Thiomicrospira hydrogeniphila sp. nov., an aerobic, hydrogen- and sulfur-oxidizing chemolithoautotroph isolated from a seawater tank containing a block of beef tallow

Tomo-o Watsuji, Emi Hada, Masayuki Miyazaki, Masako Ichimura and Ken Takai

A moderately psychrophilic, aerobic, hydrogen- and sulfur-oxidizing bacterium, designated strain MAS2\textsuperscript{T}, was isolated from a tank containing coastal seawater from Tokyo Bay and a block of beef tallow added as organic material. Growth occurred under aerobic chemolithoautotrophic conditions in the presence of molecular hydrogen, thiosulfate, tetrathionate, elemental sulfur or sulfide as the sole energy source and bicarbonate as a carbon source. The isolate represented a Gram-staining-negative rod with a single polar flagellum and grew in artificial seawater medium with thiosulfate at 2–40 °C (optimum 30 °C). The isolate grew in media with thiosulfate at Na\textsuperscript{+} concentrations between 30 and 1380 mM (optimum 270 mM). MAS2\textsuperscript{T} possessed C\textsubscript{16:0}, C\textsubscript{16:1} and C\textsubscript{18:1} as the major fatty acids. The G+C content of the genomic DNA was 39.6 mol%. The 16S rRNA gene sequence similarity analysis showed that the isolate represented a member of the genus Thiomicrospira within the class Gammaproteobacteria and was most closely related to Thiomicrospira frisia JB-A2\textsuperscript{T}. On the basis of phenotypic and molecular properties, the isolate represents a novel species of the genus Thiomicrospira, for which the name Thiomicrospira hydrogeniphila sp. nov. is proposed (type strain, MAS2\textsuperscript{T} = JCM 30760\textsuperscript{T} = DSM 100274\textsuperscript{T}).

