Pelotomaculum propionicicum sp. nov., an anaerobic, mesophilic, obligately syntrophic, propionate-oxidizing bacterium

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An anaerobic, mesophilic, syntrophic, propionate-oxidizing bacterium, strain MGP^T, was isolated as a defined co-culture with *Methanospirillum hungatei* from the methanogenic sludge of a mesophilic upflow anaerobic sludge blanket (UASB) reactor. The strain grew in the presence of propionate, but only in co-culture with methanogens, suggesting that it is an obligately syntrophic bacterium. The optimum temperature for growth was 37 °C, and the optimum pH was between 6.5 and 7.2. Based on comparative 16S rRNA gene sequence analysis, strain MGP^T was affiliated with subcluster 1h of 'Desulfotomaculum cluster I', in which it was found to be moderately related to known species of the genera *Pelotomaculum* and *Cryptanaerobacter*. Similar to known species of the genus *Pelotomaculum*, strain MGP^T could degrade propionate in syntrophy, but had no ability to reduce sulfate, sulfite and thiosulfate. Further phenotypic and genetic studies supported the affiliation of the strain as a novel species in this genus, for which the name *Pelotomaculum propionicicum* sp. nov. is proposed. The type strain is MGP^T (=DSM 15578^T=JCM 11929^T). The strain has been deposited in the DSM and JCM culture collections as a defined co-culture with *Methanospirillum hungatei*.

Propionate is one of the important intermediates in the anaerobic degradation of complex organic matter under methanogenic conditions (Gujer & Zehnder, 1983; Kaspar & Wuhrmann, 1978; Mah *et al.*, 1990). Under methanogenic conditions, propionate degradation is carried out by a syntrophic association of propionate-oxidizing, hydrogen (and/or formate)-producing fermentative bacteria and hydrogenotrophic methanogens, because the oxidation of propionate is thermodynamically unfavourable in such

environments unless the consumption of the reducing equivalents (hydrogen and/or formate) is coupled with the oxidation (Schink, 1997). Due to this thermodynamic limitation, propionate-degrading anaerobes can gain only a small amount of energy through the syntrophic reaction, and thus these organisms grow very slowly. Because of these traits, isolation of propionate-degrading bacteria has been difficult. To date, eight species have been isolated and described. Five species belong to the class *Deltaproteobacteria*; these include *Syntrophobacter wolinii* (Boone & Bryant, 1980), *Syntrophobacter pfennigii* (Wallrabenstein *et al.*, 1995), *Syntrophobacter fumaroxidans* (Harmsen *et al.*, 1998), *Syntrophobacter sulfatireducens* (Chen *et al.*, 2005) and *Smithella propionica* (Liu *et al.*, 1999). The remaining three species belong to a Gram-positive bacterial lineage 'Desulfotomaculum cluster I' in the phylum *Firmicutes*: i.e. *Desulfotomaculum thermobenzoicum* subsp. *thermosyntrophicum*

Abbreviation: 2-BES, 2-bromoethanesulfonic acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and *dsrAB* gene sequences of strain MGP^T are AB154390 and AB154391, respectively.

A figure showing the effect of temperature, pH and NaCl concentration on the specific growth rate of strain MGP^T is available with the online version of this paper.

(Plugge *et al.*, 2002), *Pelotomaculum thermopropionicum* (Imachi *et al.*, 2002) and *Pelotomaculum schinkii* (de Bok *et al.*, 2005). All the deltaproteobacterial species and *P. schinkii* are mesophiles; the others are thermophiles.

Recently, we reported the isolation of a novel anaerobic, mesophilic, propionate-oxidizing bacterium, designated strain MGP^T, as a defined co-culture with a hydrogenotrophic methanogen, *Methanospirillum hungatei* (Imachi *et al.*, 2006). 16S rRNA gene sequence analysis revealed that the strain was affiliated with 'Desulfotomaculum subcluster 1h', which consists of the genera *Pelotomaculum* and *Cryptanaerobacter*. In addition, strain MGP^T was found to have *dsrAB* genes encoding the α and β subunits of dissimilatory (bi)sulfite reductase, although the strain could not reduce sulfate, sulfite or organosulfonates, which are typical electron acceptors for dissimilatory sulfate-reduction. In this report, we describe detailed morphological and physiological characteristics of strain MGP^T and propose that this strain is a novel species of the genus *Pelotomaculum*.

