24 The Family *Peptostreptococcaceae*

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Abstract
Peptostreptococcaceae, a family within the order *Clostridiales*, includes the genera *Peptostreptococcus*, *Acetoanaerobium*, *Filifactor*, *Proteocatella*, *Sporacetigenium*, and *Tepidibacter*. Genera *Acetoanaerobium*, *Proteocatella*, and *Sporacetigenium* are monospecific. Representatives of the family have different cell morphology which varies among the genera from cocci to rods and filaments. Species of *Filifactor*, *Proteocatella*, *Sporacetigenium*, and *Tepidibacter* form endospores. All members of the family are anaerobes with fermentative type of metabolism. The genus *Tepidibacter* contains moderately thermophilic species. Members of *Peptostreptococcaceae* are found in different habitats including human body, manure, soil, and sediments. Species of *Peptostreptococcus* and *Filifactor* are components of the human oral microbiome. *Tepidibacter* spp. inhabit deep-sea hydrothermal vents. Strains of *Filifactor* are pathogenic.

Taxonomy: Historical and Current

Short Description of the Family

Pep.to.strep.to.coc.ca'ce.ae. N.L. n. *Peptostreptococcus* a bacterial genus, the type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Peptostreptococcaceae* the family of *Peptostreptococcus* (from *Bergey's Manual*). The emendation of the original description (Ezaki 2009a) is given bellow.

Phylogenetically a member of the order *Clostridiales* (Prévot 1953), phylum Firmicutes. The family contains the type genus *Peptostreptococcus*, and genera *Acetoanaerobium, Filifactor*, *Proteocatella, Sporacetigenium*, and *Tepidibacter* (see comment below). Morphology of the cells varies from cocci to rods and filaments. Motile by means of peritrichous flagella or nonmotile. Some species form round or ovoid subterminal or terminal endospores. Anaerobic. Fermentative type of metabolism. Utilize proteinaceous substrates and carbohydrates; some species are asaccharolytic. Some species grow on amino acids using Stickland reactions. Catalase-negative or occasionally weakly positive. Mesophilic, moderately thermophilic or phychrotolerant. Neutrophilic. G+C values of DNA range between 24 and 54 mol%. Isolated from human and animal clinical samples, soil, sediments, manure, anaerobic sludge, and deep-sea hydrothermal vents.

Comment: At the time of the original description (Ezaki 2009a) the family contained the type genus *Peptostreptococcus* (Kluyver and van Neil 1936; emended by Ezaki et al. 2001), *Filifactor* (Collins et al. 1994), and *Tepidibacter* (Slobodkin et al. 2003; emended by Tan et al. 2012). Since then, ribosomal RNA sequence databases, SILVA (Pruesse et al. 2007; http://www.arb-silva.de), Ribosomal Database Project II (Cole et al. 2009; http://rdp.cme.msu.edu), and EzTaxon-e (Kim et al. 2012; http://eztaxon-e.ezbiocloud.net) classified three other genera—*Acetoanaerobium* (Sleat et al. 1985), *Sporacetigenium* (Chen et al. 2006), and *Proteocatella* (Pikuta et al. 2009) as members of *Peptostreptococcaceae*. These databases also include to *Peptostreptococcaceae* several validly published *Clostridium* and *Eubacterium* species: *Clostridium bartlettii*, *C. bifermentans, C. difficile, C. ghoni, C. glycolicum, C. hiranonis, C. irregulare, C. litorale, C. lituseburense, C. mangenotii, C. mayombei, C. paradoxum, C. sordellii, C. thermoalcaliphilum* and *Eubacterium acidaminophilum, E. tenue*, and *E. yurii*. Inclusion of these microorganisms to *Peptostreptococcaceae* was also proposed by Ludwig et al. 2009. Abovementioned species of

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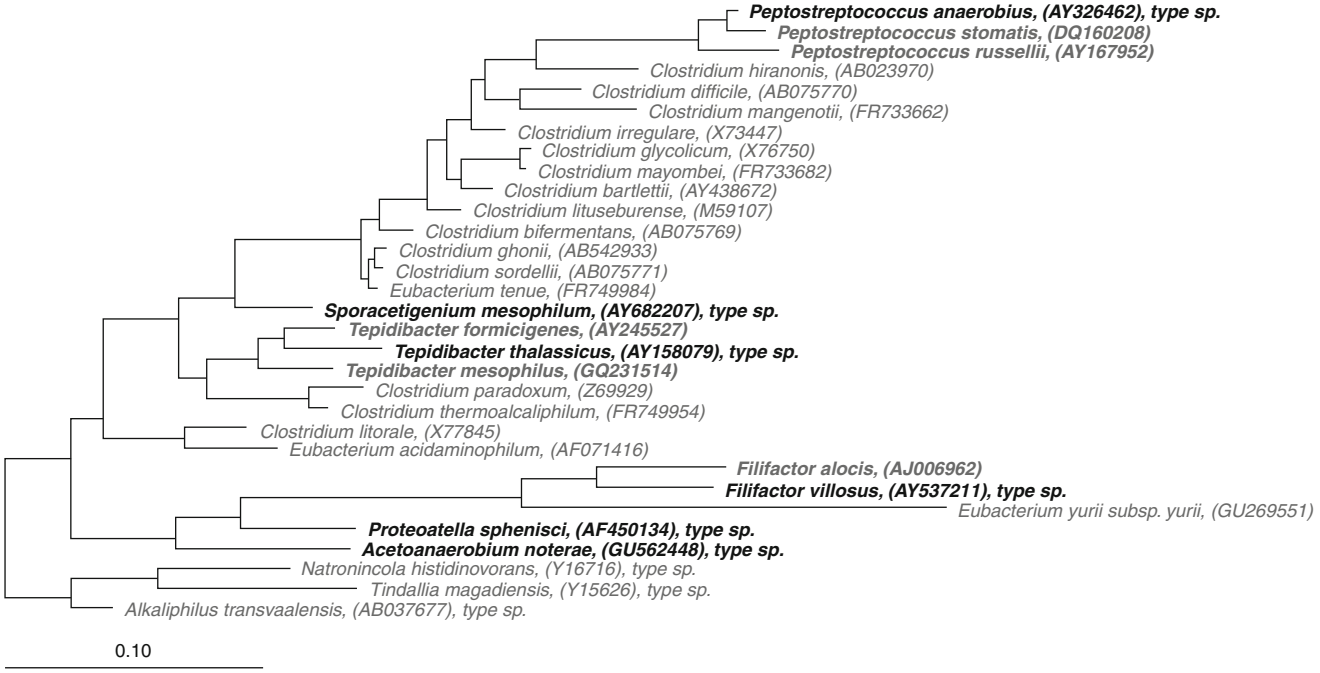

Fig. 24.1
Dendrogram showing the phylogenetic structure of the family *Peptostreptococcaceae* and the closest phylogenetic neighbors

Clostridium and *Eubacterium* need taxonomic revision and will not be covered in this chapter.

Phylogenetic Structure of the Family and Its Genera

Peptostreptococcaceae forms separate, well-defined clade in the order *Clostridiales*. The closest phylogenetic neighbors are genera *Alkaliphilus, Natronincola, and Tindallia* belonging to "Clostridiaceae 2" (LTP nomenclature; http://www.arb-silva.de) (*Fig. 24.1*). Within the family the genera *Acetoanaerobium, Filifactor*, and *Proteocatella* (and *Eubacterium yurii*) form deeply branching cluster; another cluster embrace the genera *Peptostreptococcus*, *Sporacetigenium*, and *Tepidibacter* (and 14 misclassified *Clostridium* species together with *Eubacterium acidaminophilum and Eubacterium tenue*). The genus *Filifactor* is most distantly related to other members of the family (83–87 % 16S rRNA gene sequence similarity).

