Magnetospira thiophila gen. nov., sp. nov., a marine magnetotactic bacterium that represents a novel lineage within the Rhodospirillaceae (Alphaproteobacteria)

Timothy J. Williams,1 Christopher T. Lefèvre,2,3 Weidong Zhao,4 Terry J. Beveridge5 and Dennis A. Bazylinski2

1School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, NSW 2052, Australia
2School of Life Sciences, University of Nevada at Las Vegas, 4505 Maryland Parkway, Las Vegas, NV 89154, USA
3Laboratoire de Bioénergétique Cellulaire UMR 6191, CEA Cadarache, DSV, IEBEB, Saint-Paul-lez-Durance 13108, France
4Northeast Ohio Medical University, 4209 SR 44, PO Box 95, Rootstown, OH 44272, USA
5Department of Molecular and Cellular Biology, College of Biological Science, University of Guelph, Guelph, ON N1G 2W1, Canada

A marine, magnetotactic bacterium, designated strain MMS-1T, was isolated from mud and water from a salt marsh in Woods Hole, Massachusetts, USA, after enrichment in defined oxygen-concentration/redox-gradient medium. Strain MMS-1T is an obligate microaerophile capable of chemoorganoheterotrophic and chemolithoautotrophic growth. Optimal growth occurred at pH 7.0 and 24–26 °C. Chemolithoautotrophic growth occurred with thiosulfate as the electron donor and autotrophic carbon fixation was via the Calvin–Benson–Bassham cycle. The G+C content of the DNA of strain MMS-1T was 47.2 mol%. Cells were Gram-negative and morphologically variable, with shapes that ranged from that of a lima bean to fully helical. Cells were motile by means of a single flagellum at each end of the cell (amphitrichous). Regardless of whether grown in liquid or semi-solid cultures, strain MMS-1T displayed only polar magnetotaxis and possessed a single chain of magnetosomes containing elongated octahedral crystals of magnetite, positioned along the long axis of the cell. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain MMS-1T belongs to the family Rhodospirillaceae within the Alphaproteobacteria, and is distantly related to species of the genus Magnetospirillum. Strain MMS-1T is therefore considered to represent a novel species of a new genus, for which the name Magnetospira thiophila gen. nov., sp. nov. is proposed. The type strain of Magnetospira thiophila is MMS-1T (=ATCC BAA-1438T=JCM 17960T).

The magnetotactic bacteria are a phylogenetically and physiologically diverse group that share the ability to passively align and actively migrate along magnetic field lines (Bazylinski & Frankel, 2004). In nature, these lines are caused by the Earth’s geomagnetic field. Magnetotaxis is dependent upon the presence of intracellular, single-magnetic-domain crystals of the magnetic minerals magnetite (Fe₃O₄) or greigite (Fe₃S₄) that are usually arranged as a chain or chains within the cell (Bazylinski & Frankel, 2004). Each crystal is enclosed within a lipid bilayer membrane, to form the individual magnetosome (Gorby et al., 1988). The formation and organization of magnetosomes within the cell is under genetic control, and the molecular mechanisms behind this biomineralization process have been the subject of extensive investigation (e.g. Schübbe et al., 2003; Komeili et al., 2006; Scheffel et al., 2006).
Most magnetotactic bacteria exist at their highest densities at or near the oxic–anoxic transition zone (OATZ) or redoxcline of chemically stratified aquatic environments (Simmons et al., 2004; Moskowitz et al., 2008). Thus, it has been hypothesized that magnetotaxis facilitates the search for the optimal concentrations of oxidants and reductants within the water column by simplifying a three-dimensional search to a linear search, following geomagnetic field lines (Frankel et al., 1997; Bazylinski & Frankel, 2004). Few species of magnetotactic bacteria have been cultivated in pure culture. Of those that have been isolated and cultivated axenically, most have been phylogenetically pure culture. Of those that have been isolated and cultivated axenically, most have been phylogenetically assigned to the Alphaproteobacteria, including: the freshwater genus Magnetospirillum (including Magnetospirillum magnetotacticum and Magnetospirillum gyrophilwosaldense); the marine magnetic cocoid strains MC-1 (‘Candidatus Magnetococcus marinus’; Bazylinski et al., 2012) and MO-1; the marine magnetic vibrio MV-1 (‘Candidatus Magnetovibrio blakemorei’) (Bazylinski et al., 1988); and the small marine magnetic spirilla strains MMS-1 and QH-2 (Bazylinski & Williams, 2007; Lefèvre et al., 2009; Zhu et al., 2010). Strain MMS-1 (‘marine magnetic spirillum 1’) is the subject of the current study. This strain has been studied previously under the name ‘MV-4’ (‘magnetic vibrio number 4’; Meldrum et al., 1993), but the morphology is best characterized as a spirillum rather than a vibrioid, albeit the cell morphology is variable.