Strain MGP^T was originally isolated in co-culture with *Methanospirillum hungatei* from the methanogenic sludge of a mesophilic upflow anaerobic sludge blanket (UASB) reactor used to treat artificial wastewater, which was mainly composed of sucrose, propionate and acetate, as described by Sekiguchi *et al.* (1998, 1999). *Methanospirillum hungatei* strain JF1 (DSM 864) was purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). We also used a hydrogen- and formate-utilizing methanogen, *Methanothermobacter thermautotrophicus* strain type-II, isolated in our laboratory. The media for cultivation of strain MGP^T were prepared as described previously (Sekiguchi *et al.*, 2000). *Methanospirillum hungatei* and *Methanothermobacter thermautotrophicus* were cultivated using the same medium as for strain MGP^T, except that hydrogen (approx. 100–150 kPa) and acetate (1 mM) were added to the vials as the energy and carbon sources, respectively. All incubations were performed at 37 °C in 50 ml serum vials containing 20 ml medium (pH at 25 °C, 7.2) under an atmosphere of N₂/CO₂ (80:20, v/v) without shaking, unless otherwise noted. Neutralized substrates were added to the vials from stock solutions prior to inoculation. Growth and substrate utilization were determined by monitoring turbidity visually and by the production of methane, hydrogen and acetate. All incubations for the substrate tests were performed using growing cultures (10%, v/v, inoculum) at 37 °C for over 3 months. The effects of pH, temperature and NaCl concentration on the growth of strain MGP^T in co-culture with *Methanospirillum hungatei* were determined by using 20 mM propionate plus 0.01% yeast extract medium with co-cultured cells of strain MGP^T in exponential phase used as the inoculum. For determination of the optimum pH for growth, the pH value of the propionate medium was adjusted at room temperature to 5.5–8.0 with HCl or NaOH solutions under 100% N₂ gas prior to inoculation. To

determine the temperature range for growth, cultures were incubated at 20–60 °C (pH 7.2). To evaluate the effect of NaCl concentration on growth, an autoclaved NaCl solution was added to the medium to give final concentrations of 1–20 g NaCl l⁻¹. All measurements were performed in triplicate, and the specific growth rate was calculated based on methane production. Cell morphology was examined under a fluorescent microscope (Olympus BX50F). The Gram-staining reaction was performed by Hucker's method (Doetsch, 1981). Transmission electron microscopy was performed with a Hitachi H7000 transmission electron microscope as described by Hattori *et al.* (2000). Short-chain fatty acids, sulfate, sulfite, alcohols, methane, hydrogen and carbon dioxide were measured as described previously (Imachi *et al.*, 2000, 2002, 2006). Comparative 16S rRNA sequence phylogenetic analysis was performed as described previously (Imachi *et al.*, 2006). Bootstrap resampling analysis was performed with neighbour-joining, maximum-parsimony and maximum-likelihood methods

Fig. 1. Transmission electron micrograph of strain MGP^T grown on propionate (20 mM) medium. Arrows indicate *Methanospirillum hungatei* cells. Bar, 0.5 µm.

to estimate the confidence of tree topologies as described previously (Sekiguchi *et al.*, 2006).

