Magnetospirillum caucaseum sp. nov., Magnetospirillum marisnigri sp. nov. and Magnetospirillum moscoviense sp. nov., freshwater magnetotactic bacteria isolated from three distinct geographical locations in European Russia

Marina Dziuba,¹ Veronika Koziaeva,¹ Denis Grouzdev,¹ Ekaterina Burganskaya,¹ Roman Baslerov,¹ Tatjana Kolganova,¹ Alexander Chernyadyev,² Georgy Osipov,³ Ekaterina Andrianova,¹ Vladimir Gorlenko⁴ and Boris Kuznetsov¹

Correspondence
Marina Dziuba
dzyubamv@gmail.com

¹Institute of Bioengineering, Federal Research Center of Biotechnology of the Russian Academy of Sciences, Leninsky Ave 33, bld. 2, Moscow 119071, Russia
²Education and Research Center Nanotechnology, Vyatka State University, Moscovskaya str. 36, Kirov 610000, Russia
³Bakulev Center of Cardiovascular Surgery, Russian Academy of Medical Sciences, Rublevskoe shosse 135, Moscow 121552, Russia
⁴Winogradsky Institute of Microbiology, Research Center of Biotechnology of the Russian Academy of Sciences, Leninsky Ave 33, bld. 2, Moscow 119071, Russia

Three strains of helical, magnetotactic bacteria, SO-1T, SP-1T and BB-1T, were isolated from freshwater sediments collected from three distinct locations in European Russia. Phylogenetic analysis showed that the strains belong to the genus Magnetospirillum. Strains SO-1T and SP-1T showed the highest 16S rRNA gene sequence similarity to Magnetospirillum magnetotacticum MS-1T (99.3 and 98.1%, respectively), and strain BB-1T with Magnetospirillum gryphiswaldense MSR-1T (97.3%). The tree based on concatenated deduced amino acid sequences of the MamA, B, K, M, O, P and T proteins, which are involved in magnetosome formation, was congruent with the tree based on 16S rRNA gene sequences. The genomic DNA G+C contents of strains SO-1T, SP-1T and BB-1T were 65.9, 63.0 and 65.2 mol%, respectively. As major fatty acids, C18:1ω9, C16:1ω7c, C16:0 and C18:0 were detected. DNA–DNA hybridization values between the novel strains and their closest relatives in the genus Magnetospirillum were less than 51.7±2.3%. In contrast to M. magnetotacticum MS-1T, the strains could utilize butyrate and propionate; strains SO-1T and BB-1T could also utilize glycerol. Strain SP-1T showed strictly microaerophilic growth, whereas strains SO-1T and BB-1T were more tolerant of oxygen. The results of DNA–DNA hybridization and physiological tests allowed genotypic and phenotypic differentiation of the strains from each other as well as from the two species of Magnetospirillum with validly published names. Therefore, the strains represent novel species, for which we propose the names Magnetospirillum caucaseum sp. nov. (type strain SO-1T=DSM 28995T=VKM B-2936T), Magnetospirillum marisnigri sp. nov. (type strain SP-1T=DSM 29006T=VKM B-2938T) and Magnetospirillum moscoviense sp. nov. (type strain BB-1T=DSM 29455T=VKM B-2939T).

Abbreviations: ANI, average nucleotide identity; DDH, DNA–DNA hybridization; MTB, magnetotactic bacteria.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains SO-1T, SP-1T and BB-1T are JX502622, KC252630 and KF712468, respectively. The accession numbers for the cbbM, nifH, mamA, mamB, mamK, mamM, mamO, mamP, mamQ and mamT gene sequences reported in this paper are respectively KF787146, KP886489, KF787131, KF787133, KF787135, KF787137, KF787139, KF836513, KF787142 and KF787144 (SO-1T) and KF712469, KP886488, KF787124, KF787127, KF787128, KF787126, KF787130, KF787129, KF787122 and KF787123 (BB-1T); sequences of these genes from strain SO-1T were taken from the draft genome sequence, contigs of which have been deposited in GenBank under accession numbers AONQ0000001–AONQ001000236. Two supplementary tables and a supplementary figure are available with the online Supplementary Material.
Magnetotactic bacteria (MTB) are a morphologically, physiologically and phylogenetically diverse group of motile, aquatic prokaryotes that share the ability to orientate passively and swim actively along the Earth’s magnetic field lines (Bazylinski & Frankel, 2004). This behaviour, termed magnetotaxis, is facilitated by the presence of intracellular magnetic crystals of magnetite (Fe₃O₄) and/or greigite (Fe₃S₄) enveloped by a lipid membrane. These unique organelles, called magnetosomes, are arranged into one or more chains within the cytoplasm that operate as magnetic dipoles, making the cells behave like magnetic compass needles (Bazylinski & Frankel, 2004). MTB inhabit chemically stratified aquatic habitats with redox gradients in the water column and sediments (Bazylinski & Williams, 2007). The basic hypothesis is that magnetotaxis serves MTB to increase the efficiency of aerotaxis in vertical concentration gradients by reducing a three-dimensional search to a linear one (Frankel et al., 1997).

