Syntrophothermus lipocalidus gen. nov., sp. nov., a novel thermophilic, syntrophic, fatty-acid-oxidizing anaerobe which utilizes isobutyrate

Yuji Sekiguchi,1 Yoichi Kamagata,2 Kazunori Nakamura,2 Akiyoshi Ohashi1 and Hideki Harada1

Author for correspondence: Yuji Sekiguchi. Tel: +81 258 47 9623. Fax: +81 258 47 9623. e-mail: skgc@vos.nagaokaut.ac.jp

1 Department of Environmental Systems Engineering, Nagaoka University of Technology, Nagaoka, Kamitomioka 1603-1, Niigata 940-2188, Japan
2 National Institute of Bioscience and Human Technology, Agency of Industrial Science and Technology, Tsukuba, Ibaraki 305-8566, Japan

A new anaerobic, thermophilic, syntrophic, fatty-acid-oxidizing bacterium designated strain TGB-C1T was isolated from granular sludge in a thermophilic upflow anaerobic sludge blanket (UASB) reactor. The cells were slightly curved rods and were weakly motile. Spore formation was not observed. The optimal temperature for growth was around 55 °C and growth occurred in the range 45 to 60 °C. The pH range for growth was 5.5–7.5, and the optimum pH was 6.5–7.0. Crotonate was the only substrate that allowed the strain to grow in pure culture. However, in co-culture with the thermophilic, hydrogenotrophic Methanobacterium thermoautotrophicum strain ∆H, the isolate could syntrophically oxidize saturated fatty acids with 4–10 carbon atoms, including isobutyrate. During the degradation of isobutyrate by the co-culture, isobutyrate was isomerized to butyrate, which was then oxidized. The strain was not able to utilize sulfate, sulfite, thiosulfate, nitrate, fumarate or Fe(III) as electron acceptor. The DNA base composition was 51.0 mol%. 16S rDNA sequence analysis revealed that the strain belongs to the family Syntrophomonadaceae, but it was only distantly related to other known species of β-oxidizing syntrophs. Hence, the name Syntrophothermus lipocalidus is proposed for TGB-C1T as a new species of a new genus.

Keywords: anaerobe, fatty acid oxidation, syntroph, thermophilic, Syntrophothermus lipocalidus

INTRODUCTION

Conversion of complex organic matter to methane and carbon dioxide in anaerobic ecosystems is performed by cooperations of different trophic groups of microbes. In this type of ecosystem, fatty acids such as propionate and butyrate are frequently formed as intermediates. The oxidation of these compounds is thermodynamically unfavourable under such environments unless the consumption of the reducing equivalents, i.e. hydrogen and/or formate, is coupled with the oxidation reaction (Schink, 1992, 1997). Consequently, fatty acid oxidation requires syntrophic interactions between two different microbes, a fatty acid β-oxidizing, hydrogen-producing bacterium and a hydrogenotrophic microbe such as a methanogen.

To date, several mesophilic anaerobes which perform syntrophic fatty acid β-oxidation have been isolated in co-culture with hydrogen-utilizing microbes. Syntrophomonas wolfei (subsp. wolfei) was the first strain isolated in co-culture with a hydrogenotrophic methanogen or sulfate-reducer (McInerney et al., 1981, 1979). In addition, Syntrophospora bryantii (Stieb & Schink, 1985; Zhao et al., 1990), Syntrophomonas sapovorans (Roy et al., 1986), Syntrophomonas wolfei subsp. saponavida (Lorowitz et al., 1989), ‘Syntrophus aciditrophicus’ (Jackson et al., 1999) and several unnamed mesophilic β-oxidizing syntrophs have been isolated and characterized (Shelton & Tiedje, 1984; Wu et al., 1992; Zhao et al., 1993). Since Beaty & McInerney (1987) reported that Syntrophomonas

Abbreviation: UASB, upflow anaerobic sludge blanket.