Colony isolation was first attempted by the roll tube method on propionate medium in the presence of *Methanospirillum hungatei*, but the attempt was unsuccessful. Therefore, the culture was purified by repetitive serial dilutions and heat treatments. The purity of the strain MGP^T culture was checked on several different test media (Imachi *et al.*, 2006). Moreover, we also tested the purity by 16S rRNA gene-based cloning analyses using the universal bacterial primer pair EUB338*/1490R (Hatamoto *et al.*, 2007; Weisburg *et al.*, 1991) and the universal archaeal primer pair Ar109f/1490R (Imachi *et al.*, 2002; Weisburg *et al.*, 1991). Thirty rRNA gene clones were randomly selected and sequenced from respective clone libraries. The result of the molecular survey also demonstrated that the MGP^T culture consisted of strain MGP^T and *Methanospirillum hungatei*. The co-cultured cells converted propionate to methane and acetate nearly stoichiometrically (1:0.75:1; molar ratio), indicating that strain MGP^T may use the methylmalonyl-CoA pathway, which is known to be one of the propionate-oxidizing pathways (Kosaka *et al.*, 2006). To investigate whether other substrates support growth of and are utilized by strain

MGP^T, various substrates were tried with the co-cultured cells as the inoculum. The following substrates did not support growth and were not utilized (substrates were added at a final concentration of 20 mM unless otherwise indicated): Casamino acids (0.1 %), yeast extract (0.4 %), tryptone (0.1 %), crotonate, H₂/CO₂ (80:20, v/v, head space) plus acetate (1 mM) plus 2-bromoethanesulfonic acid (2-BES) (5 mM), betaine (10 mM), glucose, ribose, xylose, lactate, glycerol (5 mM), formate (40 mM) plus acetate (1 mM) plus 2-BES (5 mM), acetate, butyrate, valerate (5 mM), caproate (5 mM), heptanoate (5 mM), malate, fumarate, succinate, ethanol (10 mM), methanol, 1-propanol, 1-butanol, 1-pentanol (10 mM), acetoin (10 mM), acetaldehyde (0.1 %), 1,2-butanediol (10 mM), 2,3-butanediol (10 mM), 1,3-propanediol (10 mM), ethylene glycol (10 mM), arabinose, 2-propanol, fructose, galactose, mannose, raffinose, sucrose, starch (5 g l⁻¹), xylan (5 g l⁻¹), pectin (5 g l⁻¹), 2-oxoglutarate (10 mM), proline (10 mM), glycine (10 mM), aspartate (10 mM), serine (10 mM), alanine (10 mM), glutamate (10 mM), 3-hydroxybutyrate (10 mM), 4-hydroxybutyrate (10 mM), 1,3-benzenediol (1 mM), benzoate (5 mM), 2,3-dihydroxybenzoate (1 mM), 2,4-dihydroxybenzoate (1 mM), 2,5-dihydroxybenzoate (1 mM), hydroquinone (1 mM), *m*-hydroxybenzoate

Fig. 2. Phylogenetic relationship among strain MGP^T and representatives of 'Desulfotomaculum cluster I'. The tree was constructed by a distance matrix analysis of 16S rRNA gene sequences (neighbour-joining tree). The 16S rRNA gene sequence of *Arthrobacter globiformis* (X80736) was used to root the tree (not shown). Branching points supported by probabilities above 90% according to all the analyses (based on 1000 replicates, estimated using neighbour-joining, maximum-parsimony and maximum-likelihood methods) are indicated by solid circles, whereas nodes with open circles indicate >75% bootstrap probability support by the three analyses. The accession number of each reference sequence is shown in parentheses. Bar, number of nucleotide changes per sequence position.

(1 mM), *p*-hydroxybenzoate (1 mM), vanillate (1 mM), syringate (1 mM), acrylate (1 mM), *o*-methoxybenzoate (1 mM), phthalate and its isomers (1 mM), *p*-toluic acid (1 mM), 3-chlorobenzoate (1 mM) and phenol (1 mM). In addition, the following electron acceptors were not utilized for growth in the presence of propionate and 2-BES (5 mM): sulfate, sulfite (2 mM), thiosulfate, elemental sulfur, taurine (10 mM), isethionate (10 mM), cysteate (5 mM), nitrate, ferric ion [Fe(III)-nitrilotriacetate (NTA): 5 mM], fumarate, anthraquinone disulfonic acid (10 mM), crotonate, proline, glycine, 4-hydroxybenzoate (10 mM) or oxygen. Therefore, we used co-cultured cells of strain MGP^T for further physiological and genetic characterizations.

