**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{a}).

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 *Thiomicrospira hydrogeniphila* sp. nov. is proposed for MAS2T.

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 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 in the tank. At 10 months of incubation, the scum-like layer was formed on the surface of the seawater, and during growth (Oremland & Taylor, 1978) and that sulfur-oxidizing bacteria would be concomitantly enriched in the tank. When the sampling was conducted, the conditions of seawater in the tank. At 10 months of incubation, the scum-like layer was formed on the surface of the seawater. The concentration of sulfide in the seawater was measured by the methylene blue method (Fogo & Popowsky, 1949) and was found to be 2.9 mM. Dissolved oxygen (DO) concentration in the seawater was measured with an oxygen sensor (Endress + Hauser) and was below detection limit (<0.01 mg l⁻¹). The scum-like material was suspended in 1 ml sterilized M1 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% N₂/25% H₂/5% CO₂/1% O₂ (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 & Hori-koshi, 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 K₂HPO₄, 0.29 g CaCl₂, 1.0 g NH₄Cl, 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 NaHCO₃ and 5.0 g Na₂S₂O₃·5H₂O. 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% N₂/25% H₂/5% CO₂/1% O₂ (200 kPa). This culture was designated strain MAS2T. 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 O₂) 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 MAS2T 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 MAS2T were thus similar to those of *Thiomicrospira frisia* JB-A2T, *Thiomicrospira chilensis* Ch-1T, *Thiomicrospira arctica* SVAL-E² and *Thiomicrospira psychrophila* SVAL-D² and these organisms form a phylogenetic subcluster within the genus *Thiomicrospira* (Fig. 1).

Spor formation was not observed under any of the growth conditions examined.

MAS2T was routinely cultivated in MMJH medium. MMJH medium consists of (per litre of distilled, deionized water) 20.0 g NaCl, 0.14 g K₂HPO₄, 0.8 g CaCl₂, 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 Na₂SeO₃·5H₂O, 0.1 mg Na₃WO₄·0.01 g Fe(NH₄)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 NaHCO₃. To prepare MMJH medium, materials other than the vitamin solution and NaHCO₃ 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 NaHCO₃ was added to the medium under gas purging with 80% N₂/20% CO₂, 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% N₂/25% H₂/5% CO₂/1% O₂ (200 kPa). MAS2T was also cultivated in MMJS medium. MMJS medium consists of MMJH medium with 5 mM Na₂S₂O₃·5H₂O, and the gas phase in the vials was adjusted to 94% N₂/5% CO₂/1% O₂ (200 kPa).

Growth of MAS2T 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, MAS2T 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. MAS2T, when tested in MMJS medium with variable NaCl content, grew over the Na+ concentration range of 30–1380 mM (optimum 270 mM) at 30°C and pH 6.0.

The effect of O2 in the gas phase on growth of MAS2T was tested with MMJS medium under a gas mixture of 95% N2/5.0% CO2, 94.9% N2/5% CO2/0.1% O2, 94.5% N2/5% CO2/0.5% O2, 94% N2/5% CO2/1% O2, 90% N2/5% CO2/5% O2, 85% N2/5% CO2/10% O2, 75% N2/5% CO2/20% O2 or 55% N2/5% CO2/40% O2 at 200 kPa and with MMJH medium under a gas mixture of 70% N2/25% H2/5% CO2, 69.9% N2/25% H2/5% CO2/0.1% O2, 69.5% N2/25% H2/5% CO2/0.5% O2, 69% N2/25% H2/5% CO2/1% O2, 65% N2/25% H2/5% CO2/5% O2, 60% N2/25% H2/5% CO2/10% O2, 50% N2/25% H2/5% CO2/20% O2 or 30% N2/25% H2/5% CO2/40% O2 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 MAS2T in both media was observed under a gas phase in the presence of 0.1–40% O2 and the maximum cell yield after 2 weeks of cultivation in MMJS and MMJH medium was under a gas phase with 5–40% O2 and 5–20% O2, respectively. These results indicated that strain MAS2T was able to grow using thiosulfate or H2 as an energy source under fully aerobic conditions.

Heterotrophic growth was tested in MMJS medium without NaHCO3 under a gas phase of 99% N2/1% O2 (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) maltose 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% N2/5% CO2/1% O2 (200 kPa). However, none of the organic compounds sustained the growth of MAS2T. In an attempt to determine potential electron donors other than thiosulfate and H2 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% N2/5% CO2/1% O2 (200 kPa). MAS2T grew autotrophically on sulfide, elemental sulfur and tetrathionate, but not on sulfate or cysteine. To test for the utilization of electron acceptors, nitrate (10 mM), nitrite (1 or 5 mM), ferric citrate (20 mM), ferricyanide (20 mM), selenate (5 mM) or fumarate (10 mM) was tested with MMJS medium under 95% N2/5% CO2 (200 kPa). None of the electron acceptors other than O2 supported the growth of MAS2T 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 MAS2T. Potential inorganic nitrogen sources (NH4Cl,
NaNO₂, NaNO₃ or N₂) for growth were also examined with MMJS medium as the sole nitrogen source. MAS2ᵀ utilized ammonium as the inorganic nitrogen source but could not utilize nitrate, nitrite and molecular nitrogen. These results indicated that MAS2ᵀ was a chemolithoautotroph, utilizing the reduced sulfur compounds such as thiosulfate, tetrathionate, elemental sulfur, sulfide and H₂ as the energy sources and O₂ as the sole electron acceptor.

