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Desulfotomaculum thermobenzoicum subsp. *thermosyntrophicum* subsp. nov., a thermophilic, syntrophic, propionate-oxidizing, spore-forming bacterium

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From granular sludge from a laboratory-scale upflow anaerobic sludge bed reactor operated at 55 °C with a mixture of volatile fatty acids as feed, a novel anaerobic, moderately thermophilic, syntrophic, spore-forming bacterium, strain TPO, was enriched on propionate in co-culture with *Methanobacterium thermoautotrophicum* Z245. The axenic culture was obtained by using pyruvate as the sole source of carbon and energy. The cells were straight rods with pointed ends and became lens-shaped when sporulation started. The cells were slightly motile. The optimum growth temperature was 55 °C and growth was possible between 45 and 62 °C. The pH range for growth of strain TPO was 6–8, with an optimum at pH 7–7·5. Propionate was converted to acetate, CO_2 and CH_4 by a co-culture of strain TPO with *Methanobacterium thermoautotrophicum* Z245. In pure culture, strain TPO could grow fermentatively on benzoate, fumarate, H_2/CO_2, pyruvate and lactate. Sulphate could serve as inorganic electron acceptor when strain TPO was grown on propionate, lactate, pyruvate and H_2/CO_2. The G+C content was 53·7 mol%. Comparison of 16S rDNA sequences revealed that strain TPO is related to *Desulfotomaculum thermobenzoicum* (98%) and *Desulfotomaculum thermoacetoxidans* (98%). DNA–DNA hybridization revealed 88·2% reassociation between strain TPO and *D. thermobenzoicum* and 83·8% between strain TPO and *D. thermoacetoxidans*. However, both organisms differ physiologically from strain TPO and are not capable of syntrophic propionate oxidation. It is proposed that strain TPO should be classified as new subspecies of *D. thermobenzoicum* as *D. thermobenzoicum* subsp. *thermosyntrophicum*.

Keywords: *Desulfotomaculum thermobenzoicum* subspecies *thermosyntrophicum* subsp. nov., propionate oxidation, syntrophy, interspecies hydrogen transfer

INTRODUCTION

The methanogenic degradation of complex organic acids is brought about via a complex food chain, which ultimately ends up as CH_4, CO_2 and NH_4^+ (Zehnder, 1978; Zeikus, 1983). Propionate is an important intermediate in this conversion. To convert this compound completely to methane and CO_2, different physiological groups of micro-organisms are needed (Stams, 1994; Schink, 1997). Propionate is oxidized by acetogenic bacteria to acetate and CO_2, whereas reducing equivalents are released as H_2 and/or formate. The energetics of this reaction are highly unfavourable and are only possible when an external sink is present for the removal of H_2 and/or formate. Methanogenic archaea usually serve as hydrogen- and formate-scavengers in syntrophic co-cultures with propionate oxidizers.

Several mesophilic, syntrophic propionate-oxidizing cultures have been described previously. *Syntropho-*

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain TPO is AY007190.

bacter wolinii (Boone & Bryant, 1980) was the first strain described. Other examples of syntrophic cultures are *Syntrophobacter pfennigii* and *Syntrophobacter fumaroxidans* (Wallrabenstein *et al*., 1995; Harmsen *et al*., 1998). These *Syntrophobacter* species are phylogenetically related and belong to a physiological heterogeneous group of sulphate-reducers. In the presence of sulphate, *Syntrophobacter* strains all couple propionate oxidation to sulphate reduction. Recently, Liu *et al*. (1999) described a syntrophic propionate-degrading organism, *Smithella propionica*. This organism is phylogenetically more related to syntrophic benzoate-degraders such as *Syntrophus buswellii*.

To date, very little has been known about thermophilic syntrophic propionate degradation. *Desulfotomaculum thermocisternum* was described as being capable of syntrophic growth on propionate in co-culture with a methanogen (Nilsen *et al*., 1996). Imachi *et al*. (2000) described the enrichment and phylogenetic position of a moderate thermophilic bacterium, strain SI, which can grow syntrophically on propionate.

A thermophilic consortium enriched by us on propionate (Stams *et al*., 1992) was able to degrade pyruvate and lactate without the presence of methanogens, indicating that the propionate-oxidizing bacterium was able to grow by fermentation. In this paper, we describe the isolation and characterization of a novel thermophilic, spore-forming, syntrophic, propionate-oxidizing bacterium, strain TPO, from that enrichment culture.

