Syntrophomonas erecta sp. nov., a novel anaerobe that syntrophically degrades short-chain fatty acids

Chunyang Zhang,^{1,2} Xiaoli Liu¹ and Xiuzhu Dong¹

Correspondence

Xiuzhu Dong

dongxz@sun.im.ac.cn

¹State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, No. 13, North 1st Ave, Zhong Guan Cun, Beijing 100080, PR China

²Graduate School, Chinese Academy of Sciences, Beijing 100080, PR China

Two novel anaerobes, strains GB4-38^T and SB9-1, were isolated from an upflow anaerobic sludge blanket reactor for treating bean-curd farm wastewater and lotus field mud, respectively. The strains degraded straight-chain fatty acids with 4–8 carbon atoms in syntrophic association with methanogens and converted 1 mol butyrate into about 2 mol acetate and presumably 2 mol H₂. None of the branched-chain fatty acids tested could be degraded. Benzoate was not degraded. Fumarate, sulfate, thiosulfate, sulfur and nitrate did not serve as electron acceptors for butyrate degradation. In the absence of a methanogen partner, strain GB4-38^T grew on crotonate in pure culture; the generation time was about 5 h at 37 °C. However, strain SB9-1 grew on butyrate plus pentenoate, but not crotonate, in pure culture and the generation time was 18 h at 37 °C. Cells of GB4-38^T and SB9-1 were straight rods and stained Gram-negative. The major cellular fatty acids of GB4-38^T were C_{14:0} (29.74 %), C_{16:0} (17.00 %), C_{16:1ω5c} (16.63 %) and isoC_{17:1} (15.34 %). LL-Diaminopimelic acid existed in the cellular peptidoglycan. The genomic DNA G + C content of strain GB4-38^T was 43.2 mol%. Phylogenetic analysis based on 16S rRNA gene sequences supported clustering of the two strains with syntrophic bacterial species of the genus *Syntrophomonas* (89.6–92.4 % sequence similarity), but phenotypic, chemotaxonomic and genetic characters differentiated the two strains from members of this genus. Therefore, it is proposed that the two strains are representatives of a novel species, *Syntrophomonas erecta* sp. nov. The type strain is GB4-38^T (= CGMCC 1.5013^T = DSM 16215^T).

It is believed that the degradation of intermediates such as short-chain fatty acids (SCFAs), primary alcohols and aromatic acids is the speed-limiting step during mineralization of organic complexes in anoxic environments. These intermediates have to be degraded by consortia containing at least two trophic groups (syntrophic acetogenic bacteria and hydrogen scavengers) because of the unfavourable energetics of the reactions (Schink, 1997). So far, eight species of syntrophic anaerobes have been described that oxidize SCFAs with more than 4 carbon atoms and long-chain fatty acids of up to 18 carbon atoms via synergetic metabolism with hydrogen-consuming partners (McInerney *et al.*, 1979, 1981; Stieb & Schink, 1985; Roy *et al.*, 1986;

Lorowitz *et al.*, 1989; Zhao *et al.*, 1990; Svetlitsnyi *et al.*, 1996; Jackson *et al.*, 1999; Sekiguchi *et al.*, 2000; Zhang *et al.*, 2004). Physiological characterization and phylogenetic analysis clustered these syntrophic bacteria into two lineages: the family *Syntrophomonadaceae* (McInerney, 1992; Zhao *et al.*, 1993), within the low-G + C-containing Gram-positive bacteria, and the family 'Syntrophaceae' in the subclass δ -Proteobacteria (Jackson *et al.*, 1999).

In the present study, two novel syntrophic bacterial strains, GB4-38^T and SB9-1, are described. They degraded straight-chain fatty acids with 4–8 carbon atoms into propionate, acetate and methane in co-culture with a methanogen. 16S rRNA gene sequence analysis indicated their affiliation to the family *Syntrophomonadaceae*; however, morphological, chemotaxonomic and genetic characters distinguished them from known species. Therefore, a novel species of *Syntrophomonas* is proposed.

Methanospirillum hungatei DSM 864^T was kindly provided by A. Stams (Department of Microbiology, Wageningen University, The Netherlands). *Syntrophomonas wolfei* subsp. *wolfei* DSM 2245B^T and *Syntrophomonas sapovorans* DSM 3441^T were purchased from the DSMZ (Braunschweig,

Published online ahead of print on 29 October 2004 as DOI 10.1099/ijs.0.63372-0.

