# 63 The Family Porphyromonadaceae

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#### Abstract

Porphyromonadaceae, a family within the order Bacteroidales, comprises the genera Porphyromonas, Barnesiella, Butyricimonas, Dysgonomonas, Macellibacteroides, Odoribacter, Paludibacter, Parabacteroides, Petrimonas, Proteiniphilum, and Tannerella. The type genus is the genus Porphyromonas. At first, this family comprised the genera Porphyromonas, Dysgonomonas, and Tannerella (Garrity GM, Bell IA, Liburn T (2005) The revised road map to the manual. In: Brenner DJ, Krieg NR, Staley JT (eds) Bergey's Manual of Systematic Bacteriology, The Proteobacteria, Part A, Introductory Essays, vol 2. 2nd edn New York, Springer pp 159-206). This family comprises five monospecific genera represented by Macellibacteroides fermentans, Paludibacter propionicigenes, Petrimonas sulfuriphila. Proteiniphilum acetatigenes, and Tannerella forsythia. Macellibacteroides fermentans and Parabacteroides chartae are phylogenetically highly related and should be considered strains of the same species, with Macellibacteroides fermentans having page priority. Members of the family are defined by a variety of cellular morphologies and chemotaxonomic characteristics. Many species of the family Porphyromonadaceae are part of the indigenous microbiota of the human and animal gastrointestinal tract and oral cavity, but some species in this family are commonly associated with a variety of human and animal infections.

#### **Taxonomy: Historical and Current**

#### **Short Description of the Family**

Por.phy.ro.mo.na.da.céa.e. N.L. fem. n. *Porphyromonas* type genus of the family; suff. *–aceae* ending to denote a family; N.L. fem. pl. n. *Porphyromonadaceae* the *Porphyromonas* family.

The family *Porphyromonadaceae* (Krieg 2011) consists of a phenotypically diverse group of genera that was based on phylogenetic analysis of 16S rRNA gene sequences. The family contains the genera *Porphyromonas* (type genus) (Shah and Collins 1988; emended by Willems and Collins 1995), *Barnesiella* (Sakamoto et al. 2007b; emended by Morotomi et al. 2008), *Butyricimonas* (Sakamoto et al. 2009a), *Dysgonomonas* (Hofstad et al. 2000), *Macellibacteroides* (Jabari et al. 2012), *Odoribacter* (Hardham et al. 2008), *Paludibacter* (Ueki et al. 2006), *Parabacteroides* (Sakamoto and Benno 2006), *Petrimonas* (Grabowski et al. 2005), *Proteiniphilum* (Chen and Dong 2005), and *Tannerella* (Sakamoto et al. 2002). Differential characteristics are shown in **●** *Table* 63.1.

#### Phylogenetic Structure of the Family and Its Genera

According to the All-Species Living Tree (Release LTPs108) (Released: July 2012), the family is related to the families

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🗖 Table 63.1

Differential characteristics of the genera of the family Porphyromonadaceae

Characteristic	Porphyromonas <sup>a-f</sup>	Barnesiella <sup>g, h</sup>	Butyricimonas <sup>i</sup>	Dysgonomonas <sup>i-m</sup>	Macellibacteroides <sup>n</sup>	Odoribacter <sup>i, o, p</sup>	Paludibacter <sup>q</sup>	Parabacteroides <sup>r-u</sup>	Petrimonas <sup>v</sup>	Petrimonas <sup>v</sup> Proteiniphilum <sup>w</sup> Tannerella <sup>x</sup>	Tannerella <sup>×</sup>
Morphology	Short rods or coccobacilli	Rods	Rods	Coccobacilli to short rods	Rods	Fusiform cells or pleomorphic rods	Rods with ends usually round to slightly tapered	Rods	Rods	Rods	Fusiform cells
Growth in bile	-	-	-	٧	ND	٨	-	+	ND	_	Ι
Aerobic growth	-			+	Ι	-	-	I	Ι	-	Ι
<i>N-</i> Acetylmuramic acid required	-	I	1	1	I	1	-	-	I	-	Ŷ
Pigment produced	z+	Ι		1	I	+	1	1	I	1	1
Metabolism	NF <sup>aa</sup>	F	F	Ŀ	F	٨	H	ш	ш	NF	NF
Major end- products <sup>bb</sup>	A, B, IV, P, PA, S	A, S	B, IB	A, P, L, S	L, A, B, IB	A, S	A, P	A, S	A, H <sub>2</sub>	A, P	A, B, IV, P, PA
Major cellular fatty acids	iso-C <sub>15:0</sub> cc, <sup>dd</sup>	anteiso-C <sub>15.0</sub>	iso-C <sub>15,0</sub>	iso-C <sub>14:0</sub> , anteiso- C <sub>15:0</sub> , iso-C <sub>16:0</sub> 3- OH	anteiso-C <sub>15:0</sub> , C <sub>15:0</sub>	iso-C <sub>15:0</sub>	anteiso-C <sub>15:0</sub> , C <sub>15:0</sub> , anteiso- C <sub>17:0</sub> 3-OH	anteiso-C <sub>15.0</sub>	anteiso- C <sub>15:0</sub>	anteiso-C <sub>15:0</sub>	anteiso- C <sub>15:0</sub>
Ratio of anteiso-C <sub>15:0</sub> to iso-C <sub>15:0</sub>	1>	2.3–2.5	$\overline{\nabla}$	4.7-9.7	12.9	1>	28	3.1–10.3	4.44.6	12.3	22.8–95.2
Ratio of iso- C <sub>15:0</sub> to anteiso- C <sub>15:0</sub>	1.1-40.5	$\overline{}$	30.0-35.3	~	~	2.9-6.0	$\overline{\lor}$	~	$\overline{}$	~	7
Predominant menaquinone (s)	MK-9, MK-10	MK-11, MK-12	MK-10	DN	MK-9, MK-9(H <sub>2</sub> ), MK-10	MK-9	MK-8	MK-9, MK-10	MK-8	DN	MK-10, MK- 11
Growth at 37 $^\circ\text{C}$	+	+	+	+	+	+	I	+	+	+	+
DNA G+C content (mol%)	40–58	45–52	46	37.5–38.5	41.4	42.4–46	39.3	37.2-46	40.8	46.6–48.9	44–48
Isolation source	Oral infections and various other clinical specimens of human and animal origin	Chicken cecum and human feces	Rat feces	Human clinical specimens, feces and microbial fuel cell	Upflow anaerobic filter treating abattoir wastewaters	Human feces and canine oral cavity	Rice plant residue in irrigated rice- field soil	Human feces, human blood cultures and wastewater of a paper mill	Oilfield well head	UASB reactor treating brewery wastewater	Periodontal pockets

Data were taken from

Brondz and Olser (1991); <sup>b</sup>Collins et al. (1994); <sup>c</sup>Love et al. (1992); <sup>d</sup>Love et al. (1994); <sup>s</sup>Cummanen et al. (2005); <sup>d</sup>Sakamoto et al. (2005); <sup>5</sup>Sakamoto et al. (2005); <sup>b</sup>Sakamoto et al. (2005); <sup>b</sup>Collins et al. (2008); <sup>d</sup>Sakamoto et al. (2012); <sup>b</sup>Low et al. (2005); <sup>d</sup>Sakamoto et al. (2012); <sup>b</sup>Low et al. (2012); <sup>d</sup>Sakamoto et al. (2012); <sup>d</sup>Sakamoto et al. (2012); <sup>b</sup>Collins et al. (2008); <sup>d</sup>Sakamoto et al. (2012); <sup>d</sup>Sakamoto et al <sup>m</sup>uawson et al. (2013); <sup>n</sup>Jabari et al. (2013); <sup>n</sup>Hardham et al. (2006); <sup>n</sup>Geki et al. (2006); <sup>s</sup>Sakamoto and Benno (2006); <sup>s</sup>Sakamoto et al. (2007a); <sup>s</sup>Sakamoto et al. (2012); <sup>v</sup>Grabowski et al. (2005); <sup>w</sup>Chen and Dong (2005); <sup>s</sup>Sakamoto et al. (2002); <sup>x</sup>The bite-wound isolates do not require N-acetylmuramic acid for growth; <sup>zP</sup>. catoniae does not produce a black pigment on blood agar