Despite the fact that MTB are ubiquitous and abundant in various natural aquatic habitats, relatively few species have been cultivated as axenic cultures. Most of them belong to the genus Magnetospirillum, whose known representatives inhabit freshwater and are helical in shape and bipolarly flagellated. Two species of the genus have validly published names, Magnetospirillum magnetotacticum (Maratea & Blakemore, 1981) and Magnetospirillum gryphiswaldense (Schleifer et al., 1991). The type strains of these species and the strain ‘Magnetospirillum magneticum’ AMB-1 are well characterized in terms of their phylogeny, magnetosome biomineralization and genomics (Matsunaga et al., 1991, 2005). M. gryphiswaldense MSR-1T and ‘M. magneticum’ AMB-1 are of great importance as model strains for magnetosome formation studies and as potential producers of bacterial magnetic nanoparticles for a variety of biotechnological applications (Murat et al., 2010; Lohße et al., 2011; Yan et al., 2012; Alphandery, 2014; Araujo et al., 2015). The genus Magnetospirillum includes both magnetotactic strains and those that do not produce magnetosomes, namely ‘Magnetospirillum bellicus’ VDY and ‘Magnetospirillum aberrantis’ SpK, the names of which have been not yet validly published (Thrash et al., 2010; Gorlenko et al., 2011). Despite the fact that strain AMB-1 is assigned to a species the name of which is not validly published, quite a comprehensive description of the strain is available from the literature. Therefore, ‘M. magneticum’ AMB-1 was also included in our polyphasic analysis in this study. Here, we describe the morphology, physiology, chemotaxonomy and phylogeny of one previously reported strain, SO-1T (Dziuba et al., 2013), and two novel magnetotactic, helical bacteria, strains SP-1T and BB-1T, isolated from three distinct freshwater aquatic habitats. We propose the assignment of all three strains to novel species of the genus Magnetospirillum.

