38 The Family *Sutterellaceae*

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Abstract

Sutterellaceae, a family within the order *Burkholderiales* in the lineage of phylum Proteobacteria (class *Betaproteobacteria*), includes the genera *Sutterella* and *Parasutterella*. The genus *Sutterella* contains three validly described species: *Sutterella wadsworthensis* (the type species), *Sutterella parvirubra*, and *Sutterella stercoricanis*. In contrast, the genus *Parasutterella* comprises the species *Parasutterella excrementihominis* (the type species) and *Parasutterella secunda*. Members of the family are mainly found in the intestinal tract of humans and some animals as members of the indigenous intestinal microbiota, and can be isolated from both the intestinal tract and from infections of gastrointestinal origin (*S. wadsworthensis*). The cells are Gram-negative rods or coccobacilli, and grow under anaerobic conditions or in a microaerophilic atmosphere. They are asaccharolytic and negative for oxidase and catalase activities. The main isoprenoid quinone is methylmenaquinone-5 (MMK-5) or MMK-6. The type genus is *Sutterella* (Wexler et al. Int J Syst Bacteriol 46:252–258, 1996).

Taxonomy, Historical and Current

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).

The genus *Sutterella* was first described by Wexler et al. (1996) and was placed within the family *Alcaligenaceae* in the second edition of *Bergey's Manual of Systematic Bacteriology* (Wexler 2005). The genus *Parasutterella* was described by Nagai et al. (2009) as the closest neighbor of genus *Sutterella*. Morotomi et al. (2011) proposed the new family *Sutterellaceae* to accommodate these two genera after finding that the genera *Sutterella* and *Parasutterella* formed a separate line of descent within the order *Burkholderiales*. In their research, this lineage could not be associated with any of the four known families (*Alcaligenaceae*, *Burkholderiaceae*, *Comamonadaceae*, and *Oxalobacteraceae*) in the order *Burkholderiales* (◗ *Fig. 38.1*). The rationale for the new family was based both on the distinct phylogenetic positions and on the biological and biochemical differences of the genera *Sutterella* and *Parasutterella* from known genera in the family *Alcaligenaceae*, which is phylogenetically the nearest neighboring family of the *Sutterellaceae*. All known members of the genera *Sutterella* and *Parasutterella* are oxidase- and catalase negative, and no aerobic growth has been observed, although some species may grow in a microaerophilic atmosphere. In contrast, species in the *Alcaligenaceae* are oxidase- and catalase positive, and can generally grow aerobically (◗ *Table 38.1*). All type strains of the *Sutterellaceae* contain $C_{18:1}\omega9c$ (32–68 % of the total) and $C_{16:0}$ (9–23 %) as the predominant fatty acids (◗ *Tables 38.1* and ◗ *38.2*). However, $C_{18:1}\omega9c$ has not been reported to be a major component in any species of the family *Alcaligenaceae* (◗ *Table 38.1*). The major respiratory quinone of the members of the *Sutterellaceae* is methylmenaquinone-5 (MMK-5) or MMK-6, whereas ubiqinones have not been detected (◗ *Tables 38.1* and ◗ *38.3*). In contrast, species of the family *Alcaligenaceae* have, in general, been characterized by the presence of ubiquinone-8 (Q-8) as the major isoprenoid quinone (◗ *Table 38.1*; Fletcher et al. 1987; Oyaizu-Masuchi and Komagata 1988).

Molecular Analyses

Phylogeny

On the basis of 16S rRNA gene sequence similarities, members of the family *Sutterellaceae* are members of the order *Burkholderiales*, with the nearest neighboring family being the *Alcaligenaceae* (◗ *Fig. 38.1*). The phylogenetic distance between type species of the family is relatively high. The 16S rRNA gene sequence of the type strain of *Sutterella wadsworthensis* shares 94.6 % similarities with the sequences of *Sutterella parvirubra* and *Sutterella stercoricanis*. The type strains of *S. parvirubra* and