Strain MMS-1T is a magnetotactic bacterium isolated from mud and water collected from School Street Marsh, Woods Hole, Massachusetts, on the north-east coast of the United States (Meldrum et al., 1993). It displays polar magnetotaxis (i.e. uses the magnetic field for both an axis and a direction for migration; Frankel et al., 1997; Bazylinski & Frankel, 2004). For isolation of strain MMS-1T in fresh culture was 49.7 ± 10.6 μm s⁻¹ (n = 229). The magnetosomes within cells of strain MMS-1T contained elongated prismatic crystals of magnetite (elongate cuboctahedra) and were arranged linearly as a chain along the long axis of the bacterial cell (Fig. 1c) (Meldrum et al., 1993). A magnetosome membrane was clearly present (Fig. 1d).

Cells of strain MMS-1T grown in semi-solid medium under chemolithoautotrophic conditions with thiosulfate as the electron donor contained 17 ± 5 magnetosomes per cell (mean ± SD, range 8–31, n = 85) as found by Meldrum et al. (1993). The latter compares well with 7–28 magnetosomes per cell (average 16) for the similar, related magnetotactic bacterium strain QH-2 (Zhu et al., 2010). Interestingly, despite the numerous transfers of strain MMS-1T in culture since its isolation, cultures never became mainly non-magnetotactic, unlike the situation previously reported for other magnetotactic bacteria such as ‘Ca. Magnetovibrio blakemorei’ (Dubbels et al., 2004). This observation might suggest a higher stability of the magnetosome genes or the magnetosome gene island, if present, in strain MMS-1T.

Energy-filtered transmission electron microscopy demonstrated that strain MMS-1T grown under chemolithoautotrophic conditions with thiosulfate as the electron donor produces crystals composed solely of iron and oxygen, consistent with the iron oxide magnetite (Fe₃O₄); there was no evidence of sulfur in the magnetosome crystals (Fig. 2). The same elemental maps also revealed the presence of polyphosphate bodies and sulfur-rich inclusions stored intracellulary by strain MMS-1T (Fig. 2), the latter being typical of many sulfur-oxidizing bacteria.

To achieve a sufficient yield of biomass for DNA extraction, cells of strain MMS-1T were grown chemolithoautotrophically in 2-litre glass bottles containing 850 ml of the medium described above with some modifications. Thiosulfate (S₂O₃²⁻) was the electron donor, and O₂ was the terminal electron acceptor. After the basal vitamin solution (Frankel et al., 1997) and 2.5 ml of 0.01 M FeSO₄ dissolved in 0.2 M HCl. The medium was dispensed into sterile, screw-capped test tubes. All cultures were incubated at 25–28 °C and cells grew as a micro-aerophilic band at the OATZ (pink/colourless interface) of the tubes. Cells also grew in this same medium but with 3.7 mM sodium succinate instead of thiosulfate. Separate colonies were obtained in a serial dilution of a culture in solid agar (13 g per litre) shake tubes of the same medium with succinate as the electron donor. Colonies were removed aseptically and the process was repeated three times. Purity of the cultures was determined by light microscopy and by amplification and sequencing of the 16S rRNA gene.