Samples of water and the upper layer of sediment were taken from three rivers located in distinct areas of European Russia: the O’lk’kova in Kislovodsk, Caucasian (43° 53’ 38.6” N 42° 43’ 15.1” E); the Pshada, Krasnodar region (44° 23’ 21.5” N 38° 20’ 10.5” E); and the Moskva, Moscow (55° 47’ 33.7” N 37° 24’ 35.8” E). Sediment samples were collected at a water depth of about 1 m. Three-litre glass jars were filled to about one-fifth to one-third of their volumes with sediment and the remaining volume was filled with sampled water. The jars were covered with aluminium foil and stored under dim light at room temperature. MTB were concentrated by using a permanent magnet attached to the jar wall near the sediment–water interface. After 1–3 h, a spot containing MTB cells became visible near the magnet and could be collected with a pipette. These cells were further purified using a capillary magnetic racetrack method (Wolfé et al., 1987). The contents of the capillary tips were used as inocula in the isolation medium. The isolation medium was similar to Magnetospirillum medium (medium no. 380, recommended by the DSMZ) with some modifications and contained (per litre): 1.00 ml of each vitamin elixir solution; 1.00 ml trace elements solution (vitamin elixirs and trace elements solution were prepared as described in Bryantseva et al., 1999); 2.00 ml 10 mM ferric citrate solution; 0.50 mg resazurin; 0.68 g KH₂PO₄; 0.12 g NaNO₃; 0.05 g sodium thioglycolate; 0.37 g sodium tartrate; 0.37 g sodium succinate; 0.05 g sodium acetate; 1.50 g agar (Difco Laboratories); pH 6.8. The medium was prepared according to http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium380.pdf. Media with an established redox gradient were inoculated using a hypodermic syringe. The isolates were incubated without shaking at 28 °C. After several weeks of incubation of the inoculated redox gradient isolation medium, growth was observed in several tubes as microaerophilic bands at a depth of 0.5–1.0 cm below the medium surface. Microscope examination revealed homogeneous populations of helical cells that exhibited polar magnetotaxis. To obtain pure cultures, serial dilutions (in the case of the isolates obtained from the O’lk’kova and Pshada) or plating on modified solid ACAM medium (for the isolate from the Moskva) were carried out (Schultheiss & Schüler, 2003). The modified ACAM medium contained (per litre): 2.38 g HEPES; 3.00 g sodium succinate; 0.10 g yeast extract; 3.00 g soybean peptone; 0.34 g NaNO₃; 0.10 g KH₂PO₄; 0.15 g MgSO₄ . 7H₂O; 3.00 g activated charcoal; 15.00 g agar; 50.00 ml 10 mM ferric citrate solution; 0.05 g sodium thioglycolate; pH 7.0. Plates were incubated in anaerobic jars under a gas atmosphere containing 99 % nitrogen and 1 % oxygen for 10–14 days. The strain isolated from the O’lk’kova was designated SO-1T, that from the Pshada, SP-1T, and that from the Moskva, BB-1T.

To determine magnetotaxis, a local magnetic field was applied by placing a large stirring bar magnet onto the microscope stage. Cell morphology and the presence and morphology of magnetosomes were determined using JEM 100CXII and JEOL JEM 2100 transmission electron microscopes, with accelerating voltages of 80 and 200 kV, respectively. Drops of the concentrated cell suspension were placed on a carbon-coated copper grid and dried in air.
Cells were rinsed with distilled water and negatively stained with 2 % (w/v) ammonium molybdate for 5 s.

Cells of the isolated strains were helical. Cells of strain SO-1T were 0.3 μm wide and 1.2–3.0 μm long, cells of strain SP-1T were 0.3–0.4 × 2.5–4.0 μm and cells of strain BB-1T were 0.3 × 2.0–4.0 μm. The strains were motile and bipolarly flagellated and demonstrated magnetotaxis. All isolated strains synthesized a single chain of magnetosomes, similar to those found in species of *Magnetospirillum* (Fig. 1).

DNA was isolated from bacterial biomass as described previously (Bulygina et al., 2002). Fragments of the 16S rRNA gene were amplified from genomic DNA using universal primers 27F and 1492R (Lane, 1991). 16S rRNA gene PCR products were purified using a Wizard PCR Preps reagent kit (Promega) and cloned using a pGEM-T Easy System reagent kit (Promega). Ten clones for each isolate were sequenced to ensure that the cultures were pure. Sequencing of the PCR products was carried out on an ABI 3730 automatic sequencer (Applied Biosystems) using the BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems) according to the manufacturer’s recommendations. Primary comparison of 16S rRNA gene sequences with sequences from the GenBank database was carried out using BLAST (Altschul et al., 1990). Alignment of 16S rRNA gene and *mam* gene sequences was carried out using the CLUSTAL W (Thompson et al., 1994) multiple-alignment accessory application in the BioEdit sequence editor (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Nearly full-length 16S rRNA gene sequences (≥1440 bp) of strains SO-1T, SP-1T and BB-1T were deposited in GenBank. Phylogenetic trees were reconstructed using MEGA version 6 (Tamura et al., 2013). The evolutionary history was inferred using the neighbour-joining algorithm (Saitou & Nei, 1987). Bootstrap values were calculated with 1000 replicates. Analysis of 16S rRNA gene sequences indicated that the strains belong phylogenetically to the family *Rhodospirillaceae* of the class *Alphaproteobacteria*. On the phylogenetic tree based on 16S rRNA gene sequences (1366 bp), the strains clustered into two distinct groups within the genus *Magnetospirillum* (Fig. 2). In addition to strain BB-1T, the first group also contained three previously described strains of *Magnetospirillum*, *M. gryphiswaldense* MSR-1T, ‘*M. bellicus*’ VDY and ‘*M. aberrantis*’ SpK. In the second group, strain SO-1T clustered with ‘*M. magneticum*’ AMB-1 and *M. magnetotacticum* MS-1T, whereas strain SP-1T formed a separate branch. Strain SO-1T showed the highest 16S rRNA gene sequence similarity to *M. magnetotacticum* MS-1T (99.3 %) and ‘*M. magneticum*’ AMB-1 (99.5 %). The closest relatives of strain SP-1T were also *M. magnetotacticum* MS-1T (98.1 %) and ‘*M. magneticum*’ AMB-1 (97.9 %). The most closely related strain to strain BB-1T was *M. gryphiswaldense* MSR-1T (97.3 %); 16S rRNA gene