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain TGB-C1T is AB021305.
Braunschweig, Germany.

lung von Mikroorganismen und Zellkulturen (DSMZ), detailed taxonomical information on neutrophilic ther-

mophilic anaerobic wastewater treatment sludge. In this paper, we describe the isolation and char-

mophilic anaerobic sludge blanket (UASB) reactor which had been

enrichment, isolation and maintenance of strain TGB-C1 contained (l

based on that of Widdel & Pfennig (1981). The medium

The culture medium used for en-

Strain TGB-C1 was isolated from an alkaline hot spring of Lake Bogoria (Kenya). This is the first and only report on a named thermophilic β-oxidizing syntroph to date; there is no detailed taxonomical information on neutrophilic ther-

mophilic anaerobic wastewater treatment sludge. Several new aspects of the strain and its taxonomic position are discussed.

METHODS

Sources of micro-organisms. Strain TGB-C1 was isolated from granular sludge of a thermophilic (55°C) upflow anaerobic sludge blanket (UASB) reactor which had been fed with an artificial wastewater containing succrose, acetate and propionate as the major carbon sources (Sekiguchi et al., 1998). Methanobacterium thermoautotrophicum strain AH (= DSM 1053) was obtained from the Deutsche Samm-

lung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany.

Cultivation conditions. The culture medium used for enrich-

ment, isolation and maintenance of strain TGB-C1 was based on that of Widdel & Pfennig (1981). The medium contained (l−1): KH₂PO₄, 0.15 g; NH₄Cl, 0.5 g; MgCl₂, 6H₂O, 0.2 g; CaCl₂, 2H₂O, 0.15 g; NaHCO₃, 2.5 g; Na₃S, 9H₂O, 0.3 g; cysteine, HCl, 0.3 g; trace elements solution, 1 ml; vitamin solution, 1 ml; and resazurin solution (1 ml; 1 mg ml−1). The trace elements solution was based on medium 318 of DSMZ (1983) with the exceptions that NaCl was eliminated and Na₂WO₄ · 3H₂O (3 mg l−1) was added. The vitamin solution was based on the vitamin mixture in medium 141 of DSMZ (1983) with a slight modification: all components were mixed at a concentration of 20 μmol l−1. All cultures were carried out at 55 °C in 50 ml serum vials containing 20 ml medium (pH 5.3 ± 0.2) under an atmosphere of N₂/CO₂ (80:20, v/v) unless other-

wise mentioned. Neutralized substrates were added to the vials from stock solutions prior to inoculation. Fe(III)nitritoltriacetate was prepared as described by Roden & Lovley (1993).

M. thermoautotrophicum was cultivated at 55 °C using the same medium mentioned above except that hydrogen (approx. 0.5–1.0 atm) was added to the gas phase in the vials as an energy source.

Solid medium was prepared by adding purified agar (Noble agar, Difco) to the medium described above at a final concentration of 20 g l−1.

Effect of pH and temperature. To determine the optimum pH for growth, the pH value of the above medium containing 10 mM crotonate was adjusted at room temperature to 5.5–9.2 by adding HCl or NaOH under a 100% N₂ atmosphere. Duplicate cultures (1% inoculum) were incubated at 55 °C, and OD₅₇₀ and acid production were measured. To evaluate the effect of temperature on growth, duplicate cultures were incubated at 28–75 °C using crotonate medium (pH5.3–6.8).

Growth and substrate utilization. To test growth and substrate utilization, autoclaved or filter-sterilized substrates were added to crotonate medium. When using fatty acids longer than C₆ and their triacylglycerols, the medium was supplemented with equimolar amounts of CaCl₂. All cul-

tures were incubated at 55 °C, pH5.3–6.8 for 4 weeks. Growth and substrate utilization were determined by monitoring increase in OD₅₇₀ and the production of acetate and hydrogen, respectively. In syntrophic growth/substrate utili-

zation tests, M. thermoautotrophicum strain AH was added to the medium (2% inoculum), and growth and substrate utilization were checked by measuring OD₅₇₀ and methane production, respectively.