Cells of strain MMS-1T ranged from lima bean-shaped to partially bean-shaped, partially helical to fully helical (Fig. 1a–c). Cells displayed a single, unshathed flagellum at or near the oxic–anoxic transition zone (OATZ) or redoxcline of chemically stratified aquatic environments (Simmons et al., 2004; Moskowitz et al., 2008). Thus, it has been hypothesized that magnetotaxis facilitates the search for the optimal concentrations of oxidants and reductants within the water column by simplifying a three-dimensional search to a linear search, following geomagnetic field lines (Frankel et al., 1997; Bazylinski & Frankel, 2004). Few species of magnetotactic bacteria have been cultivated in pure culture. Of those that have been isolated and cultivated axenically, most have been phylogenetically assigned to the Alphaproteobacteria, including: the freshwater genus Magnetospirillum (including Magnetospirillum magnetotacticum and Magnetospirillum gyrophilwosaldense); the marine magnetic cocoid strains MC-1 (‘Candidatus Magnetococcus marinus’; Bazylinski et al., 2012) and MO-1; the marine magnetic vibrio MV-1 (‘Candidatus Magnetovibrio blakemorei’) (Bazylinski et al., 1988); and the small marine magnetic spirilla strains MMS-1 and QH-2 (Bazylinski & Williams, 2007; Lefèvre et al., 2009; Zhu et al., 2010). Strain MMS-1 (‘marine magnetic spirillum 1’) is the subject of the current study. This strain has been studied previously under the name ‘MV-4’ (‘magnetic vibrio number 4’; Meldrum et al., 1993), but the morphology is best characterized as a spirillum rather than a vibrioid, albeit the cell morphology is variable.

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medium was prepared, 1.26 g NaHCO₃ was added per litre and the vessel was sealed. The medium was then sparged with 7.5% CO₂ in N₂ for 1 h at a flow rate of approximately 100 ml min⁻¹ and then sterilized by autoclaving. After the medium had cooled to room temperature, the following solutions were injected (per litre) into the medium bottles, in order, from oxygen-free stocks (except for cysteine, which was made fresh and filtered-sterilized directly into the medium): 1.5 ml of 0.5 M KHPO₄ buffer, pH 6.9, neutralized cysteine·HCl·H₂O to give a final concentration of 0.04 g l⁻¹, 10 ml of 25% (w/v) Na₂S₂O₃·5H₂O, and 0.5 ml vitamin solution (Frankel et al., 1997). The medium was allowed to become reduced (i.e. colourless), after which 2.5 ml of 0.01 M FeSO₄ dissolved in 0.2 M HCl was injected. The medium was inoculated with several bands of cells from semi-solid medium, after which sterile O₂ was introduced (0.4% of the final headspace), and carefully placed at 25 °C, so as not to disturb the nascent oxygen concentration ([O₂]) gradient. The [O₂] gradient that became established was clearly evident as indicated by the surface of the medium becoming pink while the remaining medium remained colourless. Growth was initiated at the oxic–anoxic interface near the surface and, as growth increased, O₂ was replenished in the headspace. In the initial growth stage (<5 days), observable growth was restricted to a thin white pellicle close to the surface of the medium. The amount of O₂ introduced was increased to a maximum of 4% of the headspace (delivered every 24–48 h). Nevertheless, the medium could not be mixed without adversely disrupting growth of the culture, and growth was restricted almost entirely to the surface, which was visible as a white pellicle.

Genomic DNA was isolated from cell pellets of strain MMS-1ᵀ by using the extraction protocol described by Bazylnski et al. (2004). For PCR amplification of the 16S rRNA gene, we initially used domain Bacteria-specific primers 27F (5’-AGAGTTTGATCCTGGCTC-3’) and 1387R (5’-GGGCGGWGTGTACAAGGC-3’) (Weisburg et al., 1991; Marchesi et al., 1998). Once identified as a member of the Alphaproteobacteria based on the sequence obtained, a further sequence was then obtained in the 3’ direction by using primer 27F in conjunction with primer 16SR1 (5’-CGGCTACCTTGTTACGACTTC-3’), the latter targeting the 16S rRNA gene of Alphaproteobacteria, giving the nearly full-length 16S rRNA gene. Based on 16S rRNA gene sequence analysis, strain MMS-1ᵀ was considered to represent a member of a novel genus of the family Rhodospirillaceae, not closely related to any previously described genus or species of magnetotactic bacterium (Fig. 3). However, strain MMS-1ᵀ was closely related to the uncharacterized marine magnetotactic spirillum strain QH-2, isolated from an intertidal zone of the China Sea (Zhu et al., 2010) (Fig. 3), and with which it shared highest 16S rRNA gene sequence similarity (97%), which we therefore interpret as congeneric with strain MMS-1ᵀ. Thus, strains MMS-1ᵀ and QH-2 represent a new lineage of magnetotactic bacteria within the Alphaproteobacteria. The next closest match (92.6% 16S rRNA gene sequence

