***Thermosipho geolei* sp. nov., a thermophilic bacterium isolated from a continental petroleum reservoir in Western Siberia**

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Three strictly anaerobic, thermophilic bacteria (SL31^T, SL30 and MLM39636) were isolated from a deep continental oil reservoir in Western Siberia (Russia). Following the mid-exponential phase of growth, the non-motile rod-shaped organisms were surrounded by a sheath-like structure. As DNA–DNA hybridizations showed that these strains were highly related genomically, only strain SL31^T was studied in detail. The temperature range for growth of strain SL31^T was between 45 and 75 °C, with optimum growth at 70 °C. Its optimum pH and NaCl concentration for growth were pH 7.5 and 20–30 g l⁻¹, respectively. The novel isolate reduced elemental sulfur and cystine, but not thiosulfate or sulfate, to hydrogen sulfide. The G+C content of the genomic DNA was 30.0 mol %. As determined by 16S rDNA sequence analysis, this organism belonged to the genus *Thermosipho*. DNA–DNA hybridization levels between strain SL31^T and type strains of the previously described species of *Thermosipho* were less than 10%. On the basis of physiological and molecular properties, it is proposed that this organism should be placed in a new species, *Thermosipho geolei* sp. nov. The novel organism represents the first species of the genus *Thermosipho* that has been isolated from a petroleum reservoir. The type strain is SL31^T (= DSM 13256^T = JCM 10986^T).

Keywords: oilfield, thermophiles, 'Thermotogales', *Thermosipho*, *Thermosipho geolei*

INTRODUCTION

Members of the order 'Thermotogales' are rod-shaped bacteria that are morphologically characterized by an outer sheath-like structure called a toga (Huber & Stetter, 1992). These strictly anaerobic thermophiles represent, along with *Desulfurobacterium thermolithotrophum* and the 'Aquificales', one of the deepest phylogenetic branches in the bacterial line of evolutionary descent (Achenbach-Richter *et al.*, 1987; Burgraff *et al.*, 1992; L'Haridon *et al.*, 1998). At present, the order 'Thermotogales' includes only one family, the 'Thermotogaceae', which consists of five genera: *Fervidobacterium* (Patel *et al.*, 1985), *Thermotoga* (Huber *et al.*, 1986), *Thermosipho* (Huber *et al.*, 1989; Ravot *et al.*, 1996a), *Geotoga* and *Petrotoga* (Davey *et al.*, 1993). These obligately heterotrophic

bacteria have been isolated from and/or detected using molecular techniques in shallow and deep marine hydrothermal vents as well as in continental springs and petroleum reservoirs (Huber *et al.*, 1986, 1989, 1990; Jannasch *et al.*, 1988; Windberger *et al.*, 1989; Davey *et al.*, 1993; Stetter *et al.*, 1993; Jeanthon *et al.*, 1995; L'Haridon *et al.*, 1995; Ravot *et al.*, 1995; Andrews & Patel, 1996; Grassia *et al.*, 1996; Magot, 1996; Antoine *et al.*, 1997; Fardeau *et al.*, 1997; Koch *et al.*, 1997; Harmsen *et al.*, 1997; Lien *et al.*, 1998; Slobodkin *et al.*, 1999; Corre, 2000; Orphan *et al.*, 2000; Takahata *et al.*, 2000; Takai & Horikoshi, 2000). The genus *Thermosipho* contains three validly described species, *Thermosipho africanus* (Huber *et al.*, 1989; Ravot *et al.*, 1996a), *Thermosipho melanesiensis* (Antoine *et al.*, 1997) and *Thermosipho japonicus* (Takai & Horikoshi, 2000). These three species have been isolated from coastal and deep marine hydrothermal systems.

In this paper, the first isolation of a species of the genus

The GenBank accession number for the 16S rRNA gene sequence of strain SL31^T is AJ272022

Thermosipho from oilfield water is described. The phenotypic and genetic characteristics of the novel strain are consistent with its placement in a new species of the genus *Thermosipho*, which is named *Thermosipho geolei* sp. nov.

METHODS

Source of organisms. The novel strain was isolated from an oil/water mixture taken directly from a production well-head of a deep continental petroleum reservoir in Western Siberia (Russia). *Thermosipho africanus* Ob7^T (= DSM 5309^T) was obtained from the DSMZ. *Thermosipho melanesiensis* BI429^T (= DSM 12029^T) was kindly provided by the Laboratoire de Caractérisation des Micro-organismes Marins (Ifremer, Brest, France).