Analytical methods. Short-chain fatty acids were determined with a gas chromatograph (Shimadzu GC-14A; detector type, FID; packing material, FAL-M; column temperature, 125 °C). Determination of alcohol and other compounds was performed by HPLC using a RSPak KC-811 column (Shodex; eluent, 3 mM HClO₄; column temperature, 30 °C) and a UV detector (210 nm, Shimadzu SPD-10A). Methane, hydrogen and carbon dioxide were determined by GC (GL Science model 370; detector type, TCD; packing material, Unibeads C; column temperature, 145 °C).

Effect of antibiotics. Effect of antibiotics on growth was evaluated at a final concentration of 50 μg of each antibiotic ml−1 on 10 mM crotonate medium (pH5.3–6.8). All in-

cubations were conducted at 55 °C using 2% inocula.

Microscopy. The Gram staining reaction was performed by Hucker’s method (Doetsch, 1981). Phase-contrast micro-

graphs were taken by using wet mounts of exponential-phase cultures on agar-coated slides (Pfennig & Wagener, 1986). Scanning electron micrographs were obtained as described previously (Sekiguchi et al., 1999). Cells for thin-section electron microscopy were fixed with 5% (v/v) glutaraldehyde for 6 h and then postfixed in 2% osmium tetroxide at 4 °C for 3 h. The fixed cells were stained with uranyl acetate for 1 h at room temperature, dehydrated and embedded in Spurr low-viscosity resin. Thin sections of the cells were made with an ultramicrotome (Reichert ULTRACUT N) and were examined with a Hitachi H7000 transmission electron microscope.

Determination of DNA base composition. DNA was ex-

tracted and purified according to the method described previously (Kamagata & Mikami, 1991). The G+C content was determined by HPLC (Shimadzu LC-6A) with a UV detector.

16S rDNA sequence analysis. DNA was recovered from strain TGB-C1 according to the method of Hiraishi (1992).
16S rDNA of the strain was amplified by PCR with Tag polymerase (Perkin Elmer) as described previously (Sekiguchi et al., 1998). The PCR primers used in the amplification were the bacterial-domain-specific primer 8F (5′-AGAGTTTGATCTTGGCTCAG-3′; positions 8–27 of the Escherichia coli 16S rDNA gene) and the prokaryote-specific primer 1490R (5′-GGTTACCTGTAGACTT-3′; positions 1491–1509) (Weisburg et al., 1991). Sequencing of the purified PCR products was conducted with the Thermo Sequenase Fluorescent Labelled Primer Cycle Sequencing kit (Amersham) and an automated sequence analyser (Shimadzu DSQ-1000L). Sequence data were aligned with the clustal w package (Thompson et al., 1994). Phylogenetic trees were constructed by both the neighbour-joining method (Saito & Nei, 1987) with the MEGA program (Kumar et al., 1993), and the maximum-parsimony method with the paup package (Swofford, 1993). Bootstrap resampling analysis (Felsenstein, 1985) for 100 replicates was performed to estimate the confidence of tree topologies.

RESULTS

Enrichment and isolation

Granular sludge was taken from a laboratory-scale thermophilic (55 °C) UASB reactor fed with an artificial wastewater containing sucrose, propionate and acetate as the main carbon sources (Sekiguchi et al., 1998). Gently washed and homogenized granules were used for a primary enrichment using 20 mM butyrate as substrate and an incubation temperature of 55 °C. Growth and methane production were observed within one week after inoculation. This culture was further enriched by successive transfers using 1% inoculum. The enrichment stably converted butyrate into acetate and methane over 10 transfers and contained at least 1% inoculum. The enrichment stably converted butyrate into acetate and methane over 10 transfers and contained at least two major cell morphotypes: a non-motile, F− autofluorescent rod morphologically resembling Methanobacterium, and a weakly red-fluorescent, slightly curved rod (identified later as the butyrate-oxidizing syntroph).