---

**Fig. 1.** Transmission electron micrographs of negatively stained cells of strains SP-1T (a) and BB-1T (c) and unstained cells of strain SO-1T (b). F, Flagellum; M, magnetosomes. Bars, 1 μm (a) and 500 nm (b, c).
sequence similarities of strain BB-1T to other members of *Magnetospirillum* were below 97%. 16S rRNA gene sequence similarities and distances for strains of *Magnetospirillum* are shown in Table S1, available in the online Supplementary Material.

For strains SP-1T and BB-1T, fragments of the *mamA, mamB, mamK, mamM, mamO, mamP, mamQ* and *mamT* genes required for magnetosome synthesis were amplified and sequenced with specific primers, according to the protocol described by Lefe`vre et al. (2012); *mam* gene sequences for strain SO-1T were taken from previously published genome sequence data (Grouzdev et al., 2014). The tree based on the concatenated deduced amino acid sequences of the corresponding Mam proteins is congruent with the phylogenetic tree based on the 16S rRNA gene sequences (Fig. 3), confirming the analysis based on 16S rRNA gene sequences.

The DNA G + C content was determined by thermal denaturation as described by Owen et al. (1969) or calculated directly from genomic sequence data. The genomic DNA G+C contents of strains SO-1T, SP-1T and BB-1T were 65.9, 63.0 and 65.2 mol%, respectively, close to those determined for other members of the genus *Magnetospirillum*.

DNA–DNA hybridization (DDH) was carried out spectrophotometrically as described by De Ley et al. (1970). DDH between strain SO-1T and the closely related 'M. magneti-

cum' AMB-1 was 51.7 ± 2.3%, and that between strain SO-1T and *M. magnetotacticum* MS-1T was 48.5 ± 0.5%. DDH between strains SP-1T and *M. magnetotacticum* MS-1T and between BB-1T and *M. gryphiswaldense* MSR-1T was 45.0 ± 5.0 and 40.5 ± 0.5%, respectively (Table S2). Thus, the DDH values were well below 70%, indicating strongly that strains SO-1T, SP-1T and BB-1T are members of separate species (Tindall et al., 2010).

Average nucleotide identity (ANI) was calculated for the available genome sequences of strain SO-1T,
M. magnetotacticum MS-1\textsuperscript{T}, M. gryphiswaldense MSR-1\textsuperscript{T} and ‘M. magneticum’ AMB-1 (Matsunaga et al., 2005; Grouzdev et al., 2014; Wang et al., 2014; Smalley et al., 2015) as described by Konstantinidis et al. (2006). The analysis showed ANI of \( \leq 87.5\% \), confirming that strain SO-1\textsuperscript{T} and the previously described type strains M. magnetotacticum MS-1\textsuperscript{T} and M. gryphiswaldense MSR-1\textsuperscript{T} as well as ‘M. magneticum’ AMB-1 meet the criteria for assignment to separate species (the threshold is \( \leq 95\% \); Konstantinidis et al., 2006). Detailed ANI data are shown in Table 1.