Enrichment, isolation and growth conditions. Enrichments were performed anaerobically in Hungate tubes containing 10 ml medium, according to Balch & Wolfe (1976). The enrichment medium consisted of (l⁻¹ distilled water): 20 g NaCl, 0.33 g NH₄Cl, 0.33 g KH₂PO₄, 0.33 g MgCl₂·6H₂O, 0.33 g CaCl₂·2H₂O, 1 g NaHCO₃, 2 g sodium acetate, 0.5 g yeast extract, 0.5 g Na₂S·9H₂O, 1 ml trace element solution (Kevbrin & Zavarzin, 1992) and 2 ml vitamin solution (Wolin *et al.*, 1963), and had a pH at room temperature of 6.8–7.0. It was inoculated with 1 ml oil/water sample, pressurized with N₂ (100 %; 100 kPa) and incubated without shaking at 65 °C. The medium used to grow the type strains and to isolate and to cultivate strain SL31^T routinely contained (l⁻¹ distilled water): 15 g NaCl, 0.5 g MgCl₂·6H₂O, 3.4 g PIPES, 0.2 g KCl, 1 g NH₄Cl, 0.1 g CaCl₂·2H₂O, 0.35 g K₂HPO₄, 0.35 g KH₂PO₄, 2.72 g sodium acetate trihydrate, 2 g yeast extract, 2 g bio-Trycase (bioMérieux) and 1 mg resazurin. The pH was adjusted to 7.0 using 5 M HCl before autoclaving. Prior to inoculation, maltose and Na₂S·9H₂O were injected from sterile anaerobic stock solutions to obtain final concentrations of 0.2 and 0.1 %, respectively. Single colonies were obtained and purified by streaking on the same medium that was solidified with 1 % (w/v) Phytigel (a gellan gum from Sigma). Plates were incubated in anaerobic jars at 65 °C under an N₂/CO₂ atmosphere (80:20; 100 kPa). Stock cultures of isolate SL31^T were stored in culture medium at 4 °C. For long-term storage, pure cultures were stored at –80 °C in the same medium containing 10 % (w/v) DMSO.

Determination of growth parameters. Growth was monitored by measuring the increase of OD₆₀₀ with a Spectronic 401 spectrophotometer (Bioblock). All growth experiments were performed in triplicate. The pH range for growth was determined in the culture medium with various buffers at a concentration of 10 mM (MES for pH 5.0–6.0, PIPES for pH 6.5 and 7.0, HEPES for pH 7.5, Tris for pH 8.0 and 8.5). Appropriate amounts of 1 M Na₂CO₃ were added to adjust the pH of the medium to 9.0 and 9.5. pH values were determined at room temperature. To determine the optimum NaCl range for growth, NaCl concentrations were varied while maintaining the concentrations of the other inorganic components. The effects of different pH values and concentrations of NaCl were determined at 70 °C.

Substrate utilization tests. Ability to grow lithoautotrophically was tested in the basal medium where yeast extract, bio-Trycase, acetate and maltose were omitted and with H₂/CO₂ (80:20; 100 kPa) as the gas phase. Possible growth substrates were tested in the same medium and substrates were added at a concentration of 500 mg l⁻¹ with N₂ (100 %;

100 kPa) as the gas phase. Three consequent transfers were performed on each substrate that allowed growth. The influence of yeast extract on growth with substrates was tested by adding this compound to the basal medium at a concentration of 50 mg l⁻¹. The influence of elemental sulfur on the utilization of substrates was tested in the same medium with 50 mg yeast extract l⁻¹ and 1 % elemental sulfur.

Effects of electron acceptors on growth, glucose metabolism and hydrogen and oxygen susceptibility. Elemental sulfur (1 %), cystine (1 %), sulfate (20 mM) and thiosulfate (20 mM) were tested as potential electron acceptors in the culture medium. The influence of elemental sulfur on growth and on the amounts of acetate and L-alanine produced during glucose fermentation was investigated according to the method of Ravot *et al.* (1996a,b). Amino acid concentrations were determined by liquid chromatography (Moore *et al.*, 1958). The influence of hydrogen on growth was examined in medium supplemented with or without elemental sulfur under H₂/CO₂ (80:20; 100 kPa) and under N₂/CO₂ (80:20; 100 kPa). Susceptibility to oxygen was tested by incubating the organism in culture medium at 70 °C under aerated and under microaerophilic conditions (L'Haridon *et al.*, 1998).