<sup>aa</sup>some species are weakly saccharolytic

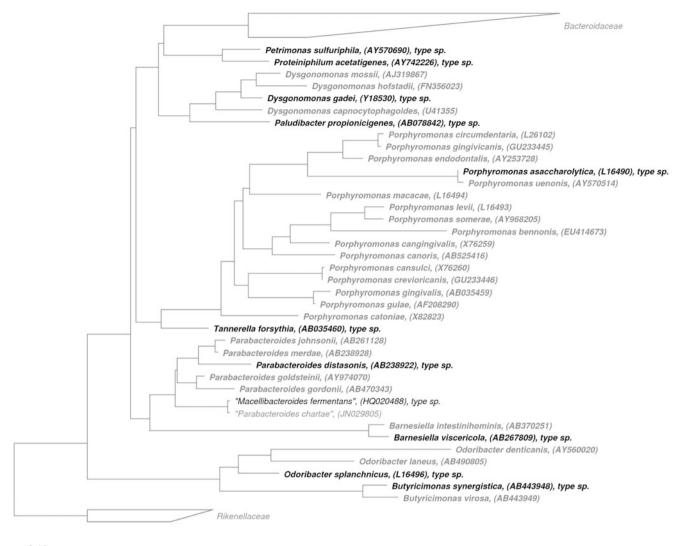
bb A, Acetic acid; B, butyric acid; IB, isobutyric acid; IV, isovaleric acid; L, lactic acid; P, propionic acid; PA, phenylacetic acid; S, succinic acid

 $^{cc}$ P. catoniae contains approximately equal amounts of iso- and anteiso-C<sub>150</sub> as it does of the predominant fatty acids

 $^{dd}$ The major cellular fatty acids of *P. bennonis* are C<sub>150</sub> DMA and/or C<sub>14:0</sub> 3-OH

Abbreviations: ND, no data available; V, variable; NF, non-fermentative; F, fermentative

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#### **Fig. 63.1**

Phylogenetic reconstruction of the family *Porphyromonadaceae* based on the maximum likelihood algorithm RAxML (Stamatakis 2006). Sequence dataset and alignments according to the All-Species Living Tree Project, release LTPs108 (Yarza et al. 2012). Representative sequences from close relative genera were used to stabilize the tree topology. In addition, a 40 % maximum frequency filter was applied to remove hypervariable positions from the alignment. Scale bar indicates estimated sequence divergence

*Bacteroidaceae* and *Prevotellaceae*. Among the genera in this family the genera, *Butyricimonas* and *Odoribacter* are phylogenetically distinct from the other members of the family *Porphyromonadaceae* (**•** *Fig.* 63.1). The genera *Butyricimonas* and *Odoribacter* will be excluded from the family *Porphyromonadaceae*, and a new family will be described for the two genera excluded.

#### **Molecular Analyses**

#### **DNA-DNA Hybridization Studies**

Parabacteroides johnsonii was closely related to Parabacteroides merdae with 98 % 16S rRNA gene sequence similarity.

DNA-DNA hybridization (DDH) experiments revealed the genomic distinction of *P. johnsonii* JCM 13406<sup>T</sup> from *P. merdae* JCM 9497<sup>T</sup> ( $\leq 60 \%$  DNA-DNA relatedness) (Sakamoto et al. 2007a).

The levels of DNA-DNA relatedness (53–65 %) and 16S rRNA gene sequence analysis (98.1 %) provided strong evidence that strains from animal biotype of *Porphyromonas gingivalis* represent a *Porphyromonas* species, *Porphyromonas gulae*, that is distinct from *P. gingivalis* (Fournier et al. 2001). The separation of *P. gulae* and *P. gingivalis* into distinct species was confirmed by the 16S-23S rRNA gene internal transcribed spacer (ITS) data (Conrads et al. 2005).

Comparison of *Porphyromonas uenonis* and *Porphyromonas asaccharolytica* showed that they are closely related (98.2–98.9 % sequence similarity); however, DDH experiment between

*P. uenonis* WAL  $9902^{T}$  and *P. asaccharolytica* ATCC  $25260^{T}$  showed that the similarity was only 54.3 % (59.9 % on repeat analysis), documenting that they are distinct species (Finegold et al. 2004).

DDH experiment has been carried out to clarify the relationship between Porphyromonas crevioricanis and Porphyromonas cansulci. The taxonomic standing of these two species was unclear so far because of high 16S rRNA gene sequence similarity level (99.9 %). The levels of DNA-DNA relatedness between P. crevioricanis JCM 15906<sup>T</sup> and P. cansulci JCM 13913<sup>T</sup> were above 91 % (91-99 %). In addition, *P. crevioricanis* JCM 15906<sup>T</sup> exhibited high *hsp60* gene sequence similarity with *P. cansulci* JCM 13913<sup>T</sup> (100 %). The *hsp60* gene sequence analysis and the levels of DNA-DNA relatedness observed demonstrated P. crevioricanis JCM 15906<sup>T</sup> and P. cansulci JCM 13913<sup>T</sup> are a single species. Consequently, P. cansulci is a later heterotypic synonym of P. crevioricanis (Sakamoto and Ohkuma 2013).

#### **Genome Analyses**

The complete genome sequences of three species have been released. The first species is P. gingivalis with the genomes of three strains (W83, ATCC 33277 and TDC60) fully sequenced (Nelson et al. 2003; Naito et al. 2008; Watanabe et al. 2011). The genome of *P. gingivalis* strain W83 (INSDC ID AE015924) is 2,343,479 bp long with a GC content of 48.3 %. The chromosome contains 1,990 open reading frames (ORFs), four ribosomal operons, and two structural RNA genes, as well as 53 tRNA genes. The genome of P. gingivalis ATCC 33277 (INSDC ID AP009380) is 2,354,886 bp long with a GC content of 48.4 %. The chromosome contains 2,090 protein-coding sequences (CDSs), four RNA operons, and 53 tRNA genes. The genome of P. gingivalis strain TDC60 (INSDC ID AP012203) is 2,339,898 bp long with a GC content of 48.34 %. The chromosome contains 2,220 CDSs, four rRNA operons, 53 tRNA sequences, and nine noncoding RNAs. Dot plot analysis comparison of TDC60 with W83 and ATCC 33277 genome sequences indicated a high degree of genome rearrangement among the three strains (Watanabe et al. 2011).

The second species is *Parabacteroides distasonis*. The genome of the type strain of *P. distasonis* ATCC 8482<sup>T</sup> (Xu et al. 2007) is 4,811,369 bp long. The chromosome contains 3,867 predicted protein-coding genes.

