***Petrotoga olearia* sp. nov. and *Petrotoga sibirica* sp. nov., two thermophilic bacteria isolated from a continental petroleum reservoir in Western Siberia**

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Strictly anaerobic, thermophilic bacteria (strains SL24^T, SL25^T, SL27, SL29 and SL32) were isolated from a deep, continental oil reservoir in Western Siberia (Russia). These motile, rod-shaped organisms were surrounded by a sheath-like structure, a feature characteristic of the *Thermotogales*. On the basis of partial 16S rDNA sequences (500 nucleotides), strains SL25^T, SL27, SL29 and SL32 were identical. Therefore, only strains SL24^T and SL25^T were studied in detail. The optimum temperature for growth of both strains was 55 °C. Their optimum pH for growth was 7.5 and their optimum NaCl concentration was between 20 and 30 g l⁻¹. The novel isolates reduced elemental sulfur and cystine, but not thiosulfate or sulfate, to hydrogen sulfide. The G+C contents of the genomic DNA of strains SL24^T and SL25^T were respectively 35 and 33 mol%. Phylogenetically, both strains are most closely related to *Petrotoga miotherma*, there being 98.9–99.4% similarity between their 16S rDNA sequences. Phenotypic properties and DNA–DNA hybridization experiments indicate that the strains belong to two novel species, for which the names *Petrotoga olearia* (type strain SL24^T = DSM 13574^T = JCM 11234^T) and *Petrotoga sibirica* (type strain SL25^T = DSM 13575^T = JCM 11235^T) are proposed.

Keywords: oilfield, thermophiles, *Thermotogales*, *Petrotoga sibirica*, *Petrotoga olearia*

INTRODUCTION

Members of the family *Thermotogaceae* (order *Thermotogales*) belong to two physiological groups: extreme thermophiles that grow at temperatures above 70 °C and moderate thermophiles that grow at lower temperatures. The extreme thermophiles encompass the genera *Fervidobacterium* (Patel *et al.*, 1985), *Thermotoga* (Huber *et al.*, 1986) and *Thermosipho* (Huber *et al.*, 1989; Ravot *et al.*, 1996a). Except in the case of *Fervidobacterium*, which is restricted to continental springs (Patel *et al.*, 1985; Andrews & Patel, 1996;

Koch *et al.*, 1997), members of these genera have been isolated and detected in diverse high-temperature environments (Huber *et al.*, 1986, 1989, 1990; Jannasch *et al.*, 1988; Windberger *et al.*, 1989; Stetter *et al.*, 1993; Jeanthon *et al.*, 1995; Ravot *et al.*, 1995; Andrews & Patel, 1996; Grassia *et al.*, 1996; Antoine *et al.*, 1997; Fardeau *et al.*, 1997; Takahata *et al.*, 2000; Takai & Horikoshi, 2000). Moderately thermophilic members of the *Thermotogales* consist of the genera *Geotoga* and *Petrotoga* (Davey *et al.*, 1993) and *Marinitoga* (Wery *et al.*, 2001). The recently discovered genus *Marinitoga* is, as far as is known, limited to deep-sea hydrothermal vents. Up to now, species of the genera *Geotoga* and *Petrotoga* have been retrieved only from deep oil reservoirs. The genus *Petrotoga* contains two described species, *Petrotoga miotherma* (Davey *et al.*, 1993) and *Petrotoga mobilis* (Lien *et al.*, 1998), isolated from petroleum reservoirs from Oklahoma and Texas and from the North Sea, respectively.

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The GenBank accession numbers for the 16S rRNA gene sequences of *Petrotoga olearia* SL24^T and *Petrotoga sibirica* SL25^T are AJ311702 and AJ311703.

Additional strains closely related to both species were isolated from sulfur-rich reservoirs in California (Orphan *et al.*, 2000).

Recent microbiological studies performed on oil/water mixtures from different high-temperature strata of the Samotlor oilfields (Western Siberia) have identified phylogenetically diverse thermophilic organisms (Slobodkin *et al.*, 1999; Jeanthon *et al.*, 2000). Among them, novel species of the genera *Thermococcus*, *Thermosipho* and *Geobacillus* have been isolated and characterized (L'Haridon *et al.*, 2001; Miroshnichenko *et al.*, 2001; Nazina *et al.*, 2001). In this paper, we describe the isolation of two novel species of the genus *Petrotoga* from oilfield waters. Although they are phylogenetically closely related to *P. miotherma*, the two strains (SL24^T and SL25^T) exhibit phenotypic and genetic characteristics consistent with their placement in two novel species of the genus *Petrotoga*.

