# *Syntrophomonas curvata* sp. nov., an anaerobe that degrades fatty acids in co-culture with methanogens

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A strict anaerobe (strain GB8-1$^T$) that degraded straight-chain fatty acids with $C_4$–$C_{18}$ in syntrophic association with methanogens was isolated from an up-flow anaerobic sludge blanket reactor treating beer wastewater. Strain GB8-1$^T$ degraded 1 mol butyrate into about 2 mol acetate and 1 mol (presumably) $H_2$ in co-culture with a methanogen. Neither branched-chain fatty acids nor benzoate could be degraded. Strain GB8-1$^T$ could grow on crotonate in pure culture and converted 1 mol crotonate to 0·5 mol butyrate and 1 mol acetate. Generation time was about 11 h when grown on crotonate at 37 °C. Fumarate, sulfate, thiosulfate, sulfur and nitrate could not serve as electron acceptors for strain GB8-1$^T$ to degrade butyrate. Cells of strain GB8-1$^T$ were curved rods with Gram-negative cell walls; no spores were observed. The DNA G+C content was 46·6 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain GB8-1$^T$ was related most closely to the fatty acid-oxidizing, syntrophic bacterium S*yntrophomonas sapovorans* DSM 3441$^T$; however, the relationship was not very close (95·4 % sequence similarity). Some phenotypic features also differentiated strain GB8-1$^T$ from *Syntrophomonas sapovorans* DSM 3441$^T$. Therefore, a novel species, *Syntrophomonas curvata* sp. nov., is proposed. The type strain is GB8-1$^T$ (=CGMCC 1.5010$^T$=DSM 15682$^T$).

In ecosystems that lack light and exogenous electron acceptors other than $CO_2$, mineralization of complex organic matter is performed by the cooperation of three major metabolic groups of micro-organisms: hydrolytic and fermenting bacteria; syntrophic, acetogenic bacteria; and methanogens. Butyric acid is an important metabolic intermediate during this mineralization. Due to the unfavourable energetics of the reaction under standard thermodynamic conditions, it can only be degraded by the synergetic reaction of syntrophic, acetogenic bacteria and hydrogen-scavenging microbes, such as methanogens (Schink, 1997).

To date, seven species and/or subspecies have been described to degrade butyric acid, as well as some long-chain fatty acids (up to $C_{18}$), syntrophically. Both physiological and genetic characterization suggested that six of them belonged to the family *Syntrophomonadaceae* (McInerney, 1992; Zhao *et al.*, 1993), within the phylum of low-G+C, Gram-positive bacteria. They are *Thermosyntropha lipolytica* DSM 11003$^T$ (Svetlitshnyi *et al.*, 1996), *Syntrophothermus lipocalidus* DSM 12680$^T$ (Sekiguchi *et al.*, 2000), *Syntrophospora bryantii* DSM 3014$^T$ (Stieb & Schink, 1985; Zhao *et al.*, 1990), *Syntrophomonas wolfei* subsp. *wolfei* DSM 2245$^T$ (McInerney *et al.*, 1979, 1981; Beaty & McInerney, 1987), *Syntrophomonas wolfei* subsp. *saponavida* DSM 4212$^T$ (Lorowitz *et al.*, 1989) and *Syntrophomonas sapovorans* DSM 3441$^T$ (Roy *et al.*, 1986). *Syntrophus aciditrophicus* ATCC 700169$^T$, a bacterium that syntrophically degrades both fatty acids ($C_4$–$C_{18}$) and benzoate, was affiliated to the *δ-Proteobacteria* and clustered with the syntrophic bacteria that oxidize propionate or benzoate (Jackson *et al.*, 1999). In the present study, we isolated a new anaerobe, strain GB8-1$^T$, that syntrophically degraded $C_4$–$C_{18}$ straight-chain fatty acids in co-culture with a hydrogenotrophic microbe. Phylogenetic analysis indicated that strain GB8-1$^T$ was a member of the genus *Syntrophomonas*; however, it was not related closely to either of the described species in this genus. Moreover, it could be distinguished from the other species by cell shape and substrate range.

Strain GB8-1$^T$ was isolated from granular sludge that was

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Abbreviations: FAME, fatty acid methyl ester; UASB, up-flow anaerobic sludge blanket.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Syntrophomonas curvata* strain GB8-1$^T$ is AY290767.