METHODS

Organisms, cultivation and isolation procedures. Strain TPO was isolated from an enrichment culture originating from thermophilic, anaerobic, granular sludge (Stams *et al*., 1992). *D. thermocisternum* (DSM 10259^T) and *Desulfotomaculum thermobenzoicum* (DSM 6193^T) were kindly provided by H. Goorissen, University of Groningen, The Netherlands. *Methanobacterium thermoautotrophicum* Z245 (DSM 3720) and *Desulfotomaculum thermoacetoxidans* (DSM 5813^T) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Braunschweig, Germany).

A bicarbonate-buffered anaerobic medium previously described by Stams *et al*. (1992) was used for the cultivation of all strains. Substrates were added from sterile anaerobic stock solutions to a final concentration of 20 mM (unless otherwise stated). Occasionally yeast extract (0·02 %, w/v) was added to stimulate growth. For isolation of strain TPO, a direct dilution method was used in liquid medium supplemented with 20 mM pyruvate as the sole source of carbon and energy, followed by dilution in soft agar media containing 0·75 % agar. Purity was checked by light microscopy and growth in soft agar media.

For syntrophic growth tests, 1 % strain TPO, *Desulfotomaculum thermoacetoxidans*, *D. thermobenzoicum* or *D. thermocisternum* were inoculated in hydrogen-pregrown cultures of *Methanobacterium thermoautotrophicum* Z245. Prior to inoculation, the gas phase of these bottles was changed to 180 kPa N_2/CO_2 (80:20, v/v) and 20 mM propionate was added.

Determination of growth parameters. The temperature optimum was determined over a temperature range of 30–75 °C in a bicarbonate-buffered medium containing 20 mM pyruvate. The pH optimum was tested in basal medium containing KH_2PO_4 (0·15 g l^{-1}), instead of sodium bicarbonate, and 20 mM pyruvate. The pH values of the medium containing 20 mM pyruvate were adjusted with NaOH or HCl. In this case, an N_2 atmosphere was applied. Duplicate bottles were incubated at 55 °C over a pH range of 4·5–9·5, and the time course of acetate production was measured.

Utilization of substrates by strain TPO in pure culture and in co-culture with *Methanobacterium thermoautotrophicum* Z245 was determined by monitoring growth, substrate depletion and product formation. All these incubations were performed at 55 °C and pH 7. The effect of electron acceptors on the growth of strain TPO was tested in basal medium with 20 mM propionate.

Morphological characterization. All methods used for the morphological characterization of strain TPO were as described previously (Plugge *et al*., 2000). Transmission electron microscopy was performed as described previously (Plugge *et al*., 2000).

G+C content. Isolation and purification of genomic DNA was carried out according to Marmur (1961). The G+C content of the DNA was determined by thermal denaturation, as described by Owen *et al*. (1969).

16S rDNA sequence analysis. DNA was extracted from strain TPO as previously described by Zoetendal *et al*. (1998). A PCR was performed with the bacterial primers 7f and 1510r by using the *Taq* DNA polymerase kit from Life Technologies to amplify the bacterial 16S rDNA. The PCR products were purified with the Qiaquick PCR purification kit (Qiagen) according to the manufacturer's instructions. Primers 515r, 1100r (Lane, 1991) and 968f (Nübel *et al*., 1996) labelled with Infrared Dye 41 (MWG-Biotech) were used for sequencing. The sequences were automatically analysed on a LI-COR DNA sequencer (4000L) and corrected manually. Phylogenetic analysis and tree construction were performed with the programs of the ARB software package (Strunk & Ludwig, 1991). The tree is based on the results of distance matrix analysis including only those sequence positions that share the same nucleotides in at least 50 % of sequences from relevant members of Gram-positive bacteria (Felsenstein, 1982). FASTA homology searches with sequences of the EMBL and GenBank DNA databases were performed and the results were compared with those obtained with the ARB programs.

DNA–DNA hybridization experiments. DNA–DNA hybridization was performed with strain TPO, *D. thermobenzoicum* DSM 6193^T, *D. thermoacetoxidans* DSM 5813^T and *D. thermocisternum* DSM 10259^T at the DSMZ. DNA was isolated by chromatography on hydroxyapatite according to the procedure of Cashion *et al*. (1977). DNA–DNA hybridization was carried out as described by DeLey *et al*. (1970), with modifications as described by Huß *et al*. (1983) and Escara & Hutton (1980) using a Gilford model 2600 spectrophotometer equipped with a Gilford model 2527-R thermoprogrammer and plotter. Renaturation rates were computed with the TRANSFER.BAS program of Jahnke (1992).