The isolation medium described above was used for testing optimal growth temperature, pH and salinity. Ranges for growth were determined by monitoring the OD\textsubscript{565}. The temperature and pH were tested within the ranges 15–50 °C (15, 18, 25, 28, 30, 32, 37, 42 and 50 °C) and pH 4.0–10.0 (pH 4.0, 5.0, 6.0, 6.5, 6.8, 7.0, 7.5, 8.0, 9.0 and 10.0). To test the salinity optimum, NaCl was added to the culture medium at 0–5 % (w/v) (0, 0.05, 0.1, 0.5, 0.7, 1, 1.5, 2, 3, 4 and 5 %). Different carbon compounds were added as sole carbon sources and electron donors to the cultivation medium, from which sodium tartrate, sodium succinate and sodium acetate were omitted. Test substances were added to a final concentration of 1 g L\textsuperscript{-1} from sterile aqueous stock solutions after autoclaving. Electron acceptors were added in anoxic medium, where nitrate was replaced with ammonium chloride. Each compound was tested in triplicate with three subsequent transfers.

<table>
<thead>
<tr>
<th>Strain</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO-1\textsuperscript{T}</td>
<td>100</td>
<td>85.63</td>
<td>79.30</td>
<td>87.50</td>
</tr>
<tr>
<td>M. magnetotacticum MS-1\textsuperscript{T}</td>
<td>85.63</td>
<td>(100)</td>
<td>78.95</td>
<td>86.56</td>
</tr>
<tr>
<td>M. gryphiswaldense MSR-1\textsuperscript{T}</td>
<td>79.30</td>
<td>78.95</td>
<td>(100)</td>
<td>79.15</td>
</tr>
<tr>
<td>‘M. magneticum’ AMB-1</td>
<td>87.50</td>
<td>86.56</td>
<td>79.15</td>
<td>(100)</td>
</tr>
</tbody>
</table>

Strain BB-1\textsuperscript{T} grew at 18–37 °C, with a broad optimum at 25–32 °C. Strains SP-1\textsuperscript{T} and SO-1\textsuperscript{T} grew at 18–42 °C, with optima at 28 and 28–30 °C, respectively. For all strains, the pH optimum was about pH 6.5–6.8. Strains SO-1\textsuperscript{T} and BB-1\textsuperscript{T} exhibited NaCl tolerance similar to that reported previously for M. magnetotacticum MS-1\textsuperscript{T}; the strains could grow at NaCl concentrations no higher than 1 %. Strain SP-1\textsuperscript{T} demonstrated the lowest tolerance of NaCl among known members of Magnetospirillum; it could not grow with more than 0.1 % NaCl. The strains were chemo-organoheterotrophic, but unable to utilize sugars or amino acids as sole carbon and energy sources. Like other members of Magnetospirillum, strains SO-1\textsuperscript{T}, SP-1\textsuperscript{T} and BB-1\textsuperscript{T} oxidized a range of fermentation end products, mainly short-chain carboxylic acids: acetate, lactate, fumarate, succinate and others. They did not utilize citrate. Unlike M. magnetotacticum MS-1\textsuperscript{T}, strains SO-1\textsuperscript{T} and SP-1\textsuperscript{T} could grow on butyrate and propionate as sole carbon sources. In contrast to M. gryphiswaldense MSR-1\textsuperscript{T}, strains SO-1\textsuperscript{T} and BB-1\textsuperscript{T} utilized glycerol and propionate. All three strains used oxygen as a terminal electron acceptor. They could also utilize nitrate as an electron acceptor, demonstrating their capacity for facultatively anaerobic growth. In contrast to ‘M. bellicus’ VDY (Thrash et al., 2010), strains SO-1\textsuperscript{T}, SP-1\textsuperscript{T} and BB-1\textsuperscript{T} were unable to use ethanol as an electron donor or perchlorate as a terminal electron acceptor.