Transmission electron micrographs of strain GB8-1$^T$ are available as supplementary material in IJSEM Online.

sampled from a lab-scale, mesophilic, up-flow anaerobic sludge blanket (UASB) reactor treating beer wastewater in Tsinghua University, Beijing, China. *Methanobacterium formicicum* DSM 1535$^T$ was kindly provided by Dr Alfons Stams in the Department of Microbiology, Wageningen University, the Netherlands, and *Syntrophomonas sapovorans* DSM 3441$^T$ was purchased from the DSMZ (Braunschweig, Germany).

The pre-reduced basal medium used in this study was prepared as described by McInerney *et al.* (1979), except that the growth-stimulating factors used were 0·05 % (w/v) yeast extract and 0·05 % (w/v) tryptone, instead of 5·0 % (v/v) rumen fluid. The gas phase was $N_2/CO_2$ (80 : 20; $1{\cdot}01 \times 10^5$ Pa), except that it comprised $H_2/CO_2$ (80 : 20; $1{\cdot}25 \times 10^5$ Pa) for cultivating *M. formicicum* DSM 1535$^T$. All inoculations and transfers were done with syringes and needles and all cultures were incubated at 37 °C in the dark unless indicated. Purity of strain GB8-1$^T$ in co-culture and pure culture was examined periodically by monitoring cell morphology under a normal bright-field microscope and clones on solid media, as well as monitoring lack of growth in a rich medium like peptone/yeast extract/glucose (PYG) medium.

To test the substrate range of strain GB8-1$^T$ in co-culture with a methanogen, the following organic acids were each used as the sole substrate: acetate, propionate, isobutyrate, butyrate, isovalerate and valerate (20 mM each); caproate, heptanoate, caprylate and benzoate (10 mM each); caprate, undecanoate, laurate, myristate, pentadecanoate, palmitate, margarate, stearate, oleate and linoleate (5 mM each, with equimolar $CaCl_2$). After inoculation, cultures were incubated for up to 60 days. Meanwhile, acetate, propionate and methane production and substrate degradation were measured. Cell growth was monitored by measurement of the $OD_{600}$ of the culture. Fatty acids and $CH_4$ were detected by GC (GC-14B, Shimadzu) as described by Touzel & Albagnac (1983); $N_2$ acted as the carrier gas. Column temperatures were 50 and 220 °C for the $CH_4$ and short-chain fatty acid assays, respectively. Fatty acids longer than $C_7$ were esterified into fatty acid methyl esters (FAMEs) before being measured and column temperatures were 200, 220 and 240 °C for $C_8$–$C_{13}$, $C_{14}$–$C_{17}$ and $C_{18}$ fatty acids, respectively. Benzoate was measured by HPLC (series 1050, Hewlett Packard) as described by Jackson *et al.* (1999). The molar growth yield of strain GB8-1$^T$ in pure culture on crotonate was determined according to the method of Jackson *et al.* (1999).

In order to determine optimum growth conditions for strain GB8-1$^T$ in co-culture and pure culture, basal medium with 20 mM butyrate and crotonate, respectively, was used. The pH of the media was adjusted to 5·5–9·5 by using 1 M HCl or 1 M NaOH. To determine the growth temperature profile, cultures were incubated at 15–55 °C (pH 7·5) in a water bath with a temperature controller. To determine NaCl tolerance, 0–1000 mM NaCl was added to the media. After cultivation for up to 60 days, growth was determined by measurement of the $OD_{600}$ of the culture and substrate degradation. All determinations were performed in duplicate with 10 % inoculum.

Exponential-phase cells of strain GB8-1$^T$ were used for morphology examination with a transmission electron microscope (H-600A, Hitachi). Before observation, negative staining was performed with uranyl acetate. Ultrathin sections were stained with uranyl acetate and lead citrate, according to Reynolds (1963).

Diamino acids of the cell wall were assayed by TLC on a cellulose plate; the solvent system of Rhuland *et al.* (1955) was used. Whole-cell fatty acids were analysed as FAMEs with the MIDI Microbial Identification system.

Genomic DNA was extracted from cells of strain GB8-1$^T$ grown on crotonate, as described by Marmur (1961). The DNA G+C content was determined by using the thermal denaturation method (Marmur & Doty, 1962) with a DU 800 spectrophotometer (Beckman). DNA from *Escherichia coli* K-12 was used as reference for determination of the thermal-melting profile ($T_m$).