Antibiotic susceptibility. Sensitivity of strain SL31^T to chloramphenicol, streptomycin, vancomycin and rifampicin (all from Sigma) was tested at 70 °C. Simultaneous experiments were performed with *Thermosipho africanus* Ob7^T and *Thermosipho melanesiensis* BI429^T at the same temperature.

Light and electron microscopy. An Olympus BX-60 microscope equipped with an Olympus OM-2 camera was used routinely to observe and to obtain photomicrographs. For negative staining, 20 µl cell suspension, fixed with 2 % (w/v) glutaraldehyde, was dropped on Formvar/carbon-coated grids (400 mesh) and stained with 2 % phosphotungstate. Thin sections were prepared as described previously (Bonch-Osmolovskaya *et al.*, 1990). Electron micrographs were taken using a model JEM-100 electron microscope.

H₂S production. H₂S production was evaluated by adding 500 µl solution containing CuSO₄ (5 mM) and HCl (50 mM) to 250 µl culture grown at 65 °C. The dark-brown precipitate demonstrating the presence of sulfide was compared with that of the uninoculated medium incubated under the same conditions. Sulfide was determined photometrically as colloidal CuS by using the method of Cord-Ruwisch (1985).

DNA extraction and base composition. Genomic DNA of strain SL31^T was isolated by using the procedure described by Charbonnier & Forterre (1994). The DNA was purified on a caesium chloride gradient (Sambrook *et al.*, 1989) and purity was checked spectrophotometrically. The G+C content of the DNA was determined from the melting point according to Marmur & Doty (1962) using as standards DNA from *Escherichia coli* (G+C content 52 mol %), *Clostridium perfringens* (24 mol %) and *Micrococcus luteus* (73 mol %) (all from Sigma).

16S rDNA-based phylogenetic analysis. Extraction of genomic DNA, PCR-mediated amplification of the 16S rDNA and sequencing of the purified PCR product were carried out according to Rainey *et al.* (1996). The sequence reaction mixtures were electrophoresed using a model 373A automatic sequencer (Applied Biosystems). The 16S rDNA sequences were aligned with published sequences of the DSMZ database using the ae2 editor (Maidak *et al.*, 1996).

and sequences retrieved from EMBL. Evolutionary distances were calculated by the method of Jukes & Cantor (1969). Distance analysis dendrograms were reconstructed as described by De Soete (1983). Bootstrap analysis was used to evaluate the tree topology after neighbour-joining by performing 500 resamplings (Felsenstein, 1985).

DNA–DNA hybridization experiments. Genetic relatedness was investigated by slot-blot DNA–DNA hybridization by using a random-prime labelling and signal amplification system (Amersham Life Sciences) following the procedure described by Kristjánsson *et al.* (1994). Increasing amounts of target DNA (50–100 ng) denatured in 0.4 M NaOH were slotted onto a nylon hybridization membrane (Bio-Rad) and probed with 200 ng labelled tracer DNA. For each duplicate of DNA–DNA association (15 h in 4×SSC buffer with formamide, 0.5% blocking agent, 5% dextran sulfate, 100 µg denatured sheared salmon sperm DNA ml⁻¹), the temperature of hybridization chosen was in the optimal range of the hybridization buffer (Johnson, 1984; Ivanova *et al.*, 1988). Final highly stringent washes and signal amplification were performed according to the manufacturer's instructions. Hybridization signals were detected with a Storm fluorescent scanner (Molecular Dynamics) and analysed by using the IMAGE-QUANT program. The signal (maximum peak area) produced by self-hybridization of the probe with homologous target DNA was set as 100% and compared with the signal generated by heterologous DNA.

RESULTS

Enrichments and isolation

Stratal fluids from different horizons of the Samotlor oilfield (1700–2500 m depth), having temperatures of 45–84 °C, were taken directly from production well-heads through a tap fitted on the production line. Stratal waters had a total mineral content of 5.7–32.9 g l⁻¹ and a pH of 5.5–7.5. Some sites of the Samotlor oilfield are exploited by flooding with a mixture of freshwater from the Vah river (about 30%) and production water. The temperature of the injection water was 4–40 °C depending on the season. The salinity of injection water was 9–17 g l⁻¹. Enrichments of thermophiles were performed by inoculating the collected production fluids into anaerobic enrichment medium and incubating at 65 °C. Within 2 d, turbidity due to cell growth was observed. This growth consisted of small, rod-shaped bacteria with a characteristic outer sheath-like structure. The sheathed bacteria were purified by streaking subcultures onto solidified medium and incubating in an anaerobic jar at 65 °C. After 3 d, whitish round colonies (1–2 mm in diameter) were obtained. Three single colonies were picked and the isolation procedure was repeated at least three times before each of the isolates, designated SL30, SL31^T and MLM39636, was considered to be pure. Isolate SL31^T was studied in detail.