In semi-solid medium, the strains formed bands at a depth of up to 1 cm below the surface, demonstrating microaerophilic behaviour. However, the degree of tolerance of oxygen varied among the strains. Strain SP-1\textsuperscript{T} was found to be less tolerant of oxygen than the others: it could grow with an oxygen concentration of no more than 5 % in the gas phase. Strains SO-1\textsuperscript{T} and BB-1\textsuperscript{T} could grow with free gas exchange between air and medium in the presence of thioglycolate, although the growth rate decreased significantly and the cells did not produce magnetosomes under these conditions. Thereby, strain SO-1\textsuperscript{T} significantly differed in its tolerance of oxygen from the very closely related M. magnetotacticum MS-1\textsuperscript{T}, which has been described as an obligate microaerophile (Blakemore et al., 1979; Heyen & Schüler, 2003). In our hands,
M. magnetotacticum MS-1T has also demonstrated strictly microaerophilic behaviour. We also compared the growth rate of strain SO-1T and another closely related strain, ‘M. magneticum’ AMB-1, which has previously been reported as able to grow aerobically (Matsunaga et al., 1991). To compare the responses to oxygen of strain SO-1T and ‘M. magneticum’ AMB-1, the strains were cultivated microaerobically and aerobically in 96-well culture plates in the basic isolation medium at 28 °C. The total volume of culture in each well was 200 μL. To create microaerobic conditions, the cells were placed in an anaerobic jar (Oxoid) under a 1 % O2/99 % N2 (v/v) gas atmosphere. The cells grown aerobically were incubated under free gas exchange with air, in the absence of sodium thioglycolate. The OD565 was measured using a 96-well microplate spectrophotometer (Picon). The growth curves in Fig. S1 show that both strains demonstrated growth under microaerobic conditions; however, strain SO-1T grew more slowly and reached a lower cell density under the same conditions. At the same time, the analysis demonstrated that, in contrast to ‘M. magneticum’ AMB-1, strain SO-1T could not grow aerobically in the absence of a reducing agent.

PCR amplification and genome analysis (for strain SO-1T) revealed the presence of the ribulose bisphosphate carboxylase/oxygenase (RubisCO) large-subunit cbbM gene in the genomes of strains SO-1T, SP-1T and BB-1T, suggesting the potential ability for autotrophic growth through the Calvin–Benson–Bassham cycle; however, CO2 fixation by these strains has not been investigated. Genes encoding the RubisCO large-subunits of form I (cbbL) and form II (cbbM) were amplified using primers described previously (Spiridonova et al., 2004), according to the following protocol: 95 °C for 4 min, 35 cycles of 95 °C for 45 s, 57 °C for 45 s and 72 °C for 45 s, followed by a final elongation at 72 °C for 10 min.

To amplify fragments of the genes encoding the essential part of the enzymic nitrogenase complex nifH, specific primers F1 and R6 from Marusina et al. (2001) were used. Nitrogenase activity was determined by the acetylene reduction method according to the procedure described by Bazylinski et al. (2000). M. gryphiswaldense MSR-1T was used as a positive control. The nifH gene essential for nitrogen fixation was found in the genomes of all the strains. Indeed, the acetylene reduction test showed that strains SO-1T, SP-1T and BB-1T, like other tested strains of Magnetospirillum (Bazylinski & Williams, 2007), displayed nitrogenase activity when a fixed nitrogen source was removed from the growth medium. These results demonstrated that the strains are capable of fixing nitrogen, at least under heterotrophic conditions.

Catalase and oxidase activities were determined using standard techniques: by adding 3 % H2O2 and 1 % tetramethyl N-phenylenediamine hydrochloride, respectively, to concentrated cell suspensions. Strains SO-1T and BB-1T demonstrated positive reactions for oxidase and negative reactions for catalase. Strain SP-1T was oxidase- and catalase-negative. Characteristics that differentiate strains SO-1T, SP-1T and BB-1T from related species of the genus Magnetospirillum are detailed in Table 2.

To determine the fatty acid composition, strains SO-1T, SP-1T and BB-1T as well as the reference strains M. magnetotacticum MS-1T, M. gryphiswaldense MSR-1T and ‘M. magneticum’ AMB-1 were grown in basal isolation medium for 3–5 days at 28 °C under microaerophilic conditions and harvested by centrifugation. Fatty acid extraction from dried samples (5 mg) was carried out using whole-biomass acid methanolysis in 0.4 ml 1.2 M HCl in methanol by heating to 80 °C for 1 h. The resulting fatty acid methyl esters were extracted twice with 0.2 ml hexane and processed on an Agilent Technologies AT-5850/5973 GC-MS system with a cross-linked methyl silicone capillary column HP-5ms. The oven temperature was programmed from 140 to 320 °C at 7 °C min⁻¹. One to two microlitres derivatized sample was injected into the gas chromatograph at 280 °C. Fatty acids and other lipid components were ionized by electron impact at 70 eV after separation in the GC column and analysed in scan mode. The quadrupole mass spectrometer has a resolution of 0.5 mass units over the whole mass range of 2–950 atomic mass units. The sensitivity of the GC-MS system is 0.01 ng methyl stearate. Each substance was confirmed by its mass spectrum and an NIST mass spectral database library search.

