Thermosyntropha lipolytica gen. nov., sp. nov., a Lipolytic, Anaerobic, Alkalitolerant, Thermophilic Bacterium Utilizing Short- and Long-Chain Fatty Acids in Syntrophic Coculture with a Methanogenic Archaeum

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Three strains of an anaerobic thermophilic organoheterotrophic lipolytic alkalitolerant bacterium, Thermosyntropha lipolytica gen. nov., sp. nov. (type strain JW/VS-265T; DSM 11003), were isolated from alkaline hot springs of Lake Bogoria (Kenya). The cells were nonmotile, non-spore forming, straight or slightly curved rods. At 60°C the pH range for growth determined at 25°C (pH25°C) was 7.15 to 9.5, with an optimum between 8.1 and 8.9 (pH25°C of 7.6 and 8.1). At a pH25°C of 8.5 the temperature range for growth was from 52 to 70°C, with an optimum between 60 and 66°C. The shortest doubling time was around 1 h. In pure culture the bacterium grew in a mineral base medium supplemented with yeast extract, tryptone, Casamino Acids, betaine, and crotonate as carbon sources, producing acetate as a major product and constitutively a lipase. During growth in the presence of olive oil, free long-chain fatty acids were accumulated in the medium but the pure culture could not utilize olive oil, triacylglycerols, short- and long-chain fatty acids, and glycerol for growth. In syntrophic coculture (Methanobacterium strain JW/VS-M29) the lipolytic bacteria grew on triacylglycerols and linear saturated and unsaturated fatty acids with 4 to 18 carbon atoms, but glycerol was not utilized. Fatty acids with even numbers of carbon atoms were degraded to acetate and methane, while from odd-numbered fatty acids 1 mol of propionate per mol of fatty acid was additionally formed. 16S rDNA sequence analysis identified Syntrophospora and Syntrophomonas spp. as closest phylogenetic neighbors.

Until now, thermophilic anaerobic lipolytic bacteria able to utilize long-chain fatty acids in syntrophic cocultures were unknown. This is surprising since a large number of obligately anaerobic thermophilic and extremely thermophilic organoheterotrophic hydrolytic (eu) bacteria and archaea have been isolated from geothermally heated and anthropogenic thermobiotic environments (41). Most of these microorganisms grow at pH values close to neutral except for a few recently isolated proteolytic anaerobic alkali(tolerant)-thermophiles (8, 20, 21). Most of the known lipolytic microorganisms are aerobes, which include some recent examples of thermophiles and alkaliphiles (7, 16, 17, 36, 38). All known anaerobic lipolytic bacteria which produce true lipases, such as Anaerovibrio lipolytica (29) and a Butyrivibrio sp. (12) from rumina, are mesophilic neutrophiles (24). They are able to grow anaerobically on triacylglycerides, hydrolyzing the acylglycerol linkage and utilizing the liberated glycerol for growth, but do not utilize the long-chain fatty acids. The short- and long-chain fatty acids under anaerobic conditions are degraded by nonlipolytic syntrophic proton-reducing microorganisms (1, 2, 4, 14, 23-26, 34, 35, 37, 43, 44) and by some nonlipolytic sulfate-reducing bacteria (32, 40); however, they depend on other lipolytic organisms, such as A. lipolytica (29). Only two nonlipolytic, neutrophilic proton-reducing mesophiles have been described, the syntrophic Syntrophomonas sapovorans and Syntrophomonas wolfei subs. saponivada, which are able to degrade long (18-carbon)-chain fatty acids via β-oxidation in syntrophic associations with H2-utilizing methanogens or sulfate reducers (23, 34).

To our knowledge, no lipolytic, obligately anaerobic neutrophilic or alkaliphilic thermophile from any natural volcanic or man-made thermobiotic environments nor an anaerobic thermophile which hydrolyzes triglycerides and utilizes the liberated short- and long-chain fatty acids for growth has been described. Recently, an anaerobic thermophilic (55°C) enrichment culture able to degrade a wide range of short- and long-chain fatty acids was described (1). Here, we report on the first thermophilic syntrophic anaerobic alkalitolerant lipolytic eu-bacterium, Thermosyntropha lipolytica gen. nov., sp. nov., isolated from alkaline volcanic environments of Lake Bogoria, Kenya. It presents a novel physiological type among the thermophiles. When incubated in the presence of triglycerides, this bacterium hydrolyzed triglycerides and grew on the liberated fatty acids in a syntrophic association with an alkali-tolerant H2-utilizing methanogen, isolated from the same environment.