The genome of the type strain of *Paludibacter propionicigenes* WB4<sup>T</sup>(INSDC ID CP002345) (Gronow et al. 2011) is 3,685,504 bp long with a GC content of 38.9 %. Of the 3,118 genes predicted, 3,054 were protein-coding genes, and 64 RNAs; 34 pseudogenes were also identified. This is the third sequence from the family *Porphyromonadaceae* and is a part of the *Genomic Encyclopedia of Bacteria and Archaea* project.

The genome-sequencing project of *Tannerella forsythia* ATCC 43037<sup>T</sup> has been completed by The Institute for Genomic

Research (TIGR). A total of 3,034 ORFs are predicted from the genomic sequence of 3,405,543 bp in length based on the annotation provided by the Oral Pathogen Sequences Databases (ORALGEN, http://www.oralgen.lanl.gov).

#### **Phenotypic Analyses**

**Porphyromonas Shah and Collins 1988, 129**<sup>VP</sup> emend. Willems and Collins 1995, 580

Por.phy.ro.mo'nas. Gr. adj. *porphyreos* purple; Gr. n. *monas* unit; N.L. fem. n. *Porphyromonas* porphyrincell.

Short rods or coccobacilli,  $0.3-1 \times 0.8-3.5 \,\mu$ m. Gram-negative, non-spore-forming, and nonmotile. Obligately anaerobic. Generally cells form brown to black colonies on blood agar due to protoheme production. Most species are asaccharolytic: Growth is not significantly affected by carbohydrates but is enhanced by protein hydrolysates such as proteose peptone or yeast extract. Major fermentation products are usually *n*-butyric acid and acetic acid; propionic, isovaleric, isobutyric, and phenylacetic acid may also be produced. The major cellular fatty acid is 13-methyltetradecanoic acid (C<sub>15:0</sub>iso). Indole is produced by most strains. Nitrate is not reduced to nitrate. Esculin is not hydrolyzed. Most species do not hydrolyze starch. Isolated from oral infections and various other clinical specimens of human and animal origin.

The mol% G+C of DNA is 40–58.

The type species is *Porphyromonas asaccharolytica* (Holdeman and Moore 1970); Shah and Collins 1988,  $128^{VP}$  [*Bacteroides asaccharolyticus* (Holdeman and Moore 1970); Finegold and Barnes 1977, 390; "*Bacteroides melaninogenicus* subsp. *asaccharolyticus*" Holdman and Moore 1970, 33]. The type strain is ATCC 25260 = CCUG 7834 = DSM 20707 = JCM 6326 = LMG 13178.

The main features of members of the genus *Porphyromonas* are listed in **●** *Table 63.2*.

*Barnesiella* Sakamoto, Lan and Benno 2007b, 344<sup>VP</sup> emend. Morotomi, Nagai, Sakon and Tanaka 2008, 2719

Bar.ne.si.el'la. N.L. dim. nem. n. *Barnesiella* named after the British microbiologist Ella M. Barnes, who contributed much to knowledge of intestinal bacteriology and to anaerobic bacteriology in general.

Rods (0.8–1.6  $\times$  1.7–11  $\mu m$ ). Non-spore-forming. Nonmotile. Gram-negative. Obligately anaerobic. On Eggerth Gagnon (EG) agar, colonies are 1–2 mm in diameter, gray to off-white-gray, circular, entire, slightly convex, and smooth. Saccharolytic, with a strictly fermentative type of metabolism. Acetic and succinic acids are the main fermentation products. Growth is inhibited on a medium containing 20 % bile. Esculin is hydrolyzed. Indole-negative. The predominant menaquinones

Table 63.2 Differentiation

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Characteristic	P. asaccharolytica	P. bennonis	P. cangingivalis	P. canoris	P. catoniae	P. circumdentaria	P. crevioricanis	P. endodontalis	P. qinqivalis	P. gingivicanis	P. qulae	P. levii	P. macacae	P. somerae	P. uenonis
Pigment production	+	M	+	+	1	+	+	+	+	+	+	+	+	+	+
Fluorescence	+	Ι	I	+	I	+	+	+	1	+	1	d	1	d	+
Hemagglutinin activity	I	QN	I	I	QN	1	+	1	+		+	1	I	QN	QN
Indole	+	I	+	+	I	+	+	+	+	+	+	1	+	-	+
Catalase	I	р	+	+	I	+	I	I	I	+	+	1	+	1	I
Lipase	I	+	I	I	I	I	I	I	I	I	I	1	+	1	I
Preformed enzyme activity:															
α-Fucosidase	+	I	I	I	+	1	1	1	1	1	Ι	I	I	I	1
α-Galactosidase	1	I	I	I	q	I	I	I	I	I	1	1	+	1	1
$\beta$ -Galactosidase	1	+	I	+	+	1	1	I	1	I	+	+	I	+	1
N-Acetyl-β- glucosaminidase	1	+	I	+	+	-	1	1	+	I	+	+	+	+	þ
Chymotrypsin	I	+	+	+	þ	+	Ι	Ι	Ι	I	ND	+	+	+	Ι
Trypsin	-	-	-	-	d	_	Ι	-	+	-	+	-	+	-	Ι
Fermentation of:															
Glucose	-	-	-		+		-	-		-		w	w	w	Ι
Lactose	1	-	-	-	+	-	Ι	-	+	-		w	w	w	Ι
Maltose	I	Ι	Ι	Ι	+		Ι	Ι	Ι	Ι	Ι	w	Ι	w	>
Glucose- 6-phosphate and 6- phosphogluconate dehydrogenases present	1	Q	+	+	+	1	1	1	1	1	QN	σ	σ	Q	QN
Major cellular fatty acids	C <sub>15:0</sub> iso	C <sub>15:0</sub> DMA and/or C <sub>14:0</sub> 3- OH	C <sub>15:0</sub> iso	C <sub>15:0</sub> iso	C <sub>15:0</sub> iso, C <sub>15:0</sub> anteiso	C <sub>15:0</sub> iso	C <sub>15.0</sub> iso	C <sub>15:0</sub> iso	C <sub>15:0</sub> iso	C <sub>15:0</sub> iso	DN	C <sub>15:0</sub> iso, C <sub>15:0</sub> anteiso	C <sub>15:0</sub> iso	C <sub>15:0</sub> iso, C <sub>15:0</sub> anteiso	C <sub>15:0</sub> iso
Major end-products	A, P, ib, B, IV, s	A, S	A, p, ib, B, IV	A, P, ib, b, IV, s	A, P, iv, I, S	A, P, ib, b, IV, s, pa	A, p, ib, B, IV, s, pa	A, P, ib, B, IV, s	A, P, ib, B, IV, s, pa	A, p, ib, B, IV, s	A, P, ib, B, IV, s, pa	A, P, ib, B, IV, s	A, P, ib, B, IV, s, pa	A, P, ib, B, IV, s	A, P, ib, B, IV, s
+ 90 % or more of the strains are positive, - 10 % or more of the strains are negative, d 11 <i>Abbreviations</i> : A, Acetic acid; B, butyric acid; IB, isobutyric acid; IV, isovaleric acid; L, lactic ac	ins are positive, – 10 d; B, butyric acid; IB,	) % or more of isobutyric acid	the strains are neg ; IV, isovaleric acid,	Jative, d 11–89 : L, lactic acid;	% of the strains P, propionic acic	–89 % of the strains are positive: v, variable; w, weak positive reaction; ND, no data available :id: P, propionic acid; PA, phenylacetic acid; S, succinic acid	ıble; w, weak pos cid; S, succinic aci	itive reaction; ND, d	no data availa	ble					

## **T**able 63.3

Differentiation of the species of the genus Barnesiella

Characteristic	B. viscericola	B. intestinihominis
Isolation source	Chicken cecum	Human feces
DNA G+C content (mol%)	52.0	45.5
Acid production from:		
Lactose	_	+
Sucrose	+	-
Rapid ID 32A:		
β-Glucuronidase	_	+
Fermentation of mannose	—	+

are MK-11 and MK-12. Isolated from chicken cecum and human feces.

