Thiomicrospira thermophila sp. nov., a novel microaerobic, thermotolerant, sulfur-oxidizing chemolithomixotroph isolated from a deep-sea hydrothermal fumarole in the TOTO caldera, Mariana Arc, Western Pacific

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A novel thermotolerant bacterium, designated strain I78T, was isolated from a self-temperature-recording in situ colonization system deployed in a hydrothermal diffusing flow (maximal temperature 78 °C) at the TOTO caldera in the Mariana Arc, Western Pacific. Cells were highly motile curved rods with a single polar flagellum. Growth was observed at 15–55 °C (optimum 35–40 °C; 60 min doubling time) and pH 5–8 (optimum pH 6–7). The isolate was a microaerobic chemolithomixotroph capable of using thiosulfate, elemental sulfur or sulfide as the sole energy source, and molecular oxygen as the sole electron acceptor. The isolate was able to grow chemolithoautotrophically with carbon dioxide. Various organic substrates such as complex proteinaceous compounds, carbohydrates, organic acids, amino acids and sugars could also support growth as the carbon source instead of carbon dioxide with sulfur oxidation. The G+C content of the genomic DNA was 43.8 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolate belonged to the genus Thiomicrospira and was most closely related to Thiomicrospira crunogena strain TH-55T and Thiomicrospira sp. strain L-12, while DNA–DNA hybridization demonstrated that the novel isolate could be genetically differentiated from previously described strains of Thiomicrospira. On the basis of its physiological and molecular properties the isolate is representative of a novel Thiomicrospira species, for which the name Thiomicrospira thermophila sp. nov. is proposed (type strain, I78T = JCM 12397T = DSM 16397T).

The genus Thiomicrospira accommodates obligate chemolithoautotrophs within the γ-Proteobacteria, except for Thiomicrospira denitrificans strain DSM 1251T, which is within the ε-Proteobacteria. Since the type species Thiomicrospira pelophila strain DSM 1534T was first isolated from the intertidal mud flats of the Dutch Wadden Sea (Kuenen & Veldkamp, 1972), six species and many strains of the genus Thiomicrospira have been isolated from various marine habitats, including deep-sea hydrothermal environments (Ruby & Jannasch, 1982; Ruby et al., 1981; Jannasch et al., 1985; Eberhard et al., 1995). In addition to the type species Thiomicrospira pelophila strain DSM 1534T, Thiomicrospira crunogena strain TH-55T was isolated from the tubes of vestimentiferan tube worms in the East Pacific Rise (EPR) deep-sea hydrothermal environment (Jannasch et al., 1985) and Thiomicrospira thyasirae strain DSM 5322T was isolated from the gill extract of the marine bivalve Thyasira flexuosa inhabiting shallow bays and cold seepage sites (Wood & Kelly, 1989, 1993). Both Thiomicrospira kuenenii strain JB-A1T and Thiomicrospira frisia strain JB-A2T were obtained from an intertidal mud flat of the German Wadden Sea (Brinkhoff et al., 1999a). The most recently described species is Thiomicrospira chilensis strain Ch-1T, from a...
sediment sample dominated by filamentous sulfur bacteria of the genus Thioloba in the Chilean coastal shelf (Brinkhoff et al., 1998). In addition to the recognized members of the genus Thiomicrospira, members of the closely related genera Hydrogenovibrio (Nishihara et al., 1989, 1991) and Thioalkalimicrobium (Sorokin et al., 2001, 2002) have also been isolated. Recent phylogenetic analysis (Sorokin et al., 2002) has revealed that Thiomicrospira pelophila strain DSM 1534T and Thiomicrospira thyasirae strain DSM 5322T are only distantly related to other Thiomicrospira species and cluster with Thioalkalimicrobium species; Hydrogenovibrio marinus strain MH-110T is phylogenetically related to Thiomicrospira kueneni strain JB-A1T and should be classified within the genus Thiomicrospira.