The 16S rRNA gene of strain GB8-1$^T$ was amplified by PCR and sequenced by using methods described by Weisburg *et al.* (1991). The 16S rRNA gene sequences of strain GB8-1$^T$ and reference strains in GenBank were aligned by using the DNAMAN program (version 4.0; Lynnon Biosoft). A phylogenetic tree was constructed with the neighbour-joining method and the topology of the phylogenetic tree was evaluated by bootstrap analysis of the sequence data with the DNAMAN program.

Granular sludge was inoculated into a medium with 20 mM butyrate, in order to enrich anaerobic, butyrate-degrading consortia. After many roll-tube isolation steps (Hungate, 1969) in the butyrate medium, in which 5 % (v/v) culture of *M. formicicum* DSM 1535$^T$ and 5 % (v/v) dilution of the enrichment culture were inoculated, a biculture that produced methane on butyrate, but not on PYG medium, was obtained. This biculture formed brownish colonies of 0·5–1·0 mm diameter in roll tubes and with green fluorescence under light at 420 nm. Only two kinds of cell shape were observed in the biculture: one was a straight, *M. formicicum*-like rod and the other was a slightly curved rod. The biculture was purified further on medium with 20 mM crotonate as the sole substrate (instead of butyrate) and 10 mM 2-bromoethanesulfonic acid to inhibit the methanogen; a monoculture, designated strain GB8-1$^T$, was obtained. Strain GB8-1$^T$ hardly grew on solid medium in pure culture; tiny white colonies of 0·1–0·2 mm diameter were formed. Strain GB8-1$^T$ neither grew on butyrate alone nor produced $CH_4$, whereas it degraded butyrate in an artificially constructed co-culture with *M. formicicum* DSM 1535$^T$.

Cells of strain GB8-1$^T$ were slightly curved rods with round to acute ends that were 0·5–0·7 μm in width and

2·3–4·0 μm in length and occurred singly, in pairs or in helical filaments. Negative staining showed the presence of one to three flagella inserted in one or both poles or subpoles (see Supplementary Figure, available in IJSEM Online). Spores were not observed. Although the Gram reaction of the cell wall was variable, a Gram-negative structure was revealed by ultrathin section (see Supplementary Figure, available in IJSEM Online).

Strain GB8-1$^T$ could degrade 1 mol butyrate into about 2 mol acetate and 1 mol (presumably) $H_2$, which was consumed by *M. formicicum* DSM 1535$^T$ in the co-culture within 3 weeks. Electron and carbon recoveries of butyrate degradation were 102·19 ± 2·23 and 96·57 ± 2·60 %, respectively. Straight-chain fatty acids with 4–18 carbons could be degraded by the co-culture (Table 1); however, neither branched-chain fatty acids, such as isobutyrate and isovalerate, nor benzoate were degraded. Among the substrates used, fatty acids with an even number of carbons were converted into acetate and $H_2$, whereas those with an odd number of carbons were converted into propionate, acetate and $H_2$, implying that $\beta$-oxidation of fatty acids was performed by the co-culture. None of the following substances could be used by strain GB8-1$^T$ as a potential electron acceptor for butyrate degradation: sodium sulfate (20 mM), sodium thiosulfate (20 mM), sulfur (20 mM), sodium nitrate (20 mM) or sodium fumarate (20 mM). These results indicate the affiliation of strain GB8-1$^T$ to the syntrophic acetogen group.

Crotonate was the only compound tested that supported growth of strain GB8-1$^T$ in pure culture; 1 mol crotonate was disproportioned into about 0·5 mol butyrate and 1 mol acetate in 7 days (with electron and carbon recoveries of 93·13 ± 1·98 and 94·19 ± 1·97 %, respectively). Doubling time of strain GB8-1$^T$ was 11 h when grown on 20 mM crotonate at 37 °C. The molar growth yield of strain GB8-1$^T$ on crotonate was 4·0–4·8 g dry wt mol$^{-1}$. Growth and acid formation were not observed on the following substrates: yeast extract (0·5 %), tryptone (1 %), glucose (20 mM), ribose (20 mM), xylose (20 mM), pyruvate (20 mM) and fumarate (20 mM).

