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# *Fretibacterium fastidiosum* gen. nov., sp. nov., isolated from the human oral cavity

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SGP1$^T$, a strain belonging to a lineage of the phylum *Synergistetes* with no previously cultivated representatives was subjected to a comprehensive range of phenotypic and genotypic tests. For good growth the strain was dependent on co-culture with, or extracts from, selected other oral bacteria. Cells of strain SGP1$^T$ were asaccharolytic and major amounts of acetic acid and moderate amounts of propionic acid were produced as end products of metabolism in peptone-yeast extract-glucose broth supplemented with a filtered cell sonicate of *Fusobacterium nucleatum* subsp. *nucleatum* ATCC 25586$^T$ (25 %, v/v). Hydrogen sulphide was produced and gelatin was weakly hydrolysed. The major cellular fatty acids were $C_{14:0}$, $C_{18:0}$ and $C_{16:0}$. The DNA G+C content of strain SGP1$^T$ was 63 mol%. Phylogenetic analysis of the full-length 16S rRNA gene showed that strain SGP1$^T$ represented a novel group within the phylum *Synergistetes*. A novel species in a new genus, *Fretibacterium fastidiosum* gen. nov., sp. nov., is proposed. The type strain of *Fretibacterium fastidiosum* is SGP1$^T$ (=DSM 25557$^T$=JCM 16858$^T$).

Strain SGP1$^T$, representing a previously uncultivated branch of the recently described phylum *Synergistetes* (Jumas-Bilak *et al.*, 2009), was isolated from the human mouth by means of colony-hybridization-directed enrichment and co-culture with other oral bacteria (Vartoukian *et al.*, 2010). The strain remains dependent on stimulation by other oral bacteria for growth in broth culture which makes phenotypic characterizations difficult. The aim of this study was to subject strain SGP1$^T$ to a range of phenotypic and genetic tests, to allow its formal description and naming. Due to the need for factors from other bacteria for growth, it was not possible to perform some tests which would ideally have been included in a description of a novel species from this group.

Strain SGP1$^T$ was isolated from a deep periodontal pocket as described previously (Vartoukian *et al.*, 2010) and *Fusobacterium nucleatum* subsp. *nucleatum* ATCC 25586$^T$ was obtained from the ATCC. The strains were cultivated on Blood Agar (BA) plates [Blood Agar Base No. 2 (LabM, UK) supplemented with 5 % horse blood] at 37 °C under anaerobic conditions in an anaerobic workstation (Don Whitley Scientific). Unless otherwise specified, cultures of strain SGP1$^T$ on BA plates were cross-streaked with *Fusobacterium nucleatum* subsp. *nucleatum* ATCC 25586$^T$ for growth stimulation. Strain SGP1$^T$ was also cultured in Nutrient Broth no. 2 (NB; Oxoid Ltd) supplemented with 1 % yeast extract and filtered culture supernatants (CF; 50 %, v/v) or filtered cell sonicates (CS; 25 %) from three-day broth cultures of *Fusobacterium nucleatum* subsp. *nucleatum* ATCC 25586$^T$. CF was prepared by centrifuging the culture and passing the supernatant through a 0.2 μm filter. For preparation of CS, the pellet (derived from centrifugation of a 20 ml broth culture) was resuspended in 5 ml PBS, sonicated for three pulses of two minutes each, centrifuged and the supernatant filtered as above. Growth in broth cultures was assessed using spectrophotometric measurements of turbidity at 550 nm up to 70 days of anaerobic incubation.

Colonial morphologies were determined using a dissecting microscope after incubation for 21 days. Cellular morphology was recorded after Gram-staining of smears prepared from BA cultures at 14 days. 14-day NB broth cultures with CF (50 %) were examined for cellular motility using phase-contrast microscopy of hanging-drop preparations. Growth on BA plates at 25, 37 and 42 °C was assessed after 21 days of anaerobic incubation. Growth in NB supplemented with CF (50 %) at pH 5, 6, 7, 8 and 9 was also assessed following up to 70 days of anaerobic incubation at 37 °C.

