Sphaerochaeta associata sp. nov., a spherical spirochaete isolated from cultures of Methanosarcina mazei JL01

Olga Troshina,1 Viktoria Oshurkova,1 Natalia Suzina,1 Andrei Machulin,1 Elena Ariskina,1 Natalia Vinokurova,1 Dmitry Kopitsyn,2 Andrei Novikov2 and Viktoria Shcherbakova1

Correspondence
Olga Troshina
olga.troshina@ibpm.pushchino.ru
1Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Prospect Nauki 5, Pushchino, Moscow region 142290, Russia
2Gubkin Russian State University of Oil and Gas, Leninskiy Prospect 65-1, 119991 Moscow, Russia

An anaerobic, saccharolytic bacterial strain designated GLS2T was isolated from aggregates of the psychrotolerant archaeon Methanosarcina mazei strain JL01 isolated from arctic permafrost. Bacterial cells were non-motile, spherical, ovoid and annular with diameter 0.2–4 μm. They were chemoorganoheterotrophs using a wide range of mono-, di- and trisaccharides as carbon and energy sources. The novel isolate required yeast extract and vitamins for growth. The bacteria exhibited resistance to a number of β-lactam antibiotics, rifampicin, streptomycin and vancomycin. Optimum growth was observed between 30 and 34 °C, at pH 6.8–7.5 and with 1–2 g NaCl l⁻¹. Isolate GLS2T was a strict anaerobe but it tolerated oxygen exposure. On the basis of 16S rRNA gene sequence similarity, strain GLS2T was shown to belong to the genus Sphaerochaeta within the family Spirochaetaceae. Its closest relatives were Sphaerochaeta globosa BuddyT (99.3 % 16S rRNA gene sequence similarity) and Sphaerochaeta pleomorpha GrapesT (95.4 % similarity). The G+C content of DNA was 47.2 mol%. The level of DNA–DNA hybridization between strains GLS2T and BuddyT was 34.7 ± 8.8 %. Major polar lipids were phosphoglycolipids, phospholipids and glycolipids; major fatty acids were C₁₄ : 0, C₁₆ : 0, C₁₆ : 0 3-OH, C₁₆ : 0 dimethyl acetal (DMA), C₁₆ : 1n9 and C₁₆ : 1 DMA; respiratory quinones were not detected. The results of DNA–DNA hybridization, physiological and biochemical tests demonstrated genotypic and phenotypic differentiation of strain GLS2T from the four species of the genus Sphaerochaeta with validly published names that allowed its separation into a new lineage at the species level. Strain GLS2T therefore represents a novel species, for which the name Sphaerochaeta associata sp. nov. is proposed, with the type strain GLS2T (=DSM 26261T=VKM B-2742T).

One of the first unusual spirochaetes, which had a coccoid morphology and was not able to demonstrate the characteristic twisting motion, was isolated from the termite hindgut and named Spirochaeta coccoides SPN1T (Dröge et al., 2006). Later, the new genus Sphaerochaeta within the family Spirochaetaceae was proposed by Ritalahti et al. (2012) to accommodate two newly isolated coccoid spirochaetes, and at present the genus Sphaerochaeta comprises four species with validly published names, Spirochaeta coccoides reclassified as Sphaerochaeta coccoides (Dröge et al., 2006; Abt et al., 2012), Sphaerochaeta globosa (Ritalahti et al., 2012), Sphaerochaeta pleomorpha (Ritalahti et al., 2012) and Sphaerochaeta multiformis (Miyazaki et al., 2014). Furthermore, some other isolates related to the genus Sphaerochaeta are known, for example strain ACE-P, isolated from hypolimnion of Antarctic Ace Lake (GenBank 16S rRNA gene accession no. M87055; Franzmann & Dobson, 1992), bacterial strains obtained from dechlorinating enrichment cultures (GenBank accession nos DQ833395–DQ833403) and strain MET-E isolated from an oilfield (AY800103) (Fig. 1). BLAST search revealed that 16S rRNA gene sequences highly homologous to Sphaerochaeta 16S rRNA genes were present ubiquitously both in nature and in anthropogenic environments like, for example, a lignocellulolytic microbial community.