For isolation of the butyrate-degrading microbe, we first conducted co-culture isolation by using the roll-tube method (Hungate, 1969) in which all tubes were inoculated beforehand with M. thermoautotrophicum cells. After two weeks incubation, white to brownish colonies 0.5–1.0 mm in diameter were formed. One colony was picked from the highest dilution which showed growth (10−6), transferred into a liquid medium containing 20 mM butyrate and incubated again. However, the culture still contained long rod-shaped bacteria which could outgrow the other morphotype in the medium supplemented with yeast extract (0.1%). This was the case even when the co-culture isolation was repeated several times. Consequently, an attempt was made to obtain a pure culture of the butyrate degrader from the highly purified enrichment by using 10 mM crotonate and 2 mM bromoethanesulfonate (BES). After a week of incubation, growth of slightly curved rods occurred. Three successive transfers into the crotonate-BES liquid medium resulted in disappearance of F−autofluorescent Methanobacterium-like rods and the cells in the culture seemed to be homogeneous. We therefore conducted roll-tube isolation using crotonate as the sole substrate. Very small colonies that were white, lens-shaped and 0.1–0.2 mm in diameter were formed after 2 weeks incubation. This step was repeated several times and the purified strain obtained was designated TGB-C1T.

Morphological characteristics

Strain TGB-C1T cells were weakly motile, slightly curved rods with round ends (Fig. 1). They were 2.0–4.0 µm long and 0.4–0.5 µm wide, occurring singly or in pairs. Some flagella were seen by electron microscopy. Gram stain was negative, although electron micrographs (Fig. 2) and 16S rRNA analysis (described below) showed that the strain was affiliated with the Gram-positive bacteria. Spore formation was never observed in any growth phase or under any

\[ \text{Fig. 1. Phase-contrast micrograph (a) and scanning electron micrograph (b) showing the cell morphology of strain TGB-C1}^\top \text{, grown on crotonate (10 mM). The phase-contrast micrograph was taken using a wet mount on an agar-coated slide (Pfennig \\ & Wagener, 1986). Bars: (a) 5 \mu\text{m}; (b) 3 \mu\text{m.} \]
growth conditions, including high or low temperatures.

Substrate utilization and growth properties in pure culture

Strain TGB-C1T was strictly anaerobic since no growth occurred in the presence of oxygen (20%, v/v, in the gas phase). Growth in pure culture was observed only on crotonate as energy source. Fermentation products from crotonate were almost equimolar amounts of acetate and butyrate.

Growth and acid formation were not observed on the following substrates: Casamino acids (0–1%), tryptone (0–1%), yeast extract (0–1%), H₂/CO₂ (80:20, v/v, head space), betaine (10 mM), glucose (20 mM), ribose (20 mM), xylose (20 mM), arabinose (20 mM), fructose (20 mM), galactose (20 mM), mannose (20 mM), raffinose (20 mM), sucrose (20 mM), starch (5 g l⁻¹), xylan (5 g l⁻¹), pectin (5 g l⁻¹), lactate (20 mM), pyruvate (20 mM), glycerol (5 mM), olive oil (5 ml l⁻¹), formate (20 mM), straight-chain fatty acids from C₂ to C₄ (20 mM), straight-chain fatty acids from C₅ to C₁₂ (5 mM), straight-chain fatty acids from C₁₄ to C₂₄ (1 mM), oleate (1 mM), linoleate (1 mM), isobutyrate (1 mM), isovalerate (1 mM), tributyryl (1 mM), trilaurin (1 mM), tripalmitin (1 mM), tristearin (1 mM), triolein (1 mM), fumarate (20 mM), malate (20 mM), succinate (20 mM), ethanol (20 mM), methanol (20 mM), 1-propanol (20 mM), 1-butanol (20 mM), benzoate (5 mM), hydroquinone (1 mM) and phenol (1 mM).

The following compounds were tested as electron acceptors with butyrate (20 mM) as electron donor, but none of them was utilized: sulfate (20 mM), nitrate (20 mM), sulfite (20 mM), thiosulfate (20 mM), fumarate (20 mM), Fe(III)-nitrilotriacetate (5 mM).