Fig. 1. Transmission electron micrographs of cells of strain MMS-1ᵀ. Negatively stained cells showing variations in morphology, from lima bean-shaped (a) to partially bean-shaped and partially helical (b). Note the presence of a single polar flagellum at each end of the cell. (c) Thin-section of a fully helical cell showing a chain of elongated prismatic magnetite magnetosomes (Meldrum et al., 1993) that traverse the cell along its long axis and the typical Gram-negative-type cell wall. (d) High-magnification thin section of magnetite magnetosomes showing the presence of a magnetosome membrane (arrows). Bars, 0.5 μm (a–c) and 50 nm (d).
MMS-1T is thus considered to represent a member of a weakly supported phylogenetic lineage within the *Rhodospirillaceae* (Fig. 3). As well as strain MMS-1T and *Ca. Magnetovibrio blakemorei*, this lineage also includes *Terasakiella pusilla* (basonym *Oceanospirillum pusillum*; Satomi et al., 2002) and *Thalassospira lucentensis* (López-López et al., 2002), and an undescribed marine biofilm bacterium (*Candidatus Koprimonimus byunsanensis*’ KOPRI 13522) (Fig. 3). Our phylogenetic analysis finds support for inclusion of these strains within the *Rhodospirillaceae*, and representing a phylogenetic lineage distinct from *Magnetospirillum*. Strain MMS-1T can be clearly differentiated from other described members of this lineage (Table 1).

Strain MMS-1T is clearly capable of chemolithoautotrophic growth, based on a number of lines of evidence, including the complete incorporation of radiolabelled CO₂ into cellular protein carbon, for cultures grown in semi-solid, [O₂]-gradient growth medium containing 10 mM Na₂S₂O₃ as the electron source and ¹⁴C-bicarbonate/CO₂ as the sole carbon source (Table S1 in IJSEM Online). Interestingly, cells did not grow with sulfide in [O₂]-gradient growth medium as the sole electron donor.

To determine the pathway for CO₂ fixation and autotrophy, we determined activities of key enzymes of the Calvin–Benson–Bassham (CBB) and the reverse or reductive tricarboxylic acid (rTCA) cycle in strain MMS-1T as some magnetotactic bacteria have previously been shown to use these cycles for autotrophy (Bazylinski et al., 2004; Williams et al., 2006). Cell-free extracts from cells of strain MMS-1T grown with thiosulfate were prepared as described by Williams et al. (2006). Ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) activity of cell extracts of strain MMS-1T was determined as described by Beudeker et al. (1980), except that the dithiothreitol concentration was changed to 5 mM and the pH was adjusted to 7.0. Cell-free extracts of strain MMS-1T showed a specific Rubisco activity of 12.3 nmol min⁻¹ (mg cell protein)⁻¹. No activity was detected when the cell-free extract was boiled prior to running the assay and when the substrate, ribulose-1,5-bisphosphate, was omitted from the reaction mix. The activities of three key enzymes of the rTCA cycle for carbon fixation were tested by using spectrophotometric kinetic assays following the methods given by Williams et al. (2006): fumarate reductase, pyruvate:acceptor oxidoreductase and 2-oxoglutarate:acceptor oxidoreductase. All reactions were carried out in oxygen-free conditions at 25 °C, in serum-stoppered cuvettes under N₂ and were initiated by injection of cell extract. Cell-free extracts of strain MMS-1T showed no activity for any of three key enzymes for the rTCA cycle that were tested. In addition, we were able to amplify a partial Rubisco type II gene (*cbbM*) by using PCR and the *cbbM*-specific primers RuIIF1 and RuIIR3 (Spiridonova et al., 2004). The closest match of the CbbM protein (90%
amino acid sequence identity) was to the CbbM of ‘Ca. Magnetovibrio blakemorei’ MV-1 (GenBank accession no. AAL76921).