Abbreviation: DMA, dimethyl acetal.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GLS2T is JN944166

Two supplementary figures and two supplementary tables are available with the online Supplementary Material.
(JF834125; Yan et al., 2012), a crude oil reservoir (JQ088436; Tang et al., 2012), leachate sediment (HQ183962; Liu et al., 2011), a methanogenic reactor (JX462552; Kobayashi et al., 2013), dechlorinating microbial consortia (AB721402; Li et al., 2013), a bio-reactor for treatment of wastewater sludge (CU923017; Rivie`re et al., 2009), freshwater sediments (Ritalahti et al., 2012), a hypersaline Antarctic lake (GQ167322; Murray et al., 2012), a canine oral cavity (JN713550; Dewhirst et al., 2012) and many other anoxic habitats.

Using molecular techniques, we detected the presence of contaminating bacteria in a culture of the methanogenic archaeon *Methanosarcina mazei* JL01 isolated from arctic permafrost (Rivkina et al., 2007). The concomitant bacteria were resistant to ampicillin and streptomycin used for isolation of archaea. These symbiotic bacteria coexisted well on the medium under conditions optimal for the archaeon. Establishment of a 16S rRNA gene clone library and sequencing revealed that these tightly associating bacteria belonged to genus *Sphaerochaeta*, a genus within the family *Spirochaetaceae*. The genus combines a group of non-spiral, pleomorphic, non-motile spirochaetes (Ritalahti et al., 2012). Here we describe morphology, physiology, chemotaxonomy and phylogeny of the newly isolated bacterium designated strain GLS2T. We suggest that strain GLS2T is a member of a novel species of the genus *Sphaerochaeta*.

Enrichment culture of the bacterial contaminant from *M. mazei* strain JL01 was obtained on the basal medium (Rivkina et al., 2007) modified by adding 1 g glucose l⁻¹ and 1 g peptone l⁻¹. Cultivation was performed under N₂ headspace at 30 °C. DNA from the enrichment culture was extracted by the method of Marmur (1961). The 16S rRNA gene fragments were amplified with universal bacterial primers (Lane, 1991) 27f (5'-AGAGTTTGATCMTGGCTCAG) and 1492r (5'-TACGGYTACCTTGTTACGACTT) and genomic DNA of the enrichment culture. PCR mix (25 µl) was composed of template DNA (up to 10 ng), 2.5 mM MgCl₂, 0.25 mM of each dNTP, 1.0 U Taq DNA polymerase, 0.1 µM of each primer in 1 × PCR buffer. PCR reagents and Taq DNA polymerase were purchased from Fermentas. The PCR conditions were 94 °C for 5 min; 30 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 2 min; and final elongation at 72 °C for 10 min. The PCR products were purified after agarose gel electrophoresis using a PCR gel purification kit with SiO₂-coated magnetic particles (Sileks). The purified fragments were ligated into
the pAL-TA plasmid (Evrogen JSC) and subsequently transformed into competent cells of *Escherichia coli* DH10B according to standard guidelines (Sambrook *et al.*, 1989). Sequencing was carried out by the dideoxy method using an ABI PRISM BigDye Terminator v.3.1 sequencing reaction kit on an Applied Biosystems 3730 DNA analyser. The sequence data obtained were submitted to GenBank to search for similar sequences using the BLAST algorithm (Altschul *et al.*, 1990, 1997). Phylogenetic trees were reconstructed using the neighbour-joining, maximum-likelihood and minimum-evolution methods implemented in MEGA6 (Tamura *et al.*, 2013). The evolutionary distances were computed using the Tamura–Nei model. The rate variation among sites was modelled with a gamma distribution (shape parameter = 5). The phylogenetic trees were evaluated by bootstrap analysis based on 1000 replications. The analysis involved 22 nt sequences. There were a total of 1372 positions in the final dataset.

Amplification of 16S rRNA gene fragments followed by cloning and sequencing allowed identification of a contaminating bacterium in the culture of *M. mazei* JL01 as a representative of the coccoid spirochaetes of the genus *Sphaerochaeta*. On the basis of information obtained from molecular experiments, a modified basal salt medium (SM) (Leadbetter & Breznak, 1996) and antibiotics (Ritalahti *et al.*, 2012) were chosen for isolation of bacterial satellites by serial dilutions in roll-tubes (Hungate, 1969). The strain isolated was named GLS2<sup>T</sup>. A nearly full-length 16S rRNA gene sequence (1504 bp) of GLS2<sup>T</sup> was deposited in GenBank (JN944166). The isolate shared 85.6–99.3 % 16S rRNA gene sequence similarity with type strains of species of the genus *Sphaerochaeta*; *Sphaerochaeta globosa* Buddy<sup>T</sup>, with the highest degree of sequence similarity (99.3 %), and *Sphaerochaeta multiformis* MO-SPC2<sup>T</sup> (85.6 %). The phylogenetic tree (Fig. 1) demonstrates the position of strain GLS2<sup>T</sup> among other isolates of the genus *Sphaerochaeta*.