Molecular Analyses

DNA–DNA Hybridization Studies

DNA–DNA hybridization studies between different species of the family were performed only for two species of the genus *Peptostreptococcus*. Hybridization between *P. anaerobius* NCTC 11460^T and *P. stomatis* strains W2278^T and W3855 was 8 % and 14 %, respectively (Downes and Wade 2006). *Intraspecies* DNA–DNA hybridization between 8 strains of *Filifactor alocis* isolated from cats and the type strain of *F. alocis* ATCC 35896^T isolated from human oral samples was in the range of 77–100 % (Love et al. 1987). For all others members of the family DNA–DNA hybridization studies were not carried out due to low values of 16S rRNA gene sequence similarity between the species (*P. russellii* and other species of *Peptostreptococcus*—93–96 %, *Filifactor* species—93 %, *Tepidibacter* species—94–95 %). *Genera Acetoanaerobium*, *Proteocatella*, and *Sporacetigenium* are represented by one species each.

Genome Comparison

The complete genomes of three species of *Peptostreptococcaceae* have been sequenced as the reference genomes for the Human Microbiome Project (Human Microbiome Jumpstart Reference Strains Consortium et al. 2010). The genome of *Peptostreptococcus anaerobius* 653-L (not a type strain of the species) (GenBank: ADJN00000000.1) has a size of 2.08 Mb, contains 1,930 genes (1,871 protein-coding genes), and its G+C content of DNA is 35.9 mol%. *Peptostreptococcus stomatis* DSM 17687^T (GenBank: ADGQ00000000.1) has the genome size of 1.99 Mb with 1,659 genes, (1,600 protein-coding genes). The genome of *Filifactor alocis* ATCC 35896^T (GenBank: CP002390.1) has a size of 1.93 Mb, contains 1,709 genes (1,641 protein-coding genes), and the mol% G+C of DNA is 35.4 %.

Phenotypic Analyses

The family *Peptostreptococcaceae* is morphologically diverse and includes cocci, rods, or long filaments. Most strains have the

Table 24.1
Morphological and chemotaxonomic characteristics of genera of *Peptostreptococcaceae*

Characteristic	*Peptostreptococcus*[a–c]	*Acetoanaerobium*[d]	*Filifactor*[e–g]	*Proteocatella*[h]	*Sporacetigenium*[i]	*Tepidibacter*[j–l]
Morphology	Cocci in pairs, irregular masses, or chains	Straight rods	Rods with rounded ends or filaments	Straight rods	Rods	Straight to slightly curved rods
Gram-stain	positive	negative	variable	positive	positive	positive
Motility (flagellation)	–	+ (peritrichous flagella)	–	+(peritrichous flagella)	+ (peritrichous flagella)	+ (peritrichous flagella)
Spore formation	–	–	–/+	+	+	+
Growth temperature (optimum) (°C)	25–45 (37)	(37)	30–45 (37)	2–37 (29)	20–42 (37–39)	10–60 (28–50)
pH range (optimum)	NR	6.6–8.4 (7.6)	NR	6.7–9.7 (8.3)	6.0–9.5 (7.5)	4.8–8.9 (6.0–7.3)
Fermentation of sugars	w	+	–	–	+	+
Peptidoglycan (position 3, bridge)	Lys, D-Asp	NR	Orn, D-Asp	NR	*meso*-DAP	NR
Major fatty acids	iso-$C_{14:0}$, iso-$C_{16:0}$, $C_{16:0}$, $C_{18:1}$ ω9c	NR	NR	$C_{14:0}$, $C_{16:0}$	$C_{14:0}$, $C_{16:1}$ ω 7c, $C_{16:0}$	iso-$C_{15:0}$
G+C content	34–36	37	34	39.5	53.9	24–30

Symbols and abbreviations: + positive, – negative, *w* weakly positive, *NR* not reported
Data from: [a]Ezaki 2009b; [b]Downes and Wade 2006; [c]Whitehead et al. 2011; [d]Sleat et al. 1985; [e]Love et al. 1979; [f]Cato et al. 1985; [g]Jalava and Eerola 1999; [h]Pikuta et al. 2009; [i]Chen et al. 2006; [j]Slobodkin et al. 2003; [k]Urios et al. 2004; [l]Tan et al. 2012

diameter of the cells in the range of 0.6–0.9 µm, irrespective of the cell shape. The family contains spore-forming as well as non-spore-forming species. Most species are Gram-stain-positive. The majority of the strains are obligately anaerobic chemo-organotrophs. The key metabolic property for all members of the family is anaerobic growth via fermentation of proteinaceous substrates and some carbohydrates. *Peptostreptococcaceae* includes mesophilic, psychrotolerant, and moderate thermophilc species. All strains grow at pH close to 7.0. The main morphological and chemotaxonomic characteristics of genera of *Peptostreptococcaceae* are listed in Table 24.1.

Peptostreptococcus Kluyver and van Niel 1936, emend. Ezaki, Kawamura, Li, Li, Zhao and Shu 2001

Pep.to.strep.to.co'ccus. Gr. adj. *peptos* cooked, digested; N.L. masc. n. *Streptococcus* a bacterial genus name; N.L. masc. n. *Peptostreptococcus* the digesting streptococcus.

Cells of all three species of *Peptostreptococcus* are non-spore-forming Gram-stain-positive cocci, 0.8–1.0 µm in diameter. Cells may occur in pairs, irregular masses, or chains. Colonies of *P. anaerobius* are convex, 2.2–4.0 mm in diameter. Colonies of *P. russellii* are 2.0–3.0 mm in diameter, convex, opaque, smooth, and whitish in color. *P. stomatis* forms circular, high convex to pyramidal, opaque, shiny, and cream to off-white in color colonies, 0.8–1.8 mm in diameter, with a narrow, gray, peripheral outer ring. Optimal cultivation temperature for all species is 37 °C (Downes and Wade 2006; Ezaki 2009b; Whitehead et al. 2011). Members of *Peptostreptococcus* are obligately anaerobic chemo-organotrophs and metabolize peptone and amino acids to acetic, butyric, isobutyric, caproic, and isocaproic acid (Holdeman Moore et al. 1986; Ezaki et al. 2006). Carbohydrates are weakly fermented by all strains (Table 24.2). Urea is not hydrolyzed and indole is not produced by all species. *P. russellii* produces prodigious amounts of ammonia (>40 mM) from various nitrogen sources (Tryptone and Casamino acids) (Whitehead et al. 2011). Diamino acid of peptidoglycan is lysine (Ezaki 2009b). Comparison of other selected characteristics of species of the genus *Peptostreptococcus* is given in Table 24.2.

Acetoanaerobium Sleat, Mah and Robinson 1985

A.ce.to.an.ae.ro'bi.um. L. n. *acetum* vinegar; Gr. pref. *an* not; Gr. n. *aer* air; Gr. n. *bios* life; M. L. neut. n. *Acetoanaerobium* vinegar anaerobe

The genus *Acetoanaerobium* includes so far only one species *A. noterae*—represented by one strain NOT-3^T (= ATCC 35199^T). Cell of *A. noterae* are straight rods, 0.8-µm wide and 1.0–5.0-µm long, motile with three or four peritrichous flagella. Cells stain gram-negative; the cell wall is atypical and is composed of two distinct layers, a darker inner layer and lighter outer layer.

Table 24.2
Comparison of selected characteristics of species of the genus *Peptostreptococcus*

Characteristic	*P. anaerobius* DSM 2949^{T} [a, b]	*P. russellii* DSM 23041^{T} [b]	*P. stomatis* DSM 17678^{T} [b, c]
Temperature range (°C)	NR	25–45	NR
Carbohydrates weakly fermented	Glucose, mannose	Glucose	Glucose, fructose, maltose
Carbohydrates not fermented	Arabinose, lactose, mannitol, raffinose, sorbitol, sucrose	Mannose, raffinose	Arabinose, cellobiose, lactose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, sucrose, trehalose
Nitrate reduction	NR	–	–
Catalase	NR	–	–
α-glucosidase	+	–	+
Proline arylamidase	+	+	–
Fermentation products	From PYG: Caproate, acetate, butyrate, iso-butyrate, iso-valerate	From glucose: Acetate, lactate, formate, citrate	From PYG: Acetate, Iso-caproate, iso-butyrate, iso-valerate
Peptidoglycan (position 3, bridge)	Lys, D-Asp	Lys, D-Asp	NR
Whole-cell-wall sugars	NR	Glucose, xylose, and traces of mannose	NR
Respiratory quinones	NR	Not detected	NR
Polar lipids	NR	Aminoglycolipid, diphosphatidylglycerol, glycolipids, phosphatidylglycerol and phospholipids	NR
Predominant cellular fatty acids (>10 %)	$C_{16:0}$, $C_{18:1}$ ω9c	iso-$C_{16:0}$	iso-$C_{14:0}$, iso-$C_{16:0}$
DNA G+C content (mol%)	34–36	35.6	36