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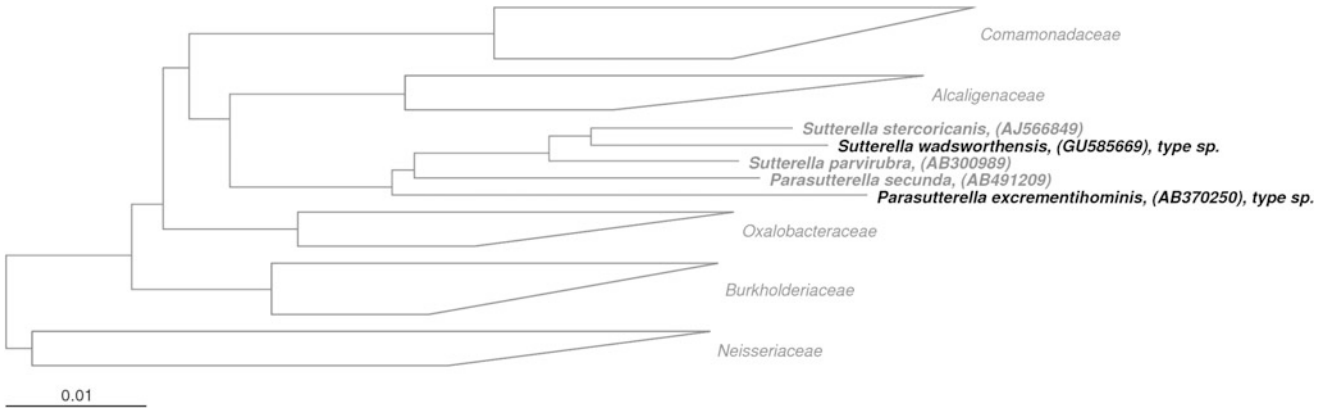

Fig. 38.1

Phylogenetic reconstruction of the family *Sutterellaceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al., 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type-strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40% maximum-frequency filter was applied in order to remove hyper variable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence. This tree was provided by Dr. Raul Muñoz of the Instituto Mediterráneo de Estudios Avanzados

S. stercoricanis share 94.7 % similarity. The type strains of the two *Parasutterella* species, *Parasutterella excrementihominis* and *Parasutterella secunda*, share 90.0 % similarity. In contrast, similarities between members of the *Sutterellaceae* and type species of the neighboring family *Alcaligenaceae* range between 88.3 % and 90.9 %. Thus, all species described so far can be unambiguously identified by their 16S rRNA gene sequence (Morotomi et al. 2011).

Genome

Sutterella parvirubra YIT 11816^T, *P. excrementihominis* YIT 11859^T, and four strains of *S. wadsworthensis* (HGA0223, HGP1, 3_1_45B, and 2_1_59BFAA) were selected for inclusion in the catalog of reference genomes by the Human Microbiome Project (http://commonfund.nih.gov/hmp/) and, at the time of writing, the assembled and annotated genomic sequences of three strains (*S. parvirubra* YIT 11816^T, *P. excrementihominis* YIT 11859^T, and *S. wadsworthensis* 3_1_45B) have been submitted to the GenBank/EMBL/DDBJ databases. The genome size, G+C content, number of predicted protein-encoding genes, and number of predicted rRNA and tRNA genes of these strains are listed in *Table 38.4*. Details are available from the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/).

DNA–DNA Hybridization

Sutterella wadsworthensis WAL 9799^T did not undergo DNA–DNA hybridization with DNA from either *Campylobacter gracilis* or other *Campylobacter* species (*Campylobacter rectus*, *Campylobacter curvus*, *Campylobacter consisus*, *Campylobacter sputorum*, and *Campylobacter showae*), which cannot be differentiated phenotypically (Wexler et al. 1996). The degree of the hybridization between *S. wadsworthensis* CCUG 42229^T (= WAL 9799^T) and *S. stercoricanis* 5BAC4^T was 35 %, which is low enough to be consistent with separate species status (Greetham et al. 2004). No hybridization studies have been done for the *Parasutterella* species, as the 16S rRNA gene sequence similarity between the type strains of the respective species was only 90.0 % that indicates separate genospecies (Morotomi et al. 2011).

Matrix-associated laser desorption/ionization—time of flight mass spectrometry (MALDI-TOF MS).

Eleven strains of *S. wadsworthensis*, including the type strain, were analyzed by MALDI-TOF MS, and the results included a dominant peak at approximately 9,400 Da that was common to all *S. wadsworthensis* strains (Mukhopadhya et al. 2011). No mass spectrometry data is available for any other species in the *Sutterellaceae*.

Neither riboprinting nor ribotyping analyses are available for any member of the *Sutterellaceae*.

