Syntrophomonas cellicola sp. nov., a spore-forming syntrophic bacterium isolated from a distilled-spirit-fermenting cellar, and assignment of Syntrophospora bryantii to Syntrophomonas bryantii comb. nov.

Chenggang Wu,1,2 Xiaoli Liu1 and Xiuzhu Dong1

1State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, P. R. China
2Graduate School, Chinese Academy of Sciences, Beijing 100049, P. R. China

A spore-forming, anaerobic, syntrophic fatty-acid-oxidizing bacterium, strain 19J-3T, was isolated from a distilled-spirit-fermenting cellar in Hebei Province, China. The cells were slightly curved rods with a spore at the end of the cell. The optimal temperature for growth was around 37 °C and growth occurred in the range 25–45 °C. The pH range for growth was 6.5–8.5 and the optimum pH was 7.0–7.5. Crotonate was the only substrate that allowed the strain to grow in pure culture. However, the strain could oxidize saturated fatty acids with four to nine carbon atoms syntrophically in co-culture with Methanobacterium formicicum DSM 1535T. The strain was not able to utilize sulfate, sulfite, thiosulfate, DMSO, nitrate, fumarate or Fe(III) as electron acceptor. The DNA base composition was 48.8 mol% G + C. Phylogenetic analysis based on the 16S rRNA gene sequence revealed that strain 19J-3T was related to members of the family Syntrophomonadaceae and most closely to Syntrophospora bryantii DSM 3014T (94.3% similarity) and Syntrophomonas wolfei subsp. wolfei DSM 2245T (93.6% similarity). Considering the phylogenetic relationship and phenotypic features, strain 19J-3T (=CGMCC 1.5041T = JCM 13582T) is designated as the type strain of a novel species of the genus Syntrophomonas, Syntrophomonas cellicola sp. nov.

Based on the close phylogenetic relationship between the genera Syntrophospora and Syntrophomonas, the presence of sporulation-specific genes in the genome of Syntrophomonas wolfei subsp. wolfei DSM 2245T and the description of a spore-forming member of Syntrophomonas, ‘Syntrophomonas erecta subsp. sporosyntropha’, we propose the assignment of Syntrophospora bryantii to the genus Syntrophomonas as Syntrophomonas bryantii comb. nov.

Butyrate is an important intermediate in the anaerobic degradation of organic matter (Mackie & Bryant, 1981) and has to be degraded by consortia of at least two trophic groups (syntrophic acetogenic bacteria and hydrogen scavengers) because of the unfavourable energetics of the reactions (Schink, 1997). Syntrophomonas wolfei subsp. wolfei (McInerney et al., 1979, 1981; Beaty & McInerney, 1987) was the first described syntrophic bacterium that degraded butyrate in co-culture with methanogens or hydrogen-utilizing sulfate-reducing bacteria. Since then, Syntrophospora bryantii (Stieb & Schink, 1985; Zhao et al., 1990), Syntrophomonas saponovorans (Roy et al., 1986), Syntrophomonas wolfei subsp. saponovida (Lorowitz et al., 1989), Thermosyntropha lipolytica (Svetlishnyi et al., 1996), Syntrophus aciditrophicus (Jackson et al., 1999), Smithella propionica (Liu et al., 1999), Syntrophothermus lipocalidus (Sekiguchi et al., 2000), Syntrophomonas curvata (Zhang et al., 2004) and Syntrophomonas erecta (Zhang et al., 2005) have been isolated and characterized. Recently, ‘Syntrophomonas erecta subsp. sporosyntropha’, which produced spores in co-culture with methanogens, was described (Wu et al., 2006). In this paper, we describe the isolation and characterization of a sporulated Syntrophospora strain with Gram-positive cell-wall structure, strain 19J-3T, which was obtained from a distilled-spirit-fermenting cellar in Hebei Province, China.

Methanobacterium formicicum DSM 1535T and Desulfovibrio strain G11 were kindly provided by Dr Alfons Stams (Department of Microbiology, Wageningen University, The Netherlands).

A pre-reduced basal medium described by McInerney et al. (1979) was used for isolation and routine cultivation. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of Syntrophomonas cellicola 19J-3T is DQ288691.
gas phase was N\textsubscript{2} gas (1\textcdot10\textsuperscript{5} Pa) except that \textit{Methanobacterium formicicum} DSM 1535\textsuperscript{T} was cultivated with H\textsubscript{2}/CO\textsubscript{2} (80:20, 1\textcdot25\textcdot10\textsuperscript{5} Pa). All inoculations and transfers were done with syringes and needles. Incubations were at 37°C and in dark unless indicated. The purity of co-cultures and pure cultures was examined periodically by monitoring the cell morphology under a bright-field microscope and the absence of growth in peptone-yeast extract-glucose (PYG) medium (Britz & Tracey, 1983).