Biochemical and physiological tests were performed using standard methods (Holdeman *et al.*, 1977;

Abbreviations: CF, filtered culture supernatants; CS, filtered culture sonicates.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Fretibacterium fastidiosum* SGP1$^T$ is GQ149247.

Two supplementary figures and a supplementary table are available with the online version of this paper.

Jousimies-Somer *et al.*, 2002), with tests for hydrogen sulphide, aesculin and nitrate performed in broth media supplemented with CF (50 %). Fermentation of sugars was tested in peptone-yeast extract (PY) broth (Holdeman *et al.*, 1977), prepared in the anaerobic workstation and supplemented with pre-reduced CF (50 %) immediately prior to inoculation, resulting in a final sugar concentration of 1 %. Strain SGP1$^T$ was grown in peptone-yeast extract-glucose (PYG) broth (Holdeman *et al.*, 1977) supplemented with CS (25 %), and short-chain volatile and non-volatile fatty acids were extracted and analysed using gas chromatography to determine the metabolic end products (Holdeman *et al.*, 1977). An uninoculated PYG broth supplemented with CS (25 %) was run as a control. Enzyme profiles were generated with the API ZYM panel (bioMérieux) and the Rapid ID 32A anaerobe identification kit (bioMérieux), tested in triplicate. BA plate cultures used to prepare the test inocula for the API ZYM, Rapid ID 32A, urease, arginine dihydrolase and indole spot tests were not cross-streaked with *Fusobacterium. nucleatum* subsp. *nucleatum* ATCC 25586$^T$ to avoid contaminating enzyme activity. Growth from 10 BA plates incubated anaerobically for 18 days was harvested to prepare sufficient inoculum for each test.

Susceptibility to amoxicillin (10 μg), ampicillin (10 μg), bacitracin (0.04 units), cefadroxil (30 μg), chloramphenicol (10 μg), ciprofloxacin (1 μg), erythromycin (5 μg), gentamicin (10 μg), metronidazole (5 μg), penicillin (1 unit), trimethoprim (2.5 μg) and vancomycin (30 μg) (all Oxoid) was determined, in triplicate, using a disc diffusion method on BA plates cross-streaked with *Fusobacterium nucleatum* subsp. *nucleatum* ATCC 25586$^T$. It was possible to test one antimicrobial per plate by this method. Zones of inhibition were recorded at 12 and 18 days incubation.

Analysis of cellular fatty acids was carried out by the Identification Service of the DSMZ, Braunschweig, Germany. Fatty acid methyl esters were obtained from 50 mg (dry weight) cells derived from 46-day CF-supplemented NB cultures by saponification, methylation and extraction using minor modifications of previously described methods (Kuykendall *et al.*, 1988; Miller, 1982). The fatty acid methyl esters mixtures were separated using the Sherlock Microbial Identification System (MIS) (version 6.1; MIDI, Microbial ID) which consisted of a gas chromatograph (6890N; Agilent) fitted with a 5 % phenyl-methyl silicone capillary column (0.2 mm × 25 m), a flame-ionization detector, an automatic sampler (7683A; Agilent), and a Hewlett Packard computer with MIDI TSBA40 database. Peaks were automatically integrated and fatty acid names and percentages calculated by the MIS Standard Software (Microbial ID). The gas chromatographic parameters were as follows: carrier gas, ultra-high-purity hydrogen; column head pressure 60kPa; injection volume 2 μl; column split ratio, 100 : 1; septum purge 5 ml min$^{-1}$; column temperature, 170 to 270 °C at 5 °C min$^{-1}$; injection port temperature, 240 °C; and detector temperature, 300 °C.

Phylogenetic analyses were performed using the 16S rRNA gene sequence previously determined for SGP1$^T$ (Vartoukian *et al.*, 2010) and the RpoB amino acid sequence obtained from the genome sequence (GenBank accession number FP929056).