Phenotypic Analyses

Members of the *Sutterellaceae* are strictly anaerobic or microaerophilic, non-motile, non-spore-forming, Gram-negative rods or coccobacilli. Biochemically, they are largely unreactive and asaccharolytic.

Differential characteristics of members of the family *Sutterellaceae* and those of the phylogenetically nearest family, the *Alcaligenaceae*, are listed in *Table 38.1*. All type strains of the genera *Sutterella* and *Parasutterella* cannot grow under aerobic conditions, and are oxidase- and catalase-negative

Table 38.1

Differential characteristics of members of the family *Sutterellaceae* and those of the phylogenetically nearest (Fig. 38.1) family, the *Alcaligenaceae*

		Aerobic growth	Oxidase	Catalase	Major fatty acids[a]	Major quinone	Reference
Sutterellaceae	1	–	–	–	$C_{18:1}\omega9c$, $C_{16:0}$, SF10	MMK-5	Morotomi et al. (2011)
	2	–	–	–	$C_{18:1}\omega9c$, $C_{16:0}$, $C_{16:1}\omega7c$	MK-5, MMK-5	Morotomi et al. (2011)
	3	–	–	–	$C_{18:1}\omega9c$, $C_{16:0}$	MK-5, MMK-5	Morotomi et al. (2011)
	4	–	–	–	$C_{18:1}\omega9c$	MK-6, MMK-6	Morotomi et al. (2011)
	5	–	–	–	$C_{18:1}\omega9c$, $C_{16:0}$, $C_{14:0}$	MK-5, MMK-5	Morotomi et al. (2011)
Alcaligenaceae	6	+	+	+	$C_{16:0}$, $C_{17:0}$cyclo, SF2	Q-8	Coenye et al. (2003), Busse and Aulling (2004b)
	7	+	+	+	$C_{18:1}\omega7C$, SF3, C16:0, SF2	ND	Coenye et al. (2005)
	8	+	+	+	$C_{16:0}$, $C_{17:0}$cyclo, $C_{14:0}$-3OH	Q-8	Lipski et al. (1992), Busse and Aulling (2004a)
	9	+	+	ND	$C_{16:1}\omega7C$, $C_{16:0}$	Q-8	Vancanneyt et al. (1995), von Wintzingerode et al. (2001), Sanden and Weyant (2004)
	10	+	+	+	$C_{18:1}\omega7C$, $C_{16:0}$, $C_{19:0}$cyclo$\omega8C$	ND	Willems et al. (2002)
	11	–	ND	ND	$C_{16:0}$, $C_{16:1}\omega7C$, SF7	Q-8	Kämpfer et al. (2006)
	12	+	+	–	$C_{18:1}\omega7C$, $C_{16:1}\omega7C$, $C_{16:0}$	Q-8	Xie and Yokota (2004)
	13	+	–	+	$C_{16:0}$, $C_{17:0}$cyclo, SF2, $C_{18:1}\omega7C$	ND	Coenye et al. (2003)
	14	+	+	+	$C_{18:1}\omega7C$, $C_{16:0}$	ND	Rossau et al. (1987)
	15	+	+	+	SF7, $C_{16:1}\omega7C$, $C_{16:0}$, SF2[b]	ND	Vandamme et al. (1998)
	16	+	+	+	$C_{16:0}$, $C_{17:0}$cyclo, $C_{19:0}$cyclo$\omega8C$	Q-8	Blümel et al. (2001)
	17	+	+	ND	$C_{17:0}$cyclo, $C_{19:1}$cyclo$\omega8C$, C16:0	Q-8	Stolz et al. (2005)
	18	–	+	+	SF7, $C_{16:0}$, SF2[b]	ND	Vandamme et al. (1998), Bleumink-Pluym and van der Zeijst (2005)
	19	+	+	+	$C_{18:1}\omega7C$, SF3, $C_{16:0}$, SF2	ND	Ghosh et al. (2005)