Preparation of cell-free extracts. Cells of strain TPO in co-culture with *Methanobacterium thermoautotrophicum* strain

Z245 grown on propionate and in pure culture grown on H_2/CO_2 plus sulphate were centrifuged (23000 ***g***, 10 min, 4 °C). The cell pellet was suspended in 0·1 M Tris buffer (pH 8·0) containing 0·4 mg Na_2EDTA per ml and 50 μg lysozyme ml^{-1} as described by Wofford *et al.* (1986). Cells were disrupted by ultrasonic disintegration (4 × 30 s, 30 kHz). Cell debris was removed by centrifugation (8000 ***g***, 15 min, 4 °C) and the supernatant was stored anaerobically at 4 °C. Protein concentrations were determined by the method of Bradford (1976), and bovine serum albumin was used as the standard.

Enzyme assays. Enzyme assays were carried out anaerobically at 55 °C by using a Hitachi U-2010 UV-VIS spectrophotometer. Cuvettes (1 ml) were closed with rubber stoppers and flushed with N_2. Hydrogenase (EC 1.12.1.2) and pyruvate dehydrogenase (EC 1.2.4.1) were assayed with methyl viologen as the electron acceptor according to Odom & Peck (1981). Fumarase (EC 4.2.1.2), malate dehydrogenase (NAD-dependent, EC 1.1.1.37, and NADH-dependent, EC 1.1.1.82), methylmalonyl-CoA:pyruvate transcarboxylase (EC 2.1.3.1), phosphotransacetylase (EC 2.3.1.8) and succinate dehydrogenase (EC 1.3.99.1) activities were assayed according to Stams *et al.* (1984). Propionate kinase and acetate kinase (EC 2.7.2.1) activities were determined as described by Aceti & Ferry (1988). Carbonmonoxide dehydrogenase (EC 1.2.99.2) and 2-oxoglutarate:methylviologen oxidoreductase (EC 1.2.7.3) activities were measured according to Schauder *et al.* (1986).

To calculate the specific activities of enzymes of the propionate-oxidizing organisms in cell-free extracts of co-cultures, a correction was made for the biomass produced by the methanogen, using the molar growth yield [2·2 g dry wt × (mol CH_4)$^{-1}$] of *Methanobacterium thermoautotrophicum* Z245 on H_2/CO_2.

Analytical methods. The concentrations of substrates and fermentation products were measured using HPLC and GC methods as described by Stams *et al.* (1993). Sulphide was measured as described by Trüper & Schlegel (1964). Anions were analysed as described by Scholten & Stams (1995).

RESULTS AND DISCUSSION

Enrichment and isolation of strain TPO

The enrichment of the thermophilic, syntrophic, propionate-oxidizing strain TPO from methanogenic granular sludge was described previously by Stams *et al.* (1992). *Methanobacterium thermoautotrophicum* Z245 was removed from the enrichment culture by pasteurizing the culture for 30 min at 90 °C and performing subsequent transfers in media with pyruvate as the carbon source. This resulted in a culture of strain TPO that was found to be pure when checked by light microscopy and on soft agar media (0·75% agar) containing pyruvate. Colonies, embedded in the soft agar, were white to brownish, lens-shaped, and reached a diameter of 0·1–0·2 mm.

Morphological characteristics

Cells of strain TPO were rod-shaped, with rounded ends, and were 1 μm × 3–11 μm in size. They occurred as single cells, but sometimes pairs were formed. When sporulation started, cells became lens-shaped, and ultimately only oval spores remained. Spores were in the centres of the cells: one spore (1·5 μm × 1·5 μm) per cell was observed. Cells stained Gram-negative but the cell wall ultrastructure resembled that of Gram-positive bacteria, as shown in Fig. 1. Weak motility was observed only in cultures growing on pyruvate.

Physiological characterization and metabolism of strain TPO in pure culture and in co-culture with *Methanobacterium thermoautotrophicum* Z245

Strain TPO was strictly anaerobic, since no growth occurred in the presence of traces of oxygen. The strain grew in pure culture on pyruvate at temperatures between 45 and 62 °C; the optimum was 55 °C. The pH optimum for growth on pyruvate was 7–7·5. Below a pH of 6 and above pH 8, no measurable growth occurred. Under optimum conditions, the growth rate of strain TPO in pure culture on pyruvate was 0·33 d^{-1}. Yeast extract (0·02%) stimulated growth but was not required. Besides pyruvate, lactate, fumarate, H_2/CO_2 and benzoate each supported growth of strain TPO in pure culture (Table 1).