The abundance of members of the genus Thiomicrospira in both deep and shallow hydrothermally active marine environments has been well characterized using not only cultures and isolates but also cultivation-independent molecular techniques (Ruby & Jannasch, 1982; Ruby et al., 1981; Jannasch et al., 1985; Eberhard et al., 1995; Brinkhoff & Muyzer, 1997; Brinkhoff et al., 1999c). However, phylogenetic and physiological diversity of members of the genus Thiomicrospira together with their biogeography in the global deep-sea hydrothermal systems were unclear because investigations had been limited to isolates from the EPR and the Mid Atlantic Ridge hydrothermal environments (Jannasch et al., 1985; Wirsen et al., 1998). Recent extensive analyses of sulfur- and hydrogen-oxidizing chemolithoautotrophs in a variety of microbial habitats in the deep-sea hydrothermal systems of the Okinawa Trough and Central Indian Ridge have provided new information regarding the phylogenetic and physiological diversity of the e-Proteobacteria. However, little has emerged with regard to deep-sea hydrothermal Thiomicrospira species potentially inhabiting similar ecological environments (but see Inagaki et al., 2003, 2004; Takai et al., 2003a, 2004a, b, c). In this study, we report the isolation of a novel Thiomicrospira strain, I78T, from a deep-sea hydrothermal field in the TOTO caldera, Mariana Arc, Western Pacific margin. It was isolated from a self-temperature-recording in situ colonization (STR-ISCS) device (Takai et al., 2003a) deployed in a hydrothermal diffusing flow (maximal temperature 78°C). We report here on the taxonomic study of strain I78T and propose that it represents a novel species for which the Thiomicrospira thermophila sp. nov. is proposed.

Sample collection, enrichment and purification

An STR-ISCS, a newly constructed microbial habitat consisting of a stainless steel pipe with numerous small holes (5 mm in diameter) and a substratum of very porous, natural pumice (Takai et al., 2003a), was deployed for 4 days in a diffusing hydrothermal flow with a maximum fluid temperature of 78°C and a pH of 5-3 at the TOTO caldera (12° 42'-8007'N, 143° 32'-3415'E), at a depth of 2922 m by means of the manned subsmersible Shinkai 6500 (Dive no. 772) in August 2003. After deployment, it was recovered to the sea surface in a sample box attached to the submersible (Dive no. 776). The TOTO caldera deep-sea hydrothermal field is characterized by highly acidic hydrothermal fluids resulting from oxidation of volatile volcanic gas (H2S) to sulfate (Gamo et al., 2004). During a series of dives, we sampled a white smoker hydrothermal vent with an unusually low pH value of 1-6 in the TOTO caldera, suggesting that the deep-sea hydrothermal activity in the TOTO caldera is a novel system driven by sub-seafloor mixing between oxygenated sea water and superheated volcanic gas, as was previously proposed for the DESMOS caldera in the Manus Basin (Gamo et al., 1997). The STR-ISCS was deployed in one of the diffusing hydrothermal flows derived from the highly acidic hydrothermal fluid with further dilution of sea water. During the 4-day deployment, the temperature of the substratum in the colonization device fluctuated between 20 and 40°C in the first 24 h, gradually increased up to 70°C for the next 24 h and remained stable at 65–70°C for the last 2 days.

Immediately after recovery of the STR-ISCS device on the ship, the substratum was suspended in 20 ml sterilized MJ synthetic sea water (Takai et al., 1999) containing 0-05% (w/v) sodium sulfide in a 100 ml glass bottle (Schott) tightly sealed with a butyl-rubber cap under a gas phase of 100% N2 (100 kPa). The suspended slurry was used to inoculate a series of media including MMJS medium (Takai et al., 2003a) under a gas phase of 80% H2/19% CO2/1% O2 (200 kPa), and the cultures were then incubated at 30°C in a dry oven.

Growth of motile, slightly curved rods was observed with production of colloidal elemental sulfur in MMJS medium after 2 days incubation at 30°C. A pure culture was obtained by using the dilution-to-extinction technique at 30°C with the same medium as used for enrichment (Takai & Horikoshi, 2000). The pure culture was streaked onto MMJS plates solidified with 1·2% (w/v) agar. After 2 days incubation at 30°C under a gas phase of 80% H2/19% CO2/1% O2 (200 kPa), only one colony type with a white to cream colour and elemental sulfur particles was noted. An isolated colony was picked and inoculated into fresh liquid MMJS medium under a gas phase of 80% H2/19% CO2/1% O2 (200 kPa). This culture was designated strain I78T. Purity was confirmed by microscopic examination and by repeated partial sequencing of the 16S rRNA gene using several PCR primers.