The SM medium for cultivation of GLS2<sup>T</sup> contained (per 1000 ml) NaCl, 1.0 g; KCl, 0.5 g; MgCl<sub>2</sub> · 6H<sub>2</sub>O, 0.4 g; CaCl<sub>2</sub> · 2H<sub>2</sub>O, 0.1 g; NH<sub>4</sub>Cl, 0.3 g; KH<sub>2</sub>PO<sub>4</sub>, 0.2 g; Na<sub>2</sub>SO<sub>4</sub>, 0.15 g; xylose, 20 mmol; yeast extract, 1.0–3.0 g; NaHCO<sub>3</sub>, 0.5 g; trace element solution SL7 (Widdel & Pfennig, 1981), 1 ml; seven vitamin solution (DSMZ medium 503), 1 ml. The medium was prepared according to the Hungate anaerobic technique (Hungate, 1969). Cysteine hydrochloride at a final concentration of 0.2 mM was added. Nitrogen-purged reduced medium was then dispensed anaerobically into 15 ml Hungate tubes or into 125 ml serum bottles. Sodium hydrogen carbonate, vitamins, xylose and antibiotics (when necessary) were added to the autoclaved medium from sterile anaerobic stock solutions. The medium was adjusted to pH 7.2–7.3 with 10 % (w/v) NaHCO<sub>3</sub> before inoculation. Cultivation was carried out at 30 °C under an atmosphere of N<sub>2</sub> without shaking. For comparative studies of strain GLS2<sup>T</sup> and reference strain *Sphaerochaeta globosa* Buddy<sup>T</sup>, both strains were cultivated on SM medium as described above.

Cell morphology was observed using a Nikon Eclipse Ci microscope with Jenoptik ProRes SpeedXT<sup>core</sup>5 camera. Gram-stain reaction was performed using the Hucker staining method (Murray *et al.*, 1994) and KOH technique (Buck, 1982). For ultrathin sectioning, cells were harvested by centrifugation and then fixed in a solution of 1.5 % (w/v) glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2) at 4 °C for 1 h. The cells were washed three times in the same buffer and post-fixed in 1 % OsO<sub>4</sub> in 0.05 M cacodylate buffer (pH 7.2) for 3 h at 20 °C. The preparation was dehydrated in a series of ethanol and embedded in Epon 812 epoxy resin. Ultrathin sections were mounted on grids and post-stained in 3 % (w/v) uranyl acetate in 70 % (v/v) ethanol for 30 min and afterwards they were additionally stained in lead citrate according to the method of Reynolds (1963). Thin sections were examined in a JEOL JEM-100B transmission electron microscope at an acceleration voltage of 80 kV.