MATERIALS AND METHODS

Isolation and culture conditions. The prereduced basal medium used for enrichment, isolation, and cultivation of lipolytic bacteria was prepared by the modified Hungate technique (22) under nitrogen gas phase. The basal medium contained (per liter) 0.3 g of K2HPO4, 0.3 g of KCl, 0.5 g of NaCl, 1.0 g of NH4Cl, 0.3 g of MgCl2·6H2O, 0.05 g of CaCl2·2H2O, 3.0 g of NaHCO3, 3.0 g of Na2CO3, 0.5 g of Na2S·9H2O, 0.15 g of cysteine, 2 ml of vitamin solution (11), and 2.5 ml of a trace element solution (11). Substrates were added in concentrations indicated below. After addition of the substrates, the pH of the medium was adjusted to values indicated below at 25°C (pH25°C) or at cultivation temperature (e.g., pH60°C).

For the enrichment of lipolytic bacteria, 20 ml of commercial olive oil (Kroger brand) per liter and 5 g of yeast extract per liter were added to the basal medium and the pH25°C was adjusted to 8.8 and 9.5. The enrichments were incubated at 60, 70, and 80°C. Serial dilutions of positive incubations were made into the same medium (pH90°C 8.0), and the mixtures were incubated at 60°C. Partially purified lipolytic cocultures obtained by serial dilutions in liquid medium were serially diluted and used to inoculate agar-shake-roll tubes containing basal medium (pH90°C 7.8) with 5 ml of emulsified olive oil, 0.5 g of yeast extract, and 3 mg of rhodamine B (19) per liter and 2% agar. The agar-shake-roll tubes were incubated...
bated at 60°C for 2 to 3 days until colonies appeared. Colonies were picked, transferred to liquid medium, serially diluted and used as inocula for subsequent agar-shake-roll tubes. Several rounds of isolating single colonies were carried out to ensure the purity of the strains with 1% (wt/vol) yeast extract-containing media.

Light microscopy. Light microscopy was performed with a model PM-10AD phase-contrast microscope (Olympus Optical Co., Ltd., Tokyo, Japan).

Determination of growth. Growth of the lipolytic bacteria and the lipolytic cocultures on media containing olive oil, triglycerides, and long-chain fatty acids was determined by direct cell counting using a phase-contrast microscope and a Petroff-Hausser bacterial counter (Hausser Scientific Partnership, Horsham, Pa.). On media with soluble substrates, growth was determined by counting and by measuring the increase in optical density at 600 nm (Spectronic 21; Bausch & Lomb, Rochester, N.Y.).

pH and temperature ranges for growth. The pH range for growth was determined for growth in the basal medium containing 30 g of yeast extract per liter as the carbon source. For determination of the pH range for growth the pH values of the medium were adjusted at 60°C (pH100°C) with growth determination by the direct counting method. The pH values were also determined at 25°C (pH25°C) to allow comparison of values from the literature (e.g., pH25°C values of 6.85, 7.5, 8.2, and 9.0 correspond to pH100°C values of 6.9, 8.0, 9.0, and 10.0, respectively). For the determination of the temperature range for growth the cultures were determined by the direct counting method.

Determination of Gram-staining reaction. Gram-staining reaction was determined by the light microscope after 60°C for 2 weeks, and substrate utilization was determined by periodical determination of cell counts, pH, and fermentation products, including hydrogen and methane, in the synthetic cultures. Medium containing only 2 g of yeast extract per liter was used as control.

Fermentation products. Determination of short-chain fatty acids and alcohols was conducted by high-performance liquid chromatography (HPLC) using a Aminex HPX-87 column (300 by 7.5 mm) (Bio-Rad, Richmond, Calif.) (elucent, 40 mM H2SO4; column temperature, 65°C; flow rate, 0.6 ml/min) and a Refractive Index Detector 156 (Altex, San Ramon, Calif.) at 35°C. The injection volume was 20 μl. Methane and hydrogen were determined by (chromatograph AGS 111 HS with a thermal conductivity detector from Carlo Erba, Loveland, Colo.) using N2 as the carrier gas for H2 determination and He for the CH4 determination.

