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

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A strict anaerobe (strain GB8-1T) that degraded straight-chain fatty acids with C₄–C₁₈ in syntrophic association with methanogens was isolated from an up-flow anaerobic sludge blanket reactor treating beer wastewater. Strain GB8-1T degraded 1 mol butyrate into about 2 mol acetate and 1 mol (presumably) H₂ in co-culture with a methanogen. Neither branched-chain fatty acids nor benzoate could be degraded. Strain GB8-1T 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 GB8-1T to degrade butyrate. Cells of strain GB8-1T 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 rDNA gene sequences revealed that strain GB8-1T was related most closely to the fatty acid-oxidizing, syntrophic bacterium Syntrophomonas sapovorans DSM 3441T; however, the relationship was not very close (95·4% sequence similarity). Some phenotypic features also differentiated strain GB8-1T from Syntrophomonas sapovorans DSM 3441T. Therefore, a novel species, Syntrophomonas curvata sp. nov., is proposed. The type strain is GB8-1T (＝CGMCC 1.5010T ＝DSM 15682T).

In ecosystems that lack light and exogenous electron acceptors other than CO₂, 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 C₁₈), 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 11003T (Svetlitshnyi et al., 1996), Syntrophothermus lipocalidus DSM 12680T (Sekiguchi et al., 2000), Syntrophosphorura bryantii DSM 3014T (Stieb & Schink, 1985; Zhao et al., 1990), Syntrophomonas wolfei subsp. wolfei DSM 2245T (McInerney et al., 1979, 1981; Beaty & McInerney, 1987), Syntrophomonas wolfei subsp. sapovorans DSM 4212T (Lorowitz et al., 1989) and Syntrophomonas sapovorans DSM 3441T (Roy et al., 1986). Syntrophus aciditrophicus ATCC 700169T, a bacterium that syntrophically degrades both fatty acids (C₄–C₁₈) 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 GB8-1T, that syntrophically degraded C₄–C₁₈ straight-chain fatty acids in co-culture with a hydrogenotrophic microbe. Phylogenetic analysis indicated that strain GB8-1T 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 GB8-1T was isolated from granular sludge that was...
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<sup>T</sup> was kindly provided by Dr Alfons Stams in the Department of Microbiology, Wageningen University, the Netherlands, and *Syntrophomonas sapovorans* DSM 3441<sup>T</sup> 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<sub>2</sub>/CO<sub>2</sub> (80 : 20; v/v) (5×10<sup>5</sup> Pa), with 1·01 × 10<sup>5</sup> Pa) for cultivating *M. formicicum* DSM 1535<sup>T</sup>. 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<sup>T</sup> 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<sup>T</sup> 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<sub>2</sub>). After inoculation, cultures were incubated for up to 60 days. Meanwhile, acetate, propionate and methane production and substrate degradation were measured. Cell growth was determined by measurement of the OD<sub>600</sub> of the culture and substrate degradation. All determinations were performed in duplicate with 10 % inoculum.

Exponential-phase cells of strain GB8-1<sup>T</sup> were used for morphological 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<sup>T</sup> 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<sub>m</sub>),

The 16S rRNA gene of strain GB8-1<sup>T</sup> was amplified by PCR and sequenced by using methods described by Weisburg *et al.* (1991). The 16S rRNA gene sequences of strain GB8-1<sup>T</sup> 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 (Hugnete, 1969) in the butyrate medium, in which 5 % (v/v) culture of *M. formicicum* DSM 1535<sup>T</sup> 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<sup>T</sup>, was obtained. Strain GB8-1<sup>T</sup> hardly grew on solid medium in pure culture; tiny white colonies of 0·1–0·2 mm diameter were formed. Strain GB8-1<sup>T</sup> neither grew on butyrate alone nor produced CH<sub>4</sub>, whereas it degraded butyrate in an artificially constructed co-culture with *M. formicicum* DSM 1535<sup>T</sup>.

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

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Strain GB8-1\textsuperscript{T} could degrade 1 mol butyrate into about 2 mol acetate and 1 mol (presumably) H\textsubscript{2}, which was consumed by \textit{M. formicicum} DSM 1535\textsuperscript{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\textsubscript{2}, whereas those with an odd number of carbons were converted into propionate, acetate and H\textsubscript{2}, implying that \textit{β}-oxidation of fatty acids was performed by the co-culture. None of the following substances could be used by strain GB8-1\textsuperscript{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\textsuperscript{T} to the syntrophic acetogen group.

Crotonate was the only compound tested that supported growth of strain GB8-1\textsuperscript{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\textsuperscript{T} was 11 h when grown on 20 mM crotonate at 37 °C. The molar growth yield of strain GB8-1\textsuperscript{T} on crotonate was 4.0–4.8 g dry wt mol\textsuperscript{−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\textsuperscript{T} and other mesophilic, syntrophic bacteria in the family \textit{Syntrophomonadaceae}

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

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>2</th>
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<th>4</th>
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<tr>
<td>Cell width (µm)</td>
<td>0.4–1.0</td>
<td>0.5–1.0</td>
<td>0.4–0.6</td>
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<td>0.5–0.7</td>
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<td>Cell length (µm)</td>
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<td>2.0–7.0</td>
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<td>2.5</td>
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<td>–</td>
<td>–</td>
<td>Variable</td>
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<td>ND</td>
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<td>25–45/35–37</td>
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was \(6.3 \pm 8.4\), 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 \(L\)-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.

**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 \(\times\) 2-3–4.0 \(\mu\)m, 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, xylene, 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**