The procedures used to examine the morphology, nutrition and growth properties, to extract genomic DNA and to determine the G+C content of the novel strain were those described by Zhang \textit{et al.} (2005).

The 16S rRNA gene was amplified by PCR and sequenced using the method described by Weisburg \textit{et al.} (1991). 16S rRNA gene sequences of strain 19J-3\textsuperscript{T} and reference strains in GenBank were aligned using CLUSTALX program (version 1.83). Phylogenetic trees were constructed using UPGMA, minimum evolution and maximum-parsimony methods implemented in the MEGA3 program and the topology of the phylogenetic trees was evaluated by bootstrap analysis of 1000 datasets.

A sample from the walls of a distilled-spirit-fermenting cellar was inoculated into pre-reduced medium with 20 mM butyrate to enrich for anaerobic butyrate-degrading consortia. After repeated roll-tube isolations (Hungate, 1969) in butyrate agar medium which contained a 5\% (v/v) culture of \textit{Methanobacterium formicicum} DSM 1535\textsuperscript{T} and 5\% (v/v) of the enrichment culture, a biculture producing methane from butyrate but not in PYG medium was obtained. This biculture formed brownish colonies of 0.6–1.0 mm in diameter in roll tubes and showed green fluorescence under 420 nm light. Only two kinds of cell morphologies were observed in the biculture, one was a straight \textit{Methanobacterium formicicum}-like rod and the other was a slightly curved, spore-forming rod. To purify the spore-forming strain, the culture was pasteurized twice at 90°C for 25 min and, after pasteurization, 0.5 ml heated culture and 0.5 ml H\textsubscript{2}/CO\textsubscript{2}-grown \textit{Methanobacterium formicicum} DSM 1535\textsuperscript{T} were inoculated into butyrate-containing basal medium and incubated at 37°C until methane was detected. The monoculture of the butyrate-degrading syntrophic strain was purified by inoculating the biculture into medium with 20 mM crotonate as sole substrate instead of butyrate and 10 mM 2-bromoethanesulfonic acid to inhibit growth of the methanogen, and the isolate was designated as strain 19J-3\textsuperscript{T}. When growing on crotonate agar medium in pure culture, tiny white colonies, 0.2–0.3 mm in diameter, were formed.

Cells of strain 19J-3\textsuperscript{T} were slightly curved rods with rounded ends, 0.4–0.5 x 3.0–10.0 \mu m, occurring singly or in clumps. An oval spore was formed at the end of the cell (Fig. 1a) and poly-\beta-hydroxyalkanoate was accumulated (Fig. 1b), which was confirmed by cytochemical stain (Burdon, 1946).

Fig. 1. Photomicrographs of cells of strain 19J-3\textsuperscript{T} grown in pure culture on crotonate. (a) Phase-contrast light micrograph. Arrows point to sporulated cells. Bar, 6 \mu m. (b) Electron micrograph of an ultrathin section showing the typical Gram-positive wall structure and the presence of poly-\beta-hydroxyalkanoate (PHA); the arrow indicates a PHA granule. Bar, 0.3 \mu m.

The Gram reaction was negative to weakly positive; however, a Gram-positive cell wall ultrastructure was observed in ultrathin sections of strain 19J-3\textsuperscript{T} (Fig. 1b). A single flagellum was inserted in the midpoint of the cell (not shown).

Strain 19J-3\textsuperscript{T} degraded 20 mM butyrate into about 40 mM acetate and 4.87 mM methane within 14 days in co-culture with \textit{Methanobacterium formicicum} DSM 1535\textsuperscript{T} and degraded 20 mM butyrate within 10.5 days in co-culture with \textit{Desulfovibrio} strain G11. The strain neither grew nor produced methane on butyrate alone, whereas it degraded butyrate in an artificially constructed co-culture with \textit{Methanobacterium formicicum} DSM 1535\textsuperscript{T}. Straight-chain fatty acids with four to nine carbons were degraded by co-cultures of strain 19J-3\textsuperscript{T} with \textit{Methanobacterium formicicum}...
The G+C content of the genomic DNA from strain 19J-3T was determined as 48.8 mol%.