Isolate GLS2<sup>T</sup> formed round, loose, pale colonies with diameter about 2–3 mm. Under the phase-contrast microscope, cells of GLS2<sup>T</sup> were non-motile and had spherical or ovoid shape with sizes of 0.2 to 1–2 μm (Fig. 2a). Many cells were associated in bubbles of various dimensions, a feature described by Ritalahti *et al.* (2012) for strain Buddy<sup>T</sup>. Such bubble-like structures filled with one to dozens of cells were particularly apparent in exponentially growing GLS2<sup>T</sup> culture. Solitary enlarged bright spherical bodies (about 4 μm) were also observed. In the stationary growth phase, the cells occurred mostly as single separate spheres. Transmission electron microscopy (TEM) images showed a Gram-negative cell wall structure (Fig. 2b–d) thereby supporting Gram staining tests (Buck, 1982; Murray *et al.*, 1994). In addition, TEM revealed another type of cell morphology, namely, annular cells (Fig. 2c). Such forms were also observed for *Sphaerochaeta multiformis* MO-SPC2<sup>T</sup> (Miyazaki *et al.*, 2014). Like its closest relatives *Sphaerochaeta globosa* Buddy<sup>T</sup> and *Sphaerochaeta pleomorpha* Grapes<sup>T</sup>, strain GLS2<sup>T</sup> was able to grow in the presence of ampicillin. Genome sequencing of Buddy<sup>T</sup> and Grapes<sup>T</sup> showed the absence of several genes coding for penicillin-binding proteins involved in transglycosylation and transpeptidation processes during the final steps of peptidoglycan synthesis (Caro-Quintero *et al.*, 2012). Apparently, Buddy<sup>T</sup> and Grapes<sup>T</sup> as well as strain GLS2<sup>T</sup> synthesize a non-rigid defective cell wall resulting in spheric and sometimes pleomorphic morphology. These strains might be considered therefore as cell-wall deficient organisms of the spheroplast type. Electron microscopy of GLS2<sup>T</sup> cells grown in SM medium with ampicillin (175 μg ml<sup>−1</sup>) showed cells without evident cell walls (Fig. S1 available in the online Supplementary Material). Some of these cells had very small sizes (about 100 nm). They resembled, in relation to morphology and sizes described previously, cell-wall-less mycoplasma in aggregates of *Methanosarcina* sp. (Zhilina & Zavarzin, 1979). Mycoplasma in methanosarcina aggregates were identified by electron microscopy. They were seen on
ultrathin sections as spheroid bodies of various sizes between 0.15 and 1 μm and without cell walls. Mycoplasma were located in places of depolymerization of the methanochondroitin matrix of Methanosarcina sp. As can be seen from Fig. S1a, these extremely small cells were possibly generated by a mechanism of membrane extrusion or blebbing. Such a mechanism of cell propagation was suggested for L-forms of the Gram-positive bacterium Bacillus subtilis (Leaver et al., 2009). It is suggested as a mode of cell division of early cells before cell-wall evolution. GLS2^T cells grown in the presence of ampicillin were able to pass through a 0.22 μm-pore-sized sterile membrane and to induce growth. Phase-contrast microscopy of cultures grown after filtration indicated typical GLS2^T cell morphology (data not presented).

Temperature, pH and NaCl ranges for growth were determined by monitoring the OD_600. The effect of temperature was checked within a temperature range from 4 to 55 °C. The pH range was tested from pH 5.5 to 8.5 and, when necessary, MES, PIPES, HEPES and MOPS buffers at a final concentration of 30 mM were used. NaCl ranged from 0 to 20 g l^{-1} in experiments to determine salt requirements. Substrate utilization studies were performed in basal SM medium without xylose and containing one of the following substrates: sugars (10 mM), organic acids at a final concentration of 1 and 2 g l^{-1}, galactosamine (0.5 and 1 g l^{-1}), glucuronic acid (0.5 and 1 g l^{-1}), Casamino acids (2 g l^{-1}), ethanol (17 mM), methanol (120 mM), trimethylamine (17 mM), xylan (1 g l^{-1}), cellulose (1 g l^{-1}) and H_2 + CO_2 (80 : 20, v/v). Ability to use various electron acceptors was tested in SM medium containing yeast extract (2 g l^{-1}) and xylose (10 mM). The following acceptors were tested: nitrate (20 mM), sulfate (20 mM), thiosulfate (15 mM), sulfite (2 mM), ferric citrate (3 mM) and ferric EDTA (3 mM). Reduction of Fe(III) was determined as described by Lovley & Phillips (1986), sulfide was measured by the Pachmayr method (Cline, 1969) and

![Fig. 2. Phase-contrast micrograph of cells of strain GLS2^T (a). Transmission electron micrographs of negatively stained thin sections of GLS2^T: image of coccoid cell (b), annular cell (c) and magnified cell-wall area (d). CM, Cytoplasmic membrane; OM, outer membrane. Bars, 10 μm (a), 1 μm (b), 0.5 μm (c), 0.1 μm (d).](image-url)
nitrate was determined using Griess reagent. All experiments were carried out in triplicate and with two consequent transfers.