Taxa: 1 *Sutterella parvirubra* YIT 11816^T, 2 *Sutterella stercoricanis* DSM 17807^T, 3 *Sutterella wadsworthensis* DSM 14016^T, 4 *Parasutterella excrementihominis* YIT 11859^T, 5 *Parasutterella secunda* YIT 12071^T, 6 *Achromobacter xylosoxidans* (*n* = 10 strains), 7 *Advenella incenata* (*n* = 8), 8 *Alcaligenes faecalis* DSM 30033^T, 9 *Bordetella pertussis* (*n* = 12), 10 *Brackiella oedipodis* LMG 19451^T, 11 *Castellaniella defragrans* DSM 12141^T, 12 *Derxia gummosa* IAM 13946^T (=ATCC 15594^T), 13 *Kerstersia gyiorum* (*n* = 6), 14 *Oligella urethralis* (*n* = 8), 15 *Pelistega europaea* (*n* = 13), 16 *Pigmentiphaga kullae* K24^T, 17 *Pusillimonas noertemannii* BN9^T, 18 *Taylorella equigenitalis* LMG 6222^T, 19 *Tetrathiobacter kashmirensis* WT001^T

Symbols: + positive, – negative, *ND* no data available, *Q* ubiquinone

[a]Includes fatty acids that account for >10 % of the total, listed in descending order. Summed feature 2 (SF2) comprises 14:0 3-OH, 16:1 iso I, an unidentified fatty acid with an equivalent chain length (ECL) of 10.928 or 12:0 aldehyde. SF3 comprises 16:1$\omega7c$ or 15:0 iso 2-OH. SF7 comprises $C_{17:1}\omega9c$ or an unknown fatty acid of ECL 16.760. SF10 comprises $C_{18:1}\omega7c$ or an unknown fatty acid of ECL 17.834. SF12 contains iso-$C_{19:0}$ or an unknown fatty acid of ECL 18.622.

[b]Referred to as SF3 by Vandamme et al. (1998)

(Table 38.1). In addition, they are negative for urease and indole production and for hydrolysis of gelatin and aesculin (Greetham et al. 2004; Sakon et al. 2008; Nagai et al. 2009; Morotomi et al. 2011 and unpublished). In contrast, species of the family *Alcaligenaceae* have, in general, the opposite characteristics (Table 38.1). All type strains of the *Sutterellaceae* contain $C_{18:1}\omega9c$ (32–68 %) and $C_{16:0}$ (9–23 %) as the predominant fatty acids, and MMK-5 or MMK-6 as the major respiratory quinone (Tables 38.1 and 38.2). The typical fragmentation of a ubiquinone ring nucleus at mole peak of $m/z = 197$ was not detected in these strains, indicating that ubiquinones are not present in the known strains of the genera *Sutterella* and *Parasutterella* (Morotomi et al. 2011). In contrast, species of the family *Alcaligenaceae* are characterized by the presence of ubiquinone-8 (Q-8) as the major isoprenoid quinone, and fatty acids other than $C_{18:1}\omega9c$ are the major cellular fatty acids (Table 38.1).

All type strains of the genera *Sutterella* and *Parasutterella* do not utilize the following API 20A substrates: L-arabinose, D-cellobiose, glucose, glycerol, lactose, maltose, D-mannitol, D-mannose, D-melezitose, D-raffinose, L-rhamnose, salicin, D-sorbitol, sucrose, D-trehalose, and D-xylose (Greetham et al. 2004;

Sakon et al. 2008; Nagai et al. 2009; Morotomi et al. 2011 and unpublished). They are negative for the following API ZYM and API rapid ID 32 A reactions: N-acetyl-β-glucosaminidase, α-arabinosidase, chymotrypsin, cystine arylamidase, fermentation of mannose, fermentation of raffinose, α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, glutamyl glutamic acid arylamidase, histidine arylamidase, indole production, lipase (C14), α-mannosidase, 6-phosphate β-galactosidase, proline arylamidase, pyroglutamic acid arylamidase, trypsin, urease, and valine arylamidase (Greetham et al. 2004; Sakon et al. 2008; Nagai et al. 2009; Morotomi et al. 2011 and unpublished). Diagnostic phenotypic differences for the fatty acid compositions of the type strains of the genera *Sutterella* and *Parasutterella* are listed in *Table 38.2*.

The fatty acid and isoprenoid quinone compositions of the type strains of the genera *Sutterella* and *Parasutterella* are listed in *Tables 38.2* and *38.3*, respectively. *Table 38.5* provides additional phenotypic details.