A remarkable feature of strain TPO is its ability to grow fermentatively on benzoate. In methanogenic environments, benzoate is generally oxidized to acetate and H_2. This reaction is highly unfavourable under standard conditions: $C_6H_5COO^- + 6H_2O \rightarrow 3CH_3COO^- + 2H^+ + CO_2 + 3H_2$; $\Delta G^{0'} = +49{\cdot}5$ kJ benzoate mol^{-1} ($\Delta G^{0'}$ values were taken from Thauer *et al.*, 1977). However, strain TPO does not produce 3 mol hydrogen from benzoate (Table 2). Only traces of hydrogen could be measured. Since the fermentation balance on benzoate is far from complete, an unknown reduced end-product must have been formed during its conversion. We speculate that the strain is able to reduce the aromatic ring, probably to cyclohexane carboxylate or cyclohexene carboxylate, in a manner similar to that described as occurring in anaerobic sludge (Kleerebezem, 1999). Elshahed *et al.* (2001) very recently proposed that benzoate oxidation by a syntrophic co-culture of '*Syntrophus aciditrophicus*' strain SB and *Methanospirillum hungatei* occurs via the intermediate formation of cyclohexane carboxylate and cyclohexene carboxylate (or their coenzyme A derivatives). Research is in progress to reveal the identity of the unknown compound(s) formed from benzoate fermentation by strain TPO.

In addition, the bacterium was able slowly to ferment glycine to acetate. Glycine is probably first converted via a glycine decarboxylase complex. The 'methylene' group formed may enter the homoacetogenic pathway, resulting in the formation of acetate.

In co-culture with *Methanobacterium thermoautotrophicum* strain Z245, strain TPO was able to utilize the following compounds: propionate, pyruvate, lactate, fumarate and benzoate. Fig. 2 shows the stoichiometry of propionate utilization by strain TPO in

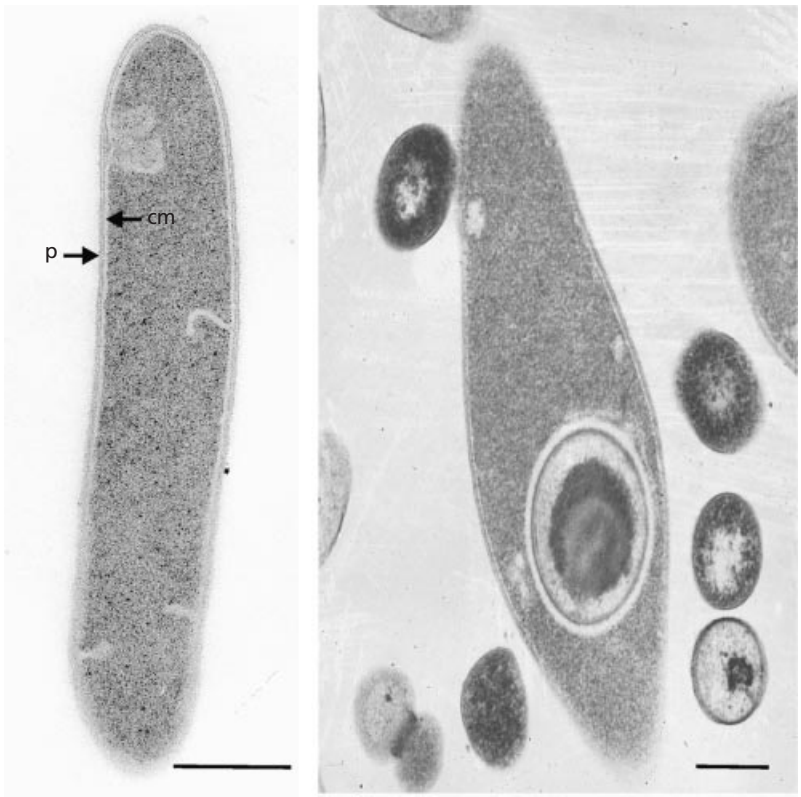

Fig. 1. Electron micrographs showing (left) the general morphology of strain TPO and the single-layered structure of the cell wall (cm, cytoplasmic membrane; p, peptidoglycan layer) and (right) spore formation. The culture was grown on propionate in the presence of *Methanobacterium thermoautotrophicum* Z245. Bars, 0·5 μm.