Cells of strain SGP1$^T$ were obligately anaerobic, motile, Gram-stain-negative, curved bacilli measuring 1–1.5 μm in width and ranging from 2–13 μm in length (mean 8 μm), with cells appearing elongated in older cultures. Colonies of strain SGP1$^T$ on BA had a circular, off-white, opaque central region with a high convex profile and smooth surface, which measured approximately 0.5 mm in diameter after 21 days incubation and was surrounded by a semi-translucent periphery with a highly irregular shape (Fig. S1a, available in IJSEM online). Variations were sometimes observed in this basic colony form and included absence of a spreading periphery, convoluted surface, and yellow pigmentation. These colonial variations were found to be unstable and colony morphology changed reversibly from one form to another on passage. However, after 3 months of serial cultivation on BA plates, a novel and distinct colonial morphotype was observed in addition to the original, and this form was stable on further subculture. This colony type was approximately 1.3 mm in diameter after 21 days incubation on BA, with a slightly irregular shape, crinkled edge, low convex profile and smooth surface, and showed a gradual variation in colour and translucency from an opaque, grey/off-white central region to a grey, semi-translucent outer region (Fig. S1b). Cells harvested from each of the two predominant colonial morphotypes of strain SGP1$^T$ were found to be of identical cellular morphology and 16S rRNA gene sequences, confirming purity. All morphotypes required co-culture with *Fusobacterium nucleatum* subsp. *nucleatum* ATCC 25586$^T$ for optimum growth. Independent culture of SGP1$^T$ on BA resulted in a variable amount of growth, ranging from no growth to, at best, a biomass equivalent to approximately a quarter of that resulting from co-culture with the feeder strain.

Strain SGP1$^T$ was unable to grow in non-supplemented NB and PY broth cultures, with $OD_{550}$ readings of less than 0.1. However, growth was achieved following the addition of CF (50 %) or CS (25 %) to broth cultures, with $OD_{550}$ readings ranging from 0.36 to 0.65 (mean, 0.52) for CF and 0.11 to 0.70 (mean, 0.30) for CS after 46 days incubation at 37 °C. Addition of 10 mM glycine to NB did not stimulate growth. The optimum temperature for growth was 37 °C with only very minimal growth (1–2 colonies) at 25 °C and 42 C. Optimal growth was achieved at pH 6 (maximum $OD_{550}$, 0.42 at 35 days) and pH 7 (maximum $OD_{550}$, 0.81 at 64 days) with minimal growth at pH 8 ($OD_{550}$ 0.14) and no growth at pH 5 and pH 9 ($OD_{550}$ <0.1).

Acid was not produced from any of the sugars tested (for details see species description). Major amounts of acetic acid and moderate amounts of propionic acid were produced as end products of metabolism in PYG

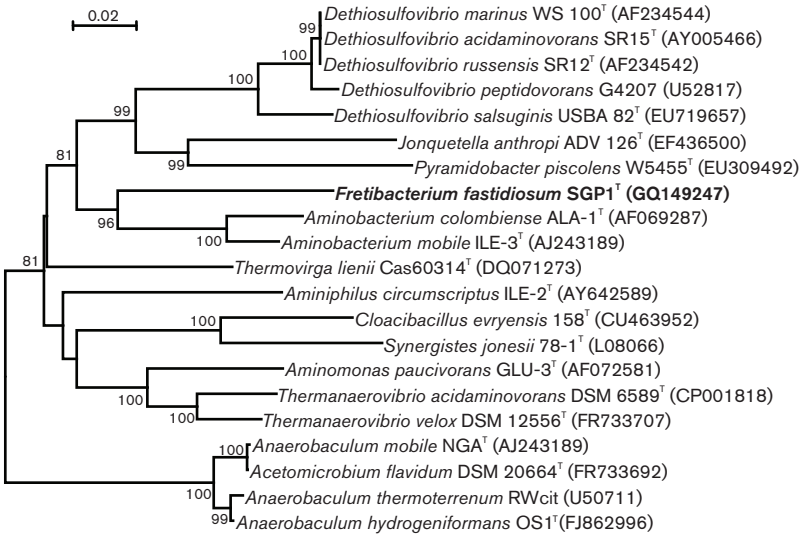


**Fig. 1.** Phylogenetic tree based on 16S rRNA gene sequence comparisons over 1178 aligned bases showing relationships between strain SGP1$^T$, and representatives of other genera within the phylum *Synergistetes*. The tree was constructed using the neighbour-joining method following distance analysis of aligned sequences. Numbers represent bootstrap values for each branch based on data for 1000 trees. Bar, 0.02 nucleotide substitutions per site.