The mol% G+C of DNA is 45–52.

The type species is *Barnesiella viscericola* Sakamoto, Lan and Benno 2007b,  $345^{VP}$ . The type strain is C46 = DSM 18177 = JCM 13660.

The genus contains two species, *Barnesiella viscericola* and *Barnesiella intestinihominis*. Major characteristics that differentiate *B. intestinihominis* from *B. viscericola* are listed in **•** *Table 63.3.* 

#### *Butyricimonas* Sakamoto, Takagaki, Matsumoto, Kato, Goto and Benno 2009a, 1751<sup>VP</sup>

Bu.ty.ri.ci.mo'nas. N.L. n. *acidum butyricum* butyric acid; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Butyricimonas* a butyric-acid-producing monad.

Cells are Gram-negative, obligately anaerobic, non-sporeforming, nonmotile, rod-shaped, and 1.0 x 1.5  $\mu$ m. Colonies on EG agar plates after 72 h incubation at 37 °C under anaerobic conditions are 0.5–1.0 mm in diameter, opalescent to yellowish, circular, entire, slightly convex, and smooth. Saccharolytic. The major end-products are butyric and isobutyric acids; small amounts of acetic, propionic, and succinic acids are produced. Growth is inhibited on medium containing 20 % bile. Esculin is not hydrolyzed. Indole and hydrogen are produced. The principal respiratory quinone is MK-10. Both non-hydroxylated and 3-hydroxylated long-chain fatty acids are present. The nonhydroxylated acids are predominantly of the iso-methyl branched type (iso- $C_{15:0}$ ), with smaller amounts of straightchain saturated acids. Isolated from rat feces.

The mol% G+C of DNA is 46.

The type species is *Butyricimonas synergistica* Sakamoto, Takagaki, Matsumoto, Kato, Goto and Benno 2009a,  $1751^{VP}$ . The type strain is MT01 = CCUG 56610 = JCM 15148.

The genus contains two species, *Butyricimonas synergistica* and *Butyricimonas virosa*. Phenotypic and biochemical characteristics of *B. synergistica* and *B. virosa* are listed in **O** *Table 63.4*. All strains produced acid from glucose, but failed to produce acid from L-arabinose, cellobiose, lactose, maltose, D-mannitol,

### Table 63.4

Differentiation of the species of the genus Butyricimonas

Characteristic	B. synergistica	B. virosa
Catalase production	-	+
Gelatin digestion	+	-
Acid production from:		
Glycerol	-	+
D-Mannose	+	-
Rapid ID 32A:		
α-Galactosidase	+	-
$\beta$ -Galactosidase	_	+
α-Fucosidase	+	-
Pyroglutamic acid arylamidase	+	-
Mannose fermentation	+	_

melezitose, raffinose, D-rhamnose, salicin, D-sorbitol, sucrose, trehalose, and D-xylose. All strains were positive for indole production and negative for esculin hydrolysis and urease activity. All strains were positive in Rapid ID 32A tests for  $\beta$ -*N*acetylglucosaminidase, alkaline phosphatase, glutamic acid decarboxylase, alanine arylamidase, glutamyl glutamic acid arylamidase, and leucyl glycine arylamidase activities, and indole production. All strains were negative in Rapid ID 32A tests for  $\alpha$ -arabinosidase, arginine dihydrolase,  $\beta$ -galactose-6-phosphate,  $\alpha$ -glucosidase,  $\beta$ -glucosidase,  $\beta$ -glucuronidase, urease, arginine arylamidase, glycine arylamidase, histidine arylamidase, leucine arylamidase, phenylalanine arylamidase, proline arylamidase, serine arylamidase and tyrosine arylamidase activities, nitrate reduction, and raffinose fermentation.

*Dysgonomonas* Hofstad, Olsen, Eribe, Falsen, Collins and Lawson 2000, 2194<sup>VP</sup>

Dys.go.no.mo'nas. Gr. pref. *dys*- with notion of hard, bad, unlucky; Gr. n. *gonos* that which is begotten, reproduction; Gr. fem. n. *monas* a monad, unit; N.L. fem. n. *Dysgonomonas* intended to mean a weakly growing monad.

Coccobacilli to short rods. Nonmotile. Gram-negative. Facultatively anaerobic. Colonies are 1–2 mm in diameter, nonadherent, entire, gray-white, smooth, and nonhemolytic and have a slight aromatic odor. Growth is not observed on MacConkey agar. Requires X factor for growth. May be catalasepositive or -negative. Oxidase-negative. Glucose in fermented, producing acid but no gas. Alkaline phosphatase is generated but not arginine dihydrolase. Nitrate is not reduced. H<sub>2</sub>S and acetoin are not produced. Esculin may not be hydrolyzed; gelatin and urea are not hydrolyzed. Indole may be produced. Long-chain cellular fatty acids include straight-chain saturated, anteiso- and iso-methyl branched and 3-hydroxy types. Isolated from human clinical specimens, stools, and microbial fuel cell.

#### Table 63.5

Differentiation of the species of the genus Dysgonomonas

Characteristic	D. gadei	D. capnocytophagoides	D. hofstadii	D. mossii	D. oryzarvi
Isolation source	Human clinical sample (infected gall bladder)	Human clinical specimens	Human clinical sample (abdominal wound)	Human clinical sample (abdominal drain)	Microbial fuel cell
DNA G+C content (mol%)	ND	38	ND	38.5	37.5
Resistance to bile	+	+	ND	+	_
Requirement for heme	+	ND	+	+	+
Aerobic growth	+ (microaerobic)	+	+	+	+
Production of:					
Indole	+	+/	+	+	+/-
$\alpha$ -Arabinosidase	+	+	+	+/	+
$\alpha$ -Fucosidase	+	-	+	+	+/-
β-Galactosidase 6-phosphate	-	+	ND	W	+
Glutamyl glutamic acid arylamidase	+	+	-	_	+
Fermentative growth on:					
Trehalose	+	-	+	-	-
L-Arabinose	+	+	-	-	+
∟-Rhamnose	+	ND	-	-	+
Raffinose	+	+	ND	_	+
Major end- products	ND	P, L, S	ND	L, A, P	L, A
Major fatty acids (%)	anteiso-C <sub>15:0</sub> (23.9), C <sub>16:0</sub> (15.2), iso-C <sub>14:0</sub> (12.9)	iso-C <sub>14:0</sub> (19.8), anteiso- C <sub>15:0</sub> (19.6), iso-C <sub>16:0</sub> 3OH (12.3)	anteiso-C <sub>15:0</sub> (34.0), iso- C <sub>14:0</sub> (24.0), iso-C <sub>16:0</sub> 3OH (9.4)	anteiso-C <sub>15:0</sub> , (22.6), C <sub>15:0</sub> (18.5), iso-C <sub>14:0</sub> (9.7)	anteiso-C <sub>15:0</sub> (31.3), iso- C <sub>17:0</sub> 3OH (15.0), C <sub>16:0</sub> 3OH (7.8)

Abbreviations: A, acetic acid; L, lactic acid; ND, no data available; P, propionic acid; S, succinic acid; W, weakly positive

The mol% G+C of DNA is 37.5–38.5.