Table 38.2
Fatty acid compositions of the type strains of the genera *Sutterella* and *Parasutterella*

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

Taxa: 1 *Sutterella parvirubra* YIT 11816^T, 2 *Sutterella stercoricanis* DSM 17807^T, 3 *Sutterella wadsworthensis* DSM 14016^T, 4 *Parasutterella excrementihominis* YIT 11859^T, 5 *Parasutterella secunda* YIT 12071^T. Values are percentages of total fatty acids; only those fatty acids that make up more than 1 % of the total are shown. Data are from Morotomi et al. (2011)

[a]Data for *Sutterella stercoricanis* DSM 17807^T are slightly different from those of Greetham et al. (2004); the difference may have resulted from the different culture conditions

[b]Summed feature 1 (SF1) contained $C_{13:1}\omega12c$ or $C_{14:0}$ aldehyde. SF2 contained $C_{12:0}$ 3-OH or $C_{13:0}$ DMA. SF5 contained $C_{15:0}$ DMA or $C_{14:0}$ 3-OH. SF7 contained $C_{17:1}\omega9c$ or an unknown fatty acid of equivalent chain length (ECL) 16.760. SF10 contained $C_{18:1}\omega7c$ or an unknown fatty acid of ECL 17.834. SF12 contained iso-$C_{19:0}$ or an unknown fatty acid of ECL 18.622

Sutterella Wexler, Reeves, Summanen, Molitoris, Mcteague, Duncan, Wilson, and Finegold 1996a, 257[VP]

Sut.ter.el'la. M.L. dim. Fem. n. *Sutterella* named in memory of Vera Sutter, respected colleague and director of the Wadsworth Anaerobe Laboratory for 20 years.

The data described for *Sutterella* species in this section are from Sakon et al. (2008), Greetham et al. (2004), Wexler et al. (1996), Wexler (2005).

Cells of *Sutterella* species are straight rods (*S. wadsworthensis* and *S. stercoricanis*), 0.5–1.0 μm × 1.0–3.0 μm, or are coccoid to coccobacillary (*S. parvirubra*), approximately 0.4–1.0 μm × 0.4–2.0 μm. They grow under anaerobic conditions or in a microaerophilic atmosphere. Colonies of *S. wadsworthensis* on *Brucella* blood agar with 5 % lysed sheep blood, 1 μg/mL Vitamin K1, 1 μg/mL hemin, and 1 % w/w formate/fumarate after 48 h at 37 °C in anaerobic chamber are circular, entire, convex, yellow to brown, translucent to opaque, 1–1.5 mm in diameter. Colonies of *S. parvirubra* on GAM agar after 48 h anaerobic incubation are 0.2–1.1 mm in diameter, circular, flat, and translucent. No culture data has been reported for *S. stercoricanis* in the literature. *Sutterella wadsworthensis* and *S. stercoricanis* are resistant to 20 % (v/v) bile; no data are available for *S. parvirubra*. Other biological and biochemical characteristics of the type strains of the genus *Sutterella* are listed in *Tables 38.1–38.3* and *38.5*.

The G + C values for DNA of the species determined by HPLC for *S. parvirubra* YIT 11816^T and *S. stercoricanis* 5BAC4^T are 64.4 and 60.0 mol%, respectively. This value for *S. parvirubra* YIT 11816^T (64.4 mol%) is slightly lower than that determined by the genome analysis (65.3 mol%, *Table 38.4*). The value for *S. wadsworthensis* 3_1_45B determined by genome analysis is 55.1 mol% (*Table 38.4*), and that for the type strain has not been reported.

The type species is *S. wadsworthensis*.

The type strains are *S. parvirubra* YIT 11816^T (= DSM 19354^T = JCM 14724^T); *S. stercoricanis* 5BAC4^T (= CCUG 47620^T = CIP 108024^T); *S. wadsworthensis* WAL 9799^T (= ATCC 51579^T = CCUG 42229^T = CIP 104799^T = DSM 14016^T).

Parasutterella Nagai, Morotomi, Sakon, and Tanaka 2009, 1795[VP]

Pa.ra.sut.te.rel'la. Gr. prep. *para* besides, next to; N.L. fem. n. *Sutterella* name of a bacterial genus; N.L. fem. n. *Parasutterella* a genus similar to *Sutterella*.