Abbreviations: SCFAs, short-chain fatty acids; UASB, upflow anaerobic sludge blanket.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Syntrophomonas erecta* strains GB4-38^T and SB9-1 are AY536889 and AY643536, respectively.

Electron micrographs of GB4-38^T cells are available as supplementary material in IJSEM Online.

Germany). *Syntrophomonas curvata* DSM 15682^T was preserved in our laboratory. Strain GB4-38^T was isolated from the granular sludge of an upflow anaerobic sludge blanket (UASB) reactor for treating wastewater from a bean-curd farm in Beijing, China, and strain SB9-1 was isolated from lotus-growing mud in a Beijing suburb.

A pre-reduced basal medium described by McInerney *et al.* (1979) was used for isolation and routine cultivation. The gas phase was normally N₂/CO₂ (80:20; 1.01 × 10⁵ Pa), although H₂/CO₂ (80:20; 1.25 × 10⁵ Pa) was used for cultivation of *M. hungatei* DSM 864^T. Inoculation and transfer of the cultures were done with a syringe and needle. The purity of strain GB4-38^T in co-culture and pure culture was examined periodically by monitoring cell morphology using normal bright field microscopy and colonies on solid media, as well as the absence of growth in a rich medium like peptone-yeast extract-glucose (PYG) liquid.

Substrate utilization and potential electron acceptors for butyrate degradation were examined according to Zhang *et al.* (2004). Cell growth was monitored by determining the OD₆₀₀ of the culture. Acetate, propionate and methane production and substrate degradation were measured by GC (GC-14A/B; Shimadzu) and HPLC (series 1050; Hewlett Packard) as previously described (Touzel & Albagnac, 1983; Jackson *et al.*, 1999; Zhang *et al.*, 2004). The molar growth yield of strain GB4-38^T in pure culture was determined in 50 ml basal medium with 20 mM crotonate; growth in basal medium without crotonate was used as a control. Cell mass was obtained by centrifuging 72 h cultures at 7000 g and resuspending the cell pellet in 10 mM monobasic potassium phosphate buffer (pH 7.0). The cell suspension was dried at 100 °C overnight and dry cell mass was determined according to Jackson *et al.* (1999).

Exponential-phase cells of strain GB4-38^T were used for morphology examination with a TEM (H-600A; Hitachi). Before observation, negative staining with uranyl acetate was performed. Ultrathin sections were stained with uranyl acetate and lead citrate according to Reynolds (1963).

Cellular fatty acids of strain GB4-38^T were analysed as fatty acid methyl esters with the MIDI microbial identification system. Diamino acids of the cell wall were assayed using TLC on a cellulose plate and the solvent system of Rhuland *et al.* (1955).

Genomic DNA of strain GB4-38^T grown on crotonate was extracted as previously described (Marmur, 1961). The G+C content was determined using the thermal denaturation method (Marmur & Doty, 1962) with a DU 800 spectrophotometer (Beckman). DNA from *Escherichia coli* K-12 was used as a reference for the thermal melting profile (*T*_m). DNA relatedness was determined on the basis of DNA–DNA liquid reassociation rate (De Ley *et al.*, 1970) at 65 °C using a DU 800 spectrophotometer (Beckman).

The 16S rRNA gene was amplified by PCR and then

sequenced using the method described by Weisburg *et al.* (1991). Sequences of GB4-38^T, SB9-1 and reference strains (obtained from GenBank) were aligned using the program DNAMAN (version 4.0; Lynnon Biosoft). A phylogenetic tree was constructed by the neighbour-joining method and the topology of the tree was evaluated by bootstrap analysis of 1000 datasets with DNAMAN.