A phylogenetic tree including strain 19J-3T and other members of the family Syntrophomonadaceae was constructed (Fig. 2) based on a consensus length of 1421 bp of the 16S rRNA genes. Phylogenetically, strain 19J-3T is most closely related to Syntrophospora bryantii DSM 3014T (94.3 % similarity) and Syntrophomonas wolfei subsp. wolfei DSM 2245T (93.6 % similarity), with 91.8–94.0 % similarity to other Syntrophomonas species.

By a combination of phylogenetic relationships and phenotypic characteristics, strain 19J-3T could represent a novel species of the genus Syntrophomonas. Morphologically, strain 19J-3T formed spores in both co-culture and monoculture, while most other Syntrophomonas species did not form spores in the same medium and growth conditions. Comparison of substrate utilization patterns with those of other Syntrophomonas strains showed that strain 19J-3T differed from Syntrophomonas wolfei subsp. saponivida DSM 4212T, Syntrophomonas sapovorans DSM 3441T and Syntrophomonas curvata DSM 15682T by using only fatty acids with four to nine carbon atoms, from Syntrophomonas wolfei subsp. wolfei DSM2245T by not using isohethanoate and from Syntrophomonas erecta DSM 16215T and 'Syntrophomonas erecta subsp. sporosynthia' by using pelargonate. Strain 19J-3T differed from Syntrophospora bryantii by a difference of 11.2 mol% in G+C content and by not utilizing caprate, undecanoate or 2-methylbutyrate. Table 1 shows the characteristics of strain 19J-3T that distinguish it from other taxa. Therefore, we propose strain 19J-3T as the type strain of a novel species of the genus Syntrophomonas, Syntrophomonas celllicola sp. nov.

Clostridium bryantii was transferred to the new genus Syntrophospora by Zhao et al. (1990) on the basis of sporulation and the presence of a Gram-positive cell-wall ultrastructure, which differentiated it from Syntrophomonas wolfei subsp. wolfei, a non-spore-former with a Gram-negative cell wall ultrastructure. Although Syntrophospora species possess a complex cell-wall ultrastructure indicative of a Gram-negative cell wall, the lack of lipo polysaccharides confirms their Gram-positive cell-wall structure. The recently completed genome sequence of Syntrophomonas wolfei subsp. wolfei DSM 2245T (http://genome.ornl.gov/microbial/swol/) showed the presence of sporulation-specific genes in the genome, such as spo0A, a master regulator in bacterial sporulation found exclusively in spore-forming bacteria (Brown et al., 1994). We found that all

DSM 1535T or Desulfovibrio strain G11; however, neither branched-chain fatty acids such as isobutyrate and isovalerate nor benzoate were degraded. Among the substrates used, fatty acids with even numbers of carbons were converted into acetate and H₂, while those with odd numbers of carbons were converted into propionate, acetate and H₂, implying that β-oxidation of fatty acids occurred. None of the following substrates were used by strain 19J-3T as potential electron acceptors for butyrate degradation: sodium sulfate (20 mM), sodium thiosulfate (20 mM), sulfur (20 mM), DMSO (20 mM), sodium nitrate (20 mM) and sodium fumarate (20 mM).

Crotonate was the only compound tested that supported growth of strain 19J-3T in pure culture, and 24.5 mM crotonate was degraded to about 10.4 mM butyrate and 23.3 mM acetate in 4–5 days, with electron and carbon recoveries of 90.0±1.3 % and 89.4±1.6 %, respectively. The doubling time of strain 19J-3T was 6.5 h when grown on 20 mM crotonate at 37°C. The molar growth yield of strain 19J-3T with crotonate was 5.0–5.6 g dry weight 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).

The temperature range for growth of both the co-culture on butyrate and pure culture on crotonate was 25–45°C, with optimal growth at 37–40°C, and the pH for growth of both ranged from 6.5 to 8.5 with an optimal initial pH of 7.0–7.5. The NaCl concentration range tolerated was 0–450 mM (better growth below 100 mM). No growth was observed in the NaCl concentration range tolerated was 0–450 mM (better growth below 100 mM). No growth was observed in air.

The G+C content of the genomic DNA from strain 19J-3T was determined as 48.8 mol%.
recognized species of the genus *Syntrophomonas* contain a homologue of the spo0A gene of *Clostridium* and *Bacillus* species (C. Wu, X. Liu and X. Dong, unpublished data). Furthermore, sporulating *Syntrophomonas* strains were reported recently (Wu et al., 2006). Therefore, *Syntrophomonas* species all have the potential to form spores, which is in agreement with their phylogenetic placement within the low-G+C-content Gram-positive bacteria. The inability to detect sporulation with previously isolated *Syntrophomonas* strains is probably due to the lack of suitable culture conditions. There is growing recognition that sporulation per se is not necessarily a good indicator of relatedness (Cook et al., 1991; Farrow et al., 1995). In addition, Collins et al. (1994) proposed to merge the genera *Syntrophospora* and *Syntrophomonas*, as the level of evolutionary divergence between *Syntrophospora bryantii* and *Syntrophomonas wolfei* subsp. *wolfei* was only about 6%. Therefore, we propose to assign *Syntrophospora bryantii* to the genus *Syntrophomonas* as *Syntrophomonas bryantii* comb. nov.