The strain grew on crotonate between 45 and 60 °C with an optimum at 55 °C (Fig. 3a). The pH range for growth was 5.8–7.5, with an optimum at pH 6.5–7.0 (Fig. 3b). Under optimum conditions (pH 6.8, 55 °C), the growth rate of the strain in pure culture on crotonate (10 mM) was 0.93 ± 0.01 d⁻¹. Neither yeast extract (0–1%) nor tryptone (0–1%) significantly stimulated growth. NaCl was slightly inhibitory at 5 g l⁻¹ (growth rate, 0.66 ± 0.05 d⁻¹) and 15–20 g l⁻¹ NaCl completely inhibited growth.

Substrate utilization and growth properties in co-culture with M. thermoautotrophicum

Strain TGB-C1T was able to oxidize the following saturated fatty acids in co-culture with M. thermoautotrophicum strain ΔH: butyrate (20 mM), straight-chain fatty acids from C₄ to C₁₀ (5 mM) and iso-butyrate (5 mM).

Growth, methane production and acid formation were never observed in the medium containing acetate (20 mM), propionate (20 mM), straight-chain fatty
Syntrophothermus lipocalidus gen. nov., sp. nov.

![Graph](image1)

Fig. 3. Growth properties of strain TGB-C1\textsuperscript{T} on crotonate medium. (a) Effect of temperature on specific growth rate (pH\textsubscript{25} °C 6-8). (b) Effect of pH on specific growth rate (temperature 55 °C). Data shown are means of duplicate determinations and error bars represent standard deviations.

![Graph](image2)

Fig. 4. Utilization of butyrate (○) and production of acetate (●), methane (▲) and hydrogen (▼) by strain TGB-C1\textsuperscript{T} in co-culture with M. thermoautotrophicum strain ∆H. Data shown are from single determinations; the experiments were repeated with similar results.

Acids from C\textsubscript{11} to C\textsubscript{24} (1 mM), oleate (1 mM), linoleate (1 mM), isovalerate (1 mM), tributyrin (1 mM), triauroin (1 mM), tripalmitin (1 mM), tristearin (1 mM), trilaurin (1 mM), ethanol (20 mM), benzoate (20 mM), lactate (20 mM) or olive oil (0-5 ml l\textsuperscript{-1}) even after 4 weeks incubation.

Under optimum conditions (pH 6-8, 55 °C), the growth rate of the strain in co-culture with M. thermoautotrophicum strain ∆H on butyrate (20 mM) was 1-06 ± 0-03 d\textsuperscript{-1} (determined by measuring methane production). This culture converted 423 μmol butyrate to 806 μmol acetate and 203 μmol methane (95% carbon recovery, 96% electron recovery). Fatty acids with even numbers of carbon atoms were changed to acetate and methane (Fig. 4), whilst fatty acids having odd numbers of carbon atoms were degraded to acetate, propionate and methane. In addition, isobutyrate was degraded to form acetate and methane. During the conversion of isobutyrate, butyrate was detected as an intermediate, indicating that isobutyrate is isomerized to butyrate in the first step of the pathway.

**Effects of antibiotics**

The effect of antibiotics on the growth of strain TGB-C1\textsuperscript{T} was tested under the optimum conditions using 10 mM crotonate as substrate. Growth was inhibited by ampicillin, chloramphenicol, kanamycin, neomycin, rifampin or vancomycin (all at 50 μg ml\textsuperscript{-1}).

**DNA base composition**

The G+C composition of strain TGB-C1\textsuperscript{T} was calculated to be 51-0 mol%.