Species of the genus Thiomicrospira have been detected in various environments, including deep-sea hydrothermal vents, continental shelf sediments, intertidal mud flats and freshwater ponds (Brinkhoff & Muyzer, 1997). The previously described species are sulfur-oxidizing chemolithoautotrophic bacteria and are able to utilize reduced sulfur compounds, such as sulfide, thiosulfate and elemental sulfur, as the energy sources and CO\textsubscript{2} as a carbon source (Brinkhoff et al., 1999a; Jannasch et al., 1985). In addition, Thiomicrospira thermophila 178\textsuperscript{T}, Thiomicrospira crunogena TH-55\textsuperscript{T} and Thiomicrospira sp. strain L-12 are capable of chemolithomixotrophic growth with reduced sulfur compounds (Takai et al., 2004). Although it was described as a member of a different genus, Hydrogenovibrio marinus MH-110\textsuperscript{T} should be classified as a member of the genus Thiomicrospira (Takai et al., 2004) and can use both reduced sulfur compounds and molecular hydrogen (H\textsubscript{2}) as the energy sources (Nishihara et al., 1991). It is also a unique hydrogen-oxidizing bacterium with high O\textsubscript{2} tolerance and is able to grow even under a gas phase of 40 % O\textsubscript{2} (Nishihara et al., 1989), because a hydrogenase, a membrane-bound respiratory [NiFe]-hydrogenase (MBH), of this strain exhibits extraordinarily high tolerance to O\textsubscript{2} (Yoon et al., 2011). The X-ray crystallographic analysis revealed that the proximal iron–sulphur (Fe–S) cluster of MBH had a [4Fe–3S] structure (Shomura et al., 2011). It has been proposed that O\textsubscript{2} reduction in the unique Fe–S cluster prevents the enzyme from entering the inactive state (Shomura et al., 2011). The hydrogenotrophic growth of H. marinus MH-110\textsuperscript{T} has been regarded as an exceptional physiological feature within the genus Thiomicrospira. Nevertheless, it has been known that the genome sequence of T. crunogena strain XCL-2 contains a full genetic repertoire of MBH although the strain cannot grow with H\textsubscript{2} under the laboratory culture conditions (Scott et al., 2006). Thus, it remains unclear whether the capability of hydrogenotrophic growth is more broadly distributed among the members of genus Thiomicrospira in their natural habitats. In this study, we report the isolation of a novel H\textsubscript{2}- and sulfur-oxidizing Thiomicrospira strain, MAS2\textsuperscript{T}, using CO\textsubscript{2} as a carbon source from a sulfidic tank with coastal...
seawater containing a block of beef tallow. The name *Thi-microspira hydrogeniphila* sp. nov. is proposed for MAS2.<sup>T</sup>. Surface seawater of Tokyo bay (35° 19.175’N, 139° 39.070’E) was collected from the dock of JAMSTEC in July 2013. A 140 l sample of natural seawater was incubated at 10 °C in a 200 l tank (105×46×42 cm). The natural seawater was supplemented with 5 kg of beef tallow. Seawater contains abundant sulfate and it is known that long-chain fatty acids can be degraded by sulfate-reducing bacteria (SRB) in marine environments under anoxic conditions (Aeckersberg et al., 1998). Thus, during the incubation of natural seawater supplemented with beef tallow, we expected that certain SRB populations would grow with beef tallow and sulfate in relatively anoxic microhabitats in the tank to produce sulfide during growth (Oremland & Taylor, 1978) and that sulfur-oxidizing bacteria would be concomitantly enriched in the tank. To investigate the natural microbial communities associated with the sulfur cycle driven by long long-chain fatty acids, the enrichment culture was conducted. The seawater in the tank was continuously agitated and filtered using a canister filter (Mega Power 9012; GEX). Water temperature was controlled with a water cooler (FZ-401AY; Rei-Sea). Once every 3 months, ammonium nitrate and potassium phosphate were added to the tank at a final concentration of 100 µM. After 2 months of incubation, a scum-like layer was formed on the surface of the seawater. To investigate the natural microbial communities associated with the sulfur cycle driven by long long-chain fatty acids, the enrichment culture was conducted. The seawater in the tank was continuously agitated and filtered using a canister filter (Mega Power 9012; GEX). Water temperature was controlled with a water cooler (FZ-401AY; Rei-Sea). Once every 3 months, ammonium nitrate and potassium phosphate were added to the tank at a final concentration of 100 µM. After 2 months of incubation, a scum-like layer was formed on the surface of the seawater. At 10 months of incubation, the scum-like layer was excitedly formed on the surface of the seawater. After 5 months of incubation, the scum-like material was suspended in 1 ml sterilized MJ synthetic seawater (Takai et al., 1999). The suspended slurry was used to inoculate a series of media including MMJH medium (described below) under a gas phase of 69% N<sub>2</sub>/25% H<sub>2</sub>/5% CO<sub>2</sub>/1% O<sub>2</sub> (200 kPa), and the cultures were then incubated at 15 °C for 2 weeks. The well-grown culture was further enriched by using the dilution-to-extinction technique at 15 °C with the same medium (Takai & Horikoshi, 2000). A pure culture was obtained by using an agar plate medium at 15 °C under atmosphere. The agar plate medium consisted of (per litre of distilled, deionized water) 25.0 g NaCl, 0.42 g K<sub>2</sub>HPO<sub>4</sub>, 0.29 g CaCl<sub>2</sub>, 1.0 g (NH₄)₂SO₄, 1.5 g MgSO₄·7H₂O, 0.3 g KCl, 10 ml trace mineral solution (Balch et al., 1979), 10 ml vitamin solution (Balch et al., 1979), 0.2 g NaHCO<sub>3</sub> and 5.0 g Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H₂O. After 1 week of incubation, only one colony type with a creamy white color was observed, in which elemental sulfur particles was not observed. An isolated colony was picked and successfully grew with fresh liquid MMJH medium under a gas phase of 69% N<sub>2</sub>/25% H<sub>2</sub>/5% CO<sub>2</sub>/1% O<sub>2</sub> (200 kPa). This culture was designated strain MAS2<sup>T</sup>. Purity was confirmed by microscopic examination and by repeated partial sequencing of the 16S rRNA gene using several PCR primers.