Morphology

Cells of isolate SL31^T were rod-shaped with a length of about 2–3 µm and a width of about 0.4–0.6 µm (Fig. 1a). In the very early exponential phase of growth, no

sheaths could be distinguished clearly around single cells by phase-contrast or transmission electron microscopy (Fig. 1a). Although motility has not been observed under phase microscopy, some of the negatively stained, unsheathed cells examined by transmission electron microscopy possessed single polar flagella (Fig. 1a). After the mid-exponential phase of growth, an envelope surrounding the cells was readily visible by phase-contrast and electron microscopy and cells became longer (Fig. 1b). No flagella could be detected on sheathed cells (data not shown). In the stationary growth phase, the sheaths and cells grew longer and envelopes containing four to six cells were observed. In addition, some of the rods became spheres. Thin sections revealed the Gram-negative structure of the cell wall, with an outer membrane (Fig. 1c and d).

Determination of growth parameters

Strain SL31^T grew between 45 and 75 °C, with optimum growth around 70 °C; no growth was observed at 40 or 80 °C (Fig. 2a). Growth was observed between pH 6.0 and 9.4, with optimum growth around pH 7.5 (Fig. 2b). Growth occurred at NaCl concentrations of 5–70 g l⁻¹, with an optimum between 20 and 30 g l⁻¹ (Fig. 2c); no growth was observed at 0 or 80 g l⁻¹. Under optimal growth conditions (temperature, pH and NaCl), the doubling time of strain SL31^T was approximately 115 min.

Substrates used for growth

Strain SL31^T was a strictly anaerobic, organotrophic organism. Its growth was prevented in the presence of low levels of oxygen (0.2–1.0%, v/v) and under the autotrophic culture conditions tested. Strain SL31^T showed good growth with peptides like beef extract, peptone, papaic digest of soybean meal and yeast extract. Growth occurred on glucose in the presence of yeast extract (50 mg l⁻¹). Casein, fructose, galactose, ribose, arabinose, xylose, maltose, sucrose, starch, xylan, cellulose, pyruvate, succinate, formate, acetate and methanol did not support growth, even when combined with yeast extract (50 mg l⁻¹) or sulfur.

Metabolic products

Molecular hydrogen, acetate, alanine and CO₂ were the main metabolic products detected after glucose fermentation, irrespective of the presence of sulfur (Table 1). In the absence of sulfur, small amounts of fructose (0.6 mM) and isovalerate (1.1 mM) were produced. These products were not detected in the presence of sulfur. Other volatile fatty acids, including branched-chain ones, and alcohols were not detected. In all cases, about 1 mol CO₂ (mol acetate produced)⁻¹ was produced.

Fig. 1. (a) Electron micrograph of a negatively stained unsheathed cell of strain SL31^T, showing a polar flagellum. Bar, 500 nm. (b) Phase-contrast micrograph of strain SL31^T in late exponential phase of growth, showing chains of cells surrounded by the typical outer sheath-like structure (arrows). Bar, 5 µm. (c, d) Thin sections of strain SL31^T showing a typical Gram-negative cell wall ultrastructure. om, Outer membrane; im, inner membrane; cm, cytoplasmic membrane. Bars, 100 nm.

Influence of sulfur compounds on growth and on glucose metabolism

Growth of SL31^T was inhibited completely by H₂ in the headspace [gas phase H₂/CO₂ (80:20; 100 kPa)]. Inhibition of growth by H₂ was overcome by the addition of sulfur. Addition of sulfur stimulated growth, with the concomitant production of H₂S (Table 1). Similar results were obtained with *Thermosipho melanesiensis* BI429^T and *Thermosipho africanus* Ob7^T, used for comparative studies. Strain SL31^T, *Thermosipho melanesiensis* BI429^T and *Thermosipho africanus* Ob7^T were unable to reduce sulfate to H₂S in the presence of H₂. Of these strains, only *Thermosipho africanus* Ob7^T was able to reduce thiosulfate. The

presence of sulfur as an electron acceptor, lowering the hydrogen partial pressure of the medium, also had an effect on the alanine/acetate and alanine/glucose ratios produced during glucose fermentation. When elemental sulfur was absent, the alanine/acetate and alanine/glucose ratios were about 0.14 and 0.16, respectively. In the presence of elemental sulfur, both ratios decreased to values of about 0.07–0.08 (Table 1).