**Emended description of the genus *Syntrophomonas* Mcinerney et al. 1982**

The genus description is the same as that given by Lorowit et al. (1989) except that some strains form spores in co-culture with methanogens on fatty acids or in pure culture on crotonate. Isolated from anaerobic environments such as aquatic sediments, digester sludge, rumen digest, rice field mud and shallow marine sediments.

**Description of *Syntrophomonas cellicola* sp. nov.**

*Syntrophomonas cellicola* (celli.co’la. L. n. *cella* a storeroom for wine and food; L. suff. -cola from L. *ncola* an inhabitant, dweller; N.L. *cellicola* an inhabitant of a storeroom, indicating that the type strain was originally isolated from a distilled-spirit-fermenting cellar).

Cells are Gram-variable, slightly curved rods, 0·4–0·5×4–7 μm, spore-forming and with one laterally inserted flagellum. The type strain grows in pure culture only on crotonate. In syntrophic association with *Methanobacterium formicicum* DSM 1535T or *Desulfovibrio* strain G11, the organism uses saturated fatty acids with four to nine carbon atoms by β-oxidation. Cells grow between 25 and 45 °C (optimum 37 °C) and at pH 6·5–8·5 (optimum pH 7·0–7·5). The genomic DNA G+C content is 48·8 mol%.

The type strain is 19J-3T (=CGMCC 1.5041T = JCM 13582T), isolated from a distilled-spirit-fermenting cellar in Hebei Province, China.

---

**Table 1. Characteristics of strain 19J-3T and other mesophilic syntrophic bacteria in the family *Syntrophomonadaceae***

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell width (μm)</td>
<td>0·4–0·5</td>
<td>0·5–1·0</td>
<td>0·4</td>
<td>0·5</td>
<td>0·5–0·7</td>
<td>0·6–0·9</td>
<td>0·5–0·7</td>
<td>0·4–0·6</td>
</tr>
<tr>
<td>Cell length (μm)</td>
<td>3·0–10·0</td>
<td>2·0–7·0</td>
<td>3·0–6·0</td>
<td>2·5</td>
<td>2·3–4·0</td>
<td>2·0–8·0</td>
<td>4–14</td>
<td>2·0–4·0</td>
</tr>
<tr>
<td>Gram type</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Flagella</td>
<td>1</td>
<td>2–8</td>
<td>0</td>
<td>2–7</td>
<td>1–3</td>
<td>2–5</td>
<td>0</td>
<td>2–4</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>48·8</td>
<td>45·1*</td>
<td>37·6</td>
<td>ND</td>
<td>46·6</td>
<td>43·2</td>
<td>40·6</td>
<td>ND</td>
</tr>
<tr>
<td>Optimum pH</td>
<td>7·0–7·5</td>
<td>ND</td>
<td>6·6–7·5</td>
<td>7·3</td>
<td>7·5</td>
<td>7·8</td>
<td>7·0</td>
<td>ND</td>
</tr>
</tbody>
</table>

Spore formation
- Co-culture: + + + + + + + +
- Monoculture: + + + + + + + +

Substrate utilization in co-culture with methanogens
- 2-Methylobutyrate: – – – – – – – –
- Pelargonate: + ND + + + – – +
- Caprate: – ND + + + + – +
- Undecanoate: – ND + + + – – +
- Laurate: – – – + + + – +
- Myristate: – – – + + + – +
- Isohexanoate: – – – + + + – +

*Data from Zhang et al. (2004).
Description of Syntrophomonas bryantii comb. nov.

Basonym: Clostridium bryantii Stieb and Schink 1985.

The description of Syntrophomonas bryantii is that given for Syntrophospora bryantii by Zhao et al. (1990). The type strain is strain CuCalT [= DSM 3014AT (co-culture with Desulfovibrio sp. E70) = DSM 3014B(T (co-culture with Methanospirillum hungatei M11h)].

Acknowledgements

This work has been supported by the National Nature Science Foundation of China under grant no. 30025001.

References