Cells were observed under a phase-contrast BX53 microscope (Olympus) equipped with a CCD camera system. Transmission electron microscopy of negatively stained cells was carried out as described previously (Zillig et al., 1990). Cells grown in MMJS medium (described below) at 30 °C under microaerobic conditions (1% partial pressure of O<sub>2</sub>) in the mid-exponential phase of growth were negatively stained with aqueous solution of 1% phosphotungstic acid (pH7.2) and were observed under a G2 20 electron microscope (FEI Tecnai) at an accelerating voltage of 200 kV. Cells of MAS2<sup>T</sup> were Gram-staining-negative, rods about 0.3–0.5 µm in diameter, 0.9–1.8 µm in length (Fig. S1, available in the online Supplementary Material), and were motile by means of a polar flagellum (Fig. S1). Morphological features of MAS2<sup>T</sup> were thus similar to those of *Thi-microspira frisia* JB-A2<sup>T</sup>, *Thi-microspira chilenis* Ch<sub>1</sub><sup>T</sup>, *Thi-microspira arctica* SVAL-E<sup>T</sup> and *Thi-microspira psychrophila* SVAL-D<sup>T</sup> and these organisms form a phylogenetic subcluster within the genus *Thi-microspira* (Fig. 1). Spore formation was not observed under any of the growth conditions examined.

MAS2<sup>T</sup> was routinely cultivated in MMJH medium. MMJH medium consists of (per litre of distilled, deionized water) 20.0 g NaCl, 0.14 g K<sub>2</sub>HPO<sub>4</sub>, 0.8 g CaCl<sub>2</sub>, 1.0 g NH₄Cl, 4.0 g MgSO₄·7H₂O, 3.0 g MgCl₂·6H₂O, 0.33 g KCl, 0.5 mg NiCl₂·6H₂O, 0.5 mg Na₂SeO<sub>3</sub>·5H₂O, 0.1 mg Na₂WO₄·0.01 g Fe(NO₃)₂(SO₄)₂·6H₂O, 10 ml trace mineral solution (Balch et al., 1979), 10 ml vitamin solution (Balch et al., 1979) and 0.3 g NaHCO<sub>3</sub>. To prepare MMJH medium, materials other than the vitamin solution and NaHCO<sub>3</sub> were dissolved, and the pH of the medium was adjusted to pH 5.5 with HCl before autoclaving. After autoclaving under an air atmosphere, a filter-sterilized and concentrated solution of vitamins and NaHCO<sub>3</sub> was added to the medium under gas purging with 80% N<sub>2</sub>/20% CO<sub>2</sub>, and the pH was readjusted to pH 6.0 with HCl at room temperature, if necessary. The 20 ml medium was anaerobically dispensed in vials (V-50; Nichiden-Rika Glass) with gas purging and was tightly sealed with a butyl-rubber stopper under a gas phase of 69% N<sub>2</sub>/25% H<sub>2</sub>/5% CO<sub>2</sub>/1% O<sub>2</sub> (200 kPa). MAS2<sup>T</sup> was also cultivated in MMJS medium. MMJS medium consists of MMJH medium with 5 mM Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H₂O, and the gas phase in the vials was adjusted to 94% N<sub>2</sub>/5% CO<sub>2</sub>/1% O<sub>2</sub> (200 kPa).