The major fatty acids for the novel strains as well as for the other strains analysed were C16:0, C16:1ω7c, C16:1ω9c, C18:1ω9c and C18:1ω7c. Only minor differences could be identified in comparison with those of the other strains of the most closely related species of Magnetospirillum. The detailed fatty acid patterns are given in Table 3.

Although the 16S rRNA gene sequence analysis demonstrated high sequence similarities of strains SO-1T, SP-1T and BB-1T with the type strains of species of Magnetospirillum, DDH experiments, ANI analysis and physiological data justify the placement of strains SO-1T, SP-1T and BB-1T in the genus Magnetospirillum as the type strains of three novel species, for which we propose the names Magnetospirillum caucaseum sp. nov., Magnetospirillum marisnigri sp. nov. and Magnetospirillum moscovienne sp. nov.

**Description of Magnetospirillum caucaseum sp. nov.**

*Magnetospirillum caucaseum* (cau.ca’se.un. L. neut. adj. caucaseum of or pertaining to the Caucasus, referring to the geographical region where the type strain was isolated).

Morphology of the cells in their basic features is the same as described for the genus (Schleifer et al., 1991): cells are spiral-shaped, 0.3 × 1.2–3.0 μm, motile, bipolarly flagellated and contain a single chain of magnetosomes. Grows best under microaerophilic conditions; relatively tolerant
of high (up to 21%) oxygen concentrations, but not aerobic (capable of growing in liquid medium with free exchange to the air in the presence of thioglycolate), facultative anaerobe. Optimum growth at pH 6.5–6.8 and 28 °C. Grows chemo-organoheterotrophically on a range of short-chain carboxylic acids (acetate, fumarate, butyrate, succinate, tartrate, malate and lactate) and glycerol; does not use citrate, sugars or alcohols. The dominant fatty acids are C₁₆:₁, ε₁₀₋₇𝜔, C₁₆:₀, C₁₈:₁, ε₁₀₋₉ and C₁₈:₀. Oxidase-positive and catalase-negative. The dominant fatty acids are C₁₆:₁, ε₁₀₋₇𝜔, C₁₆:₀, C₁₈:₁, ε₁₀₋₉ and C₁₈:₀.

The type strain is SO-1ᵀ (DSM 28995ᵀ = VKM B-2936ᵀ), isolated from a sediment sample from the Ol’khovka river, Kislovodsk, Caucasus, Russia. The DNA G+C content of the type strain is 65.9 mol% (calculated from the genome sequence).

**Description of Magnetospirillum marisnigri** sp. nov.

*Magnetospirillum marisnigri* (ma.ris.ni’gri. L. neut. n. mare, *maris* sea; L. adj. niger black; N.L. gen. n. *marisnigri* of the Black Sea, referring to the place where the type strain was isolated).

Cells morphology is as described for *M. caucaseum* sp. nov., with cell size 0.3–0.4 × 2.5–4.0 μm. Microaerophilic; grows best in a narrow range of oxygen concentrations (1–5% in the gas atmosphere), facultative anaerobe. Optimum growth at pH 6.5–6.8 and 28–30 °C. Grows under very low concentrations of NaCl (<0.1%). Utilizes the same range of electron donors as *M. caucaseum* SO-1ᵀ; does not use citrate, sugars or alcohols. The dominant fatty acids are C₁₆:₁, ε₁₀₋₇𝜔, C₁₆:₀, C₁₈:₁, ε₁₀₋₉ and C₁₈:₀. Oxidase- and catalase-negative.