Temperature ranges for the co-culture on butyrate and pure culture on crotonate were both 20–42 °C, with optimal growth at 35–37 °C; the pH range for both types of culture

**Table 1.** Characteristics of strain GB8-1$^T$ and other mesophilic, syntrophic bacteria in the family *Syntrophomonadaceae*

Species: 1, *Syntrophospora bryantii* DSM 3014$^T$; 2, *Syntrophomonas wolfei* subsp. *wolfei* DSM 2245$^T$; 3, *Syntrophomonas wolfei* subsp. *saponavida* DSM 4212$^T$; 4, *Syntrophomonas sapovorans* DSM 3441$^T$; 5, *Syntrophomonas curvata* GB8-1$^T$. Data are from Stieb & Schink (1985), Zhao *et al.* (1990), McInerney *et al.* (1979, 1981), Beaty & McInerney (1987), Lorowitz *et al.* (1989), Roy *et al.* (1986) and this study. All species were positive for production of caprylate from butyrate. Abbreviation: ND, not determined.

| Characteristic | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Cell width (μm) | 0·4 | 0·5–1·0 | 0·4–0·6 | 0·5 | 0·5–0·7 |
| Cell length (μm) | 3·0–6·0 | 2·0–7·0 | 2·0–4·0 | 2·5 | 2·3–4·0 |
| Gram reaction | Variable | − | − | − | Variable |
| Motility | − | + | + | + | + |
| DNA G+C content (mol%) | 37·6 | ND | ND | ND | 46·6 |
| Growth pH (range/optimum) | 6·6–7·5 | ND | ND | 6·3–8·1/7·3 | 6·3–8·4/7·5 |
| Growth temperature (range/optimum) (°C) | 28–34 | ND/35–37 | ND | 25–45/35–37 | 20–42/35–37 |
| Spore formation | + | − | − | − | − |
| Substrate utilization in pure culture: | | | | | |
| Crotonate | + | + | + | − | + |
| Substrate utilization in co-culture with methanogens: | | | | | |
| Acetate | ND | − | ND | − | − |
| Propionate | − | − | − | − | − |
| Pelargonate | + | ND | + | ND | ND |
| Caprate | + | ND | + | + | + |
| Laurate | − | ND | + | + | + |
| Myristate | − | ND | + | + | + |
| Palmitate | − | − | + | + | + |
| Stearate | − | − | + | + | + |
| Oleate | ND | − | − | + | + |
| Linoleate | ND | ND | − | + | − |
| Isobutyrate | − | − | − | − | − |
| Isovarelate | ND | − | − | − | − |
| Triacylglycerides | ND | ND | − | − | ND |

was 6·3±8·4, with an optimal pH of 7·5; and the NaCl tolerance concentration ranges were both 0–100 mM (better growth occurred below 50 mM NaCl). No growth was observed when oxygen was present.

Peptidoglycan of strain GB8-1$^T$ contained LL-diaminopimelic acid. The major fatty acids were as follows: $C_{14:0}$ (27·82 %), $C_{15:0}$ (19·24 %), $C_{14:0}$ 3-OH (10·90 %) and an unknown structure component, with quite a high percentage (21·44 %), with an equivalent chain-length (ECL) of 14·503. The fatty acid profile was not similar to those of anaerobic bacteria in the MIDI database, as $C_{16:0}$ was one of the predominant fatty acids in many *Clostridium* spp. and *Syntrophus aciditrophicus* (Jackson *et al.*, 1999; Broda *et al.*, 2000); however, it occurred at a very low level (2·79 %) in strain GB8-1$^T$.

The genomic DNA G+C content of strain GB8-1$^T$ was determined to be 46·6 mol%; this is different from those reported for the members of the *Syntrophomonadaceae* by 2·4–9 mol% (Matthies *et al.*, 2000; Sekiguchi *et al.*, 2000).

A phylogenetic tree including strain GB8-1$^T$ and other members of the family *Syntrophomonadaceae* was constructed (Fig. 1), based on a consensus length of 1395 bp of their 16S rRNA genes. Sequence similarity showed that strain GB8-1$^T$ was related most closely to *Syntrophomonas sapovorans* DSM 3441$^T$ (95·4 % similarity); similarities of <93 % were revealed with the other described species in the genus *Syntrophomonas* and other genera within the family *Syntrophomonadaceae*.

Although strain GB8-1$^T$ was relatively closely related to *Syntrophomonas sapovorans* DSM 3441$^T$ phylogenetically, some phenotypic characters were different between the two strains. *Syntrophomonas sapovorans* DSM 3441$^T$ had two to four lateral flagella inserted in the concave side of the cell, whereas strain GB8-1$^T$ had one to three polar or subpolar flagella. Furthermore, *Syntrophomonas sapovorans* DSM 3441$^T$ did not grow on crotonate in pure culture, but degraded linoleate in co-culture (Table 1). Considering the phenotypic and phylogenetic distinctions, we propose strain GB8-1$^T$ as the type strain of a novel species of the genus *Syntrophomonas*, *Syntrophomonas curvata* sp. nov.