Growth of MAS2<sup>T</sup> was measured by direct cell counting after staining with 4,6-diamidino-2-phenylindole (DAPI) (Porter & Feig, 1980). Triplicate cultures were examined under each condition. With MMJS medium, MAS2<sup>T</sup> grew over a temperature range of about 2–40 °C and showed optimal growth at 30 °C. The effect of pH on growth was tested at 30 °C, using MMJS medium adjusted to various pH values with 30 mM acetate/acetic acid buffer (pH 4–5), MES (pH 5–6), PIPES (pH 6–7), HEPES (pH 7–7.5) and Tris (pH 8–9.5) at room temperature. Growth in MMJS media was optimal at pH 7.5 with HCl before autoclaving. After autoclaving under an air atmosphere, a filter-sterilized and concentrated solution of vitamins and NaHCO<sub>3</sub> was added to the medium under gas purging with 80% N<sub>2</sub>/20% CO<sub>2</sub>, and the pH was readjusted to pH 6.0 with HCl at room temperature, if necessary. The 20 ml medium was anaerobically dispensed in vials (V-50; Nichiden-Rika Glass) with gas purging and was tightly sealed with a butyl-rubber stopper under a gas phase of 69% N<sub>2</sub>/25% H<sub>2</sub>/5% CO<sub>2</sub>/1% O<sub>2</sub> (200 kPa). MAS2<sup>T</sup> was also cultivated in MMJS medium. MMJS medium consists of MMJH medium with 5 mM Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H₂O, and the gas phase in the vials was adjusted to 94% N<sub>2</sub>/5% CO<sub>2</sub>/1% O<sub>2</sub> (200 kPa). Growth of MAS2<sup>T</sup> was measured by direct cell counting after staining with 4',6-diamidino-2-phenylindole (DAPI) (Porter & Feig, 1980). Triplicate cultures were examined under each condition. With MMJS medium, MAS2<sup>T</sup> grew over a temperature range of about 2–40 °C and showed optimal growth at 30 °C. The effect of pH on growth was tested at 30 °C, using MMJS medium adjusted to various pH values with 30 mM acetate/acetic acid buffer (pH 4–5), MES (pH 5–6), PIPES (pH 6–7), HEPES (pH 7–7.5) and Tris (pH 8–9.5) at room temperature. Growth in MMJS
medium occurred at pH 5.0–8.0, with the optimum growth at about pH 6.0. The pH was found to be stable during the cultivation period and no apparent inhibitory effect on growth was seen with any of the buffer systems. MAS2\textsuperscript{T}, when tested in MMJS medium with variable NaCl content, grew over the Na\textsuperscript{+} concentration range of 30–1380 mM (optimum 270 mM) at 30 °C and pH 6.0.

The effect of O\textsubscript{2} in the gas phase on growth of MAS2\textsuperscript{T} was tested with MMJS medium under a gas mixture of 95% N\textsubscript{2}/5% CO\textsubscript{2}, 94.9% N\textsubscript{2}/5% CO\textsubscript{2}/0.1% O\textsubscript{2}, 94.5% N\textsubscript{2}/5% CO\textsubscript{2}/0.5% O\textsubscript{2}, 94% N\textsubscript{2}/5% CO\textsubscript{2}/1% O\textsubscript{2}, 90% N\textsubscript{2}/5% CO\textsubscript{2}/5% O\textsubscript{2}, 85% N\textsubscript{2}/5% CO\textsubscript{2}/10% O\textsubscript{2}, 75% N\textsubscript{2}/5% CO\textsubscript{2}/20% O\textsubscript{2} or 55% N\textsubscript{2}/5% CO\textsubscript{2}/40% O\textsubscript{2} at 200 kPa and with MMJH medium under a gas mixture of 70% N\textsubscript{2}/25% H\textsubscript{2}/5% CO\textsubscript{2}, 69.9% N\textsubscript{2}/25% H\textsubscript{2}/5% CO\textsubscript{2}/0.1% O\textsubscript{2}, 69.5% N\textsubscript{2}/25% H\textsubscript{2}/5% CO\textsubscript{2}/0.5% O\textsubscript{2}, 69% N\textsubscript{2}/25% H\textsubscript{2}/5% CO\textsubscript{2}/1% O\textsubscript{2}, 65% N\textsubscript{2}/25% H\textsubscript{2}/5% CO\textsubscript{2}/5% O\textsubscript{2}, 60% N\textsubscript{2}/25% H\textsubscript{2}/5% CO\textsubscript{2}/10% O\textsubscript{2}, 50% N\textsubscript{2}/25% H\textsubscript{2}/5% CO\textsubscript{2}/20% O\textsubscript{2} or 30% N\textsubscript{2}/25% H\textsubscript{2}/5% CO\textsubscript{2}/40% O\textsubscript{2} at 200 kPa. In the absence of oxygen, either 10 mM nitrate or 10 mM fumarate was added to MMJS medium as a potential, alternative electron acceptor. Growth of MAS2\textsuperscript{T} in both media was observed under a gas phase in the presence of 0.1–40% O\textsubscript{2} and the maximum cell yield after 2 weeks of cultivation in MMJS and MMJH medium was under a gas phase with 5–40% O\textsubscript{2} and 5–20% O\textsubscript{2}, respectively. These results indicated that strain MAS2\textsuperscript{T} was able to grow using thiosulfate or H\textsubscript{2} as an energy source under fully aerobic conditions.