Cells of strain MGP^T are sausage-shaped rods, non-motile, 2.0–4.0 µm long and 1.0 µm wide, and occur singly or in pairs (Fig. 1). The cells often form aggregates with cells of *Methanospirillum hungatei*. A similar finding was also reported for a syntrophic, propionate-degrading culture of *Pelotomaculum thermopropionicum* (Ishii *et al.*, 2005). The cells are Gram-positive. Spores are spherical and central. Spore formation was observed during the late-exponential growth phase.

Optimum growth of strain MGP^T was observed at 37 °C (see supplementary Fig. S1a, available with the online version of this paper). No growth (and no substrate utilization) occurred below 20 °C or above 50 °C within 2 months of cultivation. *Methanothermobacter thermautotrophicus* cells were added into cultures for cultivation above 45 °C because *Methanospirillum hungatei* cannot grow above 42 °C (Harmsen, 1996). The pH range for growth and substrate utilization of strain MGP^T was estimated to be 6.5–7.5 with an optimum of 6.5–7.2 (supplementary Fig. S1b). Under optimum conditions (pH 7.0, 37 °C), the specific growth rate on 20 mM propionate medium was approx. 0.2 per day (calculated by measuring methane production). The strain could grow at NaCl concentrations ranging from 0 to 5 g l⁻¹ (supplementary Fig. S1c), while *Methanospirillum hungatei* could grow with 0–15 g NaCl l⁻¹.

For strain MGP^T, 1571 bp of the 16S rRNA gene sequence was determined. On the basis of the phylogenetic analysis described previously (Imachi *et al.*, 2006), strain MGP^T was affiliated with subcluster 1h of 'Desulfotomaculum cluster I'. We reconstructed a phylogenetic tree that included 16S rRNA gene sequences from each subcluster containing

Table 1. Characteristics of strain MGP^T and members of subcluster 1h of 'Desulfotomaculum cluster I'

1, *Pelotomaculum propionicum* sp. nov. MGP^T; 2, *Pelotomaculum schinkii* HH^T (de Bok *et al.*, 2005); 3, *Pelotomaculum thermopropionicum* SI^T (Imachi *et al.*, 2002); 4, *Pelotomaculum terephthalicum* JT^T (Qiu *et al.*, 2006); 5, *Pelotomaculum isophthalicum* JI^T (Qiu *et al.*, 2006); 6, *Cryptanaerobacter phenolicus* LR7.2^T (Juteau *et al.*, 2005). ND, Not determined. The numbers in parentheses in optimum temperature and optimum pH indicate the range of growth. All strains are negative for sulfate reduction.

Characteristic	1	2	3	4	5	6
Cell width (µm)	1.0	1.0	0.7–0.8	0.8–1.0	0.8–1.0	1.0
Cell length (µm)	2.0–4.0	2.0–2.5	1.7–2.8	2–3	2–3	2.0
Gram reaction	+	ND	–	–	–	+
DNA G+C content (mol%)	ND	ND	52.8	53.6	ND	51
Optimum temperature (°C)	37 (25–45)	37	55 (45–65)	37 (30–40)	37 (25–45)	30–37
Optimum pH	6.5–7.2 (6.5–7.4)	7.0	7.0 (6.5–8.0)	7.0 (6.5–7.5)	7.0 (6.8–7.2)	7.5–8.0
Spore formation	+	+	+	+	+	–*
<i>dsrAB</i> genes†	+	–	–	–	–	ND
Substrate utilization in pure culture						
Pyruvate	–	–	+	–	–	ND
Fumarate	–	–	+	–	–	–
Crotonate	–	–	–	+	–	–
Phenol	–	–	–	–	–	+
4-Hydroxybenzoate	–	–	–	–	–	+
Substrate utilization in co-culture with hydrogenotrophic methanogens:						
Propionate	+	+	+	–	–	ND
Lactate	–	–	+	–	–	ND
Ethanol	–	–	+	–	–	ND
Benzoate	–	–	–	+	+	ND
Terephthalate	–	ND	–	+	+	ND
Isophthalate	–	ND	–	+	+	ND

*Spore formation was not observed, but *C. phenolicus* could survive in heat-treated culture.