The type species is *Dysgonomonas gadei* Hofstad, Olsen, Eribe, Falsen and Lawson 2000,  $2194^{VP}$ . The type strain is ATCC BAA-286 = CCUG 42882 = CIP 106420 = JCM 16698.

The genus contains five species, *Dysgonomonas gadei*, *Dysgonomonas capnocytophagoides*, *Dysgonomonas hofstadii*, *Dysgonomonas mossii*, and *Dysgonomonas oryzarvi*. Differences between the five *Dysgonomonas* spp. are listed in **Table 63.5**.

*Macellibacteroides* Jabari, Gannoun, Cayol, Hedi, Sakamoto, Falsen, Ohkuma, Hamdi, Fauque and Fardeau 2012, 2525<sup>VP</sup>

Ma.cel.li.bac.te.ro'i.des. L. n. *macellum* a butcher's stall, meat-market, slaughterhouse; N.L. masc. n. *Macellibacteroides* a relative of the genus *Bacteroides* isolated from slaughterhouse.

Cell stain Gram-positive but have a Gram-negative type of cell wall. Nonmotile, non-spore-forming, mesophilic rods with a fermentative and obligately anaerobic type of metabolism. The major respiratory quinones are MK-9 and MK-9 (H<sub>2</sub>) and the major fatty acids are anteiso- $C_{15:0}$  and  $C_{15:0}$ . The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol and several unidentified phospholipids. Isolated from upflow anaerobic filter treating abattoir wastewaters.

The mol% G+C of DNA is 41.4.

The type species is *Macellibacteroides fermentans* Jabari, Gannoun, Cayol, Hedi, Sakamoto, Falsen, Ohkuma, Hamdi, Fauque and Fardeau 2012,  $2525^{VP}$ . The type strain is LIND7H = CCUG 60892 = DSM 23697 = JCM 16313.

The genus contains a single species *Macellibacteroides* fermentans.

In addition to properties listed in the genus description, the species is characterized as follows: Cells are approximately 2.0-3.0 µm in length and 0.5-1.0 µm in diameter, occurring singly or in pairs. Colonies are vellowish and circular with entire edges, 1.0-2.0 mm in diameter after 3-5 days of incubation at 37 °C. Growth occurs at 20-45 °C (optimum 35-40 °C) and at pH 5.0-8.5 (optimum pH 6.5-7.5). Does not require NaCl for growth, but tolerates up to 2 % NaCl. Yeast extract is required for growth. Catalase-negative. Cellobiose, glucose, lactose, mannose, maltose, peptone, rhamnose, raffinose, sucrose, and xylose are used as electron donors, but not arabinose, glycerol, mannitol, casamino acids, acetate, lactate, pyruvate, H<sub>2</sub>/CO<sub>2</sub>, or H<sub>2</sub>/CO<sub>2</sub> in the presence of acetate. Sodium sulfate, sodium thiosulfate, elemental sulfur, sodium sulfite, sodium nitrate, and sodium nitrite are not used as terminal electron acceptors. The main fermentation products from glucose metabolism are lactate, acetate, butyrate, and isobutyrate. In the API 20A system, the following reactions are positive: hydrolysis of gelatin and esculin and production of acid from glycerol, glucose, mannitol, lactose, sucrose, maltose, xylose, arabinose, cellobiose, mannose, melezitose, raffinose, sorbitol, rhamnose, and trehalose. The following reactions in the API 20A system are negative: urease activity and production of acid from salicin. In addition to the major fatty acids and respiratory quinones listed in the genus description, significant amounts (>5 %) of iso-C<sub>17:0</sub> 3-OH, iso-C<sub>15:0</sub>, a summed feature consisting of C18:006,9c and/or anteiso C18:0 and MK-10 are also present. In addition to the major polar lipids listed in the genus description, one unidentified glycolipid and several unidentified phospholipids are present. Susceptible to ampicillin (10  $\mu$ g ml<sup>-1</sup>) and vancomycin (10  $\mu$ g ml<sup>-1</sup>) but resistant to kanamycin  $(400 \ \mu g \ ml^{-1}).$ 

*Odoribacter* Hardham, King, Dreier, Wong, Strietzel, Eversole, Sfintescu and Evans 2008, 108<sup>VP</sup>

O.do.ri.bac'ter. L. n. *odor* smell; N.L. masc. n. *bacter* rod; N.L. masc. n. *Odoribacter* rod of (bad) smell.

Cells are anaerobic, Gram-negative, non-spore-forming, nonmotile, fusiform in shape and catalase-valiavle. Colonies demonstrate pigmentation after >7 days of anaerobic growth at 37 °C on medium containing blood. Isolated from human feces and canine oral cavity.

The mol% G+C of DNA is 42.4–46.

The type species is *Odoribacter splanchnicus* (Werner et al. 1975); Hardham, King, Dreier, Wong, Strietzel, Eversole, Sfintescu and Evans 2008,  $108^{VP}$ . The type strain is ATCC 29572 = CCUG 21054 A = CIP 104287 = DSM 20712 = JCM 15291 = LMG 8202 = NCTC 10825.

The genus contains three species, *Odoribacter splanchnicus*, "*Odoribacter denticanis*" and *Odoribacter laneus*. Hardham et al. (2008) wrote the following: "The type strain B106<sup>T</sup>of *Odoribacter denticanis* is the subject of the US patent application US20030228328 and the worldwide patent application WO2003054755. The strain is now available to the public." However, according to Rule 30(4), the name *Odoribacter denticanis* Hardham et al. 2008 is not validly published. Differences between the three *Odoribacter* spp. are listed in **♦** *Table 63.6*.

*Paludibacter* Ueki, Akasaka, Suzuki and Ueki 2006, 43<sup>VP</sup>

Pa.lu.di.bac'ter. L. n. *palus- udis* a swamp, marsh; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Paludibacter* rod living in swamps.

Rods (0.5–0.6 × 1.3–1.7 µm), with the ends usually round to slightly tapered. Nonmotile, Non-spore-forming. Gram-negative. Strictly anaerobic. Chemo-organotrophic. Optimum growth temperature, 30 °C. No growth occurs at 37 °C. Oxidase- and catalase-negative. Nitrate is not reduced. Various sugars are fermented, and acetate and propionate are the major fermentation end-products, with succinate as a minor product. Major cellular fatty acids are  $C_{15:0}$  anteiso,  $C_{15:0}$ , and  $C_{17:0}$  anteiso 3-OH. The major respiratory quinone is MK-8 (H<sub>4</sub>). Isolated from a rice plant residue (rice straw), sample collected from irrigated rice-field soil.

The mol% G+C of DNA is 39.3.

The type species is *Paludibacter propionicigenes* Ueki, Akasaka, Suzuki and Ueki 2006,  $43^{VP}$ . The type strain is WB4 = DSM 17365 = JCM 13257.

The genus contains a single species *Paludibacter* propionicigenes.

In addition to properties listed in the genus description, the species is characterized as follows: Elongated cells are occasionally formed both singly and in chains of the short cells. Spherical cells sometimes develop after storage of slant cultures at 4 °C. Growth occurs at pH 5.0-7.6 (optimum, 6.6) and at 15-35 °C. Growth at 33 °C is much slower than at 30 °C. The NaCl concentration range for growth is 0-0.5 % in PYG medium. The following compounds are used for growth and acid production: arabinose, cellobiose, fructose, galactose, glucose, mannose, maltose, melibiose, glycogen, soluble starch, and xylose. The following compounds are not used: cellulose, dulcitol, ethanol, fumarate, glycerol, inositol, lactate, lactose, malate, mannitol, melezitose, pyruvate, rhamnose, raffinose, ribose, salicin, sorbitol, sorbose, succinate, sucrose, trehalose, and xylan. Esculin is hydrolyzed, but gelatin and urea are not. H<sub>2</sub>S and indole are not produced. No growth occurs in the presence of 0.01 % bile salts.