METHODS

Source of organisms. The novel strains were isolated from oil/water mixtures taken directly from production well-heads of a deep, continental petroleum reservoir in Western Siberia (Russia). *P. miotherma* strain 42-6^T (= ATCC 51224^T) was obtained from the ATCC (Manassas, VA, USA).

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 the following (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, 1.5 g peptone, 1.5 g tryptone, 1.5 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. It was inoculated with 1 ml of the oil/water sample, pressurized with N₂ (100 %; 100 kPa) and incubated without shaking at 58 °C. The medium used for growth, isolation and routine cultivation of the novel strains contained the following (l⁻¹ distilled water): 25 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, 1.5 g peptone, 1.5 g tryptone, 1.5 g yeast extract and 1 mg resazurin. The pH was adjusted to 7 using 5 M HCl before autoclaving. Prior to inoculation, maltose and Na₂S·9H₂O were injected from sterile, anaerobic stock solutions to obtain respective final concentrations of 0.2 % (w/v) and 0.1 %. Single colonies were obtained and purified by streaking on the same medium that was solidified with 1 % (w/v) Phytagel (a gellan gum from Sigma). Plates were incubated in anaerobic jars at 55 °C under N₂/CO₂ (80:20, 100 kPa). Stock cultures of isolates SL24^T and SL25^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 in the OD₅₄₀ with a Spectronic 401 spectrophotometer (Bioblock). All growth experiments were performed in triplicate. The pH range for growth was determined in culture medium with various buffers, each 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, 8.5 and 9). Appropriate amounts of 1 M Na₂CO₃ were added to adjust the pH of the medium to 9.0–10. To determine the optimum NaCl range for growth, the NaCl concentrations were varied while concentrations of the other inorganic components were maintained. The effects of different pH values and concentrations of NaCl were determined at 55 °C.

Substrate-utilization tests. Ability to grow lithoautotrophically was tested in routine medium from which yeast extract, peptone, tryptone and maltose had been omitted, and with H₂/CO₂ (80:20; 100 kPa) as the gas phase. Possible growth substrates were tested in the same medium in the presence of sulfur: substrates were added at a concentration of 500 mg l⁻¹ and with N₂ (100 %; 100 kPa) as the gas phase. Three further 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 200 mg l⁻¹.

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 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 the culture medium at 55 °C under aerated and microaerophilic conditions (L'Haridon *et al.*, 1998).

Antibiotic susceptibility. The sensitivity of strains SL24^T and SL25^T to ampicillin, chloramphenicol, streptomycin, vancomycin and rifampicin (all from Sigma) at 10, 25, 50 and 100 µg ml⁻¹ was tested at 55 °C.

Light and electron microscopy. An Olympus BX-60 microscope equipped with an Olympus OM-2 camera was used routinely for observation 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 % (w/v) phosphotungstate. Thin sections were prepared as described previously (Bonch-Osmolovskaya *et al.*, 1990). Electron micrographs were taken using a model JEM-100 electron microscope (JOEL).

H₂S production. H₂S production was evaluated by adding 500 µl of a solution of CuSO₄ (5 mM) and HCl (50 mM) to 250 µl culture grown at 55 °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. The genomic DNA of strains 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 *Escherichia coli* DNA

(52 mol% G + C), *Clostridium perfringens* DNA (24 mol% G + C) and *Micrococcus luteus* DNA (73 mol% G + C) (all from Sigma) as standards.

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) according to the procedure described by Kristjánsson *et al.* (1994). Increasing amounts of target DNA (50–100 ng) denatured in 0.4 M NaOH were blotted 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 for the hybridization buffer (Johnson, 1989; 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 was compared with the signal generated by heterologous DNA.

RESULTS AND DISCUSSION

Enrichments and isolation

Stratal fluids from different horizons of the Samotlor oilfield (1700–2500 m depth), having temperatures of 45 to 84 °C, were taken directly from production well-heads through a tapping fitted on the production line. Stratal waters had total mineral contents ranging from 5.7 to 32.9 g l⁻¹ and pH values ranging from 5.5 to 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 varied from 4 to 40 °C depending on the season. The salinity of the injection water varied from 9 to 17 g l⁻¹. Enrichments of thermophiles were performed by inoculating the collected production fluids into anaerobic enrichment medium and incubating at 58 °C. Within 4 days, turbidity due to cell growth was observed. This growth consisted of small, rod-shaped bacteria with a characteristic outer sheath-like structure, a feature typical of the *Thermotogales*. The sheathed bacteria were purified by streaking subcultures onto solidified medium and

Fig. 1. Thin section of cells of strain SL24^T, showing the typical sheath-like structure (arrow). Bar, 500 nm.

incubating them in an anaerobic jar at 55 °C. After 4 days, whitish, round colonies (1–2 mm in diameter) were obtained. Five single colonies were picked and the isolation procedure was repeated at least three times before each of the isolates, designated SL24, SL25, SL27, SL29 and SL32, was considered pure. Isolates SL24^T and SL25^T were studied in detail.