Morphology

Cells were observed under a phase-contrast Olympus BX51 microscope with the SPOT RT Slider CCD camera system (Diagnostic Instruments Inc.). Transmission electron microscopy of negatively stained cells was carried out as described by Zillig et al. (1990). Cells grown in MMJS medium (described below) under microaerobic conditions (1% partial pressure of O2) at 30°C in the mid-exponential phase of growth were negatively stained with 2% (w/v) uranyl acetate and observed under a JEOL JEM-1210 electron microscope at an accelerating voltage of 120 kV.
Cells of strain I78\textsuperscript{T} were Gram-negative, slightly curved rods about 0.4–0.7 µm in diameter, 0.8–1.5 µm in length (Fig. 1), and were motile with a polar flagellum (Fig. 1). In contrast to other \textit{Thiomicrospira} isolates, we observed neither spiral-shaped cells (Kuenen & Veldkamp, 1972) nor the formation of aggregates (Brinkhoff \textit{et al}., 1999a, b). When strain I78\textsuperscript{T} was heterotrophically grown in MMJS medium with organic substrates such as yeast extract and sugars instead of carbon dioxide, the cells were enlarged straight rods (0.6–1.0 µm in diameter and 1.0–2.0 µm in length). No spore formation was observed in any of the growth conditions examined. Morphological features of strain I78\textsuperscript{T} were thus very similar to those of \textit{Thiomicrospira crunogena} strain TH-55\textsuperscript{T} (Jannasch \textit{et al}., 1985) and \textit{Thiomicrospira} sp. strain L-12 (Ruby & Jannasch, 1982), which were examined using the same medium and conditions as used in this study.

\textit{Thiomicrospira crunogena} strain TH-55\textsuperscript{T} (Jannasch \textit{et al}., 1985) and \textit{Thiomicrospira} sp. strain L-12 (Ruby & Jannasch, 1982) were kindly donated by Jan Kuever, Institute of Material Testing, Bremen, Germany, and by Stefan Sievert, Woods Hole Oceanic Institute, Woods Hole, MA, USA. These strains were routinely cultivated with MMJS medium (described below) under microaerobic conditions (1% partial pressure of O\textsubscript{2}) at 30°C.

**Growth characteristics**

Strain I78\textsuperscript{T} was routinely cultivated in MMJS medium. MMJS medium consists of (per litre of distilled, deionized water) 20.0 g NaCl, 0.14 g K\textsubscript{2}HPO\textsubscript{4}, 0.8 g CaCl\textsubscript{2}, 1.0 g NH\textsubscript{4}Cl, 4.0 g MgSO\textsubscript{4}.7H\textsubscript{2}O, 3.0 g MgCl\textsubscript{2}.6H\textsubscript{2}O, 0.33 g KCl, 0.5 mg NiCl\textsubscript{2}.6H\textsubscript{2}O, 0.5 mg Na\textsubscript{2}SeO\textsubscript{3}.5H\textsubscript{2}O, 0.1 mg Na\textsubscript{2}WO\textsubscript{4}, 0.01 g Fe(NH\textsubscript{4})\textsubscript{2}(SO\textsubscript{4})\textsubscript{3}.6H\textsubscript{2}O, 10 ml trace mineral solution (Balch \textit{et al}., 1979), 10 ml vitamin solution (Balch \textit{et al}., 1979), 2.0 g NaHCO\textsubscript{3} and 5 mM Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3}.5H\textsubscript{2}O. To prepare MMJS medium, materials other than vitamin solution (Balch \textit{et al}., 1979) and NaHCO\textsubscript{3} were dissolved, and the pH of the medium was adjusted to about 6.0 with HCl before autoclaving. After autoclaving under an air atmosphere, a concentrated solution of vitamins and NaHCO\textsubscript{3} was added to the medium under gas purging of 80% N\textsubscript{2}/20% CO\textsubscript{2}, and the pH was readjusted to 6.0 with HCl at room temperature if necessary. A concentrated sodium bicarbonate solution was separately sterilized by autoclaving and the vitamin solution was filter-sterilized. The medium was dispensed at 20% of the bottle (Schott Glasswerke, Mainz, Germany) or tube (Iwaki glass, Tokyo, Japan) volume, and tightly sealed with a butyl-rubber stopper under a gas phase consisting of 80% N\textsubscript{2}/19% CO\textsubscript{2}/1% O\textsubscript{2} at 200 kPa unless stated otherwise.