Table 1. Growth rates and optical density (OD_{600}) measurements of strain TPO grown under different conditions

Substrate (mmol l^{-1})	Products (mmol l^{-1})	Growth rate (d^{-1})	OD_{600}
Pyruvate (15·0)	Acetate (16·0) and H_2 (0·3)	0·33	0·067
Fumarate (18·9)	Succinate (13·9), acetate (4·0), propionate (1·4) and H_2 (0·065)	0·247	0·114
Lactate (12·4)	Acetate (11·3) and H_2 (0·15)	0·138	0·039
Propionate (7·8) + SO_4^{2-} (20)	Acetate (6·8), HS^- (4·1)	0·099	0·060
Benzoate (10)	Acetate (13), propionate (0·5) and succinate (1·5)	ND	ND
H_2/CO_2 (10^5 Pa)	Acetate (9·7)	ND	ND
Propionate (18·0) + *Methanobacterium thermoautotrophicum* Z245	Acetate (18·9), CH_4 (14·1)	0·115	0·144

ND, Not determined.

the presence of the methanogen *Methanobacterium thermoautotrophicum* Z245. In co-culture, malate, alanine and glycine were also slowly converted.

The following single substrates were tested and found not to be utilized for growth either by the pure culture or by the co-culture with strain TPO: glucose, fructose, ribose, sucrose, xylose, xylitol, acetate (10 mM), citrate (10 mM), succinate (10 mM), butyrate, tartrate, malonate, glutamate, aspartate, methanol, ethanol, propanol (10 mM), butanol, 2,3-butanediol (10 mM), acetoin (10 mM), Casamino acids (0·2 %, w/v), peptone (0·2 %, w/v).

The following Stickland mixtures did not support growth: alanine + arginine, alanine + proline, leucine + proline. In the case of glycine-containing incubations (propionate + glycine, leucine + glycine) no more acetate was formed than with glycine alone, indicating that glycine could not serve as an electron acceptor. Therefore it appeared that strain TPO was incapable of performing Stickland reactions.

Table 2. Characteristics that differentiate strain TPO from other closely related organisms

Characteristic	TPO	*Desulfotomaculum thermobenzoicum*	*Desulfotomaculum thermocisternum*	*Desulfotomaculum thermoacetoxidans*
Gram stain	−	+	ND	−
Cell wall structure	+	ND	+	ND
Cell morphology	Rod shaped with pointed ends to lens-shaped	Rod-shaped	Rod-shaped	Rod-shaped with pointed ends
Colony formation	On soft agar	On agar	On Gelrite	Prepurified agar
Motility	Weak	Weak	+	Weak
Spore formation	+	+	+	+
Temperature optimum (°C)	55	62	62	55–60
Temperature range (°C)	45–62	40–70	41–75	45–65
pH optimum	7·0	7·2	6·7	6·5
G+C content (mol%)	53·7	52·8	56–57	49·7
Fermentative growth	Pyruvate, lactate, fumarate, glycine, benzoate	Pyruvate, lactate	Pyruvate	Pyruvate
Substrate oxidation coupled to sulphate reduction	Incomplete	Complete	Incomplete	Complete
Other electron acceptors:				
SO_3^{2-}	−	+	+	−
$S_2O_3^{2-}$	−	+	+	+
S^0	ND	−	−	−
NO_3^-	−	+		−
Syntrophic growth on propionate	+	−	−*	−

ND, Not determined.

* Nilsen *et al.* (1996) reported syntrophic growth on propionate by *D. thermocisternum*. However, we could not reproduce these results, nor could Imachi *et al.* (2000).

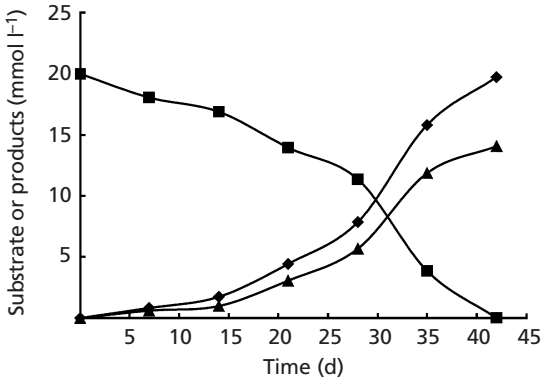

Fig. 2. Propionate degradation (■) and formation of acetate (◆) and methane (▲) by strain TPO in co-culture with *Methanobacterium thermoautotrophicum* strain Z245 measured over time.

Sulphate was able to serve as the electron acceptor for growth on propionate, lactate, pyruvate and H_2/CO_2. Acetate was formed as the end-product. Acetate could not be used by strain TPO in the presence of sulphate. The presence of sulphate had no effect on the fermentation of fumarate and benzoate.