GLS2T was an anaerobic chemoorganoheterotroph with fermentative metabolism, and used mono-, di- and trisaccharides as sources of carbon and energy for growth. It grew well on arabinose, xylose, maltose, galactose, raffinose and cellobiose, but weaker growth was observed on glucose, lactose, sucrose and starch and it did not grow on fructose, cellulose, xylan or yeast extract as the only carbon source (Table 1). Strain GLS2T grew on lactate and gluturonic acid but it could not grow on a number of other organic acids like malate, citrate, pyruvate, benzoate, fumarate, propionate, butyrate or acetate as well as on ethanol, methanol or trimethylamine. Strain GLS2T did not grow on H2 + CO2. The isolate could use for growth the components of methanochondroitin of methanosarcina, namely glucuronic acid and glucose but not galactosamine. Casamino acids did not support growth of GLS2T bacteria. Yeast extract was required for growth on all substrates and it could not be replaced by vitamin mixture. Similar to strain BuddyT, isolate GLS2T was able to reduce Fe (III) citrate and Fe (III) EDTA to Fe (II) during cultivation with xylose. Strain GLS2T could not reduce the following electron acceptors: sulfate, thiosulfate, sulfite and nitrate, when cells were grown with xylose. Sulfite inhibited growth of GLS2T completely.

Isolate GLS2T was a strict anaerobe but tolerated exposure to air. It was able to grow anaerobically after 4 days of exposure to air. The optimal growth temperature was 30–34 °C and the temperature range for growth was 20–40 °C. Optimal pH for growth was pH 6.8–7.5, with growth occurring between pH 5.7 and 8.2. The bacterium required NaCl at a concentration of 1–2 g l−1 for optimal growth. The growth was inhibited by 5 g NaCl l−1.

The effect of antibiotics on strain GLS2T was determined on SM medium using antibiotics at the following concentrations (1−1): ampicillin (200 mg), carbenicillin (250 mg), rifampicin (30 mg), vancomycin (50 mg), cefepime (1 g), streptomycin (100 mg), kanamycin (150 mg), erythromycin, (12.5 mg) and tetracycline (10 mg). As for other bacteria of the genus Sphaerochaeta, strain GLS2T was resistant to ampicillin, cefepime, rifampicin and vancomycin and it was sensitive to kanamycin, erythromycin and tetracycline. GLS2T grew in the presence of streptomycin.

Catalase activity was determined by bubble formation in 3 % (v/v) H2O2 mixed with the cell suspension. Oxidase activity was determined by using an oxidase reagent (bioMérieux). An API ZYM test (bioMérieux) was used according to manufacturer’s protocol for characterizing the enzymic profiles of isolate GLS2T and the close relative Sphaerochaeta globosa BuddyT. Strain GLS2T was catalase- and oxidase-negative. As determined with the API ZYM test, both GLS2T and Sphaerochaeta globosa BuddyT had high activities of acid and alkaline phosphatas, naphthol-AS-BI-phosphohydrolase and α-galactosidase as well as weaker activity of β-galactosidase. The two strains differed by the presence of esterase and esterase lipase activities in BuddyT while GLS2T showed highly active valine arylamidase (Table S1).

The G+C content of the DNA was determined as described by Mesbah et al. (2011) and Owen & Pitcher (1985) on a DU800 spectrophotometer (Beckman Coulter) using as a control the DNA of the reference strain Sphaerochaeta globosa BuddyT. DNA hybridization was carried out with DNA of Sphaerochaeta globosa BuddyT according to the method of Rosselló-Móra et al. (2011). Despite the high degree of 16S rRNA gene sequence similarity, strains GLS2T and BuddyT were characterized by a low level of DNA–DNA hybridization, only 34.7 ± 8.8% (SD).

Polar lipids and isoprenoid quinones were extracted from lyophilized cells (approx. 100 mg) according to procedures described by Minnikin et al. (1979, 1984). The separation of lipids was carried out by two-dimensional TLC on Silica Gel 60F TLC-plates (Merck) using the following solvent systems: chloroform/methanol/water (65 : 25 : 4, by vol.) in the horizontal dimension and chloroform/acetic acid/methanol/water (80 : 15 : 12 : 4, by vol.) in the vertical dimension. A 5 % (w/v) solution of phosphomolybdc acid in ethanol was used for detection of all lipids. Phospholipids were detected by molybdenum blue and glycolipids were detected by λ-naphthol (Minnikin et al., 1984). The polar lipid analysis showed that isolate GLS2T had glycolipids, phospholipids and phosphoglycolipids, typical for members of the genus Sphaerochaeta. However, the polar lipid profile of strain GLS2T differed significantly in both qualitative and quantitative terms from other described species of the genus Sphaerochaeta (Miyazaki et al., 2014) (Fig. S2). Isoprenoid quinones were absent. Cellular fatty acids profiles were determined by GC-MS as described previously (Slobodkina et al., 2013). Fatty acid content was determined as the percentage of the total ion current peak area.