Phenotypic characteristics of strains SL24^T and SL25^T

Cells of strains SL24^T and SL25^T were rod-shaped, about 0.9–2.5 µm long and about 0.3–0.6 µm wide. Each negatively stained unsheathed cell examined by transmission electron microscopy possessed a single polar flagellum (data not shown). During the growth phase, one or two cells per sheath were observed and the sheaths became longer. In the stationary growth phase, the cells became large spheres surrounded by the sheath. Thin sections of cells of strains SL24^T (Fig. 1) and SL25^T (data not shown) revealed the Gram-negative structure of the cell wall with outer membrane.

The two organisms had different temperature, pH and salinity ranges (Fig. 2). Details are given in the species descriptions below and in Table 1. Under optimal growth conditions (optimal temperature, pH and

Fig. 2. Effects of temperature, pH and concentration of NaCl on growth of strains SL24^T (●) and SL25^T (◆). Final OD₅₄₀ measured after 24–36 h incubation for strain SL24^T and after 36–48 h for strain SL25^T are plotted as a function of temperature (in the presence of 25 g NaCl l⁻¹ at pH 8) (a), pH (at 55 °C in the presence of 25 g NaCl l⁻¹) (b) and NaCl concentration (at 55 °C and pH 8) (c).

NaCl), the doubling time of strain SL24^T was approximately 115 min.

Both strains were strictly anaerobic, organotrophic organisms. Their growth was prevented by the presence of low levels of oxygen (0.2–1 %, v/v) and under the autotrophic culture conditions tested. In the absence of yeast extract, strain SL24^T was able to grow with pyruvate, peptone and a papaic digest of soybean meal. Its growth on pyruvate and peptone was stimulated by the addition of 0.02 % (w/v) yeast extract. In

the presence of yeast extract, strain SL24^T was able to grow with a wide range of substrates, such as arabinose, xylose, cellobiose, dextrin, sucrose, glucose, fructose, maltose, ribose, trehalose, xylan, pyruvate, peptone and papaic digests of soybean meal and starch. Strain SL25^T was unable to grow in the absence of yeast extract. In the presence of yeast extract, growth was observed with sucrose, glucose, fructose, maltose, ribose, trehalose, xylan, pyruvate, peptone and a papaic digest of soybean meal and galactose. Casein, cellulose, formate, acetate, ethanol, methanol, mannitol, rhamnose, Casamino acids, casein, beef extract and an amino acid mixture did not support growth of either strain, even when combined with yeast extract (200 mg l⁻¹).

Molecular hydrogen, acetate, lactate and alanine were the main metabolic products detected after glucose fermentation, irrespective of the presence of sulfur. In the absence of sulfur, small amounts of ethanol (0.17 mM) were produced. In all cases, about 1 mol CO₂ was produced per mol acetate produced.

Both strains were completely inhibited 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. The addition of sulfur resulted in the production of H₂S and a decrease in the cell yield. Sulfate, cystine and thiosulfate were unable to serve as alternative electron acceptors. The presence of sulfur as 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 by both strains. As an example, when S⁰ was absent, the alanine/acetate and alanine/glucose ratios were respectively about 0.6 and 0.5 after growth of strain SL24^T. In the presence of S⁰, the two ratios decreased to respective values of 0.05 and 0.02 (data not shown).

Strain SL25^T was sensitive to chloramphenicol (10 µg ml⁻¹), ampicillin (10 µg ml⁻¹), vancomycin (50 µg ml⁻¹), streptomycin (100 µg ml⁻¹) and rifampicin (100 µg ml⁻¹). Strain SL24^T was resistant to the antibiotics tested at 100 µg ml⁻¹.