Thiosulphate, sulphite, nitrate and fumarate could not be utilized as electron acceptors for propionate oxidation. When strain TPO was cultivated in the presence of propionate+fumarate, fumarate was fermented (Table 1).

Specific enzyme activities measured in cell-free extracts varied in different cell preparations. The lysozyme treatment followed by ultrasonic treatment resulted in extracts with the highest activity. Results of the measurements with cells grown in co-culture with *Methanobacterium thermoautotrophicum* strain Z245 on propionate and in pure culture on H_2/CO_2 +sulphate are shown in Table 3. Enzymes from the methylmalonyl-CoA pathway of propionate conversion were present at high to moderately high activities in cell extracts of propionate-grown cells. Extracts from cells grown with $H_2+SO_4^{2-}$ showed moderately high activities of carbon monoxide dehydrogenase, whereas no 2-oxoglutarate dehydrogenase activity could be detected, indicating the presence of the acetyl-CoA pathway during autotrophic growth.

Phylogeny and G+C content

A total of 1551 bp of the 16S rDNA gene from strain TPO were sequenced. To determine the phylogenetic position of strain TPO, we compared the data with

Table 3. Enzyme activities in cell-free extracts of strain TPO

Data are for strain TPO grown on propionate in co-culture with *Methanobacterium thermoautotrophicum* Z245 and on H_2/CO_2 plus sulphate in pure culture. Activities are expressed in μmol min^{-1} (mg protein)$^{-1}$. MV^{++}, Methyl viologen (as electron acceptor).

Enzyme	Propionate*	H_2/CO_2 + sulphate
Propionate kinase	0·93	
Methylmalonyl CoA : pyruvate transcarboxylase	0·56	
Succinate dehydrogenase	< 0·001	
Fumarase (disappearance of fumarate)	4·8	
Fumarase (formation of fumarate)	0·16	
Malate dehydrogenase (NAD)	0·58	
Malate dehydrogenase (NADH)	0·33	
Pyruvate dehydrogenase (MV^{++})	1·55	0·8
Phosphotransacetylase	1·50	
Acetate kinase	0·66	
Hydrogenase (MV^{++})	0·96	
2-Oxoglutarate dehydrogenase	ND	Not detected
Carbon-monoxide dehydrogenase (MV^{++})	ND	0·6–0·7

*Activities were calculated after correction for the protein content of the methanogen (see Methods); ND, not determined.

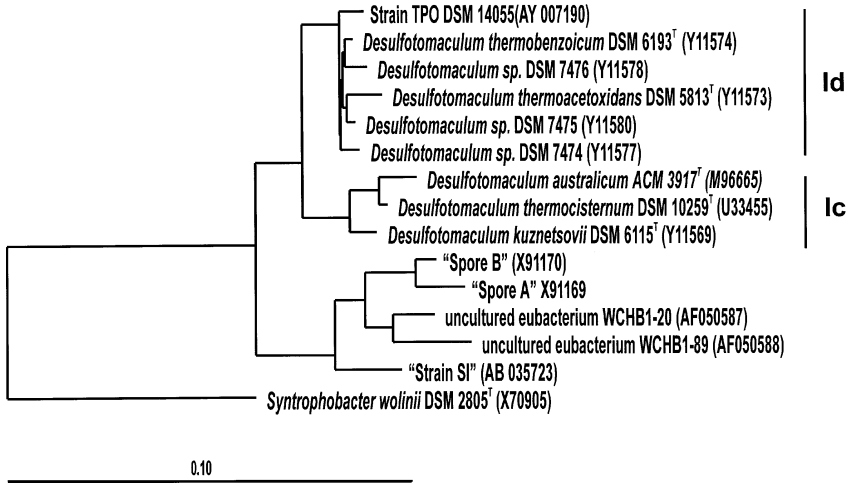

Fig. 3. Neighbour-joining tree, based on 16S rDNA sequences, showing the phylogenetic position of strain TPO among representatives of the genus *Desulfotomaculum*. The bar (0·1) represents evolutionary distance. The phylogenetic subclusters of Cluster I described previously by Stackebrandt *et al.* (1997) are indicated.

known bacterial 16S rDNA sequences (Fig. 3). Sequence analysis showed that strain TPO fell within the phylogenetic subcluster Id of the genus *Desulfotomaculum*, as defined by Stackebrandt *et al.* (1997), the closest neighbours being *D. thermoacetoxidans* (Min & Zinder, 1990) and *D. thermobenzoicum* (Tasaki *et al.*, 1991), each of which showed 98 % similarity. DNA–DNA hybridization of strain TPO with *D. thermoacetoxidans*, *D. thermobenzoicum* and *D. thermocisternum* revealed 83·8, 88·2 and 43·4 % reassociation values, respectively. The G+C content of strain TPO was 53·7 mol %.