Symbols and abbreviations: + positive, – negative, *NR* not reported. Fermentation products: starting with upper-case letter = major product, starting with lower-case letter = minor product
Data from: [a]Ezaki 2009b; [b]Whitehead et al. 2011; [c]Downes and Wade 2006

Colonies are rhizoid, opaque, and granular. Young colonies are white, but older colonies are brownish and up to 2 cm in diameter after 1 month of incubation. Obligately anaerobic. Yeast extract, maltose, and glucose are used for heterotrophic growth. Vitamins are not required. Compounds not supporting growth include arabinose, rhamnose, ribose, xylose, fructose, galactose, cellobiose, lactose, mannose, sucrose, melezitose, trehalose, erythritol, adonitol, arabitol, dulcitol, inositol, mannitol, sorbitol, formate, acetate, pyruvate, lactate, malate, fumarate, succinate, citrate, glutamate, methylamine, trimethylamine, and methanol. The strain produces acetate, propionate, iso-butyrate, butyrate, and iso-valerate (and little or no H_2) during growth on yeast extract alone. When either glucose or maltose serves as a substrate, acetate is the only fermentation product. The main physiological feature that distinguishes *Acetoanaerobium* from other *Peptostreptococcaceae* is its capacity for lithotrophic acetogenic growth with H_2:CO_2. Yeast extract is required for growth and H_2 utilization. Growth on yeast extract and H_2:CO_2 is biphasic, with an initial rapid growth phase independent of the presence of H_2. This is followed by H_2-dependent acetate production during the second slower growth phase (Sleat et al. 1985). Temperature and pH ranges and optima for growth and G+C content of DNA are presented in *Table 24.1*.

Filifactor Collins, Lawson, Willems, Cordoba, Fernández-Garayzábal, Garcia, Cai, Hippe and Farrow 1994

Fi.li.fac'tor. L. n. *filum* thread; L. masc. n. *factor* maker; N.L. masc. n. *Filifactor* thread-maker.

Cells of *Filifactor* are rods 0.4–0.7 μm in diameter and 1.5–7.0 μm in length with rounded to tapered ends that occur singly, in pairs, and occasionally in chains or filaments. *F. villosus* can form filaments up to 30 μm in length. Cells of *F. villosus* have variable Gram-staining properties—positive in young (24–48 h) cultures and negative in old (7 d) cultures. However, thin-section electron microscopy studies have shown that the

Table 24.3
Comparison of selected characteristics of species of the genus *Filifactor*

Characteristic	*F. villosus* NCTC 11220^{T} [a–c]	*F. alocis* ATCC 35896^{T} [b–d]
Gram-stain	variable	negative
Spore formation	+	–
Temperature range (°C)	NR	30–45
Optimum temperature (°C)	37	37
Utilization of pyruvate	+	–
Fermentation products	Acetate, iso-butyrate, butyrate, iso-valerate, formate and traces of lactate, methylmalonate, succinate, and H_2[e]	Butyrate, acetate, H_2[f]
DNA G+C content (mol%)	NR	34

Symbols and abbreviations: + positive, – negative, *NR* not reported
Data from: [a]Love et al. 1979; [b]Cato et al. 1985, [c]1986; [d]Jalava and Eerola 2009
[e]From cooked meat-carbohydrate and peptone-yeast extract cultures supplemented with 5 % horse serum
[f]From PYG broth

structure of the cell wall and the mode of the division are consistent with the Gram-positive bacteria. Cells of *F. alocis* stain Gram-negative. Cells of both *Filifactor* species are nonmotile and do not have flagella, but some of the strains have been shown to have twitching or end-over-end type of motility. *F. villosus* forms oval subterminal endospores that caused a slight distention of the sporangium. Formation of endospores by *F. alocis* was not observed. Colonies of *Filifactor* are small (0.5–1.0 mm) and nonhemolytic. Members of *Filifactor* are obligately anaerobic chemo-organotrophs. All strains produce acetate, butyrate, and H_2 as fermentation products (Table 24.3). Both *Filifactor* species do not utilize sugars; no acid is produced from esculin, fructose, glucose, maltose, mannitol, mannose, melibiose, ribose, sucrose, or xylose. Threonine and lactate are not utilized. Nitrate is not reduced. Indole, lecithinase and lipase are not produced. Esculin is not hydrolyzed. Milk and meat are not digested (Love et al. 1979; Cato et al. 1985, 1986; Jalava and Eerola 2009). Peptidoglycan (position 3, bridge) is Orn, D-Asp (Ezaki 2009a). Comparison of other selected characteristics of species of the genus *Filifactor* is given in Table 24.3.

Proteocatella Pikuta, Hoover, Marsic, Whitman, Lupa, Tang, and Krader 2009

Pro.te.o.ca.tel'la. N.L. n. *proteinum* protein; N.L. pref. *proteo-* prefix referring to protein used in compound words; L. fem. n. *catella* small chain; N.L. fem. n. *Proteocatella* a small chain using proteins.

The genus *Proteocatella* includes so far only one species, *P. sphenisci*, represented by one strain PPP2^{T} (=ATCC BAA-755^{T}). Cells of *P. sphenisci* are flexible, motile rods, 0.7–0.8 × 3.0–5.0 μm, that tend to form long chains. Multiplies by fission, sometimes unequally with the formation of terminally round mini-cells. Motile by means of flagella. Forms spherical endospores in non-swollen sporangia. Cell wall has Gram-positive structure. Colonies are creamy yellow and rounded lens-shaped with a diameter of 1–2 mm. Obligate anaerobe with fermentative type of metabolism. Catalase-negative. Grows with peptone, bacto-tryptone, Casamino acids, oxalate, starch, chitin, and yeast extract. The strain grows on sodium oxalate only in medium supplemented with selenium as a trace element; cell morphology on this substrate was atypical, with a tendency for the cells to appear swollen and with a hexagonal crystalline shape. No growth is observed on formate, acetate, lactate, pyruvate, propionate, butyrate, citrate, methanol, ethanol, glycerol, acetone, D-mannitol, D-glucose, D-fructose, D-ribose, trehalose, D-arabinose, maltose, D-mannose, lactose, sucrose, cellobiose, pectin, N-acetylglucosamine, urea, trimethylamine, triethylamine, or betaine. Separate amino acids on mineral medium supplemented with yeast extract (0.1 g/l) do not support growth. The Stickland reaction is negative. End products of peptone fermentation are acetate, butyrate, ethanol, and minor amounts of hydrogen and carbon dioxide. NaCl range for growth is 0–4 % (w/v); optimal growth at 0.5 % (w/v) NaCl. Alkalitolerant, psychrotolerant mesophile (Pikuta et al. 2009). Temperature and pH ranges and optima for growth, major cellular fatty acids and G+C content of DNA are presented in Table 24.1.

Sporacetigenium Chen, Song and Dong 2006

Spo.ra.ce.ti.ge'ni.um. Gr. n. *spora* seed; L. n. *acetum* vinegar; Gr. v. *gennao* to produce; N.L. neut. n. *Sporacetigenium* spored vinegar (acetate) producer.

