inference package), version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.

Finegold, S. M., Attebery, H. R. & Sutter, V. L. (1974). Effect of diet on human fecal flora: comparison of Japanese and American diets. *Am J Clin Nutr* **27**, 1456–1469.

Fletcher, M. T., Blackall, P. J. & Doheny, C. M. (1987). A note on the isoprenoid quinone content of *Bordetella avium* and related species. *J Appl Bacteriol* **62**, 275–277.

Garrity, G. M., Bell, J. A. & Lilburn, T. (2005). Order I. *Burkholderiales* ord. nov. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, The Proteobacteria, part C, The Alpha-, Beta-, Delta- and Epsilonproteobacteria, p. 575. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Ghosh, W., Bagchi, A., Mandal, S., Dam, B. & Roy, P. (2005). *Tetrathiobacter kashmirensis* gen. nov., sp. nov., a novel mesophilic, neutrophilic, tetrathionate-oxidizing, facultatively chemolithotrophic betaproteobacterium isolated from soil from a temperate orchard in Jammu and Kashmir, India. *Int J Syst Evol Microbiol* **55**, 1779–1787.

Guindon, S. & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* **52**, 696–704.

Holdeman, L. V., Good, I. J. & Moore, W. E. (1976). Human fecal flora: variation in bacterial composition within individuals and a possible effect of emotional stress. *Appl Environ Microbiol* **31**, 359–375.

Holdeman, L. V., Cato, E. P. & Moore, W. E. C. (1977). *Anaerobe Laboratory Manual*, 4th edn. Blacksburg, VA: Virginia Polytechnic Institute and State University.

Kämpfer, P., Denger, K., Cook, A. M., Lee, S.-T., Jäckel, U., Denner, E. B. M. & Busse, H.-J. (2006). *Castellaniella* gen. nov., to accommodate the phylogenetic lineage of *Alcaligenes defragrans*, and proposal of *Castellaniella defragrans* gen. nov., comb. nov. and *Castellaniella denitrificans* sp. nov. *Int J Syst Evol Microbiol* **56**, 815–819.

Katsuta, A., Adachi, K., Matsuda, S., Shizuri, Y. & Kasai, H. (2005). *Ferrimonas marina* sp. nov. *Int J Syst Evol Microbiol* **55**, 1851–1855.

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.

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

Kuykendall, L. D., Roy, M. A., O'Neill, J. J. & Devine, T. E. (1988). Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* **38**, 358–361.

Leser, T. D., Amenuvor, J. Z., Jensen, T. K., Lindecrona, R. H., Boye, M. & Møller, K. (2002). Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. *Appl Environ Microbiol* **68**, 673–690.

Lipman, D. J. & Pearson, W. R. (1985). Rapid and sensitive protein similarity searches. *Science* **227**, 1435–1441.

Lipski, A., Klatte, S., Bendinger, B. & Altendorf, K. (1992). Differentiation of Gram-negative, nonfermentative bacteria isolated from biofilters on the basis of fatty acid composition, quinone system, and physiological reaction profiles. *Appl Environ Microbiol* **58**, 2053–2065.

Miller, L. T. (1982). Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* **16**, 584–586.

Moore, W. E. & Holdeman, L. V. (1974). Human fecal flora: the normal flora of 20 Japanese-Hawaiians. *Appl Microbiol* **27**, 961–979.

Morotomi, M., Nagai, F., Sakon, H. & Tanaka, R. (2008). *Dialister succinatiphilus* sp. nov. and *Barnesiella intestinihominis* sp. nov., isolated from human faeces. *Int J Syst Evol Microbiol* **58**, 2716–2720.

Morotomi, M., Nagai, F., Sakon, H. & Tanaka, R. (2009). *Paraprevotella clara* gen. nov., sp. nov. and *Paraprevotella xylaniphila* sp. nov., members of the family '*Prevotellaceae*' isolated from human faeces. *Int J Syst Evol Microbiol* **59**, 1895–1900.

Morotomi, M., Nagai, F., Watanabe, Y. & Tanaka, R. (2010). *Succinatimonas hippei* gen. nov., sp. nov., isolated from human faeces. *Int J Syst Evol Microbiol* **60**, 1788–1793.

Nagai, F., Morotomi, M., Sakon, H. & Tanaka, R. (2009). *Parasutterella excrementihominis* gen. nov., sp. nov., a member of the family *Alcaligenaceae* isolated from human faeces. *Int J Syst Evol Microbiol* **59**, 1793–1797.

Nagai, F., Morotomi, M., Watanabe, Y., Sakon, H. & Tanaka, R. (2010a). *Alistipes indistinctus* sp. nov. and *Odoribacter laneus* sp. nov., common members of the human intestinal microbiota isolated from faeces. *Int J Syst Evol Microbiol* **60**, 1296–1302.

Nagai, F., Watanabe, Y. & Morotomi, M. (2010b). *Slackia piriformis* sp. nov. and *Collinsella tanakaei* sp. nov., new members of the family *Coriobacteriaceae*, isolated from human faeces. *Int J Syst Evol Microbiol* **60**, 2639–2646.

Oyaizu-Masuchi, Y. & Komagata, K. (1988). Isolation of free-living nitrogen-fixing bacteria from the rhizosphere of rice. *J Gen Appl Microbiol* **34**, 127–164.