supplemented with CS (25 %). Gelatin was weakly hydrolysed and hydrogen sulphide was produced. All other tests gave negative results (see genus and species descriptions). API ZYM tests for α-chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase were strongly positive while weak positive reactions were seen for C4 esterase and C8 esterase lipase. In the Rapid ID 32A panel, tests for leucyl glycine arylamidase and glycine arylamidase were positive while the remaining 27 tests were negative, resulting in a profile of 0000 0404 00. The DNA G+C content of strain SGP1$^T$ was 63 mol%, as determined from analysis of the genome sequence.

Fatty acid methyl ester analysis revealed a predominance of $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$ fatty acids (Table S1), which together made up 68 % of the total fatty acids, a profile unlike that of other members of the phylum *Synergistetes* previously studied (Downes *et al.*, 2009). This is further confirmation of the phenotypic heterogeneity of taxa within this phylum.

SGP1$^T$ was susceptible to amoxicillin, ampicillin, cefadroxil, chloramphenicol, metronidazole and penicillin (with zones of inhibition measuring greater than 35 mm in diameter); moderately susceptible to erythromycin, gentamicin and vancomycin (zone diameters of 13–22 mm); and resistant to bacitracin, ciprofloxacin and trimethoprim (no zone).

Phylogenetic analyses of the 16S rRNA gene and RpoB amino acid sequences (Figs 1 and S2, respectively) confirmed the s identity of strain SGP1$^T$ as a member of the phylum *Synergistetes*. The strain belongs to oral taxon 363 in the Human Oral Microbiome database (www.homd.org). The most closely related species with validly published name was *Aminobacterium colombiense* ALA-1$^T$ with a 16S rRNA gene sequence similarity of 86.7 % to strain SGP1$^T$. This clearly indicated that strain SGP1$^T$ represented a novel species in a new genus, for which the name *Fretibacterium fastidiosum* is proposed. A summary of characteristics which differentiate the genus *Fretibacterium* from other genera of the phylum *Synergistetes* is presented in Table 1.

## Description of *Fretibacterium* gen. nov.

*Fretibacterium* (Fre.ti.bac.te′ri.um. L. adj. *fretus* depending, depending on; L. neut. n. *bacterium* a rod; N.L. neut. n. *Fretibacterium* depending rod, referring to the dependence of this organism on co-culture for good growth).

Cells are obligately anaerobic, motile, Gram-stain-negative, curved bacilli. Stimulation from a feeder strain such as *Fusobacterium nucleatum* subsp. *nucleatum* ATCC 25586$^T$ is required for good growth, both on agar plates and in broth cultures. Glycine does not stimulate growth. Optimum temperature for growth is 37 °C, with minimal growth at 25 °C and 42 °C. The optimum pH for growth is 6–7, with minimal growth at pH 8 and no growth at pH 5 or pH 9. Cells are asaccharolytic and major amounts of acetic acid and moderate amounts of propionic acid are produced as end products of metabolism in peptone-yeast extract-glucose broth supplemented with a filtered cell sonicate of *Fusobacterium nucleatum* subsp. *nucleatum* ATCC 25586$^T$ (25 %, v/v). Hydrogen sulphide is produced, but catalase and indole are not produced. Gelatin is weakly hydrolysed; aesculin, arginine and urea are not hydrolysed. Nitrate is not reduced. The major cellular fatty acids are $C_{14:0}$, $C_{18:0}$ and $C_{16:0}$. The DNA G+C content of *Fretibacterium fastidiosum*, the only species so far described in this genus, is 63 mol%. The type species is *Fretibacterium fastidiosum*.

## Description of *Fretibacterium fastidiosum* gen. nov., sp. nov.

*Fretibacterium fastidiosum* (fas.ti.di.o′sum L. neut. adj. *fastidiosum* fastidious, referring to the nutritional requirements of the organism).