The major fatty acids for the novel strain were C14 : 0, C16 : 0, C16 : 0 3-OH and C16 : 1n7. Dimethyl acetal (DMAs) C16 : 0 DMA and C16 : 1n9 DMA were also detected (Tables 1 and S2). Interestingly, C16 : 0 DMA accounted for about 20 % of the fatty acids content in both GLS2T and BuddyT. Another major component was C16 : 0 3OH (16 %). The fatty acid profile of strain GLS2T differed from that of strain BuddyT, its closest recognized relative, in terms of the absence or low content of different C18 fatty acids components like C18 : 0 3OH and C18 : 1 DMA as well as the absence of C20 : 4n6 (Table S2).

It was found that the physiological ranges of strain GLS2T and M. mazei JL01 were the same. This fact along with the ability of strain GLS2T to grow on components of methanochondroitin such as glucose and glucuronic acid as well as its resistance to a wide variety of antibiotics and its properties of cell-wall deficiency characteristic to many pathogens explain the selection and tight association of both GLS2T and...
Table 1. Differential characteristics of strain GLS2T and the type strains of species of the genus *Sphaerochaeta*

Strains: 1, GLS2T (data from this study); 2, *Sphaerochaeta globosa* BuddyT (Ritalahti et al., 2012); 3, *Sphaerochaeta pleomorpha* GrapesT (Ritalahti et al., 2012); 4, *Sphaerochaeta coccoides* SPN1T (Droge et al., 2006; Abt et al., 2012; Miyazaki et al., 2014); 5, *Sphaerochaeta multiformis* MO-SPC2T (Miyazaki et al., 2014). +, Positive; −, negative; (+), weakly positive; ND, no data available; DMA, dimethyl acetal. All strains were Gram-stain-negative. All strains were positive for assimilation of D-xylose. All were catalase-negative.

<table>
<thead>
<tr>
<th>Feature</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphology</strong></td>
<td>Pleomorphic (spherical, ovoid, annular)</td>
<td>Coccus</td>
<td>Pleomorphic spherical, elongated or crescent shapes</td>
<td>Coccus</td>
<td>Pleomorphic (spherical, annular, curved rod, helical)</td>
</tr>
<tr>
<td><strong>Motility</strong></td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+ (Only for helical cells)</td>
</tr>
<tr>
<td><strong>Temperature for growth (°C)</strong></td>
<td>Range 20–40</td>
<td>20–37</td>
<td>15–30</td>
<td>15–40</td>
<td>0–17</td>
</tr>
<tr>
<td></td>
<td>Optimum 30–34</td>
<td>30</td>
<td>20–25</td>
<td>30</td>
<td>9</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>Range 5.7–8.2</td>
<td>ND</td>
<td>ND</td>
<td>5.5–9.5</td>
<td>6–8</td>
</tr>
<tr>
<td></td>
<td>Optimum 6.8–7.5</td>
<td>6.5–7.5</td>
<td>6.5–7.5</td>
<td>7.4</td>
<td>6.8–7.2</td>
</tr>
<tr>
<td><strong>NaCl optimum</strong> (g l(^{-1}))</td>
<td>1–2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>20–30</td>
</tr>
<tr>
<td><strong>Major fatty acids</strong></td>
<td>(\text{C}<em>{14}:0); (\text{C}</em>{16}:0); (\text{C}<em>{16}:0)-3-OH; (\text{C}</em>{16}:0) DMA; (\text{C}<em>{16}:1); (\text{C}</em>{16}:1) DMA</td>
<td>(\text{C}<em>{14}:0); (\text{C}</em>{16}:0); (\text{C}<em>{16}:1); (\text{C}</em>{16}:1); (\text{br}-\text{C}<em>{17}:1); (\text{C}</em>{14}:0); (\text{C}<em>{16}:0); (\text{iso}-\text{C}</em>{16}:0^*)</td>
<td>(\text{C}<em>{14}:0); (\text{C}</em>{16}:0); (\text{C}<em>{16}:1); (\text{br}-\text{C}</em>{17}:1); (\text{C}<em>{14}:0); (\text{C}</em>{16}:0); (\text{C}_{16}:1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DNA G+C content (mol%)</strong></td>
<td>47.2</td>
<td>48.9</td>
<td>46.2</td>
<td>50.6</td>
<td>32.3</td>
</tr>
<tr>
<td><strong>Cytochrome oxidase</strong></td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>( + )</td>
</tr>
<tr>
<td><strong>Carbon substrates</strong></td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>(\text{D}-\text{Arabinose})</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>(\text{D}-\text{Glucose})</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(\text{D}-\text{Galactose})</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(\text{D}-\text{Fructose})</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(\text{Gellobiose})</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(\text{Lactose})</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>(\text{Maltose})</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>(\text{Raffinose})</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>(\text{Sucrose})</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>(\text{Starch})</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>(\text{Yeast extract})</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Isolation source</strong></td>
<td><em>M. mazei</em> JL01</td>
<td>Fresh water sediments</td>
<td>Fresh water sediments</td>
<td>Hindgut of termite <em>Neotermes castaneus</em></td>
<td>Marine subsurface sediments</td>
</tr>
</tbody>
</table>