Genomic DNA G+C content was determined based on genomic DNA thermal denaturation curves at 260 nm, by using a Beckman DU650 spectrophotometer (Beckman-Thalassospira lucentensis DSM 14000T (AM294944)

Terasakiella pusilla IFO 13613T (AB006768)

‘Candidatus Koprimonas byunsanensis’ KOPRI 13522 (DQ167245)

**Magnetospira thiophila** gen. nov., sp. nov.

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**Table 1.** Differential characteristics of strain MMS-1T and related members of the family *Rhodospirillaceae*

<table>
<thead>
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<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>Helical–spiral</td>
<td>Vibrioid–helical</td>
<td>Spiral</td>
<td>Vibrioid–spiral</td>
</tr>
<tr>
<td>Flagella</td>
<td>Two (one per pole)</td>
<td>Single polar</td>
<td>Polar tuft</td>
<td>Single polar</td>
</tr>
<tr>
<td>Magnetotaxis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>O2 requirement</td>
<td>Obligately microaerophilic</td>
<td>Facultatively anaerobic, microaerophilic</td>
<td>Obligately aerobic</td>
<td>Obligately aerobic</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Chemolithoautotrophic, chemoorganoheterotrophic</td>
<td>Chemolithoautotrophic, chemoorganoheterotrophic</td>
<td>Chemoorganoheterotrophic</td>
<td>Chemoorganoheterotrophic</td>
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<tr>
<td>Catalase</td>
<td>−</td>
<td>−</td>
<td>V</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>47.2</td>
<td>53.5</td>
<td>48.0</td>
<td>54.7</td>
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</tbody>
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Genomic DNA G + C content was determined based on genomic DNA thermal denaturation curves at 260 nm, by using a Beckman DU650 spectrophotometer.

**Fig. 3.** Neighbour-joining tree based on 16S rRNA gene sequences showing the position of strain MMS-1T in the family *Rhodospirillaceae*, class Alphaproteobacteria. ‘Candidatus Magnetobacterium bavaricum’ (member of the ‘Nitrospirae’) and *Bacteroides uniformis* JCM 5828T (member of the Bacteroidetes) were used as outgroups. Accession numbers of 16S rRNA gene sequences retrieved from the databases are given in parentheses. Bootstrap values at nodes are percentages of 1000 replicates; only values >50% are shown. Bar, 5% sequence divergence.
The DNA G+C content of strain MMS-1T was 47.2 ± 0.2 mol% (mean ± SD of six replicates).

Phospholipid ester-linked fatty acid profiles of strain MMS-1T and their isotopic compositions are shown in Table S2. Although it is difficult to determine absolutely what stage of growth cells of strain MMS-1T are in when grown in liquid based on the observed growth (described above), cells for these analyses were harvested about 24 h after a large injection of oxygen to the culture and thus the culture was probably in late exponential growth. The concentration of head gas CO₂ was determined by using a Trace GC (Finnigan MAT) equipped with a Carboxen 1010 plot column (Supelco) under isothermal conditions at 100 °C. Carbon-isotope compositions of CO₂ in gas samples were determined on a ThermoQuest Delta plus XL isotope ratio mass spectrometer (Finnigan MAT) interfaced to a Trace GC-combustion unit. Lipid extraction of cells of strain MMS-1T was performed according to Zhang et al. (2004). GC analyses of fatty acid methyl esters were performed on an Agilent 6890 GC with flame-ionization detector, equipped with a 30 m DB-5 column (5 % phenyl) and programmable temperature vaporizing inlet. The column temperature programme for samples in hexane was an initial temperature of 60 °C, which was ramped at 10 °C min⁻¹ to 180 °C, and then 4 °C min⁻¹ to 320 °C at which the temperature was programmed to remain constant for 20 min. The injector and detector were held isothermally at 220 and 275 °C, respectively. GC-MS analyses of fatty acid methyl esters were performed on an HP5890 GC equipped with a 30 m, HP5-MSI column and programmable temperature vaporizing inlet, coupled to an HP5972 MS. Phospholipid ester-linked fatty acids of strain MMS-1 were 14 : 0 (1.2 %), 16 : 1o7t (31.2 %), 16 : 1o7c (1.4 %), 16 : 0 (26.6 %) and 20 : 0 (39.7 %). Fractionation between fatty acids and CO₂ ranged from −34.1 to −34.6‰, values consistent with those observed for bacteria that utilize the CBB cycle (Zhang et al., 2004). The combined results clearly show that strain MMS-1T uses the CBB cycle for CO₂ fixation and autotrophy.