†Data from Imachi *et al.* (2006). The information is derived from a PCR survey using a specific primer set for the genes.

cultured representatives (Fig. 2). The closest relatives of the 16S rRNA gene of strain MGP^T were the *rrnB* 16S rRNA gene sequence of *Pelotomaculum schinkii* (de Bok *et al.*, 2005) and that of *Cryptanaerobacter phenolicus* (Juteau *et al.*, 2004) (both with similarity values of 95 %).

Based on its morphological, physiological and molecular phylogenetic traits, strain MGP^T is certainly a member of subcluster 1h of 'Desulfotomaculum cluster I', which is composed of two genera, *Pelotomaculum* and *Cryptanaerobacter*. They share common phenotypic features (Table 1), particularly the following two traits: (1) all members of the subcluster lack the ability to reduce sulfate, as does strain MGP^T, although they belong to the sulfate-reducing *Desulfotomaculum* cluster; and (2) like almost all *Pelotomaculum* spp., strain MGP^T grows syntrophically with hydrogenotrophic methanogen (*C. phenolicus* was not tested for syntrophic growth; Juteau *et al.*, 2004). The most striking feature of strain MGP^T is its obligately syntrophic life style with hydrogenotrophic methanogens. Obligate syntrophy is also found in some members of the genus *Pelotomaculum*. *P. schinkii* can grow only on propionate in co-culture with hydrogenotrophic methanogens and was thus reported as the 'first true obligately syntrophic propionate-oxidizing bacterium' (de Bok *et al.*, 2005). Also, *Pelotomaculum isophthalicum* grows only with aromatic compounds in co-culture with hydrogenotrophic methanogens (Qiu *et al.*, 2006). Strain MGP^T shares these phenotypes. However, 16S rRNA gene sequence similarity values support the novelty of strain MGP^T at least at species level (Stackebrandt & Goebel, 1994). In addition to this genetic difference, one notable feature that distinguishes strain MGP^T from the known members of the genus *Pelotomaculum* may be that strain MGP^T possesses *dsrAB* genes (Table 1). These genotypic differences support the conclusion that strain MGP^T is a novel species in the genus *Pelotomaculum* for which we propose the name *Pelotomaculum propionicicum* sp. nov.

Description of *Pelotomaculum propionicicum* sp. nov.

Pelotomaculum propionicicum (pro.pi.o.ni'ci.cum. N.L. neut. adj. *propionicicum* referring to the substrate propionic acid, which can be metabolized by the species).

Strictly anaerobic. The organism only grows syntrophically on propionate with hydrogenotrophic methanogens. Although the organism does not demonstrate sulfate reduction, it has *dsrAB* genes that are essential for sulfate respiration. Cells are 2.0–4.0 µm long and 1.0 µm wide, occurring singly or in pairs. Spores are spherical and central. Gram-positive. Temperature range for growth is 25–45 °C (optimum: 37 °C). The pH range for growth is 6.5–7.5 (optimum: 6.5–7.2). Growth occurs in the presence of 0–0.5 % NaCl, but does not occur in the presence of greater than 1.0 % NaCl. Habitat is an anaerobic, mesophilic granular sludge.

The type strain is MGP^T (=DSM 15578^T=JCM 11929^T). The strain is stored in the DSM and JCM culture collections as a defined co-culture with *Methanospirillum hungatei*.