The genus *Sporacetigenium* includes so far only one species, *S. mesophilum*, represented by two strains. The physiological characteristics of the type strain ZLJ115^{T} (=DSM 16796^{T}) are described below. Cells of *S. mesophilum* are rods 0.9–1.0 × 3.6–7.3 μm in size, occurring singly or in short chains and motile by peritrichous flagella. The cells have Gram-positive wall structure; the peptidoglycan of the cell wall contains meso-DAP. Ovoid endospores are formed in the ends of cells, resulting in swollen cells. Colonies on PYG agar are milk white, smooth, circular, entire and translucent, slightly convex, and reaches 1 mm in diameter after cultivation at 37 °C for 48 h. Obligately anaerobic and chemo-organotrophic. Oxidase and catalase are not produced. Acid is produced from D-glucose, D-fructose, L-arabinose, D-xylose, and D-maltose. D-Galactose, D-mannose, cellobiose, sucrose, rhamnose, trehalose, melibiose, melezitose, and raffinose are fermented weakly. Acid is not produced from sorbose, starch, inulin, glycogen, salicin, amygdalin, glycerol, adonitol, dulcitol, erythritol, inositol, mannitol, or sorbitol. Fermentation of D-lactose and ribose is variable. The following

compounds are not utilized: methanol, ethanol, 1-propanol, citrate, fumarate, malate, succinate, malonic acid, hippurate, sodium gluconate, butanedioic acid, b-hydroxybutyric acid, phenylacetic acid, cellulose, and xylan. The major fermentation products from glucose are acetate, ethanol, hydrogen, and carbon dioxide. Peptone may serve as nitrogen source. Starch and aesculin are hydrolyzed, whereas gelatin is not. Milk is not curdled. Urease, lecithinase, and lipase are not produced. Methyl red test is positive while Voges–Proskauer test is negative. Nitrate, sulfate, and sulfur are not reduced. H_2S and NH_3 are produced from PYG. The strain could grow in the presence of 0–4 % (w/v) NaCl (Chen et al. 2006). Temperature and pH ranges and optima for growth, major cellular fatty acids and G+C content of DNA are presented in *Table 24.1*.

Tepidibacter Slobodkin, Tourova, Kostrikina, Chernyh, Bonch-Osmolovskaya, Jeanthon and Jones 2003, emend. Tan, Wu, Zhang, Wu and Zhu 2012

Te.pi.di.bac'ter. L. adj. *tepidus* warm; N.L. *bacter* masc. equivalent of Gr. neut. dim. n. *bakterion* rod; N.L. masc. n. *Tepidibacter* a warm rod.

Cells of *Tepidibacter* are straight to slightly curved rods 0.7–1.6 μm in diameter and 2.3–6.0 μm in length occurring singly, in pairs, or in short chains. Cells exhibit tumbling motility due to peritrichous flagellation. All three species of *Tepidibacter* have Gram-positive type cell wall and form round or ovoid refractile terminal or subterminal endospores. In the late-exponential phase of growth, up to 30 % of the cells of *T. thalassicus* contain spores. All *Tepidibacter* species form colonies in anaerobic agar. *T. thalassicus* and *T. formicigenes* are moderate thermophiles with temperature range for growth 30–60 °C; *T. mesophilus* is a mesophile with upper temperature limit of 40 °C. *T. thalassicus* and *T. formicigenes* grow at marine salinity; *T. mesophilus* shows the best growth with 0.5–1.0 % of NaCl but can tolerate up to 9 % of NaCl or sea salts. Members of *Tepidibacter* are anaerobic or aerotolerant (*T. mesophilus*) chemo-organotrophs. They ferment a number of proteinaceous substrates and carbohydrates and are able to perform the Stickland reaction. The best growth of *T. thalassicus* can be obtained on complex proteinaceous substrates such as tryptone, casein, and peptone or on starch. Carbohydrates, in the presence of yeast extract, slightly stimulate growth of *T. thalassicus*. Growth of *T. formicigenes* and *T. mesophilus* on sugars is more efficient. Differences in substrates utilization by *Tepidibacter* species are shown in *Table 24.4*. *T. thalassicus* and *T. mesophilus* reduce elemental sulfur to hydrogen sulfide, but sulfur reduction does not stimulate growth. All strains of *Tepidibacter* do not use nitrate, nitrite, Fe(III), sulfate, sulfite, and thiosulfate as electron acceptors. Oxidase and catalase activities are negative for all strains. All *Tepidibacter* species produce ethanol and acetate from glucose; in addition *T. thalassicus* forms moderate amounts of H_2 and CO_2, and *T. formicigenes* produces formate as a main fermentation product (Slobodkin et al. 2003; Slobodkin 2009; Urios et al. 2004; Tan et al. 2012). The major cellular fatty acid in the three species of *Tepidibacter* is iso-$C_{15:0}$, it constitutes 97.0, 77.7, and 51.8 % of the total fatty acids in *T. thalassicus, T. formicigenes*, and *T. mesophilus*, respectively (Tan et al. 2012). Differentiating characteristics of species of the genus *Tepidibacter* is given in *Table 24.4*.

Isolation, Enrichment, and Maintenance Procedures

Members of the family *Peptostreptococcaceae* can be enriched and isolated at anaerobic conditions in media that are rich in proteinaceous substances. All species have complex growth requirements, which may include vitamins, cofactors, and amino acids.

Peptostreptococcus anaerobius and *P. russellii* grow well on anaerobic chopped meat medium (DSM medium 78; http://dsmz.de). *P. stomatis* strains can be cultivated on fastidious anaerobe agar supplemented with 5 % horse blood in the atmosphere of 80 % N_2, 10 % H_2, 10 %, CO_2 or on anaerobic PYG medium supplemented with glucose (4.0 g/l), cellobiose (1.0 g/l), maltose (1.0 g/l), and soluble starch (1.0 g/l) (DSM medium 104 modified) (Downes and Wade 2006; Ezaki 2009b). Strain of *P. stomatis* was recovered from subgingival plaque using single-cell long-term cultivation method to minimize the effect of fast-growing microorganisms (Sizova et al. 2012). Colonies of *P. russellii* can be obtained on agar plates incubated in an anaerobic chamber in an atmosphere of CO_2:H_2 (96:4) on the medium containing buffer, salts, yeast extract (0.3 %), Bacto-Tryptone (1 %), and Casamino Acids (1 %) (Whitehead et al. 2011). All strains of *Peptostreptococcus* also grow on brain heart infusion medium under anaerobic conditions. In most cases, the temperature for enrichment, isolation, and cultivation of *Peptostreptococcus* species was 37 °C.

Acetoanaerobium noterae was isolated using anaerobic reduced mineral medium with H_2:CO_2 in the gas phase supplemented with 2.0 g/l of yeast extract. In the complete absence of yeast extract, H_2 was not utilized; at least 0.5 g/l was required for sustainable growth. *A. noterae* can also be cultivated without molecular hydrogen, on yeast extract (2.0 g/l) alone, or on maltose or glucose in the presence of 0.5 g/l of yeast extract. Vitamins are not required for the growth of this microorganism (Sleat et al. 1985).

Filifactor villosus can be isolated and grown on sheep blood agar plates and brain heart infusion agar plates incubated anaerobically. Cooked meat plus peptic digest of meat broth alone or supplemented with 0.4 % glucose, 0.1 % cellobiose, 0.1 % maltose, and 0.1 % starch can be used for cultivation of pure cultures (Love et al. 1979). Cultures of this microorganism can also be maintained on anaerobic chopped meat medium or chopped meat medium with carbohydrates (DSM medium 78 and DSM medium 110; http://dsmz.de). Fastidious anaerobic agar plates with and without 7 % (w/v) bovine blood have been used for cultivation of pure cultures of *F. alocis* under anaerobic conditions (Jalava and Eerola 1999).