Growth of strain I78\textsuperscript{T} was measured by direct cell counting after staining with 4',6-diamidino-2-phenylindole (DAPI) (Porter & Feig, 1980) using a phase-contrast Olympus BX51 microscope. Cultures were prepared in duplicate. The cultures were grown in 100 ml glass bottles (Schott Gläserwerke) with shaking (100 r.p.m.) in a temperature-controlled dry incubator. With MMJS medium, strain I78\textsuperscript{T} grew over the temperature range of about 15–55°C, showing optimal growth at 35–40°C; the generation time at 40°C, pH 6.0, was about 60 min (supplementary figure available in IJSEM Online). The effect of pH on growth was studied at 35°C, using MMJS medium adjusted to various pH values with 30 mM acetate/acetate 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 (supplementary figure available in IJSEM Online). Growth occurred at pH 5.0–8.0, with 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. Strain I78\textsuperscript{T}, when tested in MMJS medium with variable NaCl content, grew over the NaCl concentration range 3–70 g l\textsuperscript{-1}, with optimum growth at 12–20 g l\textsuperscript{-1} at 30°C and pH 6.0 (supplementary figure available in IJSEM Online). The optimum temperature and the temperature range for growth of strain I78\textsuperscript{T} were considerably higher than those of any previously described species of the genus \textit{Thiomicrospira} (Kuenen & Veldkamp, 1972; Jannasch \textit{et al}., 1985; Wood & Kelly, 1989, 1993; Brinkhoff \textit{et al}., 1999a, b) and for \textit{Thiomicrospira} sp. strain L-12 (Ruby & Jannasch, 1982) (Table 1). The optimum pH for growth of the strain I78\textsuperscript{T} was lower than those of \textit{Thiomicrospira crunogena} strain TH-55\textsuperscript{T} (Jannasch \textit{et al}., 1985) and \textit{Thiomicrospira} sp. strain L-12 (Ruby & Jannasch, 1982) from deep-sea hydrothermal environments (Table 1).

The effect of oxygen concentration in the gas phase on growth of strain I78\textsuperscript{T} was tested with MMJS medium under a gas mixture of 80% N\textsubscript{2}/20% CO\textsubscript{2}, 80% N\textsubscript{2}/19–9% CO\textsubscript{2}/0–1% O\textsubscript{2}, 80% N\textsubscript{2}/19–5% CO\textsubscript{2}/0–5% O\textsubscript{2}, 80% N\textsubscript{2}/19% CO\textsubscript{2}/1% O\textsubscript{2}, 80% N\textsubscript{2}/15% CO\textsubscript{2}/5% O\textsubscript{2}, 75% N\textsubscript{2}/15% O\textsubscript{2}
Table 1. Comparison of properties among *Thiomicrospira thermophila* strain I78T, *Thiomicrospira crunogena* strain TH-55T, *Thiomicrospira* sp. strain L-12, *Thiomicrospira kunenii* strain JB-A1T and *Hydrogenovibrio marinus* strain MH-110T

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
<td>Source of isolate</td>
<td>Deep-sea hydrothermal fumarole in the West Pacific</td>
<td>Deep-sea hydrothermal vent chimneys and animals in the East Pacific</td>
<td>Deep-sea hydrothermal vent chimneys and animals in the East Pacific</td>
<td>Intertidal mud flat in the German Wadden Sea</td>
<td>Coastal sea water in Japan</td>
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<td>Temperature for growth (°C): Range (optimum)</td>
<td>15–55 (35–40)</td>
<td>14–38·5 (28–32)</td>
<td>10–35 (25)</td>
<td>3·5–42 (29–33·5)</td>
<td>&gt; 5 to &lt; 45 (37)</td>
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<td>pH for growth: Range (optimum)</td>
<td>5·0–8·0 (6)</td>
<td>5·0–8·5 (7·5–8·0)</td>
<td>6·0–8·5 (8)</td>
<td>4·0–7·5 (6)</td>
<td>ND (6·5)</td>
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<td>NaCl concentration for growth (%): Range (optimum)</td>
<td>0·3–7·0 (1·2–2·0)</td>
<td>&gt; 0·25 (ND)</td>
<td>&gt; 0·45 (1·1–2·3)</td>
<td>0·56–6·9 (2·6)</td>
<td>ND (2·9)</td>
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<td>O₂ concentration for growth (%): Optimum (upper limit)</td>
<td>0·5–1·0 (10)</td>
<td>Microaerobic (&gt; 20)</td>
<td>Microaerobic (&gt; 20)</td>
<td>4·20 ( &gt; 20)</td>
<td>5·10 (&gt; 40)</td>
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<td>Electron donor:</td>
<td>H₂</td>
<td>+</td>
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<td>Reduced sulfur compounds</td>
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<td>Accumulation of S² during growth</td>
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<td>+</td>
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<td>Heterotrophic growth</td>
<td>+</td>
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<td>Utilization of N₂ as nitrogen source</td>
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<td>–</td>
<td>ND</td>
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<td>Vitamin dependence</td>
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<td>G+C content of genomic DNA (mol%)</td>
<td>43·8</td>
<td>44·2</td>
<td>ND</td>
<td>42·4</td>
<td>44·1</td>
</tr>
</tbody>
</table>

*Property confirmed in this study.*

CO₂/10 % O₂ or 65 % N₂/15 % CO₂/20 % 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. The maximum cell yield of strain I78T was approximately 8–9 x 10⁸ cells ml⁻¹ under a gas phase in the presence of 0·5 or 1 % O₂, whereas slightly lower yields (2·5 x 10⁸ cells ml⁻¹) were seen with 0·1, 5 and 10 % O₂. No growth was observed in the absence of O₂ or with 20 % O₂ in a gas phase. These results indicated that strain I78T grew under microaerobic (up to 10 % O₂) conditions.