Determination of long-chain fatty acids. The concentrations of long-chain fatty acids in cultures grown in the presence of triacylglycerides and in lipase activity assays were measured enzymatically with an assay kit. The kit is based on the reaction of α,ω-unsaturated α,ω-unsaturated acid (CAPS) (pH100°C 9.0) or mouse 100 mM 3-cyclohexylamino-1-propanesulfonic acid (TAPS) (pH100°C 10.0) buffer and 0.2 ml of culture supernatant. The free fatty acids liberated from olive oil by lipase activity were separated on a NEFA kit. The NEFA kit was used for the reaction mixture containing 100 μl of culture supernatant, 1.08 μl of TAPS or CAPS buffer, and 12 μl of 300 mM pNP-laurate in acetone/trifluoroacetic acid (final concentration in the assay, 3 mM). After 15 to 60 min of incubation at 50°C the reaction mixture was chilled on ice to stop the reaction and the A580 of the centrifuged supernatant was measured. One unit was defined as the amount of the enzyme catalyzing the release of 1 μmol of p-nitrophenol per min from pNP-laurate at 50°C with the molar absorption coefficient of 18,200 M−1 cm−1.

Susceptibility to antibiotics. Susceptibility to antibiotics was determined at 60°C by the agar diffusion method. The minimal inhibitory concentration (MIC) was determined by well diffusion of the medium containing 10 g of yeast extract per liter and 50 μg of filter-sterilized G4-free antibiotic per ml.

Lipase-staining reaction. The Gram-staining reaction was determined by the modified Hucker method (Enhanced Gram Stain kit; Carr Scarborough Microbiology, Inc., Decatur, Ga.). Gram type was determined according to Wiegel and Quandt (42).

DNA isolation and determination of G+C content. DNA was isolated by the NaOH method of Mesbah et al. (27). The guanine-plus-cytosine content (mole percent G+C) was determined photometrically after enzymatic digestion and separation by HPLC of nucleosides as described by Whitman et al. (39) and Mesbah et al. (27).

Genes coding for 16s rDNA sequence analysis. Genomic DNA was isolated from the strains JW/VS-264, JW/VS-2655, and JW/VS-269, and the 16s rDNA of SJWNS-264, SJWNS-265T, and JW/VS-269, and the 16s rDNA sequences of the three strains were manually aligned with previously published 16s rDNA/16s rRNA sequences of representatives of the chlorofluorocarbon and related taxa. The method of Jukes and Cantor (18) was used to calculate evolutionary distances from which a phylogenetic dendrogram was reconstructed according to the algorithm of De Soete (6).

RESULTS AND DISCUSSION

Isolation of anaerobic lipolytic thermophiles. Over 80 samples taken from various volcanic areas in New Zealand, the United States, Kenya, the Far East of Russia, and different sewage plants in the United States were used as inocula for enrichments of anaerobic lipolytic thermophiles. A phosphate-based mineral medium (pH25°C 8.8 or 9.5, pH60°C 8.0 and 8.6) containing 20 ml of commercial olive oil per liter as the carbon and energy source and 0.5 g of yeast extract per liter was employed. Stable lipolytic enrichments were obtained from three samples from alkaline hot springs on the shores of the alkaline soda-containing Lake Bogoria (Kenya) and were incubated at pH25°C 8.8 (pH60°C 8.0) at 60°C. The growth on olive oil was accompanied by a strong decrease of pH25°C down to 7.5 and methane formation. After several transfers and serial dilutions, mainly two morphologically distinct types of microorganisms remained in these enrichments: slightly curved rods (later identified as the lipolytic bacterium) and long, more or less filamentous cells identified by epifluorescence microscopy as methanogens.

The lipolytic bacteria were isolated by serial dilutions and picking single colonies in agar-shake-roll tubes using medium supplemented with emulsified commercial olive oil (rendering the medium turbid) and rhodamine B for visualization of lipase activity. After about 1 week, incubations at 60°C yielded small irregular white colonies with clearing zones. When rhodamine B was present, the colonies with clearing zones formed orange fluorescent zones when irradiated with UV light at 350 nm, a specific test for lipases (19). All positive enrichments yielded morphologically similar types of colonies and bacteria. Three isolates with the highest lipase activity, strains JW/VS-264, JW/VS-2655, JW/VS-269, were selected for further studies.