**Phylogenetic analysis**

A total of 1588 nucleotides of the 16S rDNA from strain TGB-C1\textsuperscript{T} were sequenced. To determine the phylogenetic position of the strain, we compared the data with other bacterial 16S rDNAs using the neighbour-joining algorithm. According to the analysis, strain TGB-C1\textsuperscript{T} fell into the Clostridium–Bacillus subclass of the Gram-positive bacteria. More detailed phylogeny was determined by using both neighbour-joining and maximum-parsimony analyses with clostridial 16S rDNA sequences. Both showed that the strain belongs to the family Syntrophomonadaceae and that the closest neighbour is Thermosyntropha lipo-lytica. Strain TGB-C1\textsuperscript{T} was located in the deepest
branch in the family and was relatively distant from the other members of the Syntrophomonadaceae. The evolutionary distances calculated with 16S rDNA/ rRNA sequences by using the formula of Jukes & Cantor (1969) were 11.9% with Thermosyntropha lipolytica (GenBank accession no. X99980), 13.6% with Syntrophomonas wolfei subsp. wolfei (M26492), 14.8% with Syntrophomonas sapovorans (AF022249), 14.0% with Syntrophospora bryantii (M26491) (gap treatment: pairwise deletion). In addition, the neighbour-joining and maximum-parsimony analyses did not show that the strain formed a clade with Thermosyntropha lipolytica (Fig. 5).

**DISCUSSION**

To our knowledge, there has been no detailed description of thermophilic, neutrophilic, butyrate-oxidizing syntrophs in anaerobic wastewater treatment sludge to date. Two thermophilic, butyrate-oxidizing co-cultures were reported by Henson & Smith (1985) and Ahring & Westermann (1987). The co-culture isolated by Henson & Smith (1985) consisted of slightly curved rods (butyrate-oxidizer) and bent rods (*M. thermoautotrophicum*). Like our strain, the culture grew at neutral pH at 55°C. However, no other information is available to allow discussion of the difference between the two. TGB-C1T is also morphologically similar to the butyrate-oxidizing thermophile reported by Ahring & Westermann (1987). However, details of the physiological and taxonomical characteristics of this strain have not been well described either. We are therefore unable to compare these previously isolated thermophilic butyrate-oxidizers with strain TGB-C1T.

The origin of strain TGB-C1T was a UASB reactor in which sucrose, acetate and propionate had been fed as model wastewater. We found that a small amount of butyrate was formed from sucrose by the thermophilic sludge (data not shown), suggesting that strain TGB-C1T may have been involved in the oxidation of butyrate formed from sucrose. In our previous 16S rDNA cloning analysis of the microbial community members of the sludge, one clone in a total of 110 was found to be a relative of the known butyrate-oxidizing syntrophs (Sekiguchi et al., 1998). The 16S rDNA sequence of strain TGB-C1T was identical to the sequence of this clone (TUG15), suggesting that this strain actually contributed to fatty acid degradation in the digestion.

One of the most interesting features of strain TGB-C1T was that the strain was an ‘obligate’ β-oxidizing syntroph, since fatty acids were the only substrates that allowed it to grow in co-culture with a methanogen, and crotonate was the only substrate that allowed it to grow in pure culture. For the degradation of butyrate and higher homologues, strain TGB-C1T was able to associate with *M. thermoautotrophicum* strain ΔH. Considering the fact that *M. thermoautotrophicum* strain ΔH is only able to utilize hydrogen as energy source, it is likely that formate transfer (Thiele
Table 1. Characteristics of strain TGB-C1<sup>T</sup> and other related organisms in the family Syntrophmonadaceae

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>TGB-C1&lt;sup&gt;T&lt;/sup&gt;</th>
<th>T. lipolytica&lt;sup&gt;*&lt;/sup&gt;</th>
<th>S. wolfei subsp. wolfei&lt;sup&gt;†&lt;/sup&gt;</th>
<th>S. wolfei subsp. saponavida&lt;sup&gt;‡&lt;/sup&gt;</th>
<th>S. sapovorans&lt;sup&gt;§&lt;/sup&gt;</th>
<th>S. bryantii&lt;sup&gt;s&lt;/sup&gt;</th>
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<tr>
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<td>0·3–0·4</td>
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<tr>
<td>Gram reaction</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
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<td>+</td>
<td>+</td>
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<td>G+C content (mol%)</td>
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<td>ND</td>
<td>7·3</td>
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<td>Optimum temperature (°C)</td>
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**Substrate utilization in pure culture**