Chemoautotrophic growth of strain MMS-1T was tested by using semi-solid agar medium that allowed the formation of a defined [O₂]/redox gradient in the tube. The basal medium was that described above except that no electron donor was included, NH₄Cl was added to give a final concentration of only 2 mM and the agar concentration was reduced to 1.2 g l⁻¹. After autoclaving, the medium was then placed in a 44 °C water bath, and when cooled, the following reagents were added (per litre), in order: 1.5 ml of 0.5 M KH₂PO₄ buffer, pH 6.9, neutralized cysteine, HCl, H₂O₂ to give a final concentration of 0.2 g l⁻¹ and 2.4 ml of 0.8 M NaHCO₃. The pH was checked to see if it was in the range 7.0–7.1. After the temperature vaporizing inlet. The column temperature programme for samples in hexane was an initial temperature of 60 °C, which was ramped at 10 °C min⁻¹ to 180 °C, and then 4 °C min⁻¹ to 320 °C at which the temperature was programmed to remain constant for 20 min. The injector and detector were held isothermally at 220 and 275 °C, respectively. GC-MS analyses of fatty acid methyl esters were performed on an HP5890 GC equipped with a 30 m, HP5-MSI column and programmable temperature vaporizing inlet, coupled to an HP5972 MS. Phospholipid ester-linked fatty acids of strain MMS-1 were 14 : 0 (1.2 %), 16 : 1o7t (31.2 %), 16 : 1o7c (1.4 %), 16 : 0 (26.6 %) and 20 : 0 (39.7 %). Fractionation between fatty acids and CO₂ ranged from −34.1 to −34.6‰, values consistent with those observed for bacteria that utilize the CBB cycle (Zhang et al., 2004). The combined results clearly show that strain MMS-1T uses the CBB cycle for CO₂ fixation and autotrophy.

Chemosynthesis (Fukushima et al., 2002). Any microaerophilic bands of cells of strain MMS-1T began to appear after 1–2 days, at the OATZ (as demarcated by the pink–colourless interface in the medium). After 7–10 days, any carbon sources that showed visible bands were transferred further by withdrawing >100 µl cell-containing medium from the band of one of the three replicate tubes via a 1 ml syringe, and inoculating 50 µl into duplicate tubes containing the same carbon source. The inoculum was delivered approximately 150 mm below the surface, just beneath the oxic–anoxic interface. The other two tubes for each carbon source were used for direct counts: formaldehyde was added to a final concentration of 0.15 %, thoroughly mixed in the semi-solid medium by inversion and a sample was taken from both tubes. With this method, only acetate, fumarate, malate and succinate bath after inoculation. Organic compounds tested as carbon sources were added to tubes to give a final concentration of 0.1 % (w/v or v/v). Compounds were neutralized when necessary; L-enantiomers were used for amino acids, D-enantiomers for sugars. The compounds tested were: acetate, citrate, fumarate, α-ketoglutarate, lactate, malate, maleate, malonate, oxalate, oxaloacetate, propionate, pyruvate, succinate, tartrate, urea, Casamino acids, peptone, tryptone, yeast extract, alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, valine, adonitol, amygdalin, arabinose, cellobiose, glucose, dulcitol, fructose, galactose, gluconic acid, inositol, α-lactose, lyxose, maltose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, ribose, sorbitol, sorbose, sucrose, trehalose and xylose. Each organic carbon source and controls were tested in triplicate. If growth was observed, it was transferred twice further on the same media with the same carbon source, in triplicate. Thiosulfate (10 mM) and succinate (0.1 %) were used as positive controls, and sterile H₂O₂ was used as the negative control. Immediately prior to inoculation, each tube was cooled and then inoculated with 100 µl of a suspension of cells of strain MMS-1T. The inoculum was derived from a culture of strain MMS-1T grown chemolithoautotrophically and microaerophilically on thiosulfate, as described above. Cells were harvested by centrifugation (6000 × g at 4 °C for 15 min), washed twice in a buffer of 20 mM Tris-HCl (pH 7.0) in the artificial seawater base, and resuspended in this buffer to a cell density of 10⁸ cells ml⁻¹. Immediately after inoculation, each tube was gently inverted two to three times to ensure that the bacterial cells were evenly suspended, and then cooled to solidify the agar. After several minutes, the medium was placed at 25 °C under ambient light to promote formation of the oxygen-concentration gradient. High light intensities are known to photocatalyse the reduction of resazurin N-oxide in the presence of cysteine in anaerobic growth medium and we noted that it also appeared to reduce the resazurin in the anaerobic portion of the oxygen-concentration gradient medium described here (Fukushima et al., 2002).
resulted in chemooorganoheterotrophic growth of strain MMS-1T, indicating that it utilizes only a small range of organic acids as carbon and energy sources for chemooorganoheterotrophic growth.