Page, R. D. M. (1996). TREEVIEW: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* **12**, 357–358.

Patton, T. G., Scupham, A. J., Bearson, S. M. & Carlson, S. A. (2009). Characterization of fecal microbiota from a *Salmonella* endemic cattle herd as determined by oligonucleotide fingerprinting of rDNA genes. *Vet Microbiol* **136**, 285–292.

Rajilić-Stojanović, M., Smidt, H. & de Vos, W. M. (2007). Diversity of the human gastrointestinal tract microbiota revisited. *Environ Microbiol* **9**, 2125–2136.

Rossau, R., Kersters, K., Falsen, E., Jantzen, E., Segers, P., Union, A., Nehls, L. & De Ley, J. (1987). *Oligella*, a new genus including *Oligella urethralis* comb. nov. (formerly *Moraxella urethralis*), and *Oligella ureolytica* sp. nov. (formerly CDC group IVe): relationship to *Taylorella equigenitalis* and related taxa. *Int J Syst Bacteriol* **37**, 198–210.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sakon, H., Nagai, F., Morotomi, M. & Tanaka, R. (2008). *Sutterella parvirubra* sp. nov. and *Megamonas funiformis* sp. nov., isolated from human faeces. *Int J Syst Evol Microbiol* **58**, 970–975.

Sanden, G. N. & Weyant, R. S. (2005). Genus *Bordetella* Moreno-López 1952, 178[AL]. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, The Proteobacteria, part C, The Alpha-, Beta-, Delta- and Epsilonproteobacteria, pp. 662–671. Edited by D. J. Brenner, N. P. Kreig, J. T. Staley & G. M. Garrity. New York: Springer.

Scupham, A. J. (2007). Succession in the intestinal microbiota of preadolescent turkeys. *FEMS Microbiol Ecol* **60**, 136–147.

Scupham, A. J., Patton, T. G., Bent, E. & Bayles, D. O. (2008). Comparison of the cecal microbiota of domestic and wild turkeys. *Microb Ecol* **56**, 322–331.

Stolz, A., Bürger, S., Kuhm, A., Kämpfer, P. & Busse, H.-J. (2005). *Pusillimonas noertemannii* gen. nov., sp. nov., a new member of the family *Alcaligenaceae* that degrades substituted salicylates. *Int J Syst Evol Microbiol* **55**, 1077–1081.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetic analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface:

flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Vancanneyt, M., Vandamme, P. & Kersters, K. (1995). Differentiation of *Bordetella pertussis*, *B. parapertussis*, and *B. bronchiseptica* by whole-cell protein electrophoresis and fatty acid analysis. *Int J Syst Bacteriol* **45**, 843–847.

Vandamme, P., Segers, P., Ryll, M., Hommez, J., Vancanneyt, M., Coopman, R., De Baere, R., Van de Peer, Y., Kersters, K. & other authors (1998). *Pelistega europaea* gen. nov., sp. nov., a bacterium associated with respiratory disease in pigeons: taxonomic structure and phylogenetic allocation. *Int J Syst Bacteriol* **48**, 431–440.

von Wintzingerode, F., Schattke, A., Siddiqui, R. A., Rösick, U., Göbel, U. B. & Gross, R. (2001). *Bordetella petrii* sp. nov., isolated from an anaerobic bioreactor, and emended description of the genus *Bordetella*. *Int J Syst Evol Microbiol* **51**, 1257–1265.

Watanabe, Y., Nagai, F., Morotomi, M., Sakon, H. & Tanaka, R. (2010). *Bacteroides clarus* sp. nov., *Bacteroides fluxus* sp. nov. and *Bacteroides oleiciplenus* sp. nov., isolated from human faeces. *Int J Syst Evol Microbiol* **60**, 1864–1869.

Wexler, H. M. (2005). Genus VIII. *Sutterella* Wexler, Reeves, Summanen, Molitoris, McTeague, Duncan, Wilson and Finegold 1996a 257^{VP}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, The Proteobacteria, part C, The Alpha-, Beta-, Delta- and Epsilonproteobacteria, 2nd edn, vol. 2, pp. 682–684. Edited by D. J. Brenner, N. R. Kreig, J. T. Staley & G. M. Garrity. New York: Springer.

Wexler, H. M., Reeves, D., Summanen, P. H., Molitoris, E., McTeague, M., Duncan, J., Wilson, K. H. & Finegold, S. M. (1996). *Sutterella wadsworthensis* gen. nov., sp. nov., bile-resistant microaerophilic *Campylobacter gracilis*-like clinical isolates. *Int J Syst Bacteriol* **46**, 252–258.

Willems, A., Gilhaus, H., Beer, W., Mietke, H., Gelderblom, H. R., Burghardt, B., Voigt, W. & Reissbrodt, R. (2002). *Brackiella oedipodis* gen. nov., sp. nov., Gram-negative, oxidase-positive rods that cause endocarditis of cotton-topped tamarin (*Saguinus oedipus*). *Int J Syst Evol Microbiol* **52**, 179–186.

Xie, C. H. & Yokota, A. (2004). Phylogenetic analyses of the nitrogen-fixing genus *Derxia*. *J Gen Appl Microbiol* **50**, 129–135.