Heterotrophic growth was tested in MMJS medium without NaHCO\textsubscript{3} under a gas phase of 99% N\textsubscript{2}/1% O\textsubscript{2} (200 kPa), containing each of the following substrates as a potential organic carbon source: 0.1% (w/v) yeast extract, 0.1% (w/v) peptone, 0.1% (w/v) tryptone, 0.1% (w/v) casein, 0.1% (w/v) starch, 0.1% (w/v) carboxymethylcellulose, 0.1% (w/v) casamino acids, 5 mM formate, 5 mM acetate, 5 mM glycerol, 5 mM citrate, 5 mM tartrate, 5 mM fumarate, 5 mM malate, 5 mM succinate, 5 mM propionate, 5 mM lactate, 5 mM oxalate, 5 mM pyruvate, 5 mM of each of 20 amino acids, 0.02% (w/v) glucose, 0.02% (w/v) galactose, 0.02% (w/v) sucrose, 0.02% (w/v) fructose, 0.02% (w/v) lactose, 0.02% (w/v) malonate and 0.02% (w/v) trehalose. None of the organic carbon sources supported heterotrophic growth using thiosulfate as an energy source and molecular oxygen as an electron acceptor.

Utilization of these organic compounds as alternative energy sources instead of thiosulfate was also examined in MMJS medium in the absence of thiosulfate under a gas phase of 94% N\textsubscript{2}/5% CO\textsubscript{2}/1% O\textsubscript{2} (200 kPa). However, none of the organic compounds sustained the growth of MAS2\textsuperscript{T}. In an attempt to determine potential electron donors other than thiosulfate and H\textsubscript{2} for the autotrophic growth, sulfide (0.25, 0.5, 1, 2 or 5 mM), sulfate (1 or 5 mM), elemental sulfur (3%; w/v), cysteine/HCl (0.25, 0.5, 1, 2 or 5 mM) or tetrathionate (1 or 5 mM) was tested instead of thiosulfate in MMJS medium with a gas phase of 94% N\textsubscript{2}/5% CO\textsubscript{2}/1% O\textsubscript{2} (200 kPa). MAS2\textsuperscript{T} grew autotrophically on sulfide, elemental sulfur and tetrathionate, but not on sulfite or cysteine. To test for the utilization of electron acceptors, nitrate (10 mM), nitrite (1 or 5 mM), ferric citrate (20 mM), ferrihydrite (20 mM), selenite (5 mM) or fumarate (10 mM) was tested with MMJS medium under 95% N\textsubscript{2}/5% CO\textsubscript{2} (200 kPa). None of the electron acceptors other than O\textsubscript{2} supported the growth of MAS2\textsuperscript{T} in MMJS medium. The potential factors required for growth, such as selenite, tungstate and vitamins, were examined with MMJS medium with and without the specified nutrients. Selenium, tungsten and vitamins were not required for growth of MAS2\textsuperscript{T}. Potential inorganic nitrogen sources (NH\textsubscript{4}Cl,
The 16S rRNA gene sequence was analyzed using the strain JB-A2 found to be most closely related to the sequences of from a shallow-water hydrothermal vent. The 16S rRNA gene was amplified in almost complete 16S rRNA genes, purified by Teflon-lined, screw-capped tube containing 3 ml anhydrous medium with an Na\(^+\) concentration of 140 mM under a gas phase of 65 % N\(_2\)/25 % H\(_2\)/5 % CO\(_2\)/5 % O\(_2\) (200 kPa) and MMJH medium with an Na\(^+\) concentration of 430 mM under a gas phase of 90 % N\(_2\)/10 % CO\(_2\) (200 kPa) and the cultures were stirred continuously. The maximum growth rate of MAS2\(^T\) was found to be 0.4 h\(^{-1}\) in MMJS medium and 0.6 h\(^{-1}\) in MMJH medium.