Anaerobic growth was tested under chemolithoautotrophic and chemooorganoheterotrophic conditions in semi-solid media by using thiosulfate or succinate as electron donors, respectively. The headspace gas of the cultures was analyzed for CO2 as the terminal electron acceptor. Cells only grew with O2 as the terminal electron acceptor under microaerophilic conditions and did not grow anaerobically with nitrate (2 and 5 mM), nitrite (2 mM), nitrous oxide (1 atm in the headspace), DMSO (15 mM), fumarate (20 mM), sulfite (5 mM), thiosulfate (10 mM), sulfate (10 mM) or trimethylamine oxide (15 mM). These results therefore show that strain MMS-1T is an obligate microaerophile.

As with almost all cultured magnetotactic bacteria tested (Bazylinski & Williams, 2007), strain MMS-1T displayed nitrogenase activity (the ability to reduce acetylene to ethylene) under microaerophilic conditions (Table S3), when a fixed nitrogen source was absent from the growth medium (with the exception of cysteine, the reducing agent, and nitrilotriacetic acid, which was used as a chelating agent in the mineral solution). These results indicate that strain MMS-1T is capable of nitrogen fixation and that cysteine and nitrilotriacetic acid cannot be used as sources of nitrogen.

On the basis of the data presented here, strain MMS-1T is considered to represent a novel species of a new genus, for which the name Magnetospira thiophila gen. nov., sp. nov. is proposed.

Description of Magnetospira gen. nov.

Magnetospira (Ma.gne.to.spi’ra. L. n. magnes -etis a magnet; N.L. pref. magneto- pertaining to a magnet; L. fem. n. spira a spiral; N.L. fem. n. Magnetospira the magnetic spiral, with reference to the spiral morphology and magnetotactic behaviour of this bacterium). Cells are variable in morphology, ranging from a truncated spirillum (lima bean-shaped) to fully helical (Meldrum et al., 1993). Gram-negative. Assimilates inorganic carbon (as CO2) chemolithoautotrophically with S2O2- as the electron donor by using the CBB cycle. Has form II RubisCO (CbbM). Motile by means of bipolar flagella (amphitrichous), with a single flagellum at each pole. Exhibits only polar magnetotaxis, and biomineralizes a single chain of magnetosomes that contain elongated cuboctahedral magnetite crystals positioned along the long axis of the cell. Belongs to the Rhodospirillaceae, within the Alphaproteobacteria. The type species is Magnetospira thiophila.

Description of Magnetospira thiophila sp. nov.

Magnetospira thiophila [θi’o.phila.la. Gr. n. theion (Latin transliteration thium) sulfur; N.L. adj. philus -a -um (from Gr. adj. philos -ē-on) friend, loving; N.L. fem. adj. thiophila sulfur-loving, with reference to the utilization of thiosulfate as an energy source for chemolithoautotrophic growth]. Exhibits the following characteristics in addition to those given for the genus. Cells are 1–3 μm long by 0.2–0.5 μm wide. Cells occur mostly singly, but also in pairs, chains and clumps. Obligately microaerophilic. Catalase- and oxidase-negative. Mesophilic, with a growth temperature range of 5–37 °C, and optimal growth temperature of 24–26 °C. Doubling time of 4 days in liquid media with CO2 as the carbon source, S2O2- as the electron donor and O2 as the electron acceptor (chemolithoautotrophic growth). Cells produce internal sulfur globules when grown on S2O2-. Utilizes the organic acids acetate, fumarate, malate and succinate for chemooorganoheterotrophic growth.

The type strain, MMS-1T (=ATCC BAA-1438T =JCM 17960T), was isolated from mud and water from a salt marsh in Woods Hole, Massachusetts, USA. The DNA G+C content of the type strain is 47.2 mol%.

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