The growth rate of MAS2ᵀ was examined with MMJS medium with an Na⁺ concentration of 270 mM under a gas phase of 90% N₂/5% CO₂/5% O₂ (200 kPa) and MMJH medium with an Na⁺ concentration of 430 mM under a gas phase of 65% N₂/25% H₂/5% CO₂/5% O₂ (200 kPa) and the cultures were stirred continuously. The maximum growth rate of MAS2ᵀ was found to be 0.4 h⁻¹ in MMJS medium and 0.6 h⁻¹ in MMJH medium.

MAS2ᵀ in MMJS medium was sensitive to a variety of antibiotics, including chloramphenicol (50 µg ml⁻¹), streptomycin (50 µg ml⁻¹), kanamycin (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 (C₁₂:0 C₁₄:0 C₁₆:0) of MAS2ᵀ were similar to those of T. chilensis strain Ch⁻¹ᵀ and T. arctica strain SVAL-Eᵀ (Knittel et al., 2005), but three fatty acid components (C₁₂:1, C₁₄:1, C₁₄:1 3-OH) could not be detected in the case of MAS2ᵀ (Table 1).

Genomic DNA of MAS2ᵀ grown at 30 °C in MMJS was prepared as described by Marmur & Doty (1962). T. frisia strain JB-A2ᵀ 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 Showu) after digestion with nuclease P1 (Tamaoka & Komagata, 1984). The G+C content of the genomic DNA of MAS2ᵀ was found to be 39.6 mol%, which is the same as that of T. frisia strain JB-A2ᵀ (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 T. frisia strain JB-A2ᵀ (98.7%) (Brinkhoff et al., 1999b), Thiomicrospira sp. strain Art-3 (98.5%) (Brinkhoff & Muyzer, 1997), isolated from sediments of saline spring and 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 Thiomicrospira was below 97%. The 16S rRNA gene sequence was distantly related to the sequence of Hydrogenovibrio marinus MH-110ᵀ (93.7%), the only strain within the genus Thiomicrospira capable of hydrogenotrophic growth (Nishihara et al., 1998). The nearly complete sequence was applied to the phylogenetic tree construction 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 MAS2ᵀ was most closely related to T. frisia JB-H2ᵀ (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 & 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 MAS2ᵀ and T. frisia JB-A2ᵀ 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).

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

<table>
<thead>
<tr>
<th>Strains:</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>T. hydrogenophilica MAS2ᵀ (grown in MMJS)</td>
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<td></td>
<td></td>
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<tr>
<td>T. chilensis Ch⁻¹ᵀ</td>
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<tr>
<td>T. arctica SVAL-Eᵀ</td>
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<tr>
<td>Proportions of fatty acids are given as percentages of whole-cell fatty acids.</td>
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</table>

*Classification uncertain.*
The data were obtained from strain MAS2 closely related to T. frisia. The temperature range for growth is 2–30 °C in MMJS. The pH range for growth is 5.0–8.0 (optimum pH 6.0) in MMJS. The optimal Na⁺ concentration for growth is 270 mM in MMJS; growth is possible at a Na⁺ concentration range of 30–1380 mM in MMJS. Chemolithoautotrophic growth occurs with H₂ 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 O₂ 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 C₁₆ : 0, C₁₆ : 1 and C₁₈ : 1ω7c.

The type strain is MAS2T (=JCM 30760T=DSM 100274T). The DNA G+C content of the type strain is 39.6 mol% (by HPLC).

The cells of MAS2T 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 MAS2T is closely related to T. frisia JB-A₂T 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 MAS2T is similar to those of T. chilensis Ch-1T and T. arctica SVAL-DT in the phylogenetic subcluster (Table 1). However, MAS2T is able to grow with not only reduced sulfur compounds but also with H₂ as an energy source. Phylogenetic analysis based on 16S rRNA gene sequences reveals that MAS2T is distantly related to H. marinus MH-110T, which has been known as only hydrogen-oxidizing bacterium within the genus Thiomicrospira (Fig. 1). Meanwhile, MAS2T as well as H. marinus MH-110T are able to grow under a gas phase of 40% O₂ (Nishihara et al., 1989). On the basis of these physiological and genetic properties, we suggest that MAS2T is representative of a novel species of the genus Thiomicrospira, for which the name Thiomicrospira hydrogeniphila sp. nov. is proposed.

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

Thiomicrospira hydrogeniphila (hy.dro.ge.ni’phi.ila. Gr. n. hydor hydros water; Gr. v. genein to produce; Gr. adj. philos loving; N.L. 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% O₂ 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⁺ concentration for growth is 270 mM in MMJS; growth is possible at a Na⁺ concentration range of 30–1380 mM in MMJS. Chemolithoautotrophic growth occurs with H₂ 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 O₂ 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 C₁₆ : 0, C₁₆ : 1 and C₁₈ : 1ω7c.

The type strain is MAS2T (=JCM 30760T=DSM 100274T). The DNA G+C content of the type strain is 39.6 mol% (by HPLC).

**Acknowledgements**

We would like to thank Mr Katsuyuki Uematsu for assistance in preparing electron microscopy. This work was supported by JSPS KAKENHI Grant Number 25840166.

**References**


**Table 2.** Comparison of properties among *T. hydrogeniphila* MAS2T and related species

<table>
<thead>
<tr>
<th>Characteristic</th>
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<td>Cell shape</td>
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<tr>
<td>DNA G+C content (mol%)</td>
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<td>49.9</td>
<td>42.4</td>
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<td>Maximum growth rate (h⁻¹)</td>
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<td>0.14</td>
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<td>Temperature range (°C)</td>
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<td>Na⁺ concentration range (mM)</td>
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*The data were obtained from strain MAS2T cultivated in MMJS.*