**Table 1.** Differential characteristics of the genus *Fretibacterium* gen. nov. and other genera of the phylum *Synergistetes*

Adapted from Patel & Hugenholtz (2009) augmented with data from the species descriptions cited below. Genera: 1, *Fretibacterium* gen. nov. (data from this study); 2, *Aminiphilus* (Díaz *et al.*, 2007); 3, *Aminobacterium* (Baena *et al.*, 1998, 2000); 4, *Aminomonas* (Baena *et al.*, 1999a); 5, *Anaerobaculum* (Maune & Tanner, 2012; Menes & Muxí, 2002; Rees *et al.*, 1997); 6, *Cloacibacillus* (Ganesan *et al.*, 2008); 7, *Dethiosulfovibrio* (Díaz-Cárdenas *et al.*, 2010; Magot *et al.*, 1997; Surkov *et al.*, 2001); 8, *Jonquetella* (Jumas-Bilak *et al.*, 2007); 9, *Pyramidobacter* (Downes *et al.*, 2009); 10, *Synergistes* (Allison *et al.*, 1992); 11, *Thermanaerovibrio* (Baena *et al.*, 1999b; Guangsheng *et al.*, 1992; Zavarzina *et al.*, 2000); 12, *Thermovirga* (Dahle & Birkeland, 2006). +, Positive; −, negative; V, variable reaction; ND, no data available

| Character | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Habitat | Human oral cavity | Anaerobic sludge reactor | Anaerobic sludge reactor | Anaerobic sludge reactor | Petroleum reservoir; wool-scouring wastewater | Wastewater treatment plant | Saline sulfur mats, oil production water, saline spring | Human infections and oral cavity | Human oral cavity | Goat rumen | Methanogenic digester and thermophilic cyanobacterial mats | Oil production water |
| Morphology | Curved rods | Curved rods | Straight to slightly curved rods | Curved rods | Straight to slightly curved rods | Rods | Curved rods | Rods | Rods | Oval rods | Curved rods | Rods |
| Motility | + | + | V | − | V | − | + | − | − | − | + | + |
| DNA G+C content (mol%) | 63 | 53 | 44–46 | 43 | 44–52 | 56 | 51–56 | 59 | 59 | 57–59 | 55–57 | 47 |
| Utilization of carbohydrates | − | − | − | − | + | − | − | − | − | − | + | − |
| Principal cell wall FAME | $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ | ND | ND | ND | iso-$C_{15:0}$ | iso-$C_{15:0}$, iso-$C_{15:0}$ 3-OH, $C_{17:1}\omega6c$ | iso-$C_{15:0}$, $C_{18:0}$ | iso-$C_{15:0}$, $C_{16:0}$ | $C_{13:0}$, $C_{14:0}$ plus 2 unidentified | $C_{17:0}$, $C_{20:0}$ cyclo, $C_{17:1}\omega6c$ | ND | ND |

Shows the following characteristics in addition to those given for the genus. Cells are 1.0–1.5 × 2.0–13.0 μm in size. After 21 days incubation on BA plates streaked with *Fusobacterium nucleatum* subsp. *nucleatum* ATCC 25586$^T$, colonies are approximately 0.5 mm in diameter with a circular, high convex, smooth, off-white, opaque central region surrounded by an irregular, semi-translucent periphery. Occasional variations of this morphology include the absence of the spreading periphery, a convoluted surface and yellow pigmentation. After numerous subcultures a second colonial morphology is observed. These colonies are approximately 1.3 mm in diameter, low convex, smooth, slightly irregular with an opaque, grey to off-white centre and semi-translucent periphery. Acid is not produced from fructose, galactose, glucose, lactose, maltose and sucrose. Positive results in API ZYM tests for α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, C4 esterase and C8 esterase lipase. Positive result in the Rapid ID 32A panel tests for leucyl glycine arylamidase and glycine arylamidase resulting in a profile of 0000 0404 00.