Methanogenic sludge samples were inoculated into pre-reduced basal medium containing 20 mM butyrate as sole carbon source. After subculturing several times in this medium, 5 % (v/v) enrichment culture and 5 % (v/v) *M. hungatei* DSM 864^T culture were inoculated into the same medium with 1.6 % (w/v) agar and the Hungate roll-tube technique (Hungate, 1969) was performed. After repeating this procedure five to seven times, strains GB4-38^T and SB9-1 were obtained, respectively, in co-culture with *M. hungatei* DSM 864^T from the roll-tubes. 2-Bromoethanesulfonic acid (20 mM) was added to the co-cultures to inhibit the methanogen and, after subculturing 18 times, monocultures were obtained in the basal medium containing either 20 mM crotonate (GB4-38^T) or 20 mM butyrate plus 20 mM pentenoate (SB9-1). Homogeneous cell morphology was observed in the monoculture. Without a methanogen, neither of the two strains degraded butyrate alone. However, the strains were able to resume butyrate degradation in artificial co-cultures with *M. hungatei* DSM 864^T (data not shown). Hence, the two strains could be confirmed as syntrophic bacteria. In this study, a monoculture of *S. wolfei* subsp. *wolfei* DSM 2245B^T was also obtained in a dozen transfers on crotonate medium containing 2-bromoethanesulfonic acid, i.e. in the absence of *M. hungatei* DSM 864^T.

Cells of GB4-38^T and SB9-1 were Gram-negative straight rods with round ends. They were 0.6–0.8 µm in width, 2.0–8.0 µm in length and occurred singly, in pairs or in chains. Spores were never observed. Electron microscopy showed the presence of multiple flagella interspersed around the subpoles of GB4-38^T cells (micrograph available as supplementary material in IJSEM Online). Ultrathin sections of GB4-38^T revealed the atypical Gram-positive cell wall structure (micrograph available as supplementary material in IJSEM Online). The two strains formed small, round, yellow colonies on butyrate medium in co-culture with *M. hungatei* DSM 864^T.

Both GB4-38^T and SB9-1 could obtain energy from the oxidation of straight-chain fatty acids with 4–8 carbon atoms in co-culture with *M. hungatei* DSM 864^T. Benzoate, fatty acids longer than C₈ and branched-chain fatty acids such as isobutyrate and isovalerate could not be utilized by the two strains (Table 1). Among the utilized substrates, butyrate, caproate and caprylate were degraded into acetate and presumably H₂ (converted into CH₄ by the methanogen) within 2 weeks, whereas valerate and heptanoate were converted into propionate, acetate and H₂, which implied β-oxidation of fatty acids used by the strains. When 1 mol butyrate was degraded, about 2 mol acetate and 2 mol

Table 1. Differential characteristics of the two novel strains and related *Syntrophomonas* species

Strains: 1, strain GB4-38^T (data from this study); 2, strain SB9-1 (this study); 3, *S. wolfei* subsp. *wolfei* DSM 2245^T (McInerney *et al.*, 1979, 1981; Beaty & McInerney, 1987; this study); 4, *S. wolfei* subsp. *saponavida* DSM 4212^T (Lorowitz *et al.*, 1989); 5, *S. sapovorans* DSM 3441^T (Roy *et al.*, 1986; this study); 6, *S. curvata* DSM 15682^T (Zhang *et al.*, 2004; this study). All strains listed could utilize butyrate, valerate, caproate, heptanoate and caprylate as substrates; none of the strains utilized propionate, isobutyrate or isovalerate. ND, Not determined.

Characteristic	1	2	3	4	5	6
Cell morphology*	SR	SR	CR	CR	CR	CR
Cell width (µm)	0.6–0.9	0.5–0.8	0.5–1.0	0.4–0.6	0.5	0.5–0.7
Cell length (µm)	2.0–8.0	2.0–8.5	2.0–7.0	2.0–4.0	2.5	2.3–4.0
Position of flagellar insertion	Subpole	ND	Concavity	Concavity	Concavity	Subpole/pole
G + C content (mol%)	43.2	43.9	45.1	ND	ND	46.6
Growth pH (optimum)	6.0–8.8 (7.8)	6.7–9.0 (7.5–8.5)	6.2–8.0 (7.0–7.5)	ND	6.3–8.1 (7.3)	6.3–8.4 (7.5)
Growth temperature (optimum) (°C)	25–47 (37–40)	25–45 (37–40)	25–45 (35–37)	ND	25–45 (35–37)	20–42 (35–37)
Substrate utilization in pure culture:						
Crotonate	+	–	+	+	–	+
Butyrate + pentenoate	–	+	–	ND	–	+
Substrate utilization in co-culture with methanogens:						
Acetate	–	–	–	ND	–	–
Pelargonate	ND	ND	ND	+	ND	ND
Caprate	–	–	–	+	+	+
Laurate	–	–	–	+	+	+
Myristate	–	–	–	+	+	+
Palmitate	–	–	–	+	+	+
Stearate	–	–	–	+	+	+
Oleate	–	–	–	–	+	+
Linoleate	–	–	–	–	+	–
Arachidate	–	–	–	ND	–	–

*CR, Curved rod; SR, straight rod.

hydrogen (converted into CH₄) were produced; the carbon and electron recoveries were 97.76 ± 4.18 % and 99.00 ± 4.21 %, respectively. None of the following tested substances (all at 20 mM) could be used by the two strains as a potential electron acceptor in butyrate oxidation: sodium sulfate, sodium thiosulfate, sulfur, sodium nitrate and sodium fumarate.