*Data from Miyazaki et al. (2014).*
M. mazei JL01. The characteristics differentiating strain GLS2T from species of the genus Sphaerochaeta with validly published names are detailed in Table 1.

On the basis of morphological, physiological, genotypic and phylogenetic traits, strain GLS2T is considered to represent a novel species within the genus Sphaerochaeta. We propose the name Sphaerochaeta associata since the novel bacterium was isolated from a binary culture with M. mazei, coexisting together in symbiotic relationship for a long time under conditions of substrate limitation for growth.

Description of Sphaerochaeta associata sp. nov

Sphaerochaeta associata (as.so.ci.a’ta. N.L. fem. part. adj. associata united with, occurring together, referring to isolation from binary cultures with M. mazei).

Cells are non-motile, cocoid, ovoid and annular with sizes of 0.2–4 μm. Gram-stain-negative, catalase- and oxidase-negative, anaerobic chemoorganoheterotroph. Saccharolytic fermenting metabolism. Good growth occurs on mono-, di- and trisaccharides. Growth occurs in the presence of arabinose, xylose, galactose, maltose, cellobiose, raffinose, glucose, lactose, sucrose, starch, lactate and gluconic acid, but not on fructose, cellulose, xylan, yeast extract, galactosamine, methanol, ethanol, glycerol, acetate, formate, pyruvate, propionate, butyrate, benzoate, fumarate, trimethylamine, Casamino acids or H2 + CO2 alone. Yeast extract is required for growth on all substrates. Requires 1–2 g NaCl l−1 for optimal growth. Positive for the following in the API ZYM test: acid and alkaline phosphatases, naphthol-AS-BI-phosphohydrolase, β-galactosidase, leucine arylamidase, aminopeptidase, alkaline phosphatase, α-galactosidase and valine arylamidase. Mesophile with optimum growth at 30–34 °C and a growth temperature range of 20–40 °C. Neutrophile with optimum pH 6.8–7.5 and growth within the range pH 5.7–8.2. Resistant to ampicillin, carbenicillin, cepfem, vancomycin, rifampicin and streptomycin but sensitive to kanamycin, erythromycin and tetra-cycline. Fatty acid profile is mostly composed of C14:0, C16:0, C16:1 3-OH, C16:0 DMA, C16:1 ins 8, and C16:1 DMA. Major polar lipids are phosphoglycolipids, phospholipids and glycolipids. Isoprenoid quinones are absent.

Type strain is GLS2T (＝DSM 26261T＝VKM B-2742T). Isolated from a binary culture with Methanosarcina mazei JL01 (＝VKM B-2370). The genomic DNA G+C content of the type strain is 47.2 ± 0.77 mol%.

ACKNOWLEDGEMENTS

We are grateful for help with analysis of isoprenoid quinones to Boris Baskunov, Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Science. This work was supported by a grant of the Russian Foundation of Basic Research, no. 15-04-08612. The fatty acids analysis was supported by President of Russia (grant MK-4530.2015.4).

REFERENCES