 Colony and cell morphology. Colonies in agar (2%)-shake-roll tubes (mineral medium, 0.01% [wt/vol] yeast extract; 0.5% [vol/vol] olive oil; pH 8.5, 60°C) which appeared after 2 days of incubation at 60°C were small (diameter 0.5 mm), white, and lens shaped. Cells of the three strains were morphologically similar, straight or slightly curved rods 0.3 to 0.4 μm in diameter and 2 to 3.5 μm in length (Fig. 1). Chains of two and more cells were seen during the exponential growth phase. Motility of the cells was never observed, nor was the formation of heat-stable spores after growth under various culture conditions which included low and high substrate concentrations in liquid and solid medium, different temperatures (37 to 70°C) and pH values (pH 7.0 to 9.0), and several weeks of prolonged incubations.

 Cell wall. Gram-staining reaction, and Gram type. The cells of the strain JW/VS-2655 stained gram negative in both exponential and stationary growth phases, but the organism is a gram-type-positive bacterium (42). This is consistent with the 16s rRNA sequencing data which placed the organism into the Clostridium-Bacillus subphylum.

 Physiological characterization. (i) Temperature and pH ranges for growth. The temperature range for growth of the three purified strains was 52 to 70°C with an optimum at 60 to 66°C (Fig. 2A). Growth was not observed within several weeks.
at temperatures of 44°C and below or at 73°C and above. The pH optimum for growth was pH 2.5 to 8.5 (pH 7.0 to 8.0) for strains JW/VS-264 and JW/VS-265 and 8.1 to 8.5 (pH 8.6 to 7.8) for strain JW/VS-269. The strains grew slowly at pH 2.5 to 7.15 and 9.5 but not at pH 7.6 to 9.7 (Fig. 2B). With mineral medium supplemented with yeast extract (1%, vol/ vol), the doubling time under the above optimum growth conditions was about 1 h (Fig. 2), whereas in the presence of only 0.2% yeast extract and 0.5% (vol/vol) olive oil the doubling time was 3.4 h.

(ii) Substrate utilization by the lipolytic monocultures at 60°C. The three strains grew well as monocultures on liquid medium with yeast extract as the sole source of carbon and energy. The cell count increased proportionally with the yeast extract concentration (0 to 1%, wt/vol) (data not shown) and reached approximately 1.5 × 10⁷ to 1.4 × 10⁸ cells/ml with 1.0 and 10 g of yeast extract per liter, respectively. At 60°C, strain JW/VS-265 utilized the following substrates when added to the basal mineral medium (pH 2.5 to 8.8) containing 2 g of yeast extract per liter: maximal optical density (beyond the base value in the control without supplements) was reached within 1 to 2 days with 5 g (each tested separately) of tryptone, Casamino Acids, beef extract, brain heart infusion, or nutrient broth per liter. More than 7 days of incubation were required for growth on crotonate (15 mM), producing acetate and butyrate, and on betaine (10 mM), producing acetate. The cell count on crotonate was 8 × 10⁸ cells/ml. With pyruvate (15 mM), ribose (2.5 g/liter), and xylose (2.5 g/liter), weak growth with concomitant formation of small amounts of acetate acidifying the medium containing sugars was seen.