Table 24.4

Differentiating characteristics of the species of the genus *Tepidibacter*

Characteristic	*T. thalassicus* DSM 15285^{T} [a, c]	*T. formicigenes* DSM 15518^{T} [b, c]	*T. mesophilus* JCM 16806^{T} [c]
Temperature range (°C)	33–60	35–55	10–40
Optimum temperature (°C)	50	28–32	45
pH range	4.8–8.5	5.0–8.0	6.0–8.9
Optimum pH	6.5–6.8	6.0	7.3
NaCl concentration (%, w/v) range	1.5–6.0	2.0–6.0	0–9.0
Optimum NaCl concentration (%, w/v)	2.0	3.0	0.5–1.0
S^0 reduction	+	–	+
Utilization of			
Albumin	+	–	–
Casein	+	–	+
Gelatin	–	+	–
Peptone	+	w	+
Yeast extract	+	w	+
D-fructose	–	–	+
D-galactose	–	–	+
D-glucose	w	+	+
Maltose	w	+	+
Mannose	–	w	–
D-ribose	–	–	+
L-rhamnose	–	–	+
Sucrose	–	+	–
Trehalose	–	–	+
D-xylose	–	–	+
L-arginine	w	–	–
L-valine	w	+	+
D-mannitol	–	+	–
Ethanol	–	w	–
Pyruvate	w	+	–
Fermentation products from glucose	Ethanol, acetate, H_2, CO_2	Formate, acetate, ethanol	Acetate, ethanol
Predominant cellular fatty acids (>5 %)	iso-$C_{15:0}$	iso-$C_{15:0}$, $C_{16:0}$	iso-$C_{15:0}$, $C_{14:0}$, $C_{16:0}$, $C_{16:1}$ cis9
DNA G+C content (mol%)	24	29	29.8

All strains utilized beef extract, tryptone, starch, DL-alanine plus L-proline, and DL-alanine plus L-glycine

None of the strains used L-arabinose, lactose, DL-alanine, L-glycine, acetate, betaine, butyrate, formate, fumarate, glycerol, lactate, methanol, D-sorbitol, succinate, urea, chitin, filter paper, or olive oil

Symbols and abbreviations: + positive, – negative, *w* weakly positive, *NR* not reported

Data from: [a]Slobodkin et al. 2003; [b]Urios et al. 2004; [c]Tan et al. 2012

Proteocatella sphenisci was enriched and isolated in a pure culture at the temperature +2 °C. Anaerobic mineral medium used for isolation included peptone (3 g/l) and yeast extract (0.2 g/l). Initial salinity of enrichments was 30 g/l of NaCl; however, the optimal NaCl concentration for pure culture of *P. sphenisci* was 5 g/l (Pikuta et al. 2009).

Strains of *Sporacetigenium mesophilum* were isolated and routinely cultivated in pre-reduced peptone/yeast extract/glucose medium (Chen et al. 2006). Type strain could be maintained in DSM medium 104b (PYX-medium, http://dsmz.de).

Thermophilic *Tepidibacter* species can be enriched in the temperature range of 45–55 °C in anaerobic medium of marine salinity supplemented with proteinaceous substrates (Slobodkin et al. 2003; Urios et al. 2004). *T. thalassicus*, *T. formicigenes*, and *T. mesophilus* were isolated in the presence of 0.2–1.0 g/l of yeast extract with casein (10 g/l), peptone (0.5 g/l), or Casamino acids (3 g/l) as a main carbon source, respectively. *T. thalassicus* rapidly hydrolyzes casein (Hammerstein grade) that results in visual disappearance of the casein flocks and may help in the detection of growth. All *Tepidibacter* strains form colonies in 1.5 % (w/v) anaerobic agar, and Hungate roll tube or agar block

techniques can be used for the isolation of a single colony (Hungate 1969). Members of the genus *Tepidibacter* may be maintained on the medium of Slobodkin et al. (2003) with peptone or casein as a substrate or on the glucose/yeast extract/peptone medium of Urios et al. (2004). All three species of *Tepidibacter* show a good growth on DSM medium 985 with 1 % peptone (Tan et al. 2012; http://dsmz.de). Reproducible growth of *T. thalassicus* and *T. mesophilus* can be obtained in liquid anaerobic medium lacking sulfide as a reducing agent (Slobodkin et al. 2003; Tan et al. 2012). Freeze-drying of the cultures results in good recovery. *T. mesophilus* may be preserved in 25 % glycerol at −80 °C. Liquid cultures of *T. thalassicus* may be stored at +4 °C for 10–12 months without loss of viability.

Ecology

Members of the family *Peptostreptococcaceae* were isolated from various environments including clinical human and animal samples, manure, soil, marine and terrestrial sediments, and deep-sea hydrothermal vents.

Peptostreptococcus species have been found in body and feces of humans and vertebrates. Taking into consideration that the group of the anaerobic Gram-positive cocci was subjected to major revision (Ezaki et al. 2006), it is difficult to determine the exact taxonomic status of the strains referred as the members of the genus *Peptostreptococcus* in reports dated before 1990s because of the absence of 16S rRNA gene sequence data. Numerous studies point to the presence of *Peptostreptococcus* strains in human oral cavity—*P. anaerobius* is a component of the human oral microbiome (Chen et al. 2010; http://www.homd.org); eight strains of *P. stomatis*, including the type strain, have been isolated from oral cavity (Downes and Wade 2006). Different phylotypes of *Peptostreptococcaceae* related to *Peptostreptococcus* species have been detected in oral samples by culture-independent methods (Paster et al. 2001; Munson et al. 2002; Sakamoto et al. 2004; Dewhirst et al. 2010). Strains of *P. anaerobius* have been isolated also from various human non-oral infection and abscesses and from intestine, vagina, and skin of healthy individuals (Downes and Wade 2006; Ezaki 2009b; Human Microbiome Jumpstart Reference Strains Consortium et al. 2010). Besides the human body, species of the genus *Peptostreptococcus* have been found in other vertebrate hosts. The presence of *Peptostreptococcus* spp. in canine oral cavity has been proven by culture-dependent and culture-independent techniques (Elliott et al. 2005; Dewhirst et al. 2012). *Peptostreptococcus* strains have been isolated from the rumen of dairy cows, deer, and sheep (Russell et al. 1988; Paster et al. 1993; Attwood et al. 1998). Most probably in the rumen ecosystem, *Peptostreptococcus* spp., that produce very high concentration of ammonia, but are not able to hydrolyze intact proteins and do not use carbohydrates, occupy a niche of peptide- and amino acid-degrading microorganism and depend on proteolytic bacteria (Attwood et al. 1998). One isolate of *Peptostreptococcus* has been obtained from feces of the mallard duck (Murphy et al. 2005). Seven strains of *P. russellii* have been isolated from a swine manure storage pit located near Peoria, IL, USA. In this habitat, concentration of the cells of *P. russellii* was at least 10^8 cells per ml and constituted approximately 0.1–1 % of the culturable bacterial population present in the swine manure samples (Whitehead et al. 2011).

The type strain of *Acetoanaerobium noterae* has been isolated from sediment of the Notera 3 oil exploration drilling site in the Hula swamp area of Galilee, Israel. The most probable number analysis of the drilling site sample yielded 1.75×10^5 H_2-oxidizing acetogens per gram (wet weight) (Sleat et al. 1985). Uncultured *Acetoanaerobium* clones (>98 % 16S rRNA gene sequence similarity with the type strain, sequence length >1,300 bp, retrieved from NCBI databases using BLAST, http://blast.ncbi.nlm.nih.gov) have been detected in production waters and sewage of oil reservoirs (accession numbers AY570564, DQ011249), wastewater treatment systems (FJ167476, AF234746, HE576030), and dechorinating microbial consortia (AJ488068, GQ377124).

Species of the genus *Filifactor* so far have been found only in human and animal samples. Eleven strains of *F. villosus* including the type strain have been isolated from subcutaneous abscesses of cats (Love et al. 1979). *F. alocis* is associated with oral cavity, and a large number of strains of this microorganism have been isolated from human gingival sulcus of patients with gingivitis or periodontitis, from oral cavities of cats, and from soft tissue infections of cats caused by contamination from oral cavities (Cato et al 1985; Love et al. 1987; Jalava and Eerola. 2009). *Filifactor* spp. are the components of the human and canine microbiomes where they have been detected by culture-independent methods (Dewhirst et al. 2010, 2012; Kong et al. 2012).

The type strain of *Proteocatella sphenisci* has been isolated from a sample of guano of the Magellanic penguin (*Spheniscus magellanicus*) in Chilean Patagonia. The physiological characteristics of *P. sphenisci*—tolerance to low temperature (down to 2 °C), high pH, and marine concentrations of NaCl—reflect the environmental conditions of the habitat. Magellanic penguins are endemic to the southern tip of South America, a region with a very cold climate. The ability of the strain to use exclusively products of proteolysis and oxalate (but not sugars) is probably due to the restricted diet of these penguins that feed on marine fish and crustaceans (Pikuta et al. 2009). Three different 16S rRNA gene sequences belonging to *Proteocatella* have been found in canine oral microbiome (Dewhirst et al. 2012) and human skin microbiome (GenBank accession number HM266866) (Kong et al. 2012). Other uncultured *Proteocatella* clones have been detected in various wastewater treatment systems (HM467987, FJ645707, CU925306), in river estuary of Northern Taiwan (DQ234248), and in a drinking water reservoir in Greece (GQ340217) (>98 % 16S rRNA gene sequence similarity with the type strain, sequence length >1,300 bp, retrieved from NCBI databases using BLAST, http://blast.ncbi.nlm.nih.gov).