Table 38.3
Isoprenoid quinone compositions of the type strains of the genera *Sutterella* and *Parasutterella*

	% of total isoprenoid quinones				
Isoprenoid quinone	**1**	**2**	**3**	**4**	**5**
		–	–	–	–
MK-4	–	–	–	1.58	–
MK-5	–	10.19	5.59	4.35	9.01
MK-6	–	–	–	13.40	–
MMK-5	100	89.75	94.41	3.16	90.99
MMK-6	–	–	–	77.51	–

Taxa: 1 *Sutterella parvirubra* YIT 11816^T, 2 *Sutterella stercoricanis* DSM 17807^T, 3 *Sutterella wadsworthensis* DSM 14016^T, 4 *Parasutterella excrementihominis* YIT 11859^T, 5 *Parasutterella secunda* YIT 12071^T. Values are percentages of total isoprenoid quinones; only those isoprenoid quinones that make up more than 1 % of the total are shown. MK-4, MK-5, and MK-6 are menaquinones with four, five, or six isoprene units, respectively; MMK-5 and MMK-6 are methylmenaquinones with five or six isoprene units, respectively. Data are from Morotomi et al. (2011)

The data described for *Parasutterella* species in this section are from Nagai et al. (2009) and Morotomi et al. (2011).

Cells of *Parasutterella* species are cocci to coccobacilli, 0.4–1.3 μm × 0.6–2.6 μm. Colonies on modified GAM agar after 4 days of anaerobic incubation at 37 °C are translucent to beige, circular, convex, and pinpoint in size. Growth in peptone-yeast extract broth (Holdeman et al. 1977) is weak, producing no visible turbidity, and no short-chain fatty acids are detected as an end product of metabolism. Addition of glucose, lactate, or succinate does not enhance growth or result in the production of short-chain fatty acids. Other biological and biochemical characteristics of the type strains of the genus *Parasutterella* are listed in *Tables 38.1–38.3* and *38.5*.

The G+C of DNA of the species determined by HPLC for *P. excrementihominis* YIT 11859^T and *P. secunda* YIT 12071^T are 49.8 and 48.2 mol%, respectively. The value for *P. excrementihominis* YIT 11859^T (49.8 mol%) is slightly higher than that determined by genome analysis (48.1 mol%, *Table 38.4*).

The type species is *P. excrementihominis*.

The type strains are *P. excrementihominis* YIT 11859^T (= DSM 21040^T = JCM 15078^T) and *P. secunda* YIT 12071^T (= DSM 22575^T = JCM 16078^T).

Isolation, Enrichment, and Maintenance Procedures

Sutterella wadsworthensis grows under anaerobic conditions or in a microaerophilic atmosphere of 2 % or 6 % oxygen. The type strain of *S. stercoricanis* grows in a microaerophilic atmosphere of 2 % oxygen but not at 6 % oxygen, or under anaerobic conditions. Growth of the type strains of *S. parvirubra*, *P. excrementihominis*, and *P. secunda* was only observed under strict anaerobic conditions (Wexler et al. 1996; Wexler 2005; Greetham et al. 2004; Sakon et al. 2008; Nagai et al. 2009; Morotomi et al. 2011).

Members of the family *Sutterellaceae* are asaccharolytic and their colonies on agar plates are very small, ranging from pinpoint in size to 1.5 mm in diameter. Therefore, the main problem in isolating these organisms from samples of feces or intestinal contents is the exclusion of the dominant intestinal microbiota, which cover large areas of the isolation plates.

Sutterella wadsworthensis is isolated on *Brucella* blood agar with 5 % lysed sheep blood, 1 μg/mL Vitamin K1, 1 μg/mL hemin, and 1 % w/w formate/fumarate, and is mainly obtained from the intestinal tract and from infections of gastrointestinal origin (Wexler 2005).

Sutterella parvirubra YIT 11816^T was isolated from the feces of a healthy human adult on a medium 10 (Caldwell and Bryant 1966) agar plate supplemented with 40 mM succinic acid as the sole carbon source, from which the other basal carbon sources (a mixture of glucose, cellobiose, soluble starch, and volatile fatty acids) had been excluded (Sakon et al. 2008).

Sutterella stercoricanis 5BAC4^T was isolated from the feces of a healthy male Labrador Retriever dog on bacteroides agar (Holdeman et al. 1977) by Greetham et al. (2004).

Parasutterella excrementihominis YIT 11859^T was isolated from the feces of a healthy human adult on anaerobe basal agar (Oxoid), pH 6.0 (Nagai et al. 2009).