In the absence of a methanogen, the addition of olive oil (0.5 to 10 ml/liter), soybean oil (0.5 to 10 ml/liter), tributyrin (3 mM), trilaurin (1 mM), tripalmitin (1 mM), tristearin (1 mM), and trilinolein (1 mM) did not stimulate but also did not inhibit the initial growth in basal medium containing 2 g of yeast extract per liter. The triacyl glycerides (see below for details), however, were hydrolyzed by a constitutively formed lipase found in the culture supernatant, but the liberated glycerol and long-chain fatty acids were not utilized regardless of whether CaCl₂ was added (in equimolar concentrations relative to the fatty acid content of the triacyl glycerides [3 mM]) (33). Furthermore, no growth and no accumulation of any fermentation products were observed with the 2-g/liter yeast extract-containing basal medium supplemented with (tested separately) 6.8 to 68 mM glycerol; 5 mM acetate, propionate, butyrate, isobutyrate, hydroxybutyrate, valerate, isovalerate, or caproate; 2 mM heptanoate, caprylate, pelargonate, or laurate; 1 mM myristate, palmitate, stearate, oleate, linoleate, arachidate, behenate, or lignocerate; 15 mM lactate, malate, fumarate, succinate, methanol, ethanol, 1-propanol, 1-butanol (2,5); 5 g of arabinose, fructose, galactose, glucose, mannose, raffinose, sucrose, starch, xylan, or pectin per liter; 0.5 mM 1,3-benzenediol, benzoate, 2,3-dihydroxybenzoic acid, 2,4-dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid, hydroquinone, m-hydroxybenzoic acid, p-hydroxybenzoic acid, 2-hydroxybenzoic acid, or phenol; and a mixture of 80% H₂ and 20% CO₂ (vol/vol).

**FIG. 1.** Light microscopy of exponentially growing culture of *T. lipolytica* JW/VS-265 during growth in mineral medium (60°C, pH 8.6) supplemented with 1% (wt/vol) yeast extract.

**FIG. 2.** Growth of *T. lipolytica* JW/VS-265 (●), JW/VS-265 (○), and JW/VS-269 (□) in mineral medium supplemented with 10 g of yeast extract per liter at various temperatures at pH 8.5 (A) and at various pH values at 60°C (B).
reconstituted coculture grew and utilized olive oil (1 to 10 ml/liter of medium) and the tested triacyl glycerides (tributyrin [3 mM] and trilaurin, tripalmitin, tristearin, and triolein [all at 1 mM]) but required the presence of CaCl₂ in equimolar concentrations relative to the fatty acyl residues in the triglycerides (33). The best growth was observed on olive oil and tristearin. Acetate (leading to an acidification), methane, and glycerol were produced stoichiometrically. The coculture grew on the saturated and unsaturated fatty acids butyrate, valerate, and caproate (all at 5 mM, but only very slowly; detectable growth required 2 to 3 weeks of incubation); heptanoate, caprylate, pelargonate, caprate, and laurate (all at 2 mM); and myristate, palmitate, stearate, oleate, and linoleate (all at 1 mM) when equimolar concentrations of CaCl₂ were provided. Fatty acids with even numbers of carbon atoms were degraded to acetate and methanone, while odd-numbered fatty acids yielded additionally one propionate per fatty acid degraded. The coculture did not grow and did not utilize acetate, propionate, hydroxybutyrate, isobutyrate, isovalerate and the long-chain fatty acids caproate (all at 1 mM), butyrate, valerate, and oleate, only very slowly; detectable growth required 2 to 3 weeks of incubation; heptanoate, caprylate, pelargonate, caprate, and laurate (all at 2 mM); and myristate, palmitate, stearate, oleate, and linoleate (all at 1 mM) when equimolar concentrations of CaCl₂ were provided. Fatty acids with even numbers of carbon atoms were degraded to acetate and methanone, while odd-numbered fatty acids yielded additionally one propionate per fatty acid degraded. The coculture did not grow and did not utilize acetate, propionate, hydroxybutyrate, isobutyrate, isovalerate and the long-chain fatty acids caproate (all at 1 mM), butyrate, valerate, and oleate, only very slowly; detectable growth required 2 to 3 weeks of incubation; heptanoate, caprylate, pelargonate, caprate, and laurate (all at 2 mM); and myristate, palmitate, stearate, oleate, and linoleate (all at 1 mM) when equimolar concentrations of CaCl₂ were provided. Fatty acids with even numbers of carbon atoms were degraded to acetate and methano...