The type strain, SGP1$^T$ (=DSM 25557$^T$=JCM 16858$^T$), was isolated from subgingival plaque from a human with periodontitis. The DNA G+C content of the type strain is 63 mol%.

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## References

**Allison, M. J., Mayberry, W. R., McSweeney, C. S. & Stahl, D. A. (1992).** *Synergistes jonesii*, gen. nov., sp. nov.: a rumen bacterium that degrades toxic pyridinediols. *Syst Appl Microbiol* **15**, 522–529.

**Baena, S., Fardeau, M. L., Labat, M., Ollivier, B., Thomas, P., Garcia, J. L. & Patel, B. K. (1998).** *Aminobacterium colombiensegen* nov. sp. nov., an amino acid-degrading anaerobe isolated from anaerobic sludge. *Anaerobe* **4**, 241–250.

**Baena, S., Fardeau, M. L., Ollivier, B., Labat, M., Thomas, P., Garcia, J. L. & Patel, B. K. (1999a).** *Aminomonas paucivorans* gen. nov., sp. nov., a mesophilic, anaerobic, amino-acid-utilizing bacterium. *Int J Syst Bacteriol* **49**, 975–982.

**Baena, S., Fardeau, M. L., Woo, T. H. S., Ollivier, B., Labat, M. & Patel, B. K. C. (1999b).** Phylogenetic relationships of three amino-acid-utilizing anaerobes, *Selenomonas acidaminovorans*, '*Selenomonas acidaminophila*' and *Eubacterium acidaminophilum*, as inferred from partial 16S rDNA nucleotide sequences and proposal of *Thermanaerovibrio acidaminovorans* gen. nov., comb. nov. and *Anaeromusa acidaminophila* gen. nov., comb. nov. *Int J Syst Bacteriol* **49**, 969–974.

**Baena, S., Fardeau, M. L., Labat, M., Ollivier, B., Garcia, J. L. & Patel, B. K. (2000).** *Aminobacterium mobile* sp. nov., a new anaerobic amino-acid-degrading bacterium. *Int J Syst Evol Microbiol* **50**, 259–264.

**Dahle, H. & Birkeland, N. K. (2006).** *Thermovirga lienii* gen. nov., sp. nov., a novel moderately thermophilic, anaerobic, amino-acid-degrading bacterium isolated from a North Sea oil well. *Int J Syst Evol Microbiol* **56**, 1539–1545.

**Díaz, C., Baena, S., Fardeau, M. L. & Patel, B. K. (2007).** *Aminiphilus circumscriptus* gen. nov., sp. nov., an anaerobic amino-acid-degrading bacterium from an upflow anaerobic sludge reactor. *Int J Syst Evol Microbiol* **57**, 1914–1918.

**Díaz-Cárdenas, C., López, G., Patel, B. K. & Baena, S. (2010).** *Dethiosulfovibrio salsuginis* sp. nov., an anaerobic, slightly halophilic bacterium isolated from a saline spring. *Int J Syst Evol Microbiol* **60**, 850–853.

**Downes, J., Vartoukian, S. R., Dewhirst, F. E., Izard, J., Chen, T., Yu, W.-H., Sutcliffe, I. C. & Wade, W. G. (2009).** *Pyramidobacter piscolens* gen. nov., sp. nov., a member of the phylum '*Synergistetes*' isolated from the human oral cavity. *Int J Syst Evol Microbiol* **59**, 972–980.

**Ganesan, A., Chaussonnerie, S., Tarrade, A., Dauga, C., Bouchez, T., Pelletier, E., Le Paslier, D. & Sghir, A. (2008).** *Cloacibacillus evryensis* gen. nov., sp. nov., a novel asaccharolytic, mesophilic, amino-acid-degrading bacterium within the phylum '*Synergistetes*', isolated from an anaerobic sludge digester. *Int J Syst Evol Microbiol* **58**, 2003–2012.

**Guangsheng, C., Plugge, C. M., Roelofsen, W., Houwen, F. P. & Stams, A. J. M. (1992).** *Selenomonas acidaminovorans* sp. nov., a versatile thermophilic proton-reducing, anaerobe able to grow by decarboxylation of succinate to propionate. *Arch Microbiol* **157**, 169–175.