<table>
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<tr>
<th>Substrate</th>
<th>TGB-C1&lt;sup&gt;T&lt;/sup&gt;</th>
<th>T. lipolytica&lt;sup&gt;*&lt;/sup&gt;</th>
<th>S. wolfei subsp. wolfei&lt;sup&gt;†&lt;/sup&gt;</th>
<th>S. wolfei subsp. saponavida&lt;sup&gt;‡&lt;/sup&gt;</th>
<th>S. sapovorans&lt;sup&gt;§&lt;/sup&gt;</th>
<th>S. bryantii&lt;sup&gt;s&lt;/sup&gt;</th>
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<tr>
<td>Yeast extract</td>
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<td>−</td>
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<td>+</td>
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<td>+</td>
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<td>ND</td>
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<tr>
<td>Pyruvate</td>
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<td>(+)*</td>
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<td>Xylose</td>
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<td>(+)*</td>
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**Substrate utilization in co-culture with methanogens**

<table>
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<th>T. lipolytica&lt;sup&gt;*&lt;/sup&gt;</th>
<th>S. wolfei subsp. wolfei&lt;sup&gt;†&lt;/sup&gt;</th>
<th>S. wolfei subsp. saponavida&lt;sup&gt;‡&lt;/sup&gt;</th>
<th>S. sapovorans&lt;sup&gt;§&lt;/sup&gt;</th>
<th>S. bryantii&lt;sup&gt;s&lt;/sup&gt;</th>
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<tbody>
<tr>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Propionate (C&lt;sub&gt;3:0&lt;/sub&gt;)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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</tr>
<tr>
<td>Butyrate to caprylate (C&lt;sub&gt;4:0&lt;/sub&gt;–C&lt;sub&gt;8:0&lt;/sub&gt;)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pelargonate (C&lt;sub&gt;9:0&lt;/sub&gt;)</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Caprate (C&lt;sub&gt;10:0&lt;/sub&gt;)</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Laurate (C&lt;sub&gt;12:0&lt;/sub&gt;)</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Myristate (C&lt;sub&gt;14:0&lt;/sub&gt;)</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Palmitate (C&lt;sub&gt;16:0&lt;/sub&gt;)</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND</td>
</tr>
<tr>
<td>Stearate (C&lt;sub&gt;18:0&lt;/sub&gt;)</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Oleate (C&lt;sub&gt;18:1&lt;/sub&gt;)</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Linoleate (C&lt;sub&gt;18:2&lt;/sub&gt;)</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Linolenate (C&lt;sub&gt;18:3&lt;/sub&gt;)</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Triacylglycerides</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, Not determined.

* Obtained from Svetlitshnyi et al. (1996).
† Obtained from McInerney et al. (1979, 1981) and Beaty & McInerney (1987).
‡ Obtained from Lorowitz et al. (1989).
§ Obtained from Roy et al. (1986).
∥ Obtained from Stieb & Schink (1985) and Zhao et al. (1990).
* Weak growth.
# Straight-chain fatty acids with C<sub>4</sub> (butyrate) to C<sub>6</sub> (caprylate).

& Zeikus, 1988) is not involved in interspecies electron transfer between the organisms.

The other notable feature of the strain was its syntrophic isobutyrate-degrading capability. Isobutyrate-utilizing syntrophs were previously described by Shelton & Tiedje (1984), Wu et al. (1992) and Zhao et al. (1993). However, all of these strains were mesophilic and none of them was characterized taxonomically. During the degradation of isobutyrate by strain TGB-C1<sup>T</sup> with a hydrogenotrophic methanogen, butyrate was detected as an intermediate, suggesting that isomerization of isobutyrate occurred similarly to the strain reported by Wu et al. (1994).