Sensitivity to antibiotics

Strain SL31^T and *Thermosipho melanesiensis* BI429^T were sensitive to chloramphenicol (10 µg ml⁻¹), whereas *Thermosipho africanus* Ob7^T was resistant at

Fig. 2. Specific growth rates (μ) of strain SL31^T as a function of temperature in the presence of 15 g NaCl l⁻¹ and at pH 7 (a), as a function of pH at 70 $^{\circ}\text{C}$ and in the presence of 15 g NaCl l⁻¹ (b) and as a function of concentration of NaCl at 70 $^{\circ}\text{C}$ and at pH 7 (c).

Table 1. Fermentation of glucose by strain SL31^T in the presence or absence of sulfur

The results shown were obtained after 1 month of incubation at 70 $^{\circ}\text{C}$. Uninoculated controls did not exhibit H₂S production in the presence or absence of sulfur.

Culture conditions	Amount of substrate utilized (mmol)	Amount of end product formed (mmol)				Molar ratio of product produced to sugar consumed	
		H ₂ *	H ₂ S	Acetate	Alanine	Acetate	Alanine
Glucose	7.3	9.3	0.5	8.5	1.2	1.20	0.16
Glucose + S ⁰	6.0	0	12.5	7.5	0.5	1.25	0.08

* Amount of H₂ produced in millimolar equivalents.

this concentration. The three strains were resistant to vancomycin (100 $\mu\text{g ml}^{-1}$) and to streptomycin and rifampicin (10 $\mu\text{g ml}^{-1}$), but were inhibited by the latter compounds at concentrations of 100 $\mu\text{g ml}^{-1}$.

16S rDNA sequence analysis

16S rDNA sequence analysis showed that strain SL31^T was a close relative of *Thermosipho africanus* Ob7^T (96.4% similarity), *Thermosipho japonicus* IHB1^T

(96.3% similarity), strain RI 19.B2 (93.8% similarity) and *Thermosipho melanesiensis* BI429^T (92.3% similarity). Members of other genera of the 'Thermotogaceae' showed lower similarity values. Phylogenetic trees generated using distance algorithms and maximum-likelihood analysis gave the same topology. The bootstrap values from 500 samplings confirmed the affiliation of strain SL31^T to a clade (monophyletic taxon) that also included *Thermosipho africanus* Ob7^T, strain RI 19.B2, *Thermosipho japonicus* IHB1^T and

Fig. 3. 16S rDNA sequence-based phylogenetic distance analysis dendrogram showing the phylogenetic position of strain SL31^T (= DSM 13257^T) and related species in the genus *Thermosipho* and other members of the order 'Thermotogales'. The sequence of *Aquifex pyrophilus* served as an outside reference. Bar, 10 inferred nucleotide substitutions per 100 nucleotides. Numbers at branching points refer to bootstrap values (> 70%). Accession numbers are given for each sequence.

Thermosipho melanesiensis BI429^T (Fig. 3). Based upon partial 16S rDNA sequences (500 nt), strains SL31^T and SL30 were identical.

DNA base composition and DNA–DNA hybridization

The G + C content of the DNA of isolate SL31^T was 30.0 mol % as determined by the thermal denaturation method. No significant relatedness was obtained between bulk cellular DNA of the isolate SL31^T and that from *Thermosipho africanus* Ob7^T or *Thermosipho melanesiensis* BI429^T (mean hybridization values < 10 %). High relatedness (mean hybridization values > 80 %) was found between strain SL31^T and isolates SL30 and MLM39636.