Among the tested compounds, crotonate was the only substrate able to support growth of strain GB4-38^T in pure culture. The generation time of GB4-38^T was 5 h when growing on 20 mM crotonate at 37 °C and the molar growth yield was detected as 10.2–11.2 g dry wt (mol crotonate)^{–1}, which was similar to that of *S. wolfei* subsp. *wolfei* DSM 2245^T (Beaty & McInerney, 1987). Strain SB9-1 did not grow on crotonate. Growth and acid formation were not observed for the two strains on the following substrates: yeast extract (0.5 %), tryptone (1 %), peptone (1 %), maltose (10 mM), glucose (20 mM), fructose (20 mM), ribose (20 mM), xylose (20 mM), pentenoate (20 mM), fumarate (20 mM) and pyruvate (20 mM).

Temperature ranges for growth of GB4-38^T in both co-culture on butyrate and pure culture on crotonate were 25–47 °C, with optimal growth at 37–40 °C. The pH range

for growth was 6.0–8.8, with optimal growth at pH 7.8. The NaCl concentration range tolerated was 0–500 mM (optimal growth occurred below 50 mM NaCl). The profiles of temperature, pH and NaCl concentration for SB9-1 both in co-culture on butyrate and in pure culture on butyrate plus pentenoate were 25–45 °C (optimum at 37–40 °C), pH 6.7–9.0 (optimum at pH 7.5–8.5) and 0–500 mM NaCl (optimum below 50 mM NaCl). For both strains, no growth was observed in either pure culture or co-culture using air as the gas phase. The growth characteristics of *S. wolfei* subsp. *wolfei* DSM 2245^T were retested and are shown in Table 1.

The major cellular fatty acids of strain GB4-38^T were C_{14:0} (29.74 %), C_{16:0} (17.00 %), C_{16:1ω5c} (16.63 %) and isoC_{17:1} I (15.34 %), whereas C_{16:1ω7c} (38 %), C_{16:0} (16 %), C_{16:1ω9c} (16 %) and C_{14:0} (12 %) were predominant in *S. wolfei* subsp. *wolfei* DSM 2245^T (Henson *et al.*, 1988; Lorowitz *et al.*, 1989) and C_{14:0} (27.82 %), C_{15:0} (19.24 %) and C_{14:0} 3-OH (10.90 %), as well as an unknown component with an equivalent chain-length of 14.503 (21.44 %), constituted the main fatty acids of *S. curvata* DSM 15682^T (Zhang *et al.*, 2004). The peptidoglycan of GB4-38^T contained LL-diaminopimelic acid, whereas *S. wolfei* subsp. *wolfei* DSM 2245^T contained

meso-diaminopimelic acid (McInerney *et al.*, 1981; Lorowitz *et al.*, 1989).

The 16S rRNA genes of strains GB4-38^T and SB9-1 shared 98.8 % sequence similarity and these two strains had similar phenotypic characteristics, except for the substrate that supported the pure culture, indicating that the two strains could belong to one species. A phylogenetic tree including the two strains and other members of the family *Syntrophomonadaceae* was constructed (Fig. 1) and showed that the two strains clustered loosely with members of the genus *Syntrophomonas*. The 16S rRNA gene sequence similarities between strain GB4-38^T and its three nearest relatives, *S. curvata* DSM 15682^T, *S. sapovorans* DSM 3441^T and *S. wolfei* subsp. *wolfei* DSM 2245^T, were 92.4, 92.3 and 89.6 %, respectively.

The genomic DNA G+C contents of strain GB4-38^T, strain SB9-1 and *S. wolfei* subsp. *wolfei* DSM 2245^T were determined as 43.2 ± 0.2, 43.9 ± 1.2 and 45.1 mol%, respectively. DNA–DNA relatedness values between GB4-38^T and other *Syntrophomonas* species, *S. curvata* DSM 15682^T and *S. wolfei* subsp. *wolfei* DSM 2245^T, were 28.58 and 0 %, respectively, indicating that GB4-38^T is not a member of either of these two species and therefore represents a different species.