## *Parabacteroides* Sakamoto and Benno 2006, 1602<sup>VP</sup>

Pa'ra.bac.te.ro'i.des. Gr. prep. *para* beside; N.L. masc. n. *Bacteroides* a genus name; N.L. masc. n. *Parabacteroides* resembling the genus *Bacteroides*.

Cells are Gram-negative, obligately anaerobic, non-sporeforming, nonmotile and rod-shaped, and 0.8–1.6  $\times$  1.2–12  $\mu$ m in size. Colonies on EG agar plates are 1–2 mm in diameter, gray to off-white-gray, circular, entire, slightly convex, and smooth.

#### O. laneus O. splanchnicus Human feces, vagina, and occasionally Isolation source Crevicular spaces of canine periodontitis Human feces from abdominal infection patients Morphology Rods Fusiform-shaped Pleomorphic rod-shaped Cell dimensions 0.7 × 1.0–5.0 5.9<sup>a</sup> 0.4-1.9 × 1.4-19.1 DNA G+C content (mol%) ND 42.4 ND Major products from PY or PYG<sup>b</sup> S, A, P, b, iv, ib, (I) ND s, a, (ib), (iv) Sugar utilization Saccharolytic Asaccharolytic Asaccharolytic Vc Catalase ND + Hydrolysis of gelatin + ND Resistance to 20 % bile + ND Major cellular fatty acids iso-C<sub>15:0</sub>, C<sub>18:1</sub>*w*9c, iso-C<sub>15:0</sub> anteiso-C<sub>15:0</sub>

Table 63.6 Differentiation of the species of the genus Odoribacter

Abbreviations: V, variable; ND, no data available

<sup>a</sup>Cells are fusiform with tapered ends and a mean cell length of 5.9 µm (Hardham et al. 2008)

<sup>b</sup>Products from PY or PYG medium: upper-case letters, >1 meq acid per 100 ml broth; lower-case letters, <1 meq acid per 100 ml; A, acetic acid; B, butyric acid; IB, isobutyric acid; IV, isovaleric acid; L, lactic acid; P, propionic acid; S, succinic acid. Products in parentheses may or may not be detected

<sup>c</sup>Data from Holdeman et al. (1977), based on 9 strains and Sakamoto et al. (2009a)

Saccharolytic. Major end-products are acetic and succinic acids; lower levels of other acids may be produced. Grow on medium containing 20 % bile. Esculin is hydrolyzed. Indole is not produced. Glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (6PGDH), malate dehydrogenase, and glutamate dehydrogenase are present.  $\alpha$ -Fucosidase is negative. The principal respiratory quinones are menaquinones MK-9 and MK-10. Both non-hydroxylated and 3-hydroxylated long-chain fatty acids are present. The non-hydroxylated acids are predominantly of saturated straight-chain and anteiso-methyl branched-chain types. Isolated from human feces, human blood cultures, and wastewater of a paper mill.

#### The mol% G+C of DNA is 37.2–46.

The type species is *Parabacteroides distasonis* (Eggerth and Gagnon 1933); Sakamoto and Benno 2006,  $1602^{VP}$ . The type strain is ATCC 8503 = CCUG 4941 = CIP 104284 = DSM 20701 = JCM 5825 = NCTC 11152.

The six species, genus contains Parabacteroides distasonis, Parabacteroides chartae, Parabacteroides goldsteinii, Parabacteroides johnsonii Parabacteroides gordonii, and Parabacteroides merdae. Differences between the six Parabacteroides spp. are listed in **S** Table 63.7.

*Petrimonas* Grabowski, Tindall, Bardin, Blanchet and Jeanthon 2005, 1118<sup>VP</sup>

Pe.tri.mo'nas. L. fem. n. *petra* rock, stone; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Petrimonas* stone monad.

Straight rods (0.7–1  $\times$  1.5–2.0 µm) during exponential growth; some longer cells (0.5  $\times$  4.0 µm) may be observed in

old cultures. Non-spore-forming. Gram-negative. Chemoorganotrophic. Mesophilic. Strictly anaerobic, having a fermentative type of metabolism. End-products of glucose fermentation are acetate, H<sub>2</sub>, and CO<sub>2</sub>. Carbohydrates and some organic acids are fermented. Tryptone is required for growth. The predominant menaquinone is MK-8, with smaller amounts of MK-7 and MK-9. The major polar lipids are phosphatidylethanolamine, an unidentified phospholipid, two aminophospholipids, three unidentified unidentified phosphoglycolipids, a glycolipid, an aminolipid, and two additional uncharacterized lipids. The fatty acids include straightchain and branched fatty acids; in addition, 2-OH and 3-OH fatty acids are present. The major cellular fatty acids are C15:0anteiso, C13:0anteiso, C15:0iso, C15:0. The major hydroxyl fatty acids are 3-OH iso-16:0, 3-OH iso 17:0 and 2-OH 17:0. Isolated from a biodegraded oil reservoir.

The mol% G+C of DNA is 40.8.

The type species is *Petrimonas sulfuriphila* Grabowski, Tindall, Bardin, Blanchet and Jeanthon 2005,  $1119^{VP}$ . The type strain is BN3 = DSM 16547 = JCM 12565.

The genus contains a single species Petrimonas sulfuriphila.

In addition to properties listed in the genus description, the species is characterized as follows: Cells occur singly or in pairs. The temperature range for growth at pH 7.2 is 15–40 °C; optimum, 37–40 °C. Growth occurs in the presence of 0–4 % NaCl; optimum, 0 %. Yeast extract and elemental sulfur stimulate growth. The following substrates support growth when yeast extract, tryptone, and elemental sulfur are present: glucose, arabinose, galactose, maltose, mannose, rhamnose, lactose, ribose, fructose, sucrose, lactate, mannitol, glycerol, and cellobiose. Fumarate, pyruvate, and Casamino acids support weak growth. Acetate, formate, butyrate, propionate, methanol,

#### **Table 63.7**

Differentiation of the species of the genus Parabacteroides

Characteristic	P. distasonis	P. chartae	P. goldsteinii	P. gordonii	P. johnsonii	P. merdae
Isolation source	Human feces	Wastewater of a paper mill	Human feces	Human blood	Human feces	Human feces
API 20A						
Salicin	+	_	-	_	-	-
L-Arabinose	-	+	-	+	+	-
Cellobiose	+	+	w	-	-	-
D-Melezitose	+	+	-	-	-	-
∟-Rhamnose	+	+	+	-	+	-
D- Trehalose	+	+	+	-	+	+
Rapid ID 32A						
$\alpha$ -Arabinosidase	+	NT	-	+	+	+
β-Glucosidase	+	+	+	_	-	-
β-Glucuronidase	-	-	-	-	+	+
Glutamic acid decarboxylase	+	NT	-	-	+	+
Phenylalanine arylamidase	+	NT	+	-	+	+
Pyroglutamic acid arylamidase	-	NT	-	-	-	+
Tyrosine arylamidase	+	NT	+	_	+	+
Glycine arylamidase	+	NT	+	_	+	+
Histidine arylamidase	+	NT	+	_	+	+
Glutamyl glutamic acid arylamidase	+	NT	+	_	+	+
Serine arylamidase	+	NT	+	_	+	+
Distinctive major cellular fatty acids <sup>a</sup>	С <sub>18:1</sub> <i>ω9с,</i> iso-С <sub>17:0</sub> 3-ОН	C <sub>15:0</sub> , iso-C <sub>17:0</sub> 3-OH	С <sub>18:1</sub> <i>ю</i> 9с, iso- С <sub>17:0</sub> 3-ОН	C <sub>18:1</sub> @9c, Summed feature 3	iso-C <sub>17:0</sub> 3-OH	С <sub>18:1</sub> <i>ш</i> 9с, iso- С <sub>17:0</sub> 3-ОН
DNA G+C content (mol%)	47.1	37.2	43	44.6	47.6	47.2

<sup>a</sup>Anteiso-C<sub>15:0</sub> is major component of cellular fatty acids in all species

peptone, ethanol, propanol, butanol, toluene, sorbose, and cellulose are not used. Elemental sulfur is reduced to sulfide. Nitrate is reduced to  $NH_4^+$ .