Parasutterella secunda YIT 12071^T was isolated from the feces of a healthy human adult on modified Gifu anaerobic agar (GAM; Nissui Pharmaceutical) supplemented with oxacillin (4 μg/mL; Sigma) (Morotomi et al. 2011).

Some growth media suitable for cultivation of strains of the family *Sutterellaceae* and their compositions are shown in the websites of Leibniz-Institut DSMZ—Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (http://www.dsmz.de/) and Japan Collection of Microorganisms (http://www.jcm.riken.jp/JCM/JCM_Home_J.shtm).

The strains are generally maintained in anaerobic medium as broths or agar slants at 4 °C for a few days. Medium-term maintenance is as suspensions in 20 % v/v glycerol, 20 % w/v skim milk, or 10 % w/v skim milk supplemented with 1 % w/v sodium glutamate at −70 °C. Long-term preservation is by lyophilization.

Ecology

Sutterella wadsworthensis was first reported when performing biochemical characterization and susceptibility testing of *Campylobacter gracilis*–like clinical isolates from patients with diverse infections of the gastrointestinal tract (Wexler et al. 1996). Although a potential role of *S. wadsworthensis* in human gastrointestinal diseases has been documented in the past (Wexler et al. 1996; Molitoris et al. 1997), recent evaluation of the colonic mucosal isolates of this species from patients with inflammatory bowel disease has led to the conclusion that this species is probably a commensal; *S. wadsworthensis* was detected in

Table 38.4

Basic genome statistics for strains of the family *Sutterellaceae*

	Genome size (bp)	G+C content (mol%)	Number of predicted genes		
			protein	rRNA	tRNA
Sutterella parvirubra YIT 11816^T	2,374,505	65.3	2,483	2	63
Sutterella wadsworthensis 3_1_45B	2,965,437	55.1	2,373	7	64
Parasutterella excrementihominis YIT 11859^T	2,831,696	48.1	2,751	1	54

Data are from the GenBank/EMBL/DDBJ databases

Table 38.5

Diagnostic phenotypic differences for the type strains of the genera *Sutterella* and *Parasutterella*

Characteristic	1	2	3	4	5
Microaerophilic growth					
2 % oxygen	–	+	+[a]	–	–
6 % oxygen	–	–	+[a]	–	–
Nitrate reduction	–	+	+[a]	–	–
API ZYM and API rapid ID 32 A reaction					
Alkaline phosphatase	–	–	+	w	w
Acid phosphatase	–	–	+	w	–
Alanine arylamidase	–	–	+	w	–
Alkaline phosphatase	–	–	+	w	+
Arginine arylamidase	w	–	+	+	–
Arginine dihydrolase	–	–	+	–	+
Esterase lipase (C8)	w	–	+	w	+
Esterase (C4)	–	–	+	+	+
Glutamic acid decarboxylase	–	–	+	–	–
Glycine arylamidase	–	w	+	w	–
Leucine arylamidase	–	–	+	+	–
Leucyl glycine arylamidase	–	–	+	–	–
Naphthol-AS-BI-phosphohydrolase	w	–	–	w	+
Phenylalanine arylamidase	–	–	+	–	–
Serine arylamidase	–	–	+	–	–
Tyrosine arylamidase	–	–	+	–	–

Taxa: 1 *Sutterella parvirubra* YIT 11816^T (Data from Sakon et al. 2008), 2 *Sutterella stercoricanis* DSM 17807^T (Greetham et al. 2004), 3 *Sutterella wadsworthensis* DSM 14016^T (Morotomi et al. 2011), 4 *Parasutterella excrementihominis* YIT 11859^T (Nagai et al. 2009), 5 *Parasutterella secunda* YIT 12071^T (Morotomi et al. 2011)

+positive, – negative, *w* weakly positive

[a]Data from Wexler et al. (1996)

83.8 % of adult patients with ulcerative colitis as opposed to 86.1 % of the control subjects (Mukhopadhya et al. 2011). This study also indicated that *S. wadsworthensis* adheres closely to the mucosal lining and is thus more likely to be detected in biopsy samples than in feces.

Recently improved sequencing technology has led to the deposition of a large number of uncultured bacterial clones in the GenBank/EMBL/DDBJ public databases. There is evidence that *S. wadsworthensis* occurs in human feces as a common member of the human indigenous microflora, because many uncultured bacteria with highly similar 16S rRNA gene sequences (>98.7 % identity, the threshold proposed for distinguishing species by Stackebrandt and Ebers (2006) have been reported in these databases. These uncultured bacteria have been identified in fecal samples and intestinal biopsy samples from apparently healthy subjects from different countries, suggesting that *S. wadsworthensis* is a normal inhabitant of the human intestinal microbiota.