Differentiation of T. lipolytica from other thermophilic anaerobes and mesophilic syntrophic fatty acid degraders. To our knowledge, the isolated strains are the first obligately anaerobic thermophilic lipolytic bacteria, hydrolyzing triglycerides and utilizing the liberated short- and long-chain fatty acids in a syntrophic association with an H₂-utilizing methanogen but not utilizing the liberated glycerol. Until now, no thermophilic anaerobe responsible for the degradation of triglycerides in volcanic hydrothermal biotopes had been identified. The complete anaerobic degradation of triacylglycerols and short- and long-chain fatty acids in anaerobic zones of volcanic environments can be performed by syntrophic association of the here-described novel lipolytic syntrophic bacteria and H₂-consuming thermophilic methanogens (e.g., M. thermautotrophica [synonym, M. alcaliphilum]) which were always present in our lipolytic enrichments. Thus, T. lipolytica represents a new physiological group of thermophilic anaerobes, combining features of mesophilic anaerobic lipolytic bacteria and syntrophic long-chain fatty acid-fermenting bacteria. T. lipolytica differs from other anaerobic biopolymer-degrading thermophiles (40, 41), including the recently isolated glycolytic alkalithermophiles (8, 9, 20, 21), in that in cannot utilize cellulose, starch, pectin, and xylan. It differs from long-chain fatty acid-utilizing sulfate- or sulfur-reducing bacteria because it can hydrolyze triglycerides (32, 40). But unlike the mesophilic, neutrophilic, lipolytic A. lipolytica (13, 15, 29) (Table 1), the newly...

DNA base composition and 16S rDNA sequence analysis. The G+C content of the DNA was between 43 and 44 mol%. 16S rDNA sequence analysis of strains JW/VS-264, JW/VS-265, and JW/VS-269 placed them into the Clostridium-Bacillus subphylum. The three strains were indistinguishable at the 16S rDNA level. The 16S rDNA sequence of strain JW/VS-265 had 93% similarity to those of Syntrophomonas wolfei (25, 26) and Syntrophoplasma bryantii (37, 44) as the closest related species. The new isolates are clearly separated from all other anaerobic thermophilic bacteria, hydrolyzing triglycerides and utilizing the liberated short- and long-chain fatty acids in a syntrophic association with an H₂-utilizing methanogen but not utilizing the liberated glycerol. Until now, no thermophilic anaerobe responsible for the degradation of triglycerides in volcanic hydrothermal biotopes had been identified. The complete anaerobic degradation of triacylglycerols and short- and long-chain fatty acids in anaerobic zones of volcanic environments can be performed by syntrophic association of the here-described novel lipolytic syntrophic bacteria and H₂-consuming thermophilic methanogens (e.g., M. thermautotrophica [synonym, M. alcaliphilum]) which were always present in our lipolytic enrichments. Thus, T. lipolytica represents a new physiological group of thermophilic anaerobes, combining features of mesophilic anaerobic lipolytic bacteria and syntrophic long-chain fatty acid-fermenting bacteria. T. lipolytica differs from other anaerobic biopolymer-degrading thermophiles (40, 41), including the recently isolated glycolytic alkalithermophiles (8, 9, 20, 21), in that in cannot utilize cellulose, starch, pectin, and xylan. It differs from long-chain fatty acid-utilizing sulfate- or sulfur-reducing bacteria because it can hydrolyze triglycerides (32, 40). But unlike the mesophilic, neutrophilic, lipolytic A. lipolytica (13, 15, 29) (Table 1), the newly...
### TABLE 1. Characteristics that differentiate *T. lipolytica* from other, phylogenetically and physiologically related organisms

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Thermosyntropha lipolytica&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Syntrophospora bryantii&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Syntrophomonas wolfei subsp. wolfei&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Syntrophomonas wolfei subsp. sapovorans&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Syntrophomonas sapovorans&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Syntrophobacter wolinii&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Anaerovibrio lipolytica&lt;sup&gt;b&lt;/sup&gt;</th>
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<td>Optimum pH</td>
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<td>Optimum temp (°C)</td>
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<tr>
<td>Maximum temp (°C)</td>
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<td>30–37</td>
<td>30–37</td>
<td>45</td>
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<td>Spore formation</td>
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<td>Lipolytic</td>
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#### Substrates used

**In pure culture**
- Yeast extract: +
- Tryptone: +
- Casamino Acids: +
- Crotanate: +
- Betaine: +
- Pyruvate: +
- Glucose: –
- Ribose: +
- Xylose: +
- Glycerol: –
- H<sub>2</sub>CO<sub>2</sub>: +