GB4-38^T and SB9-1 also differed from other members of the genus *Syntrophomonas* in their phenotypic features. They were different from *S. curvata* DSM 15682^T and *S. sapovorans* DSM 3441^T in cell morphology, substrate range (Table 1), growth temperature and pH range and cellular fatty acid composition. Although our two strains had the same substrate range as *S. wolfei* subsp. *wolfei* DSM 2245^T in co-culture, they could be distinguished from the latter by the following: growth at higher temperature and higher

Fig. 1. Phylogenetic tree of strain GB4-38^T and related organisms based on a 1430 bp fragment of the 16S rRNA gene. The tree was rooted with *Clostridium butyricum* DSM 2478 and constructed using the neighbour-joining method with bootstrap values based on 1000 replications. Numbers at each branch point are the percentage bootstrap supports. GenBank accession numbers of 16S rRNA gene sequences are given in parentheses. Bar, 5 % sequence divergence.

pH with different cell morphology (Table 1), diaminopimelic acid type in the peptidoglycan, cellular fatty acid composition and low DNA–DNA hybridization values. Based on multiphasic taxonomic traits, a novel syntrophic species, *Syntrophomonas erecta* sp. nov., is proposed.

Description of *Syntrophomonas erecta* sp. nov.

Syntrophomonas erecta (e.rec'ta. L. fem. adj. *erecta* erect).

Cells are straight rods with Gram-negative cell wall, 0.6–0.8 × 2.0–8.0 µm, non-spore-forming and with 2–5 flagella, usually occurring in the subpole of cells. Straight-chain fatty acids with 4–8 carbon atoms can serve as substrates for the species in co-culture with *M. hungatei* DSM 864^T. Even-numbered fatty acids are degraded into acetate and presumably H₂, whereas odd-numbered ones are degraded into propionate, acetate and H₂. Straight-chain fatty acids shorter than C₄ (acetate and propionate), longer than C₈ (caprate, laurate, myristate, palmitate, stearate, oleate, linoleate and arachidate), branched-chain fatty acids (isobutyrate and isovalerate) and benzoate do not support the co-culture. Fumarate, sulfate, thiosulfate, sulfur and nitrate cannot act as electron acceptors for butyrate oxidation. Crotonate is the only tested substrate that supports growth of the type strain in pure culture, whereas butyrate plus pentenoate supports growth of SB9-1. Yeast extract, tryptone, peptone, maltose, glucose, fructose, ribose, xylose, pentenoate, fumarate and pyruvate alone do not support growth. The type strain can grow at pH 6.0–8.8, 25–47 °C and in 0–500 mM NaCl. The cellular fatty acids of the type strain contain mainly C_{14:0} (29.74 %), C_{16:0} (17.00 %), C_{16:1ω5c} (16.63 %) and isoC_{17:1} I (15.34 %). LL-Diaminopimelic acid exists in the cellular peptidoglycan. The G+C content of the genomic DNA is 43.2–43.9 mol%.

The type strain is GB4-38^T (=CGMCC 1.5013^T=DSM 16215^T), isolated from the granular sludge of a UASB reactor for treating bean-curd farm wastewater in Beijing, China.

Acknowledgements

This research has been supported by grants of the National Nature Science Foundation of China (30025001) and the Innovation program (field frontier) from the Chinese Academy of Sciences.

References

- Beaty, P. S. & McInerney, M. J. (1987). Growth of *Syntrophomonas wolfei* in pure culture on crotonate. *Arch Microbiol* **147**, 389–393.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 143–153.
- Henson, J. M., McInerney, M. J., Beaty, P. S., Nickels, J. & White, D. C. (1988). Phospholipid fatty acid composition of the syntrophic anaerobic bacterium *Syntrophomonas wolfei*. *Appl Environ Microbiol* **54**, 1570–1574.