MAS2\(^T\) in MMJS medium was sensitive to a variety of antibiotics, including chloramphenicol (50 µg ml\(^{-1}\)), streptomycin (50 µg ml\(^{-1}\)), ampicillin (50 µg ml\(^{-1}\)) and rifampicin (50 µg ml\(^{-1}\)) and was resistant to vancomycin (50 µg ml\(^{-1}\)).

The cellular fatty acid composition was analyzed using cells grown at 30 °C in MMJS medium in the late-exponential phase of growth. Lyophilized cells (100 mg) were placed in a Teflon-lined, screw-capped tube containing 3 ml anhydrous methanolic HCl and it was heated at 100 °C for 3 h. Extraction and analysis of fatty acid methyl esters were as described previously (Takai et al., 2003). The relative amounts of the major cellular fatty acids (C\(_{16:0}\) C\(_{16:1}\) C\(_{18:1}\)) of MAS2\(^T\) were similar to those of \textit{T. chilensis} strain Ch-1\(^T\) and \textit{T. arctica} strain SVAL-E\(^T\) (Knittel et al., 2005), but three fatty acid components (C\(_{12:1}\) C\(_{14:1}\) C\(_{14:1}\) 3-OH) could not be detected in the case of MAS2\(^T\) (Table 1).

Genomic DNA of MAS2\(^T\) grown at 30 °C in MMJS was prepared as described by Marmur \& Doty (1962). \textit{T. frisia} strain JB-A2\(^T\) was purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) and subjected to G+C content analysis and DNA–DNA hybridization. The G+C content of DNA was determined by reversed-phase HPLC with a DNA-GC kit (Yamasu Shoyu) after digestion with nuclease P1 (Tamaoka \& Komagata, 1984). The G+C content of the genomic DNA of MAS2\(^T\) was found to be 39.6 mol\%, which is the same as that of \textit{T. frisia} strain JB-A2\(^T\) (39.6 mol\%) (Brinkhoff et al., 1999b) (Table 2).

PCR amplification of almost complete 16S rRNA genes, purification of PCR products and subsequent sequencing analysis were performed as described previously (Watsui et al., 2010). The 16S rRNA gene sequence was analyzed using the gapped-BLAST search algorithm (Altschul et al., 1997) and was found to be most closely related to the sequences of \textit{T. frisia} strain JB-A2\(^T\) (98.7 %) (Brinkhoff et al., 1999b), \textit{Thiomicrospira} sp. strain Art-3 (98.5 %) (Brinkhoff \& Muyzer, 1997), isolated from sediments of saline spring and \textit{Thiomicrospira} sp. strain Milos-T2 (97.6 %) (Brinkhoff et al., 1999c), isolated from a shallow-water hydrothermal vent. The 16S rRNA gene sequence similarity with the other species of the genus \textit{Thiomicrospira} was below 97 %. The 16S rRNA gene sequence was distantly related to the sequence of \textit{Hydrogenovibrio marinus} MH-110\(^T\) (93.7 %), the only strain within the genus \textit{Thiomicrospira} capable of hydrogenotrophic growth (Nishihara et al., 1998). The nearly complete sequence was applied to the phylogenetic tree reconstruction with the ARB software package (Ludwig et al., 2004). Evolutionary distance matrix analysis (using the Jukes–Cantor correlation method) and neighbour-joining analysis were performed using ARB (Fig. 1). Bootstrap analysis was performed to provide confidence estimates for the phylogenetic tree topology. The phylogenetic tree indicated that MAS2\(^T\) was most closely related to \textit{T. frisia} JB-H2\(^T\) (Brinkhoff et al., 1999b) (Fig. 1).