The type strain is SP-1ᵀ (DSM 29006ᵀ = VKM B-2938ᵀ), isolated from sediments of the Pshada river, near to the coast of the Black Sea, Krasnodar region, Russia. The DNA G+C content of the type strain is 63.0 mol% (thermal denaturation method).

**Description of Magnetospirillum moscoviense** sp. nov.

*Magnetospirillum moscoviense* (mos.co.vi.en’se. N.L. neut. adj. *moscoviense* of or pertaining to Moscow, named after the place where this organism was first isolated).

Morphological description is as for *M. caucaseum* sp. nov., with cell size 0.3 × 2–4 μm. Microaerophilic; grows in a broad range of oxygen concentrations (up to 21%), but not aerobically. Optimum growth at pH 6.5–7.0 and 25–32 °C. Utilizes the same range of short-chain carboxylic acids (acetate, fumarate, butyrate, succinate, tartrate, malate and lactate) and glycerol; does not use citrate, sugars or alcohols. The DNA G+C content of the type strain is 65.9 mol% (calculated from the genome sequence).
Table 3. Fatty acid compositions of the novel strains and other representatives of the genus Magnetospirillum

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12 : 0</td>
<td>1.1</td>
<td>0.6</td>
<td>1.1</td>
<td>1.7</td>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>iso-C14 : 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14 : 0</td>
<td>0.9</td>
<td>0.6</td>
<td>0.7</td>
<td>2.7</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>iso-C15 : 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anteiso-C15 : 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C15 : 0</td>
<td>0.2</td>
<td></td>
<td>1.1</td>
<td></td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>iso-C16 : 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16 : 107c</td>
<td>28.3</td>
<td>24.7</td>
<td>25.4</td>
<td>22.8</td>
<td>25.1</td>
<td>24.2</td>
</tr>
<tr>
<td>C16 : 109</td>
<td>0.4</td>
<td>0.4</td>
<td></td>
<td></td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>C16 : 0</td>
<td>20.6</td>
<td>10.9</td>
<td>19.3</td>
<td>13.4</td>
<td>19.1</td>
<td>18.1</td>
</tr>
<tr>
<td>anteiso-C17 : 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C17 : 0 cyclo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C17 : 0</td>
<td>0.6</td>
<td>0.3</td>
<td>0.8</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>C18 : 109</td>
<td>35.9</td>
<td>45.5</td>
<td>37.3</td>
<td>46.3</td>
<td>34.8</td>
<td>31.8</td>
</tr>
<tr>
<td>C18 : 2</td>
<td>12.3</td>
<td>16.2</td>
<td>11.6</td>
<td>10.3</td>
<td>18.3</td>
<td>8.6</td>
</tr>
<tr>
<td>C18 : 1 cyclo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C19 : 0 cyclo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>C20 : 0</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td></td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>C14 : 0 3-OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14 : 0 3-OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16 : 0 3-OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C17 : 0 3-OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fatty acids other than M. caucaseum SO-1T do not use citrate, sugars or alcohols, with the exception of glycerol. The dominant fatty acids are C16 : 107c, C16 : 0, C18 : 109 and C18 : 0. Oxidase-positive and catalase-negative.

The type strain is BB-1T (= DSM 29455T = VKM B-2939T), isolated from sediments of the Moskva river, Moscow, Russia. The DNA G+C content of the type strain is 65.2 mol% (calculated from unpublished genome sequence data).

Acknowledgements

This work was supported by the RAS program 'Fundamental Basis of Technologies of Nanostructures and Nanomaterials' and by the President of the Russian Federation (grant 14.120.14.6150-NSh). We thank Dr. E. Detkova for her help in DNA–DNA hybridization analysis, Dr. O. Samylin for her assistance in analysis of nitrogenase activity and Dr. O. Bystrova (N. D. Zelinsky Institute of Organic Chemistry, International Analytical Center) for help in fatty acid analysis. Transmission electron micrographs were obtained in the Education and Research Center of Nanotechnology of Vytatka State University and Winogradsky Institute of Microbiology, Russian Academy of Sciences. We thank the Dean of the Faculty of Biology of Vytatka State University, Dr. Ekaterina Martinson, for her help with the organization of the work on transmission electron microscopy and for providing laboratory facilities.

References