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

**Jousimies-Somer, H. R., Summanen, P., Citron, D. M., Baron, E.-J., Wexler, H. M. & Finegold, S. M. (2002).** *Wadsworth-KTL Anaerobic Bacteriology Manual*. Belmont, CA: Star Publishing Company.

**Jumas-Bilak, E., Carlier, J. P., Jean-Pierre, H., Citron, D., Bernard, K., Damay, A., Gay, B., Teyssier, C., Campos, J. & Marchandin, H. (2007).** *Jonquetella anthropi* gen. nov., sp. nov., the first member of the candidate phylum '*Synergistetes*' isolated from man. *Int J Syst Evol Microbiol* **57**, 2743–2748.

**Jumas-Bilak, E., Roudière, L. & Marchandin, H. (2009).** Description of '*Synergistetes*' phyl. nov. and emended description of the phylum '*Deferribacteres*' and of the family *Syntrophomonadaceae*, phylum '*Firmicutes*'. *Int J Syst Evol Microbiol* **59**, 1028–1035.

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

**Magot, M., Ravot, G., Campaignolle, X., Ollivier, B., Patel, B. K., Fardeau, M. L., Thomas, P., Crolet, J. L. & Garcia, J. L. (1997).** *Dethiosulfovibrio peptidovorans* gen. nov., sp. nov., a new anaerobic, slightly halophilic, thiosulfate-reducing bacterium from corroding offshore oil wells. *Int J Syst Bacteriol* **47**, 818–824.

**Maune, M. W. & Tanner, R. S. (2012).** Description of *Anaerobaculum hydrogeniformans* sp. nov., an anaerobe that produces hydrogen from glucose, and emended description of the genus *Anaerobaculum*. *Int J Syst Evol Microbiol* **62**, 832–838.

**Menes, R. J. & Muxí, L. (2002).** *Anaerobaculum mobile* sp. nov., a novel anaerobic, moderately thermophilic, peptide-fermenting bacterium that uses crotonate as an electron acceptor, and emended description of the genus *Anaerobaculum*. *Int J Syst Evol Microbiol* **52**, 157–164.

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

**Patel, B. K. C. & Hugenholtz, P. (2009).** Anaerobaculum. In *Bergey's Manual of Systematic Bacteriology*, pp. 1170–1174. Edited by P. de Vos, G. M. Garrity, D. Jones, N. R. Krieg, W. Ludwig, F. A. Rainey, K.-H. Schleifer & W. B. Whitman. Heidelberg: Springer.

**Rees, G. N., Patel, B. K., Grassia, G. S. & Sheehy, A. J. (1997).** Anaerobaculum thermoterrenum gen. nov., sp. nov., a novel, thermophilic bacterium which ferments citrate. *Int J Syst Bacteriol* **47**, 150–154.

**Surkov, A. V., Dubinina, G. A., Lysenko, A. M., Glöckner, F. O. & Kuever, J. (2001).** *Dethiosulfovibrio russensis* sp. nov., *Dethosulfovibrio marinus* sp. nov. and *Dethosulfovibrio acidaminovorans* sp. nov., novel anaerobic, thiosulfate- and sulfur-reducing bacteria isolated from '*Thiodendron*' sulfur mats in different saline environments. *Int J Syst Evol Microbiol* **51**, 327–337.

**Vartoukian, S. R., Palmer, R. M. & Wade, W. G. (2010).** Cultivation of a *Synergistetes* strain representing a previously uncultivated lineage. *Environ Microbiol* **12**, 916–928.

**Zavarzina, D. G., Zhilina, T. N., Tourova, T. P., Kuznetsov, B. B., Kostrikina, N. A. & Bonch-Osmolovskaya, E. A. (2000).** *Thermanaerovibrio velox* sp. nov., a new anaerobic, thermophilic, organotrophic bacterium that reduces elemental sulfur, and emended description of the genus *Thermanaerovibrio*. *Int J Syst Evol Microbiol* **50**, 1287–1295.