The generally recommended and accepted criteria for delineating bacterial species state that strains with 16S rRNA gene sequence dissimilarity of greater than 3 % are considered to belong to separate species (Stackebrandt et al., 2002; Stackebrandt \& Goebel, 1994). The generally recognized criteria for delineating bacterial species state that strains with a DNA–DNA relatedness of less than 70 %, as measured by hybridization, represent separate species (Wayne et al., 1987). Thus, DNA–DNA hybridization between MAS2\(^T\) and \textit{T. frisia} JB-A2\(^T\) was carried out at 40.5 °C for 4 h and measured fluorometrically using the microplate reader model POWERSCAN HT (BioTek) as described by Ezaki et al. (1989). The result showed less than 20 % relatedness, and these two strains were clearly separate, representing distinct species according to the recommendations of Wayne et al. (1987).

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**Table 1. Cellular fatty acid composition of species of the genus Thiomicrospira**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Saturated fatty acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C(_{12:0})</td>
<td>2.1</td>
<td>ND</td>
<td>2.4</td>
</tr>
<tr>
<td>C(_{14:0})</td>
<td>0.8</td>
<td>ND</td>
<td>0.8</td>
</tr>
<tr>
<td>C(_{16:0})</td>
<td>16.9</td>
<td>18.9</td>
<td>12.7</td>
</tr>
<tr>
<td>C(_{18:0})</td>
<td>1.9</td>
<td>3.5</td>
<td>0.08</td>
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<tr>
<td>Unsaturated fatty acids</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C(_{12:1})</td>
<td>ND</td>
<td>3.4</td>
<td>3.2</td>
</tr>
<tr>
<td>C(_{14:1})</td>
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<tr>
<td>C(_{16:1})</td>
<td>44.4</td>
<td>43.4</td>
<td>39.1</td>
</tr>
<tr>
<td>C(_{18:1})</td>
<td>30.4</td>
<td>27.8</td>
<td>26.5</td>
</tr>
<tr>
<td>Hydroxy fatty acids</td>
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</tr>
<tr>
<td>C(_{10:0}) 3-OH</td>
<td>0.5</td>
<td>1.7</td>
<td>0.4</td>
</tr>
<tr>
<td>C(_{14:1}) 3-OH *</td>
<td>ND</td>
<td>2.1</td>
<td>1.6</td>
</tr>
</tbody>
</table>

*Classification uncertain.*

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NaNO\(_3\), NaNO\(_3\) or N\(_2\) for growth were also examined with MMJS medium as the sole nitrogen source. MAS2\(^T\) utilized ammonium as the inorganic nitrogen source but could not utilize nitrate, nitrite and molecular nitrogen. These results indicated that MAS2\(^T\) was a chemolithoautotroph, utilizing the reduced sulfur compounds such as thiosulfate, tetrathionate, elemental sulfur, sulfide and H\(_2\) as the energy sources and O\(_2\) as the sole electron acceptor.

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The cells of MAS2\textsuperscript{T} and other organisms forming a phylogenetic subcluster within the genus *Thiomicrospira* (Fig. 1) are rods and grow autotrophically with reduced sulfur compounds as electron donors (Table 2). Although MAS2\textsuperscript{T} is closely related to *T. frisia* JB-A2\textsuperscript{T} in phylogenetic analysis based on 16S rRNA gene sequences, the DNA–DNA hybridization reveals that they should be strictly assigned to different species. The major cellular fatty acids composition of MAS2\textsuperscript{T} is similar to those of *T. chilensis* Ch-1\textsuperscript{T} and *T. arctica* SVAL-E\textsuperscript{T} in the phylogenetic subcluster (Table 1). However, MAS2\textsuperscript{T} is able to grow with not only reduced sulfur compounds but also with H\textsubscript{2} as an energy source. Phylogenetic analysis based on 16S rRNA gene sequences reveals that MAS2\textsuperscript{T} is distantly related to *H. marinus* MH-110\textsuperscript{T}, which has been known as only hydrogen-oxidizing bacteria within the genus *Thiomicrospira* (Fig. 1). Meanwhile, MAS2\textsuperscript{T} as well as *H. marinus* MH-110\textsuperscript{T} are able to grow under a gas phase of 40\% O\textsubscript{2} (Nishihara et al., 1989). On the basis of these physiological and genetic properties, we suggest that MAS2\textsuperscript{T} is representative of a novel species of the genus *Thiomicrospira*, for which the name *Thiomicrospira hydrog eniphila* sp. nov. is proposed.