Regarding its physiological characteristics, strain TGB-C1<sup>T</sup> was similar to known members of the *Syntrophomonadaceae*. Phylogenetic analysis based on 16S rDNA supported the conclusion that the strain should be classified as a member of the *Syntrophomonadaceae*. The closest relative of the strain was *Thermosyntropha lipolytica* (Svetlitshnyi et al., 1996).
Strain TGB-C1\textsuperscript{T} was similar to \textit{T. lipolytica} in terms of morphology, growth temperature and syntrophic growth properties. However, strain TGB-C1\textsuperscript{T} was apparently different from \textit{T. lipolytica} in the following three major aspects: (1) \textit{T. lipolytica} exhibits heterotrophic growth on substrates such as yeast extract and tryptone as well as showing lipolytic growth, whilst strain TGB-C1\textsuperscript{T} grows only on a limited range of fatty acids (Table 1); (2) \textit{T. lipolytica} can grow at higher pH (range 7.15–9.5) whilst TGB-C1\textsuperscript{T} is nearly neutrophilic; (3) The DNA base composition of TGB-C1\textsuperscript{T} is significantly higher than that of \textit{T. lipolytica} (Table 1). On the basis of phenotypic and phylogenetic distinctiveness of the isolate, we concluded that the organism characterized here represents a new and distinct taxon for which we propose the name \textit{Syntrophothermus lipocalidus} gen. nov., sp. nov.

**Description of \textit{Syntrophothermus} gen. nov.**

\textit{Syntrophothermus} (Syn.tro.pho.ther'mus. Gr. adj. syn together with; Gr. fem. n. trophos one who feeds; Gr. adj. thermus hot; M.L. masc. n. Syntrophus thermophilic syntrophic bacterium, referring to growth in syntrophic association with hydrogenotrophic organisms at high temperatures of around 55 °C).

Cells are slightly curved rods, Gram-reaction negative, weakly motile, and obligately anaerobic. The organisms grow optimally at high temperatures of around 55 °C and at nearly neutral pH. Only fatty acids, including butyrate and higher homologues, are utilized in syntrophic association with hydrogen-utilizing microbes. On the basis of the 16S rDNA phylogeny, the genus \textit{Syntrophothermus} belongs to the family \textit{Syntrophomonadaceae}. The DNA base composition is 51.0 mol% G + C (determined by HPLC). \textit{Syntrophothermus lipocalidus} is the type species of the genus.

**Description of \textit{Syntrophothermus lipocalidus} sp. nov.**

\textit{Syntrophothermus lipocalidus} (lip.o.cal'id.us. Gr. neut. lipos fat; M.L. adj. calidus expert; M.L. adj. lipocalidus fatty-acid-specific, i.e. specifically utilizing fatty acids).

Cells are slightly curved rods with flagella. The dimensions of single cells are 2–0–4.0 by 0.4–0.5 µm. Weakly motile. Spores are never observed. The cells can grow in pure culture only on crotonate. In syntrophic association with hydrogenotrophic methanogens, the organism can utilize saturated fatty acids with 4 to 10 carbon atoms by β-oxidation. Isobutyrate is also utilized by isomerization to butyrate. The cells can grow between 45 and 60 °C (optimum 55 °C), and pH 5.8–7.5 (optimum 6.5–7.0). The DNA base composition is 51.0 mol% (determined by HPLC). The type strain is TGB-C1\textsuperscript{T} (= DSM 12680\textsuperscript{T}).

**ACKNOWLEDGEMENTS**

We thank Xian-Ying Meng for the electron micrographs of ultrathin sections of strain TGB-C1\textsuperscript{T}. We also thank Yoko Ueda for the measurement of the DNA base composition.

This study was financially supported by a research grant no. 97Ea11-011 on Proposal-Based R & D Program of the New Energy and Industrial Technology Development Organization (NEDO), Japan.

**REFERENCES**


Kumar, S., Tomura, K. & Nei, M. (1993). \textsc{mega}: molecular evolutionary genetics analysis, version 1.0. The Pennsylvania State University, University Park, PA, USA.


Syntrophothermus lipocalidus gen. nov., sp. nov.