As described above, *S. wadsworthensis* is not associated with inflammatory bowel disease, but its presence has been reported in ileal mucosal biopsy samples from children with autism and gastrointestinal dysfunction (AUT-GI) by Williams et al. (2012). They reported that the 16S rRNA gene sequences of either *S. wadsworthensis* or *S. stercoricanis* were found in 12 of 23 AUT-GI children but in none of 9 control children with GI but not autism. Further investigations of the microbiome are needed in larger cohorts of patients with AUT-GI compared to the control GI groups, as well as in patients with AUT but without GI manifestations and in normally developing children with no GI disturbances.

The two other *Sutterella* species, *S. parvirubra* and *S. stercoricanis*, were isolated as novel species of the genus from healthy human feces (Sakon et al. 2008) and from the feces of a healthy Labrador Retriever dog (Greetham et al. 2004), respectively. Although there are no subsequent reports of the isolation of these species, a number of uncultured bacteria with highly similar 16S rRNA gene sequences have been deposited in the GenBank/EMBL/DDBJ databases. For example, the most similar 16S rRNA gene sequences (99.8–99.9 % similarity) to the type strain of *S. parvirubra* were derived from studies of uncultured bacteria from human intestinal mucosal biopsies (accession nos. FJ507106, FJ507078, and FJ506786; Walker et al. 2011). In contrast, the most similar 16S rRNA gene sequence for *S. stercoricanis* (99.2 % similarity) was detected in the feces of dhole (*Cuon alpinus*, a species of canid native to southern and southeastern Asia). This sequence (accession no. JN559525) is a direct submission by Zhang et al. (Unpublished). Williams et al. (2012) reported detecting *S. stercoricanis* 16S rRNA gene sequences in ileal mucosal biopsy specimens from patients diagnosed with AUT-GI symptoms, although it remains unclear whether this species contributes to the disease or is simply a normal component of the human intestinal microbiota.

The genus *Parasutterella* contains two species, *P. excrementihominis* (Nagai et al. 2009) and *P. secunda* (Morotomi et al. 2011). These species were isolated from the feces of healthy human subjects and each was described based on a single strain. There are no subsequent reports of isolation of these species, but a number of uncultured bacteria with highly similar 16S rRNA gene sequences have been deposited in the GenBank/EMBL/DDBJ databases. For *P. excrementihominis*, 200 clones (as of August 2012) with similar 16S rRNA gene sequence (>98.7 % similarity) have been derived from feces, intestinal contents, and mucosal biopsies of healthy human subjects and of patients with gastrointestinal diseases (e.g., ulcerative colitis, Crohn's disease, and *Clostridium difficile*–associated diarrhea); from feces of the black lemur (*Eulemur macaco*), the brown rat (*Rattus norvegicus*), the wolf (*Canis lupus*), and cattle (*Bos taurus*); and from human skin samples and mattress dust. Based on these similar sources of isolation and the similar 16S rRNA gene sequences, *P. excrementihominis* is presumably common in the intestines of humans and other animals. For *P. secunda*, nine clones with a similar 16S rRNA gene sequence (>98.7 %) have been derived from human feces and the intestinal contents of turkeys and cattle.

Overall, all these data suggest that members of the family *Sutterellaceae* are common inhabitants of the intestines of humans and various animals.

Pathogenicity, Clinical Relevance

In addition to the details presented in the Ecology section, there is limited information on the antibiotic sensitivity of this family. Most strains of *S. wadsworthensis* (>95 %) are susceptible to amoxicillin/clavulanate, ticarcillin/clavulanate, cefoxitin, ceftriaxone, and clindamycin, and 85–95 % of the strains are susceptible to piperacillin, piperacillin/tazobactam, ceftizoxime, ciprofloxacin, trovafloxacin, azithromycin, clarithromycin, erythromycin, and roxithromycin (Wexler 2005). Strains of *S. wadsworthensis* (8 strains) were susceptible to kanamycin and colistin, but were resistant to vancomycin (Warren et al. 2005).

No information on antibiotic sensitivity and resistance is available for other species of the genera *Sutterella* and *Parasutterella*.

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