Comparison of strain TPO with related strains

Thus far, there is no detailed description of other thermophilic propionate-oxidizing syntrophs from anaerobic environments. Only *D. thermocisternum* (Nilsen *et al.*, 1996) was described as growing syntrophically on propionate in the presence of *Methanococcus thermolithotrophicus* (DSM 8766). However, no details were given concerning the stoichiometry and propionate-conversion rates.

D. thermobenzoicum, *D. thermoacetoxidans* and *D. thermocisternum* were all pregrown on propionate +sulphate. These cells were used as the inocula to construct syntrophic consortia with *Methanobacterium thermoautotrophicum* Z245 to degrade propionate. After 60 d incubation, neither substantial methane production nor disappearance of propionate could be detected. Experiments were also carried out in the presence of 2 mM sulphate, to stimulate the organisms. Furthermore, the organisms were incubated at their optimum growth temperatures (Table 2). In all cases propionate was depleted to a level just

sufficient to reduce all the sulphate present. Thereafter, no further propionate degradation could be measured. A third trial was done and, in this case, besides 2 mM sulphate, 0·2 mM $FeCl_2$ was also added to the cultures. The addition of $FeCl_2$ leads to the formation of FeS precipitates, to which many bacteria can adhere (Stams *et al*., 1992), and brings the acetogenic and methanogenic organisms into closer contact with each other. An additional advantage is that the methanogens remain viable over a much longer period of time. These conditions did not result in measurable propionate degradation after 60 d incubation either. Imachi *et al*. (2000) were also unsuccessful in growing *D. thermocisternum* in a syntrophic co-culture on propionate. They used *Methanobacterium thermoautotrophicum* strain ΔH and a *Methanobacterium thermoformicicum* strain as the syntrophic partner. The ability to grow syntrophically on propionate separates strain TPO from members of the Id subclass of the genus *Desulfotomaculum*.

The physiological differences between the *Desulfotomaculum* species mentioned and strain TPO are considerable (Table 2). Substrate oxidation coupled to sulphate reduction of *D. thermoacetoxidans* and *D. thermobenzoicum* is complete to CO_2. Strain TPO cannot oxidize substrates further than acetate when sulphate is present. Like *D. thermoacetoxidans* and *D. thermobenzoicum*, strain TPO is capable of autotrophic growth on H_2/CO_2, forming acetate. This is confirmed by the presence of carbon monooxide dehydrogenase activity in cell-free extracts of strain TPO grown in H_2/CO_2 + sulphate (Table 3). Strain TPO can use only sulphate as an electron acceptor, whereas *D. thermoacetoxidans* and *D. thermobenzoicum* can also use thiosulphate. *D. thermobenzoicum* can also use sulphite and nitrate.

Comparison of strain TPO with other syntrophic organisms

The phylogenetic tree in Fig. 3 shows that strain TPO is distantly related to the mesophilic spore-forming propionate-oxidizing enrichment 'Spore A' and 'Spore B' (Harmsen *et al*., 1996) and strain SI (Imachi *et al*. 2000). These organisms are unable to use sulphate as a terminal electron acceptor, although they group within the *Desulfotomaculum* genus. They form a new cluster between clusters Ia and If as defined by Stackebrandt *et al*. (1997). Further comparison with these cultures is not possible because of their limited physiological description.

Physiologically, strain TPO has similarities with almost all other mesophilic, non-sporulating, syntrophic propionate-oxidizers. The ability to use sulphate as a terminal electron acceptor is a characteristic that strain TPO shares with other mesophilic, non-sporulating, syntrophic, propionate-oxidizers (Wallrabenstein *et al*., 1995; Van Kuijk & Stams, 1995). On the basis of the enzyme measurements shown in Table 3, it seems that strain TPO uses the methylmalonyl-CoA pathway for syntrophic propionate oxidation. This pathway is used by most mesophilic, syntrophic propionate-oxidizers (Houwen *et al*., 1990; Plugge *et al*., 1993; Wallrabenstein *et al*., 1995). *Smithella propionica* (Liu *et al*., 1999) has a different stoichiometry for propionate conversion with respect to strain TPO. It forms acetate as well as butyrate as organic end-products from propionate conversion. *Smithella propionica* can grow on crotonate in pure culture, but cannot use sulphate as the terminal electron acceptor.