16S rDNA sequence analysis

16S rDNA sequence analysis based on 899 nucleotides placed strains SL24^T and SL25^T as close relatives of *P. miotherma* 42-6^T (99.4 and 98.9 % similarity) and *P. mobilis* DSM 10674^T (98.5 and 97.9 % similarity). Lower similarities were obtained with isolates vp56 and vp424 isolated from oil reservoirs in California (Orphan *et al.*, 2000) (respectively 97.9 and 97.3 % similarity between vp56 and strains SL24^T and SL25^T and 97.2 and 96.8 % between vp424 and SL24^T and SL25^T). Members of other genera of the *Thermotogaceae* showed lower similarity. Phylogenetic trees generated using distance algorithms and maximum-likelihood analysis gave the same topology. Bootstrap values from 500 samplings confirmed the affiliation of

Fig. 3. 16S rDNA sequence-based phylogenetic distance-analysis dendrogram showing the phylogenetic positions of strains SL24^T and SL25^T and related species in the genus *Petrotoga* and other members of the order *Thermotogales*. The sequence of *Aquifex pyrophilus* served as an outgroup. A mask was set to include only those positions that were occupied in each of the 14 sequences. Bar, 10 inferred nucleotide substitutions per 100 nucleotides. Numbers at branching points refer to bootstrap values (> 70 %).

both strains to a clade (monophyletic taxon) that also included *P. miotherma* 42-6^T and *P. mobilis* SJ95^T (Fig. 3). On the basis of partial 16S rDNA sequences (500 nucleotides), strains SL25^T, SL27, SL29 and SL32 were identical.

DNA base composition and DNA–DNA hybridization

The G + C contents of the DNA of isolates SL24^T and SL25^T were respectively 35.0 and 33.0 mol %, as determined by the thermal denaturation method. Low relatedness was obtained between bulk cellular DNAs of the two isolates and that from *P. miotherma* 42-6^T. Strain SL24^T exhibited hybridization values ≤ 11 % with *P. miotherma* and 32 % with strain SL25^T. The hybridization value between strain SL25^T and *P. miotherma* was ≤ 20 %.

Taxonomic position

When a number of different taxonomic parameters are compared, several phenotypic differences are apparent that can be used to differentiate strains SL24^T and SL25^T from the two *Petrotoga* species described. The novel isolates differ from them in their cell size, flagellation, maximum temperature and optimum pH for growth and inability to reduce thiosulfate (Table 1). In contrast to *P. mobilis*, strains SL24^T and SL25^T grow optimally at moderate NaCl concentrations and are resistant to low concentrations of antibiotics. They differ from their closest phylogenetic relative, *P. miotherma*, by their motility, their inability to form spherical bodies and their ability to grow on xylan. Moreover, the levels of genetic relationship between the two strains and *P. miotherma*, determined by DNA–DNA hybridization, indicate that these organisms cannot be assigned to this species (Johnson, 1989). Similarly, low levels of DNA–DNA hybridization were measured between strains SL24^T and SL25^T. Although the two strains share similar phenotypic

Table 1. Differentiating characteristics between strains SL24^T and SL25^T, *P. miotherma* and *P. mobilis*

Data for reference species were taken from Davey *et al.* (1993) (*P. miotherma*) and Lien *et al.* (1998) (*P. miotherma* and *P. mobilis*). ND, Not determined.

Characteristic	<i>P. miotherma</i>	<i>P. mobilis</i>	SL24 ^T	SL25 ^T
Cell size (µm)	0.6 × 2.0–7.5	0.5–1.5 × 1–50.0	0.3–0.6 × 0.9–2.5	0.3–0.6 × 0.9–2.5
No. of cells per sheath	1–5	1–24	1–2	1–2
Motility	–	+	+	+
Flagellation/no. flagella	–	Subpolar/some	Polar/1	Polar/1
Formation of spherical bodies	+	–	+	+
Temperature range (°C) [opt.]	35–65 [55]	40–65 [58–60]	37–60 [55]	37–55 [55]
pH range [opt.]	5.5–9.0 [6.5]	5.5–8.5 [6.5–7.0]	6.5–8.5 [7.5]	6.5–9.4 [8.0]
NaCl range (%) [opt.]	0.5–10.0 [2.0]	0.5–9.0 [3–4]	0.5–8.0 [2.0]	0.5–7.0 [1.0]
G + C content (mol %)	32,* 34.2, 40	31,* 34	35*	33*
Reduction of thiosulfate	+	+	–	–
Substrates utilized:				
Xylan	–	+	+	+
Galactose	+	+	–	+
Xylose	+	+	+	–
Arabinose	+	+	+	–
Cellobiose	ND	+	+	–

* Values obtained by the thermal denaturation method. Other values were obtained by the HPLC method.

characteristics, they differ in their temperature, pH and NaCl ranges and optima for growth and in the spectrum of substrates that they are able to oxidize. On the basis of these distinctive phenotypic, phylogenetic and genomic features, strains SL24^T and SL25^T should be considered as novel species of the genus *Petrotoga*, for which we propose the names *Petrotoga olearia* sp. nov. and *Petrotoga sibirica* sp. nov., respectively.