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References

- Boone, D. R. & Bryant, M. P. (1980). Propionate-degrading bacterium, *Syntrophobacter wolinii* sp. nov., gen. nov., from methanogenic ecosystems. *Appl Environ Microbiol* **40**, 626–632.
- Chen, S., Liu, X. & Dong, X. (2005). *Syntrophobacter sulfatireducens* sp. nov., a novel syntrophic, propionate-oxidizing bacterium isolated from UASB reactors. *Int J Syst Evol Microbiol* **55**, 1319–1324.
- de Bok, F. A. M., Harmsen, H. J. M., Plugge, C. M., de Vries, M. C., Akkermans, A. D. L., de Vos, W. M. & Stams, A. J. M. (2005). The first true obligate syntrophic propionate-oxidizing bacterium, *Pelotomaculum schinkii* sp. nov., co-cultured with *Methanospirillum hungatei*, and emended description of the genus *Pelotomaculum*. *Int J Syst Evol Microbiol* **55**, 1697–1703.
- Doetsch, R. N. (1981). Determinative methods of light microscopy. In *Manual of Methods for General Bacteriology*. Edited by P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Phillips. Washington, DC: American Society for Microbiology.
- Gujer, W. & Zehnder, A. J. B. (1983). Conversion processes in anaerobic digestion. *Water Sci Technol* **15**, 127–167.
- Harmsen, H. J. M. (1996). *Detection, phylogeny and population dynamics of syntrophic propionate-oxidizing bacteria in anaerobic granular sludge*. PhD thesis, Wageningen Agricultural University, Wageningen, The Netherlands.
- Harmsen, H. J. M., Van Kuijk, B. L. M., Plugge, C. M., Akkermans, A. D. L., De Vos, W. M. & Stams, A. J. M. (1998). *Syntrophobacter fumaroxidans* sp. nov., a syntrophic propionate-degrading sulfate-reducing bacterium. *Int J Syst Bacteriol* **48**, 1383–1387.
- Hatamoto, M., Imachi, H., Ohashi, A. & Harada, H. (2007). Identification and cultivation of anaerobic, syntrophic long-chain fatty acid-degrading microbes from mesophilic and thermophilic methanogenic sludges. *Appl Environ Microbiol* **73**, 1332–1340.
- Hattori, S., Kamagata, Y., Hanada, S. & Shoun, H. (2000). *Thermoacetogenium phaeum* gen. nov., sp. nov., a strictly anaerobic, thermophilic, syntrophic acetate-oxidizing bacterium. *Int J Syst Evol Microbiol* **50**, 1601–1609.
- Imachi, H., Sekiguchi, Y., Kamagata, Y., Ohashi, A. & Harada, H. (2000). Cultivation and in situ detection of a thermophilic bacterium capable of oxidizing propionate in syntrophic association with hydrogenotrophic methanogens in a thermophilic methanogenic granular sludge. *Appl Environ Microbiol* **66**, 3608–3615.
- Imachi, H., Sekiguchi, Y., Kamagata, Y., Hanada, S., Ohashi, A. & Harada, H. (2002). *Pelotomaculum thermopropionicum* gen. nov., sp. nov., an anaerobic, thermophilic, syntrophic propionate-oxidizing bacterium. *Int J Syst Evol Microbiol* **52**, 1729–1735.
- Imachi, H., Sekiguchi, Y., Kamagata, Y., Loy, A., Qiu, Y.-L., Hugenholtz, P., Kimura, N., Wagner, M., Ohashi, A. & Harada, H. (2006). Non-sulfate-reducing, syntrophic bacteria affiliated with *Desulfotomaculum* cluster I are widely distributed in methanogenic environments. *Appl Environ Microbiol* **72**, 2080–2091.