Strain TPO is phylogenetically different from previously described propionate-degrading bacteria (Fig. 3).

On the basis of the differences between strain TPO and known organisms, we propose to designate strain TPO as a new subspecies of *D. thermobenzoicum*, namely *Desulfotomaculum thermobenzoicum* subsp. *thermosyntrophicum* subsp. nov. This automatically creates *Desulfotomaculum thermobenzoicum* subsp. *thermobenzoicum* subsp. nov.

Emended description of *Desulfotomaculum thermobenzoicum* Tasaki *et al.* 1991

Desulfotomaculum thermobenzoicum (thermo.ben.zo′i.cum. Gr. adj. *thermos* hot; N.L. gen. n. *benzoicum* pertaining to benzoate; *Desulfotomaculum thermobenzoicum* a sausage-shaped organism that reduces sulphur compounds and oxidizes benzoate under anaerobic conditions).

Rod-shaped Gram-positive cells 1·0–2 μm × 3–11 μm in size with pointed ends, single or in pairs. Spore-forming and slightly motile. Strictly anaerobic, moderately thermophilic and neutrophilic. The substrates used coupled to sulphate reduction are lactate, pyruvate, propionate and H_2/CO_2. Acetate is not oxidized. Pyruvate and lactate are degraded without sulphate. Grows at 40–70 °C and pH 6–8; optimum conditions are at 55–62 °C and pH 7·0–7·2. Desulfoviridin is not present. The DNA G+C content is 52·8–53·7 mol %. Isolated from thermophilic methanogenic reactors. The type strain is DSM 6193^T (= ATCC 49756^T).

Description of *Desulfotomaculum thermobenzoicum* subsp. *thermobenzoicum* subsp. nov.

Desulfotomaculum thermobenzoicum subsp. *thermobenzoicum* (ther.mo.ben.zo′i.cum. Gr. adj. *thermos* hot; N.L. gen. n. *benzoicum* pertaining to benzoate; *Desulfotomaculum thermobenzoicum* a sausage-shaped organism that reduces sulphur compounds and oxidizes benzoate under anaerobic conditions).

Rod-shaped Gram-positive cells 1·5–2 μm × 5–8 μm with pointed ends, single or in pairs. Spore-forming and slightly motile. Strictly anaerobic, moderately thermophilic and neutrophilic. The substrates used coupled to sulphate reduction are benzoate, H_2/CO_2,

formate, propionate, butyrate, valerate, caproate, ethanol, propanol, butanol, 1,2-propanediol, 1,3-propanediol, crotonate, lactate, pyruvate, fumarate and malate. Acetate is not oxidized. Pyruvate and lactate are degraded without sulphate. Grows well on benzoate, alcohols, butyrate and H_2+CO_2. Electron acceptors are sulphate, sulphite, thiosulphate and nitrate. Organic supplements (yeast extract) are required for growth. Grows at 40–70 °C and pH 6–8; optimum conditions are at 62 °C and pH 7·2. Desulfoviridin is not present. The DNA G+C content is 52 mol%. Isolated from a thermophilic methane fermentation reactor treating kraft-pulp waste water. The type strain is DSM 6193^T (= ATCC 49756^T).

Description of *Desulfotomaculum thermobenzoicum* subsp. *thermosyntrophicum* subsp. nov.

Desulfotomaculum thermobenzoicum subsp. *thermosyntrophicum* (ther.mo.syn.tro′phi.cum. Gr. adj. *thermos* hot; Gr. pref. s*yn* together; Gr. v. *trephein* to eat; *syntrophos* nourished together; N.L. adj. *thermosyntrophicum* referring to the capacity of the organism to grow at elevated temperatures on propionate in the presence of a partner organism).

Spore-forming, Gram-positive rods with pointed ends and 1·0×3–11 μm in size. Weakly motile, strictly anaerobic, moderately thermophilic, neutrophilic. The substrates used coupled to sulphate reduction are propionate, lactate, pyruvate and H_2/CO_2. Ferments pyruvate, lactate, fumarate, glycine and benzoate. H_2+CO_2 is converted to acetate. No organic supplements are required for growth, but yeast extract (0·02%) enhances growth. Grows syntrophically on propionate with *Methanobacterium thermoautotrophicum* Z245. Grows at 45–62 °C and pH 6–8; optimum conditions are at 55 °C and pH 7. The DNA G+C content is 52·8 mol%. Isolated from thermophilic granular methanogenic sludge. Strain TPO is deposited at the DSMZ as DSM 14055^T.

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