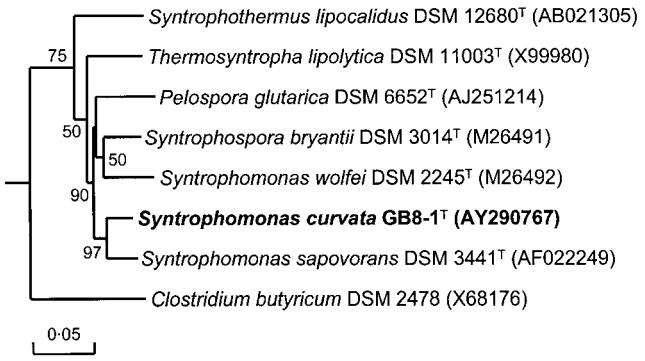


**Fig. 1.** Phylogenetic tree of strain GB8-1$^T$ and related organisms, based on a 1395 bp fragment of 16S rRNA gene sequences. The tree, rooted with *Clostridium butyricum* DSM 2478, was constructed by using the neighbour-joining method with bootstrap values based on 1000 replications. The number at each branch-point is the percentage supported by bootstrap; GenBank accession numbers of 16S rRNA gene sequences are given in parentheses. Bar, 5 % sequence divergence.

### Description of *Syntrophomonas curvata* sp. nov.

*Syntrophomonas curvata* (cur.va′ta. L. fem. adj. *curvata* curved).

Cells are Gram-negative, curved rods, 0·5–0·7 × 2·3–4·0 μm, non-spore-forming, with one to three polar or subpolar flagella. Straight-chain fatty acids with $C_4$–$C_{18}$ serve as substrates for strain GB8-1$^T$ in co-culture with *M. formicicum* DSM 1535$^T$. Even-numbered fatty acids are degraded into acetate and presumably $H_2$, whereas odd-numbered ones are degraded into propionate, acetate and $H_2$. Acetate, propionate, isobutyrate, isovalerate and benzoate do not support growth of the co-culture. Fumarate, sulfate, thiosulfate, sulfur and nitrate cannot act as electron acceptors for butyrate oxidation. Crotonate is the only substrate tested that enables growth of the strain in pure culture, whilst yeast extract, tryptone, glucose, ribose, xylose, pyruvate and fumarate do not. Cellular fatty acids comprise mainly $C_{14:0}$ (27·82 %), $C_{15:0}$ (19·24 %), $C_{14:0}$ 3-OH (10·90 %) and an unknown component of ECL 14·503 (21·44 %). Genomic DNA G+C content is 46·6 mol%.

The type strain, GB8-1$^T$ (=CGMCC 1.5010$^T$=DSM 15682$^T$), was isolated from the granular sludge of a UASB reactor treating beer wastewater in Beijing, China.

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## References

**Beaty, P. S. & McInerney, M. J. (1987).** Growth of *Syntrophomonas wolfei* in pure culture on crotonate. *Arch Microbiol* **147**, 389–393.

**Broda, D. M., Saul, D. J., Bell, R. G. & Musgrave, D. R. (2000).** *Clostridium algidixylanolyticum* sp. nov., a psychrotolerant, xylan-degrading, spore-forming bacterium. *Int J Syst Evol Microbiol* **50**, 623–631.

**Hungate, R. E. (1969).** A roll-tube method for the 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* **5**, 109–118.

**Matthies, C., Springer, N., Ludwig, W. & Schink, B. (2000).** *Pelospora glutarica* gen. nov., sp. nov., a glutarate-fermenting, strictly anaerobic, spore-forming bacterium. *Int J Syst Evol Microbiol* **50**, 645–648.

**McInerney, M. J. (1992).** The genus *Syntrophomonas* and other syntrophic anaerobes. In *The Prokaryotes, a Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications*, 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 $\alpha,\varepsilon$-diaminopimelic acid on paper chromatograms. *J Am Chem Soc* **77**, 4844–4846.

**Roy, F., Samain, E., Dubourguier, H. C. & 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.

**Svetlitshnyi, 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 characterization of *Methanococcus mazei* strain $MC_3$. *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.

**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-$\beta$-oxidizing syntrophic bacteria to *Syntrophomonadaceae* fam. nov. on the basis of 16S rRNA sequence analysis. *Int J Syst Bacteriol* **43**, 278–286.