**In coculture**
- Olive oil: +
- Tributyrin: +
- Triacylglycerol: +
- Tryptone: +
- Tryptone C<sub>2</sub>: +
- Caprate C<sub>10</sub>: +
- Myristate C<sub>14</sub>: +
- Stearate C<sub>18</sub>: +
- Oleate C<sub>18</sub>: +

<sup>a</sup>This study.<br>
<sup>b</sup>Values taken from references 2, 3, 13, 23, 26, 29, 34, 35, 37, and 44.<br>
<sup>c</sup>In pure culture.

isolated strains are alkalitolerant (pH<sup>25°C</sup> range for growth, <8.0 to 9.5) thermophiles (52 to 70°C; optimum, around 8.5) (41) and they do not ferment glycerol either in pure culture or in coculture with an H<sub>2</sub>-utilizing methanogen.

The 16S rDNA sequence analysis places the new strains clearly in the gram-type-positive subphylum and thus separates them from the gram-type-negative *Syntrophus* and related bacteria. The lipolytic nature and the syntrophic fatty acid degradation separate the novel isolates from other known thermophilic anaerobes, including our recently isolated thermoalkaliphiles (8, 9, 22, 2). The nearest neighbors in the 16S rDNA sequence-based tree (Fig. 3), the gram-positive syntrophic *Syntrophospora* and *Syntrophomonas* spp. and the homoacetogenic bacteria *Moorella thermoacetica* and *Moorella thermoautotrophicum* (5), differ in several important aspects (Table 1). *Syntrophobacter wolinii* (4) is a propionate-utilizing syntrophic bacterium, and *Syntrophus buswellii* (28) utilizes syntrophically benzoate. The long-chain fatty acid-utilizing syntrophic anaerobe *Syntrophomonas sapovorans* (34) is not lipolytic. Other differences between these organisms are summarized in Table 1. On the basis of physiological properties and 16S rDNA analysis, the organism described here constitutes a novel taxon that is most closely related to the mesophilic, neutrophilic syntrophic bacteria.

**Description of the genus Thermosyntropha gen. nov.** *Thermosyntropha* (Ther.mo.syt.ro'pha. Gr. adj. thermos, hot; Gr. prefix syn, with, together; Gr. verb. trophein, to eat; Gr. masc. n syntrophos, foster brother or sister; M.L. fem. n. thermosyntro-
pha ["foster sisters liking it hot," referring to the fact that the bacterium grows at elevated temperatures on fatty acids only in syntrophic cultures with hydrogen-utilizing microorganisms]. The Gram reaction-negative and gram-type-positive cells are generally rod shaped, obligately anaerobic, heterotrophic, and lipolytic and grow at elevated temperatures and moderately alkaline pH values. Thermodynamically unfavorable substrates leading to hydrogen formation are utilized in syntrophic associations with H2-consuming microorganisms. The DNA G+C content is 43 to 44 mol%. The lipolytic, fatty acid-degrading *T. lipolytica* is the type and, so far, only species in this genus. On the basis of the 16S rDNA sequence the genus *Thermosyntrophus* belongs to the family *Syntrophomonadaceae* (45).

Description of *Thermosyntrophus lipolytica* sp. nov. *Thermosyntrophus lipolytica* (lip.o.ly'ti.ca. Gr. n. lipos, fat; Gr. adj. lipo'tikes, able to looseen; M.L. adj. lipolytica, referring to the property of being able to hydrolyze lipids from the formation of fatty acids and glycerol). The cells are straight or slightly curved rods, 0.3 to 0.4 by 2.0 to 3.5 µm. Most cells occur in the exponential growth phase in pairs or chains. Motility or spores have not been observed. The organism is a peptidolytic and lipolytic chemoanotroph. Oleate, linolate, and the saturated fatty acids butyrate (only slowly metabolized) up to stearate are β-oxidized in syntrophic association with an H2-utilizing microorganism. All *T. lipolytica* isolates are alkali-tolerant thermophiles growing between 52 and 70°C (optimum, 60 to 66°C) and in a pH8.9 range from 7.15 to 9.5 (optimum, 8.1 to 8.9). The DNA base composition is 43 to 44 mol% G+C.

Strain JW/VS-265T (DSM 11003) is the type strain of *T. lipolytica*. It has a G+C content of 44 mol%.

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