Both strains of *Sporacetigenium mesophilum* have been isolated from the sludge of an anaerobic digester treating municipal solid waste and sewage in Zhangzhou city, Fujian province, PR China (Chen et al. 2006). Uncultured clones of *Sporacetigenium*

(>98 % 16S rRNA gene sequence similarity with the type strain, sequence length >1,300 bp, retrieved from NCBI databases using BLAST) have been detected in such different habitats such as anaerobic zones of Tinto River (JQ815621), shallow-sea hydrothermal vent Tutum Bay, Papua New Guinea (JN881597), alkaline lake Alchichica, Mexico (JN825560), tallgrass prairie soil (EU134685), and rhizosphere of reed (AB240265).

Thermophilic strains of *Tepidibacter* inhabit deep-sea hydrothermal vents. The type strain of *T. thalassicus* has been isolated from the outer wall of a actively venting hydrothermal sulfidic chimney-like deposit ("black smoker") covered with the polychetous annelid *Alvinella* spp. (13° N to the East-Pacific Rise, hydrothermal site Genesis, depth 2,650 m) (Slobodkin et al. 2003). Strain NS55-A that is closely phylogenetically related to *T. thalassicus* (16S rRNA gene sequence similarity—98.9 %) have been obtained from black exterior surface layer of hydrothermal chimney structure (North Big Chimney, Iheya North field in the Mid-Okinawa Trough, depth ca. 1,000 m) (Nakagawa et al. 2005). *T. formicigenes* has been isolated from hydrothermal fluid (the Menez-Gwen hydrothermal site, Mid-Atlantic Ridge, 37° 51′ N 31° 31′ W, depth 800–1,000 m) (Urios et al. 2004). Location of these vents, two of which are in different parts of Pacific Ocean and one is in Atlantic Ocean, suggests wide geographical distribution of *Tepidibacter* species in marine hydrothermal environments where they probably function as decomposers of organic matter produced by deep-sea biota. *Tepidibacter* spp. also inhabit terrestrial geothermal environments; *T. formicigenes* strain JB2 (99 % 16S rRNA gene sequence similarity with the type strain of *T. formicigenes*) has been obtained from Tunisian hot spring with salinity 1.8–2.0 % (Sayeh et al. 2010). Mesophilic representatives of the genus have been found in cold marine sediments and soils. Strain UXO3-5 (96.8–97.3 % 16S rRNA gene sequence similarity with *T. thalassicus* and *T. formicigenes*) has been isolated from marine sediments in unexploded ordnance disposal sites (800-m offshore Oahu Island, Hawaii, depth 10–21 m). This microorganism, obtained in pure culture under mesophilic conditions (21 °C), accounts for 4.5 % of total anaerobes in sediment (Zhao et al. 2007). *T. mesophilus* has been isolated from the completely different habitat—the soil polluted by crude oil (the Karamay Oil Field, 45° 36′ N 84° 57′ E, northwestern China). Ecological functions of *T. mesophilus* are currently unknown, but the aerotolerance of this strain suggests its adaptation to soil environments (Tan et al. 2012). Uncultured *Tepidibacter* clones (>95 % 16S rRNA gene sequence similarity with the type strains of *T. thalassicus, T. formicigenes*, and *T. mesophilus*, sequence length >1,300 bp, retrieved from NCBI databases using BLAST, http://blast.ncbi.nlm.nih.gov) have been detected in environmental samples and enrichment cultures obtained from cold and hydrothermal marine ecosystems: seaweed bed associated with marine hot springs on East Coast of Kalianda Island, Indonesia (accession number JQ670702); coastal soil of Gulf of Khambhat, India (JX240907); superficial sediments of Milazzo Harbor, Italy (AJ810557); and polluted coastal seawater in Tunisia (CU914830 to CU914837).

Pathogenicity: Clinical Relevance

Among the members of the family *Peptostreptococcaceae* pathogenicity are definitely shown only for the species of the genus *Filifactor*. Strains of this genus have been isolated from human gingival sulcus of patients with gingivitis or periodontitis, from oral cavities of cats, and from subcutaneous wound abscesses of cats; therefore, a pathogenic role of *Filifactor* in mixed anaerobic infections was suggested (Cato et al. 1985; Love et al. 1979, 1987). Association of *F. alocis* with periodontal diseases was also confirmed by culture-independent studies (Kumar et al. 2005; Siqueira and Rocas 2003). *F. alocis* is involved in the formation of periodontal biofilms in patients suffering from generalized aggressive periodontitis and chronic periodontitis and can be considered an excellent marker organism for periodontal disease. *F. alocis* predominantly colonized apical parts of the pocket in close proximity to the soft tissues and was involved in numerous structures that constitute characteristic architectural features of subgingival periodontal biofilms (Schlafer et al. 2010). *F. alocis* has virulence attributes that can enhance its persistence under oxidative stress conditions and mediate invasion of epithelial cells by *Porphyromonas gingivalis* (Aruni et al. 2011). Recently, the pathogenic mechanisms of *F. alocis* in periodontal diseases have been investigated. When infected with *F. alocis*, primary cultures of gingival epithelial cells (GECs) stimulate the secretion of the pro-inflammatory cytokines interleukin-1β, interleukin-6, and tumor necrosis factor-α. *F. alocis* also induced apoptosis in GECs through pathways that involved caspase-3 but not caspase-9. Apoptosis was coincident with inhibition of mitogen-activated protein kinase kinase activation (Moffatt et al. 2011).

Pathogenicity of the members of the genus *Peptostreptococcus* currently is difficult to assess. In the Internet-available medical literature, there are about 2,000 references about involvement of *Peptostreptococcus* in clinical cases. However, in the majority of these studies, the data on 16S rRNA gene sequence are not provided, so it is impossible to determine if the authors are dealing with the species of the genus *Peptostreptococcus sensu stricto* or with the other Gram-positive anaerobic cocci. Information about clinical relevance and pathogenicity of Gram-positive anaerobic cocci before taxonomic revision of this group is summarized in review by Murdoch (1998). Representatives of the genus *Peptostreptococcus* are frequently isolated from clinical samples of healthy and sick individuals (see section "Ecology"). Strains of *P. anaerobius* have been isolated from leg ulcer, urinary tract infection, ankle wound, buttock abscess, and vaginal infection (Downes and Wade 2006). *P. anaerobius* can cause primary sternal osteomyelitis (Chen et al. 2012). Strains of *P. stomatis* have been isolated from dento-alveolar abscesses, endodontic infections, a periodontal pocket, and from a pericoronal infection (Downes and Wade 2006). Recent culture-independent studies show that acute noma disease (gangrenous disease that leads to severe disfigurement of the face) and necrotizing gingivitis are associated with large increase in counts of members of the *Peptostreptococcus* genus (Bolivar et al. 2012). On the other hand, species of *Peptostreptococcus* are a part of the normal oral and vaginal microflora (Zhou et al. 2004; Aas et al. 2005).