#### Proteiniphilum Chen and Dong 2005, 2259<sup>VP</sup>

Pro.tei'ni.phi.lum. N.L. neut. n. proteinum protein; Gr. adj. philos-ê-on loving; N.L. neut. n. Proteiniphilum protein-loving.

Rods 0.6–0.9  $\times$  1.9–2.2  $\mu$ m. Motile by means of lateral flagella. Non-spore-forming. Gram-negative. Obligately anaerobic. Growth occurs at 20-45 °C. Oxidase- and catalasenegative. Chemo-organotrophic. Proteolytic. Yeast extract can be used and peptone as energy sources. Nonsaccharolytic. Carbohydrates, alcohols, and organic acids (except pyruvate) are not used. Gelatin is not hydrolyzed. Not resistant to 20 % bile. The major fermentation products from peptone-yeast extract (PY) medium are acetic acid and propionic acid. Nitrate is not reduced. Cellular fatty acids mainly consist of iso-branched fatty acids, predominantly  $C_{15:0}$ anteiso. Isolated from the granule sludge of an Upflow Anaerobic Blanket (UASB) reactor treating brewery wastewater.

The mol% G+C of DNA is 46.6–48.9.

The type species is *Proteiniphilum acetatigenes* Chen and Dong 2005,  $2261^{VP}$ . The type strain is TB107 = As 1.5024 = JCM 12891.

The genus contains a single species *Proteiniphilum* acetatigenes.

In addition to properties listed in the genus description, the species is characterized as follows: Milk is not curdled. Starch and esculin are hydrolyzed. Indole is not produced. Urease, lecithinase, and lipase are not produced. Methyl red and Voges-Proskauer tests are negative. H<sub>2</sub>S is not produced from peptone or thiosulfate. NH<sub>3</sub> is produced from yeast extract, peptone, and L-arginine. Acetic acid is the main product from fermentation of yeast extract, peptone, pyruvate, and L-arginine; propionic acid is also produced. The following substrates are not used: adonitol, amygdalin, L-arabinose, L-aspartate, butanedioic acid, cellobiose, cellulose, citrate, L-cysteine, dulcitol, erythritol, esculin, ethanol, D-fructose, fumarate, D-galactose, gluconate,

D-glucose, L-glutamine, glycogen, hippurate, L-histidine,  $\beta$ -hydroxybutyric acid, inositol, inulin, L-isoleucine, D-lactose, L-leucine, L-lysine, malate, malonate, D-maltose, mannitol, mannose, melibiose, methanol, L-methionine, phenylacetic acid, L-phenylalanine, L-proline, 1-propanol, raffinose, rhamnose, ribitol, ribose, salicin, D-sorbitol, sorbose, succinate, sucrose, starch, trehalose, tryptophan, L-tyrosine, L-valine, xylan, and xylose. The major cellular fatty acids are C<sub>15:0</sub>anteiso (46.21 %), C<sub>15:0</sub> (8.90 %), C<sub>17:0</sub>iso 3-OH (5.93 %), and C<sub>17:0</sub>anteiso (5.15 %).

*Tannerella* Sakamoto, Suzuki, Umeda, Ishikawa and Benno 2002, 848<sup>VP</sup>

Tan.ne.rel'la. L. dim. suffix-*ella*; N.L. fem. dim. n. *Tannerella* named after the American microbiologist Anne C. R. Tanner, for her contributions to research on periodontal disease.

Fusiform cells, generally  $0.3-0.5 \times 1-30 \,\mu\text{m}$ . Gram-negative. Nonmotile. Obligately anaerobic. N-acetylmuramic acid (NAM) is required for growth (some strains do not require NAM). Growth is inhibited in the presence of 20 % bile. The major end-products are acetic acid, butyric acid, isovaleric acid, propionic acid, and phenylacetic acid; smaller amounts of isobutyric acid and succinic acid may be produced. Esculin is hydrolyzed. Indole variable. Trypsin activity is positive. Glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (6PGDH), malate dehydrogenase, and glutamate dehydrogenase are present. The principal respiratory quinones are menaquinones MK-10 and MK-11. Both non-hydroxylated and 3-hydroxylated long-chain fatty acids are present. The non-hydroxylated acids are predominantly of the saturated straight-chain and anteiso-methyl-branched-chain types. The ratio of C<sub>15:0</sub>anteiso to C<sub>15:0</sub>iso is high (>20). Isolated from human oral cavity.

The mol% G+C of DNA is 44–48.

The type species is *Tannerella forsythia* corrig. (Tanner, Listgarten, Ebersole and Strezempko 1986); Sakamoto, Suzuki, Umeda, Ishikawa, and Benno 2002,  $848^{VP}$  (*Bacteroides forsythus* Tanner, Listgarten, Ebersole and Strezempko 1986, 216). The type strain is FDC 338 = ATCC 43037 = CCUG 21028A = CCUG 33064 = CCUG 33226 = CIP 105219 = JCM 10827.

The genus contains a single species *Tannerella forsythia*. The name *Bacteroides forsythus* was published by Tanner et al. (1986) but did not appear on a Validation List in the IJSEM. Thus, the name was illegitimate when Sakamoto et al. (2002) created the genus *Tannerella* for the organism in and renamed *Bacteroides forsythus* as *Tannerella forsythensis*. Although this name is legitimate, Maiden et al. (2003) requested an Opinion from the Judicial Commission that the original adjectival form of the specific epithet be conserved, i.e., "*Tannerella forsythia*." In Opinion 85, the Judicial Commission (2008) agreed with the proposal of Maiden et al. (2003). The characteristics are as given for the genus and as listed in **()** *Table 63.1*.

# Isolation, Enrichment, and Maintenance Procedures

A complex medium containing peptone, yeast extract, vitamin  $K_1$  or menadione, and hemin, and supplemented with 5 % blood is recommended for isolation of *Porphyromonas* from body sites. Fresh or prereduced media are recommended, as they increase isolation efficacy. For determination of pigmentation, rabbit blood (laked) is preferable to blood from other animals. Kanamycin-vancomycin laked-blood agar with reduced vancomycin concentration (2 µg ml<sup>-1</sup>) may be used as a selective medium when *Porphyromonas* species are sought (Jousimies-Somer et al. 2002). Members of the genera *Barnesiella, Butyricimonas, Dysgonomonas, Odoribacter,* and *Parabacteroides* may be grown on the above-mentioned media as do *Porphyromonas* species.