**Table 2. Comparison of properties among *T. hydrogeniphila* MAS2\textsuperscript{T} and related species**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1*</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell shape</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Vibrio</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>39.6</td>
<td>39.6</td>
<td>49.3</td>
<td>42.4</td>
<td>42.5</td>
<td>44.1</td>
</tr>
<tr>
<td>Maximum growth rate (h\textsuperscript{-1})</td>
<td>0.4</td>
<td>0.45</td>
<td>0.4</td>
<td>0.14</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Optimal pH</td>
<td>6.0</td>
<td>6.5</td>
<td>7.0</td>
<td>7.3–8.0</td>
<td>7.5–8.5</td>
<td>6.5</td>
</tr>
<tr>
<td>pH range</td>
<td>5.0–8.0</td>
<td>4.2–8.5</td>
<td>5.3–8.5</td>
<td>6.5–9.0</td>
<td>6.5–9.0</td>
<td>ND</td>
</tr>
<tr>
<td>Optimal temperature (°C)</td>
<td>30</td>
<td>32–35</td>
<td>32–37</td>
<td>11.5–13.2</td>
<td>14.6–15.6</td>
<td>37</td>
</tr>
<tr>
<td>Temperature range (°C)</td>
<td>2–40</td>
<td>3.5–39</td>
<td>3.5–42</td>
<td>–2.0–20.8</td>
<td>–2.0–20.8</td>
<td>&gt;5–&lt;45</td>
</tr>
<tr>
<td>Optimal Na\textsuperscript{+} concen- tration (mM)</td>
<td>270</td>
<td>470</td>
<td>470</td>
<td>250</td>
<td>250</td>
<td>500</td>
</tr>
<tr>
<td>Na\textsuperscript{+} concentration range (mM)</td>
<td>30–1380</td>
<td>100–1240</td>
<td>100–1240</td>
<td>40–1240</td>
<td>40–1240</td>
<td>ND</td>
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<tr>
<td>Electron donor:</td>
<td></td>
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<td></td>
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<tr>
<td>Reduced sulfur compounds</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H\textsubscript{2}</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

*The data were obtained from strain MAS2\textsuperscript{T} cultivated in MMJS.*

**Description of *Thiomicrospira hydrogeniphila* sp. nov.**

*Thiomicrospira hydrogeniphila* (hy.dro.ge.ni’phi.la. Gr. n. hydor hydros water; Gr. v. genein to produce; Gr. adj. philos loving; L.N. fem. adj. hydrogeniphila hydrogen-loving).

Cells are Gram-staining-negative, motile rods with a polar flagellum, with a mean length of 0.9–1.8 μm and a diameter of approximately 0.3–0.5 μm. Cells in MMJS medium are strictly aerobic, tolerating up to 40\% O\textsubscript{2} in the gas phase. The temperature range for growth is 2–40°C (optimum 30°C) in MMJS. The pH range for growth is 5.0–8.0 (optimum pH 6.0) in MMJS. The optimal Na\textsuperscript{+} concentration for growth is 270 mM in MMJS; growth is possible at a Na\textsuperscript{+} concentration range of 30–1380 mM in MMJS. Chemolithoautotrophic growth occurs with H\textsubscript{2} and reduced sulfur compounds such as sulfide, thiosulfate, tetrathionate and elemental sulfur as electron donors and molecular oxygen as an electron acceptor. Hydrogen oxidation exhibits resistance against high O\textsubscript{2} concentrations. Heterotrophic growth does not occur. Ammonium is utilized as nitrogen source. Vitamins, selenium and tungsten are not required for growth. The major cellular fatty acids are C\textsubscript{16:0}, C\textsubscript{16:1} and C\textsubscript{18:1}.

The type strain is MAS2\textsuperscript{T} (=JCM 30760\textsuperscript{T}=DSM 100274\textsuperscript{T}). The DNA G+C content of the type strain is 39.6 mol\% (by HPLC).

**Acknowledgements**

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**References**