- Hungate, R. E. (1969).** A roll tube method for cultivation of strict anaerobes. *Methods Microbiol* **3B**, 117–132.
- Jackson, B. E., Bhupathiraju, V. K., Tanner, R. S., Woese, C. R. & McInerney, M. J. (1999).** *Syntrophus aciditrophicus* sp. nov., a new anaerobic bacterium that degrades fatty acids and benzoate in syntrophic association with hydrogen-using microorganisms. *Arch Microbiol* **171**, 107–114.
- Lorowitz, W. H., Zhao, H. & Bryant, M. P. (1989).** *Syntrophomonas wolfei* subsp. *saponavida* subsp. nov., a long-chain fatty-acid-degrading, anaerobic, syntrophic bacterium; *Syntrophomonas wolfei* subsp. *wolfei* subsp. nov.; and emended descriptions of the genus and species. *Int J Syst Bacteriol* **39**, 122–126.
- Marmur, J. (1961).** A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Marmur, J. & Doty, P. (1962).** Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **4**, 109–118.
- McInerney, M. J. (1992).** The genus *Syntrophomonas*, and other syntrophic bacteria. In *The Prokaryotes*, 2nd edn, pp. 2048–2057. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.
- McInerney, M. J., Bryant, M. P. & Pfennig, N. (1979).** Anaerobic bacterium that degrades fatty acids in syntrophic association with methanogens. *Arch Microbiol* **122**, 129–135.
- McInerney, M. J., Bryant, M. P., Hespell, R. B. & Costerton, J. W. (1981).** *Syntrophomonas wolfei* gen. nov., sp. nov., an anaerobic, syntrophic, fatty acid-oxidizing bacterium. *Appl Environ Microbiol* **41**, 1029–1039.
- Reynolds, E. S. (1963).** The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J Cell Biol* **17**, 208–212.
- Rhuland, L. E., Work, E., Denman, R. F. & Hoare, D. S. (1955).** The behavior of the isomers of α,ϵ -diaminopimelic acid on paper chromatograms. *J Am Chem Soc* **77**, 4844–4846.
- Roy, F., Samain, E., Dubourguier, H. & Albagnac, G. (1986).** *Syntrophomonas sapovorans* sp. nov., a new obligately proton reducing anaerobe oxidizing saturated and unsaturated long chain fatty acids. *Arch Microbiol* **145**, 142–147.
- Schink, B. (1997).** Energetics of syntrophic cooperation in methanogenic degradation. *Microbiol Mol Biol Rev* **61**, 262–280.
- Sekiguchi, Y., Kamagata, Y., Nakamura, K., Ohashi, A. & Harada, H. (2000).** *Syntrophothermus lipocalidus* gen. nov., sp. nov., a novel thermophilic, syntrophic, fatty-acid-oxidizing anaerobe which utilizes isobutyrate. *Int J Syst Evol Microbiol* **50**, 771–779.
- Stieb, M. & Schink, B. (1985).** Anaerobic oxidation of fatty acids by *Clostridium bryantii* sp. nov., a sporeforming, obligately syntrophic bacterium. *Arch Microbiol* **140**, 387–390.
- Svetlitsnyi, V., Rainey, F. & Wiegel, J. (1996).** *Thermosyntropha lipolytica* gen. nov., sp. nov., a lipolytic, anaerobic, alkalitolerant, thermophilic bacterium utilizing short- and long-chain fatty acids in syntrophic coculture with a methanogenic archaeum. *Int J Syst Bacteriol* **46**, 1131–1137.
- Touzel, J. P. & Albagnac, G. (1983).** Isolation and partial characterization of *Methanococcus mazei* strain MC3. *FEMS Microbiol Lett* **16**, 241–245.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991).** 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.
- Zhang, C., Liu, X. & Dong, X. (2004).** *Syntrophomonas curvata* sp. nov., an anaerobe that degrades fatty acids in co-culture with methanogens. *Int J Syst Evol Microbiol* **54**, 969–973.
- Zhao, H., Yang, D., Woese, C. R. & Bryant, M. P. (1990).** Assignment of *Clostridium bryantii* to *Syntrophospora bryantii* gen. nov., comb. nov. on the basis of a 16S rRNA sequence analysis of its crotonate-grown pure culture. *Int J Syst Bacteriol* **40**, 40–44.
- Zhao, H., Yang, D., Woese, C. R. & Bryant, M. P. (1993).** Assignment of fatty acid- β -oxidizing syntrophic bacteria to *Syntrophomonadaceae* fam. nov. on the basis of 16S rRNA sequence analysis. *Int J Syst Bacteriol* **43**, 278–286.