Inoculated media should immediately be placed in an anaerobic environment, such as an anaerobic pouch, jar, or chamber. If anaerobic systems utilizing palladium catalysts are used, the anaerobic gas mixture must contain hydrogen to enable the palladium to reduce oxygen to water. The plates may be examined after a 48-h incubation. However, a total incubation period of at least 7 days is recommended for *Porphyromonas* species, because not all *Porphyromonas* species may be detected with shorter incubation times.

*Macellibacteroides fermentans* (Jabari et al. 2012) was isolated from an upflow anaerobic filter treating abattoir wastewaters. For enrichment and isolation, the basal medium contained the following (g per liter):  $KH_2PO_4$  (0.3),  $K_2HPO_4$  (0.3),  $NH_4Cl$ (1), NaCl (1), KCl (0.1),  $CaCl_2 \cdot 2H_2O$  (0.1),  $MgCl_2 \cdot 6H_2O$  (1), L-cysteine  $\cdot$  HCl (0.5), Difco yeast extract (2), 10-ml trace mineral element solution (Balch et al. 1979) and 1 ml of 0.1 % resazurin (pH 7.2). The pH was adjusted to 7.2 with 10 M KOH solution. Prior to inoculation, 0.1 ml of 10 % (w/v) NaHCO<sub>3</sub>, 0.1 ml of 2 % (w/v) Na<sub>2</sub>S  $\cdot$  9H<sub>2</sub>O, and 20 mM glucose from sterile solutions were injected into the tubes (5 ml).

*Paludibacter propionicigenes* was isolated using the anaerobic roll tube method. PY4S agar can be used for maintenance of the strain on agar slants (Ueki et al. 2006). *Paludibacter propionicigenes* cannot grow at 37 °C and is inhibited by bile.

*Petrimonas* enrichment was performed in Nitrate Broth medium (Difco) (Grabowski et al. 2005). Isolation of single colonies was accomplished using the anaerobic roll tube method with Nitrate Broth solidified with 2 % (w/v) purified agar (Difco).

*Proteiniphilum* was isolated from methanogenic propionatedegrading mixtures obtained from the granule sludge of an upflow anaerobic sludge blanket reactor fort treatment of brewery wastewater (Chen and Dong 2005). Isolation was accomplished by initial serial dilution in anaerobic PY medium followed by selection of colonies on anaerobic roll tubes. Repeated subculturing of colonies in PY broth followed by colony isolation is performed until purity is assured.

*Tannerella forsythia* (Sakamoto et al. 2002) was isolated from deep periodontal pockets of humans (Tanner et al. 1979). Isolates were maintained on Trypticase Soy Agar (Becton

Dickinson) plates supplemented with 5 % (v/v) sheep blood on which a piece of filter disk (Braham and Moncla 1992) containing *N*-acetylmuramic acid (NAM) was placed. *T. forsythia* requires exogenous NAM as a growth factor (Wyss 1989).

Isolates can be put into stock from broth or plate cultures. Freezing at -80 °C or lyophilization of young cultures grown in a well-buffered liquid or solid medium is satisfactory for storage of members of the family *Porphyromonadaceae*. Cryoprotective agents such as 10 % glycerol or DMSO should be added to cultures before freezing. Storage of lyophilized cultures at 4 °C is recommended. Even with the best storage conditions, only a portion of the original cell population survives. Therefore, a large inoculum in a supportive medium and minimal exposure to oxygen are recommended for recovery of viable cultures from stored material.

#### Ecology

Several of the members of *Porphyromonas* are indigenous bacterial flora in oral cavity of humans and animals. Many species are found in the urogenital and intestinal tracts. *Porphyromonas* species have been isolated from oral infections, and from many infections throughout the body, e.g., blood, amniotic fluid, umbilical cord, pleural empyema, peritoneal and pelvic abscesses, inflamed endometrium, and other infected tissues. *Porphyromonas* species of animal origin have been encountered in humans with animal bite infections.

*Dysgonomonas* species are found in clinical sources such as blood, wounds, urine, peritoneal fluid, umbilicus, stools, and gall bladder (Hofstad et al. 2000; Lawson et al. 2002).

#### **Pathogenicity: Clinical Relevance**

Some species of the genus *Porphyromonas* are considered true pathogens and are associated with human or animal infections. In particular, *P. gingivalis* is a major causative agent in the initiation and progression of severe forms of periodontal disease. *P. gingivalis* possesses a multitude of cell-surface-associated and extracellular activities that contribute to its virulence potential. Several of these factors are adhesins that interact with other bacteria, epithelial cells, and extracellular matrix proteins. Secreted or cell-bound enzymes, toxins, and hemolysins play a significant role in the spread of the organism through tissue, in tissue destruction, and in evasion of host defenses (Shan et al. 2009).

*Porphyromonas* species are generally very susceptible to most of the antimicrobial agents commonly used for treatment of anaerobic infections, such as amoxicillin-clavulanate, piperacillin-tazobactam, ampicillin-sulbactam, imipenem, cephalosporins, and metronidazole. β-lactamase production has been described in *P. asaccharolytica* (Aldridge et al. 2001), *P. catoniae* (Könönen et al. 1996), *Porphyromonas somerae* (Summanen et al. 2005), and *P. uenonis* (Finegold et al. 2004). The frequency of β-lactamase production has been reported at approximately 20 %. Animal-derived *Porphyromonas* species are more often  $\beta$ -lactamase producers than those derived from humans. Occasional resistance to clindamycin and ciprofloxacin may occur in *P. asaccharolytica* and *P. somerae*; also, ciprofloxacin resistance of *P. gingivalis* has been reported (Lakhssassi et al. 2005).

Dysgonomonas capnocytophagoides (Hofstad et al. 2000) and Dysgonomonas mossii (Lawson et al. 2002) are member of the CDC DF (dysgonic fermenter)-3 group (Daneshvar et al. 1991; Wallace et al. 1989). The DF-3 strains are resistant to penicillin, ampicillin, ampicillin-sulbactam, aztreonam, aminoglycosides, cephalosporins (including cephalotin, cefexitin, ceftriaxone, cefeperazone, and ceftazidime), erythromycin, ciprofloxacin, and vancomycin (Koneman et al. 1997). They are usually susceptible to trimethoprim-sulfamethoxazole and chloramphenicol, and variably susceptible to piperacillin, clindamycin, tetracycline, and imipenem. Dysgonomonas gadei are sensitive to metronidazole, clindamycin, doxycycline, imipenem, meropenem, and trimethoprim-sulfamethoxazole (Hofstad et al. 2000). D. gadei is resistant to cefoxitin and other cephalosporins (cefotaxime, cefpirome, ceftazidime, ceftriaxone, cefuroxime, and cephalotin), aminoglycosides (gentamicin, netilmicin, sulfadiazine), fluoroquinolones (ciprofloxacin, oxafloxacin), vancomycin, and teicoplanin. D. capnocytophagoides are susceptible to ampicillin, tetracycline, chloramphenicol, clindamycin, and trimethoprim-sulfamethoxazole, while it is resistant to penicillin, cephalosporins, metropenem, aminoglycosides, and ciprofloxacin (Hansen et al. 2005).

The minimum inhibitory concentrations of antimicrobial agents have been reported by Takemoto et al. (1997). *Tannerella forsythia* strains are most sensitive to clindamycin and metronidazole. *Tannerella forsythia* shows a comparatively lower susceptibility to ciprofloxacin. Most strains are sensitive to penicillin G, ampicillin, amoxycillin, tetracycline, doxycycline, and erythromycin.

#### Application

No information on the application is available for the family *Porphyromonadaceae*.

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