Description of *Petrotoga olearia* sp. nov.

Petrotoga olearia (o.le.a'ri.a. L. fem. adj. *olearia* pertaining to oil, referring to its site of isolation).

Cells are Gram-negative rods, about 0.9–2.5 µm long and about 0.3–0.6 µm wide. They are motile by means of a polar flagellum. A sheath-like structure is clearly visible. Cells occur singly, in pairs or in chains containing a maximum of six cells. In the stationary growth phase, the cells became large spheres surrounded by the sheath. When cultivated on Gelrite plates containing peptone, tryptone, yeast extract and maltose, white colonies are formed, about 1–2 mm in diameter. Growth occurs at temperatures between 37 and 60 °C, with an optimum at 55 °C. Growth occurs at pH values between 6.5 and 8.5, with an optimum at approximately 7.5, and at NaCl concentrations between 0.5 and 8%, with an optimum at 2%. Strictly anaerobic. H₂ inhibits growth, but this is alleviated by the addition of elemental sulfur. Under these conditions, hydrogen sulfide is produced. Thiosulfate, cystine and sulfate cannot be used as electron acceptors. Metabolism shifts to alanine production in the absence of elemental sulfur. Pyruvate, peptone and papaic digest of soybean meal support growth in the absence of yeast extract. In the presence of yeast extract, a variety of organic compounds are used, including xylose, arabinose and cellobiose. Growth is observed in the presence of ampicillin, chloramphenicol, streptomycin, vancomycin and rifampicin at final concentrations of 100 µg ml⁻¹. The DNA base composition of the type strain is 35 mol % G + C (as determined by the thermal denaturation method). Phylogenetic analysis based on the almost complete 16 rRNA sequence conclusively affiliates the strain with the *Thermotogales* and, more specifically, with the genus *Petrotoga*. Low DNA similarity to *P. miotherma* and *P. sibirica*.

The type strain is strain SL24^T (= DSM 13574^T = JCM 11234^T), which was obtained from an oil/water mixture collected from a deep, continental oil reservoir in Western Siberia (Russia).

Description of *Petrotoga sibirica* sp. nov.

Petrotoga sibirica (si.bi'ri.ca. N.L. fem. adj. *sibirica* originating from Siberia, referring to its site of isolation).

Cells are Gram-negative rods, about 0.9–2.5 µm long and about 0.3–0.6 µm wide. They are motile by means of a polar flagellum. A sheath-like structure is clearly

visible. Cells occur singly, in pairs or in chains containing a maximum of six cells. In the stationary growth phase, the cells became large spheres surrounded by the sheath. When cultivated on Gelrite plates containing peptone, tryptone, yeast extract and maltose, white colonies are formed, about 1–2 mm in diameter. Growth occurs at temperatures between 37 and 55 °C, with an optimum at 55 °C. Growth occurs at pH values between 6.5 and 9.4, with an optimum of approximately 8, and at NaCl concentrations between 0.5 and 7%, with an optimum at 1%. Strictly anaerobic. H₂ inhibits growth, but this is alleviated by the addition of elemental sulfur. Under these conditions, hydrogen sulfide is produced. Thiosulfate, cystine and sulfate cannot be used as electron acceptors. Metabolism shifts to alanine production in the absence of elemental sulfur. Yeast extract is required for growth. In the presence of yeast extract, a variety of compounds are used, but some, such as xylose, arabinose and cellobiose, are not. Growth is inhibited in the presence of ampicillin and chloramphenicol (10 µg ml⁻¹), vancomycin (50 µg ml⁻¹), streptomycin and rifampicin (each 100 µg ml⁻¹). The DNA base composition of the type strain is 33 mol % G + C (as determined by the thermal denaturation method). Phylogenetic analysis based on the almost complete 16 rRNA sequence conclusively affiliates the strain with the *Thermotogales* and, more specifically, with the genus *Petrotoga*. Low DNA similarity is found to *P. miotherma* and *P. olearia*.

The type strain is strain SL25^T (= DSM 13575^T = JCM 11235^T), which was obtained from an oil/water mixture collected from a deep, continental oil reservoir in Western Siberia (Russia).

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