DISCUSSION

The novel isolate SL31^T is an obligately anaerobic, heterotrophic bacterium with a typical Gram-negative cell wall ultrastructure. This organism exhibited characteristics similar to those described for representatives of the order 'Thermotogales', i.e. fermentative thermophiles that are rod-shaped and have the characteristic sheath-like outer structure referred to as a toga. Several features affiliated strain SL31^T with the genus *Thermosipho*, as represented by three species, *Thermosipho africanus* (Huber *et al.*, 1989), *Thermosipho melanesiensis* (Antoine *et al.*, 1997) and *Thermosipho japonicus* (Takai & Horikoshi, 2000): (i) possible growth of cells in chains, surrounded by a common sheath; (ii) a DNA G + C content of 30.0–31.4 mol %; and (iii) its 16S rRNA similarity to these organisms. However, several phenotypic characters differentiated strain SL31^T from the three described *Thermosipho* species. The novel isolate differed from the three species by the presence of a polar flagellum visible on single cells in the early exponential phase of growth. It differed from *Thermosipho africanus* by its lower temperature optimum, its higher pH and salinity maxima for growth and its inability to grow on sugars other than glucose and to reduce thiosulfate. SL31^T differed from *Thermosipho melanesiensis* by its lower temperature maximum and pH minimum for growth and its inability to utilize a wide range of sugars. It differed from *Thermosipho japonicus* by the white

colour of its colonies, its lower salinity optimum and its inability to grow on casein and sugars other than glucose and to reduce thiosulfate. 16S rDNA analysis indicated that SL31^T is most closely related to *Thermosipho africanus* Ob7^T. However, a low level of genetic relatedness between the two strains was determined by DNA–DNA hybridization studies, indicating that these organisms cannot be assigned to the same species (Johnson, 1984). Similarly, weak levels of DNA–DNA hybridization were measured between strain SL31^T and *Thermosipho melanesiensis*. It is considered that strain SL31^T is phenotypically, phylogenetically and genomically sufficiently different from the three previously described *Thermosipho* species to be proposed as a member of a new species of the genus *Thermosipho*.

Up to now, the described *Thermosipho* species have been isolated from coastal and deep-sea hydrothermal vents (Huber *et al.*, 1989; Antoine *et al.*, 1997; Takai & Horikoshi, 2000). A description of the first organism belonging to this genus that has been isolated from a deep petroleum reservoir is given here. Cultural and molecular studies have also revealed that other phylogenetically diverse members of the 'Thermotogales' thrive in the continental deep oil reservoir of Western Siberia sampled in this study (Slobodkin *et al.*, 1999; E. A. Bulygina, Y. P. Lysov, N. A. Chernyh, A. V. Lebedinski, E. A. Bonch-Osmolovskaya, H. Hippe, S. L'Haridon, C. Jeanthon & A. D. Mirzabekov, unpublished results).

Description of *Thermosipho geolei* sp. nov.

Thermosipho geolei (ge.o'le.i. Gr. n. *ge* earth; L. gen. neut. n. *olei* of oil; N.L. gen. neut. n. *geolei* from earth oil, referring to the site of isolation).

Cells are Gram-negative rods with a length of about 2–3 µm and a width of about 0.4–0.6 µm. No motility is observed under the microscope. A polar flagellum is inserted on unsheathed cells in the very early exponential phase of growth. A sheath-like structure is clearly visible after the mid-exponential phase of growth. Cells form enlarged spherical bodies in stationary phase. They occur singly, in pairs or in chains of a maximum of six cells. White colonies,

about 1–2 mm in diameter, are formed on ‘Phytigel’ plates. Growth occurs between 45 and 75 °C (optimum growth at 70 °C; doubling time of 115 min), between pH 6.0 and 9.4 (optimum growth at approximately pH 7.5) and at NaCl concentrations between 0.5 and 7.0% (optimum growth between 2 and 3%). Strictly anaerobic. H₂ inhibits growth but inhibition is alleviated by addition of elemental sulfur; under these conditions, hydrogen sulfide is produced. Neither thio-sulfate nor sulfate can be used as electron acceptors. Metabolism shifts to alanine production in the absence of elemental sulfur. Growth is inhibited by chloramphenicol (10 µg ml⁻¹), vancomycin and rifampicin (both at a final concentrations of 100 µg ml⁻¹); growth occurs in the presence of streptomycin (100 µg ml⁻¹). DNA G+C composition of the type strain is 30.0 mol% (thermal denaturation method). Phylogenetic analysis based on the complete 16S rRNA sequence affiliates the strain conclusively with the ‘*Thermotogales*’ and, more specifically, with the genus *Thermosipho*. No significant bulk DNA similarity to *Thermosipho africanus* or *Thermosipho melanesiensis*. The type strain is SL31^T (= DSM 13256^T = JCM 10986^T), obtained from an oil/water mixture collected from a deep continental oil reservoir in Western Siberia (Russia).

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