Table 24.5
Antibiotic sensitivity of the members of the family *Peptostreptococcaceae*

Microorganism	Number of strains tested	Sensitive	Resistant
Peptostreptococcus anaerobius[a]	9	Amoxicillin-clavulanic acid (0.12), cefoxitin (0.25), ciprofloxacin (0.5), clindamycin (0.06), imipenem (0.03), metronidazole (0.06), penicillin G (0.03), piperacillin-tazobactam (0.5), trovafloxacin (0.06)	NR
Acetoanaerobium noterae[b]	1, the type strain	Cephalosporin, chloramphenicol, cycloserine, erythromycin, penicillin, (all at 100)	NR
Filifactor alocis[c]	20 strains, including the type strain	Chloramphenicol (12), clindamycin (1.6), erythromycin (3), tetracycline (6)	Penicillin (2)[d]
Filifactor villosus[e]	1, the type strain	Amoxycillin (2.5), carbenicillin (100), chloramphenicol (12), doxycycline (6), erythromycin (3), penicillin (2)	NR
Proteocatella sphenisci[f]	1, the type strain	Gentamicin, kanamycin, rifampicin, tetracycline, vancomycin (250), chloramphenicol (125)	Ampicillin (250)

Concentrations of antibiotics in parentheses are given in μg/ml except for penicillin for which U/ml is used. For *Peptostreptococcus anaerobius*, minimal inhibitory concentration (MIC_{50}) is presented
NR not reported
Data from: [a]Bowker et al. 1996; [b]Sleat et al. 1985; [c]Cato et al. 1985; [d]One of 20 strains; [e]Love et al. 1979; [f]Pikuta et al. 2009

There are no reports on pathogenicity and medical relevance of the representatives of *Acetoanaerobium*, *Proteocatella*, *Sporacetigenium*, and *Tepidibacter*. No strains of these genera were found in clinical samples.

Antibiotic sensitivity of the members of genera *Peptostreptococcus*, *Acetoanaerobium*, *Filifactor*, and *Proteocatella* is shown in *Table 24.5*. Majority of the strains are susceptible to penicillin and chloramphenicol. It is worth to note that *Proteocatella sphenisci* is resistant to ampicillin, a characteristic that is rare in environmental bacterial strains.

Application

To date, microorganisms belonging to *Peptostreptococcaceae* did not find any application in industrial or bioremediation processes; however, there are a number of reports about biotechnological potential of the members of the family.

Peptostreptococcus russellii may play a role in swine manure management. It produces prodigious amounts of ammonia (> 40 mM) from different nitrogen sources (Tryptone and Casamino acids) and belongs to the so-called hyper-ammonia-producing microorganisms. This group of organisms may be important in the digestion and fermentation of proteinaceous material in the manure and the production of ammonia and other compounds (Attwood et al. 1998; Whitehead and Cotta 2004). The fermentation of amino acids such as tryptophan, phenylalanine, and tyrosine may give rise to the production of indole and phenolic compounds (such as skatole), contributing to the foul odors associated with swine facilities (Whitehead et al. 2011).

Tepidibacter thalassicus has the potential for immobilization of radionuclides such as technetium(VII). Washed cell suspensions of *T. thalassicus* completely reduced technetium [^{99}Tc(VII)], supplied as soluble pertechnetate with molecular hydrogen or peptone as an electron donor, forming highly insoluble Tc(IV)-containing grayish-black precipitate. This capacity can be used during bioremediation of thermally insulated contaminated environments and in biotechnological treatment of the heated nuclear waste streams (Chernyh et al. 2007). Under mesophilic conditions, *Tepidibacter* sp. strain UXO-3-5 can metabolize octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), a toxic explosive known to be resistant to biodegradation. This organism plays a significant role in HMX removing in sites of undersea deposition of unexploded ordnance in Hawaii (Zhao et al. 2007).

Production of acetate from molecular hydrogen by *Acetoanaerobium noterae* and formation of H_2 during fermentation of glucose by *Sporacetigenium mesophilum* also deserve attention for biotechnological applications (Sleat et al. 1985; Chen et al. 2006).

References

Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE (2005) Defining the normal bacterial flora of the oral cavity. J Clin Microbiol 43:5721–5732

Aruni AW, Roy F, Fletcher HM (2011) *Filifactor alocis* has virulence attributes that can enhance its persistence under oxidative stress conditions and mediate invasion of epithelial cells by *Porphyromonas gingivalis*. Infect Immun 79:3872–3886

Attwood GT, Klieve AV, Ouwerkerk D, Patel BK (1998) Ammonia-hyper producing bacteria from New Zealand ruminants. Appl Environ Microbiol 64:1796–1804

Bolivar I, Whiteson K, Stadelmann B, Baratti-Mayer D, Gizard Y, Mombelli A, Pittet D, Schrenze J, Geneva Study Group on Noma (GESNOMA) (2012) Bacterial diversity in oral samples of children in niger with acute noma, acute necrotizing gingivitis, and healthy controls. PLoS Negl Trop Dis 6:e1556

Bowker KE, Wootton M, Holt HA, Reeves DS, MacGowan AP (1996) The in-vitro activity of trovafloxacin and nine other antimicrobials against 413 anaerobic bacteria. J Antimicrob Chemother 38:271–281

Cato EP, Moore LVH, Moore WEC (1985) *Fusobacterium alocis* sp. nov. and *Fusobacterium sulci* sp. nov. from the human gingival sulcus. Int J Syst Bacteriol 35:475–477

Cato EP, George WL, Finegold SM (1986) Genus *Clostridium* Prazmowski 1880, 23AL. In: Sneath PHA, Mair NS, Sharpe ME, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 2. The Williams & Wilkins, Baltimore, pp 1141–1200

Chen S, Song L, Dong X (2006) *Sporacetigenium mesophilum* gen. nov., sp. nov., isolated from an anaerobic digester treating municipal solid waste and sewage. Int J Syst Evol Microbiol 56:721–725

Chen T, W-Han Yu, Izard J, Baranova OV, Lakshmanan A, Dewhirst FE (2010) The human oral microbiome database: a web accessible resource for investigating oral microbe taxonomic and genomic information. Database 2010. doi: 10.1093/database/baq013. Article ID baq013

Chen YL, Tsai SH, Hsu KC, Chen CS, Hsu CW (2012) Primary sternal osteomyelitis due to *Peptostreptococcus anaerobius*. Infection 40:195–197

Chernyh NA, Gavrilov SN, Sorokin VV, German KE, Sergeant C, Simonoff M, Robb F, Slobodkin AI (2007) Characterization of technetium(VII) reduction by cell suspensions of thermophilic *Bacteria* and *Archaea*. Appl Microbiol Biotechnol 76:467–472

Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, Kulam-Syed-Mohideen AS, McGarrell DM, Marsh T, Garrity GM, Tiedje JM (2009) The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res 37 (Database issue):D141–145

Collins MD, Lawson PA, Willems A, Cordoba JJ, Fernández-Garayzábal J, Garcia P, Cai J, Hippe H, Farrow JAE (1994) The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. Int J Syst Bacteriol 44:812–826

Dewhirst FE, Chen T, Izard J, Paster BJ, Tanner ACR, Yu W-H, Lakshmanan A, Wade WG (2010) The human oral microbiome. J Bacteriol 192:5002–5017

Dewhirst FE, Klein EA, Thompson EC, Blanton JM, Chen T, Milella L, Buckley CM, Davis IJ, Bennett ML, Marshall-Jones ZV (2012) The canine oral microbiome. PLoS One 7:e36067

Downes J, Wade WG (2006) *Peptostreptococcus stomatis* sp. nov., isolated from the human oral cavity. Int J Syst Evol Microbiol 56:751–754

Elliott DR, Wilson M, Buckley CM, Spratt DA (2005) Cultivable oral microbiota of domestic dogs. J Clin Microbiol 43:5470–5476

Ezaki T (2009a) *Peptostreptococcaceae* fam. nov. In: DeVos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman WB (eds) Bergey's manual of systematic bacteriology, vol 3, 2nd edn. Springer, New York, p 1008

Ezaki T (2009b) Genus *Peptostreptococcus*. In: DeVos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman WB (eds) Bergey's manual of systematic bacteriology, vol 3, 2nd edn. Springer, New York, pp 1008–1009

Ezaki T, Kawamura Y, Li N, Li ZY, Zhao L, Shu S (2001) Proposal of the genera *Anaerococcus* gen. nov., *Peptoniphilus* gen. nov. and *Gallicola* gen. nov. for members of the genus *Peptostreptococcus*. Int J Syst Evol Microbiol 51:1521–1528

Ezaki T, Li N, Kawamura Y (2006) The anaerobic Gram-positive cocci. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) Prokaryotes, vol 4, 3rd edn. Springer, New York, pp 785–808