- Ishii, S., Kosaka, T., Hori, K., Hotta, Y. & Watanabe, K. (2005). Coaggregation facilitates interspecies hydrogen transfer between *Pelotomaculum thermopropionicum* and *Methanothermobacter thermautotrophicus*. *Appl Environ Microbiol* **71**, 7838–7845.
- Juteau, P., Côté, V., Duckett, M.-F., Beaudet, R., Lépine, F., Villemur, R. & Bisailon, J.-G. (2005). *Cryptanaerobacter phenolicus* gen. nov., sp. nov., an anaerobe that transforms phenol into benzoate via 4-hydroxybenzoate. *Int J Syst Evol Microbiol* **55**, 245–250.
- Kaspar, H. P. & Wuhrmann, K. (1978). Kinetic parameters and relative turnovers of some important catabolic reactions in digesting sludge. *Appl Environ Microbiol* **36**, 1–7.
- Kosaka, T., Uchiyama, T., Ishii, S., Enoki, M., Imachi, H., Kamagata, Y., Ohashi, A., Harada, H., Ikenaga, H. & Watanabe, K. (2006). Reconstruction and regulation of the central catabolic pathway in the thermophilic propionate-oxidizing syntroph *Pelotomaculum thermopropionicum*. *J Bacteriol* **188**, 202–210.
- Liu, Y., Balkwill, D. L., Aldrich, H. C., Drake, G. R. & Boone, D. R. (1999). Characterization of the anaerobic propionate-degrading syntrophs *Smithella propionica* gen. nov., sp. nov. and *Syntrophobacter wolnii*. *Int J Syst Bacteriol* **49**, 545–556.
- Mah, R. A., Xun, L.-Y., Boone, D. R., Ahring, B., Smith, P. H. & Wilkie, A. (1990). Methanogenesis from propionate in sludge and enrichment systems. In *Microbiology and Biochemistry of Strict Anaerobes Involved in Interspecies Hydrogen Transfer*, pp. 99–111. Edited by J.-P. Belaich, M. Bruschi & J.-L. Garcia. New York: Plenum.
- Plugge, C. M., Balk, M. & Stams, A. J. M. (2002). *Desulfotomaculum thermobenzoicum* subsp. *thermosyntrophicum* subsp. nov., a thermophilic, syntrophic, propionate-oxidizing, spore-forming bacterium. *Int J Syst Evol Microbiol* **52**, 391–399.
- Qiu, Y.-L., Sekiguchi, Y., Hanada, S., Imachi, H., Tseng, I.-C., Cheng, S.-S., Ohashi, A., Harada, H. & Kamagata, Y. (2006). *Pelotomaculum terephthalaticum* sp. nov., and *Pelotomaculum isophthalaticum* sp. nov.: two anaerobic bacteria that degrade phthalate isomers in syntrophic association with hydrogenotrophic methanogens. *Arch Microbiol* **185**, 172–182.
- Schink, B. (1997). Energetics of syntrophic cooperation in methanogenic degradation. *Microbiol Mol Biol Rev* **61**, 262–280.
- Sekiguchi, Y., Kamagata, Y., Syutubo, K., Ohashi, A., Harada, H. & Nakamura, K. (1998). Phylogenetic diversity of mesophilic and thermophilic granular sludges determined by 16S rRNA gene analysis. *Microbiology* **144**, 2655–2665.
- Sekiguchi, Y., Kamagata, Y., Nakamura, K., Ohashi, A. & Harada, H. (1999). Fluorescence *in situ* hybridization using 16S rRNA-targeted oligonucleotides reveals localization of methanogens and selected uncultured bacteria in mesophilic and thermophilic sludge granules. *Appl Environ Microbiol* **65**, 1280–1288.
- 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.
- Sekiguchi, Y., Imachi, H., Susilorukmi, A., Muramatsu, M., Ohashi, A., Harada, H., Hanada, S. & Kamagata, Y. (2006). *Tepidanaerobacter syntrophicus* gen. nov., sp. nov., an anaerobic, moderately thermophilic, syntrophic alcohol- and lactate-degrading bacterium isolated from thermophilic digested sludges. *Int J Syst Evol Microbiol* **56**, 1621–1629.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Wallrabenstein, C., Hauschild, E. & Schink, B. (1995). *Syntrophobacter pfennigii* sp. nov., new syntrophically propionate-oxidizing anaerobe growing in pure culture with propionate and sulfate. *Arch Microbiol* **164**, 346–352.
- 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.