Holdeman Moore LV, Johnson JL, Moore WEC (1986) Genus *Peptostreptococcus*. In: Sneath PHA, Mair NS, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 2. The Williams & Wilkins, Baltimore, pp 1083–1092

Human Microbiome Jumpstart Reference Strains Consortium et al (2010) A catalog of reference genomes from the human microbiome. Science 328:994–999

Hungate RE (1969) A roll tube method for cultivation of strict anaerobes. In: Norris JR, Ribbons RW (eds) Methods in microbiology, vol 3B. Academic Press, London, pp 117–132

Jalava J, Eerola E (1999) Phylogenetic analysis of *Fusobacterium alocis* and *Fusobacterium sulci* based on 16S rRNA gene sequences: proposal of *Filifactor alocis* (Cato, Moore and Moore) comb. nov. and *Eubacterium sulci* (Cato, Moore and Moore) comb. nov. Int J Syst Bacteriol 49:1375–1379

Jalava J, Eerola E (2009) Genus *Filifactor*. In: DeVos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman WB (eds) Bergey's manual of systematic bacteriology, vol 3, 2nd edn. Springer, New York, pp 1009–1013

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA Gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721

Kluyver AJ, van Neil CB (1936) Prospects for a natural classification of bacteria. Zentbl Bacteriol Parasitenkd Infektionskr Hyg Abt II 94:369–403

Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beatson MA, Nomicos E, Polley EC, Komarow HD, Murray PR, Turner ML, Segre JA (2012) Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. Genome Res 22:850–859

Kumar PS, Griffen AL, Moeschberger ML, Leys EJ (2005) Identification of candidate periodontal pathogens and beneficial species by quantitative 16S clonal analysis. J Clin Microbiol 43:3944–3955

Love DN, Jones RF, Bailey M (1979) *Clostridium villosum* sp. nov. from subcutaneous abscesses in cats. Int J Syst Bacteriol 29:241–244

Love DN, Cato EP, Johnson JL, Jones RF, Bailey M (1987) Deoxyribonucleic acid hybridization among strains of Fusobacteria isolated from soft tissue infections of cats: comparison with human and animal type strains from oral and other sites. Int J Syst Bacteriol 37:23–26

Ludwig W, Schleifer K-H, Whitman WB (2009) Revised road map to the phylum Firmicutes. In: DeVos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman WB (eds) Bergey's manual of systematic bacteriology, vol 3, 2nd edn. Springer, New York, pp 1–13

Moffatt CE, Whitmore SE, Griffen AL, Leys EJ, Lamont RJ (2011) *Filifactor alocis* interactions with gingival epithelial cells. Mol Oral Microbiol 26:365–373

Munson MA, Pitt-Ford T, Chong B, Weightman AJ, Wade WG (2002) Molecular and cultural analysis of the microflora associated with endodontic infections. J Dent Res 81:761–766

Murdoch DA (1998) Gram-positive anaerobic cocci. Clin Microbiol Rev 11:81–120

Murphy J, Devane ML, Robson B, Gilpin BJ (2005) Genotypic characterization of bacteria cultured from duck faeces. J Appl Microbiol 99:301–309

Nakagawa S, Takai K, Inagaki F, Chiba H, Ishibashi J, Kataoka S, Hirayama H, Nunoura T, Horikoshi K, Sako Y (2005) Variability in microbial community and venting chemistry in a sediment-hosted backarc hydrothermal system: impacts of subseafloor phase-separation. FEMS Microbiol Ecol 54:141–155

Paster BJ, Russell JB, Yang CMJ, Chow JM, Woese CR, Tanner R (1993) Phylogeny of the ammonia-producing ruminal bacteria *Peptostreptococcus anaerobius*, *Clostridium sticklandii*, and *Clostridium aminophilum* sp. nov. Int J Syst Bacteriol 43:107–110

Paster BJ, Boches SK, Galvin JL, Ericson RE, Lau CN, Levanos VA, Sahasrabudhe A, Dewhirst FE (2001) Bacterial diversity in human subgingival plaque. J Bacteriol 183:3770–3783

Pikuta EV, Hoover RB, Marsic D, Whitman WB, Lupa B, Tang J, Krader P (2009) *Proteocatella sphenisci* gen. nov., sp. nov., a psychrotolerant, spore-forming anaerobe isolated from penguin guano. Int J Syst Evol Microbiol 59:2302–2307

Prévot AR (1953) In: Hauduroy P, Ehringer G, Guillot G, Magrou J, Prévot CAR, Rosset D, Urbain A (eds) Dictionnaire des Bactéries Pathogènes, 2nd edn. Paris, Masson

Pruesse E, Quast C, Knittel K, Fuchs B, Ludwig W, Peplies J, Glöckner FO (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nuc Acids Res 35:7188–7196

Russell JB, Strobel HJ, Chen G (1988) Enrichment and isolation of a ruminal bacterium with a very high specific activity of ammonia production. Appl Environ Microbiol 54:872–877

Sakamoto M, Huang Y, Ohnishi M, Umeda M, Ishikawa I, Benno Y (2004) Changes in oral microbial profiles after periodontal treatment as determined by molecular analysis of 16S rRNA genes. J Med Microbiol 53:563–571

Sayeh R, Birrien JL, Alain K, Barbier G, Hamdi M, Prieur D (2010) Microbial diversity in Tunisian geothermal springs as detected by molecular and culture-based approaches. Extremophiles 14:501–514

Schlafer S, Riep B, Griffen AL, Petrich A, Hübner J, Berning M, Friedmann A, Göbel UB, Moter A (2010) Filifactor alocis–involvement in periodontal biofilms. BMC Microbiol 10:66

Siqueira JF, Rocas IN (2003) Detection of *Filifactor alocis* in endodontic infections associated with different forms of periradicular diseases. Oral Microbiol Immunol 18:263–265

Sizova MV, Hohmann T, Hazen A, Paster BJ, Halem SR, Murphy CM, Panikov NS, Epstein SS (2012) New approaches for isolation of previously uncultivated oral bacteria. Appl Environ Microbiol 78:194–203

Sleat R, Mah RA, Robinson R (1985) *Acetoanaerobium noterae* gen. nov., sp. nov.: an anaerobic bacterium that forms acetate from H_2 and CO_2. Int J Syst Bacteriol 35:10–15

Slobodkin A (2009) Genus *Tepidibacter*. In: DeVos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman WB (eds) Bergey's manual of systematic bacteriology, vol 3, 2nd edn. Springer, New York, pp 1013–1015

Slobodkin AI, Tourova TP, Kostrikina NA, Chernyh NA, Bonch-Osmolovskaya EA, Jeanthon C, Jones BE (2003) *Tepidibacter thalassicus* gen. nov., sp. nov., a novel moderately thermophilic, anaerobic, fermentative bacterium from a deep-sea hydrothermal vent. Int J Syst Evol Microbiol 53:1131–1134

Tan HQ, Wu XY, Zhang XQ, Wu M, Zhu XF (2012) *Tepidibacter mesophilus* sp. nov., a mesophilic fermentative anaerobe isolated from soil polluted by crude oil, and emended description of the genus *Tepidibacter*. Int J Syst Evol Microbiol 62:66–70

Urios L, Cueff V, Pignet P, Barbier G (2004) *Tepidibacter formicigenes* sp. nov., a novel spore-forming bacterium isolated from a Mid-Atlantic Ridge hydrothermal vent. Int J Syst Evol Microbiol 54:439–443

Whitehead TR, Cotta MA (2004) Isolation and identification of hyper-ammonia producing bacteria from swine manure storage pits. Curr Microbiol 48:20–26

Whitehead TR, Cotta MA, Falsen E, Moore E, Lawson PA (2011) *Peptostreptococcus russellii* sp. nov., isolated from a swine-manure storage pit. Int J Syst Evol Microbiol 61:1875–1879

Zhao JS, Manno D, Hawari J (2007) Abundance and diversity of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)-metabolizing bacteria in UXO-contaminated marine sediments. FEMS Microbiol Ecol 59:706–717

Zhou X, Bent SJ, Schneider MG, Davis CC, Islam MR, Forney LJ (2004) Characterization of vaginal microbial communities in adult healthy women using cultivation-independent methods. Microbiology 150:2565–2573