Heterotrophic growth was tested in MMJS medium without NaHCO₃ under a gas phase of 99 % N₂/1 % O₂ (200 kPa), containing the following as potential organic carbon sources: 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 (CMC), 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. It was able to grow with any of yeast extract, peptone, tryptone, casein, starch, CMC, Casamino acids, glycerol, fumarate, glycine, leucine, isoleucine, lysine, glucose, galactose, sucrone, fructose, lactose, maltose and trehalose, providing a maximum cell yield of 3·0–5·0 x 10⁸ cells ml⁻¹. A somewhat lower yield (1–2 x 10⁸ cells ml⁻¹) was obtained from heterotrophic growth on malate, citrate, alanine, valine, cysteine, methionine, arginine, histidine, asparagine, glutamine, aspartate and glutamate.

No other organic carbon source supported the heterotrophic growth using thiosulfate as an energy source and molecular oxygen as an electron acceptor. Simultaneous experiments with *Thiomicrospira crunogena* strain TH-55T (Jannasch et al., 1985) and *Thiomicrospira* sp. strain L-12 (Ruby & Jannasch, 1982) in MMJS medium indicated that both were able to grow heterotrophically on any of the organic carbon compounds, such as yeast extract, tryptone, casein, starch, Casamino acids, glucose, galactose, sucrose, fructose, lactose, maltose, xylitol and trehalose.

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...
Thiomicrospira thermophila sp. nov.

The time-course of oxidation of thiosulfate and concomitant bacterial growth of Thiomicrospira thermophila strain 178T was examined in MMJS medium with a gas phase of 80 % N2/19 % CO2/1 % O2 (200 kPa). The concentrations of thiosulfate, sulfite and sulfate were analysed by ion chromatography using a Shim-pack IC column (Shimadzu, Kyoto, Japan) and the production of elemental sulfur during growth was monitored as described by Takai et al. (2001). Thiosulfate was consumed and sulfate and elemental sulfur were produced during the growth of strain 178T (Fig. 2). Production of sulfite was not observed during the growth, in contrast to growth in Thiomicrospira strain L-12 (Ruby & Jannasch, 1982). In addition, the accumulation of elemental sulfur during growth was a distinct characteristic of strain 178T and Thiomicrospira crunogena strain TH-55T (Jannasch et al., 1985) and Thiomicrospira sp. strain L-12 (Ruby & Jannasch, 1982); other Thiomicrospira species show the nearly complete oxidation of thiosulfate to sulfate (Brinkhoff et al., 1999a, b). The control (uninoculated) medium showed no oxidation of thiosulfate and no production of either elemental sulfur or sulfate. Thus, strain 178T was found to be a respiratory sulfur-oxidizing, oxygen-reducing chemolithomixotroph.

Strain 178T was found to be 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), vancomycin (50 µg ml−1) and rifampicin (50 µg ml−1) (data not shown).

Fatty acid analysis

The cellular fatty acid composition was analysed from cells grown in MMJS medium at 35 °C in the late-exponential phase of growth. The cellular fatty acid composition of Thiomicrospira crunogena strain TH-55T (Jannasch et al., 1985) was also determined from cells grown in MMJS medium at 30 °C 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 that was heated at 100 °C for 3 h. Extraction and
analysis of fatty acid methyl esters were as described by Takai et al. (2003b). The major cellular fatty acids of strain I78T were found to be C14:0 (7.4%), C16:0 (16.3%), C16:1 (37.2%), anteiso-C17:0 (4.0%), C18:0 (21.3%) and C18:1 (13.8%), whereas those of Thiomicrospira crunogena strain TH-55T were C14:0 (2.0%), C16:0 (49.2%), C16:1 (21.4%), anteiso-C17:0 (0.9%), C18:0 (15.2%) and C18:1 (11.8%). As compared with Thiomicrospira crunogena strain TH-55T, strain I78T contained higher proportions of unsaturated fatty acids and longer chains of fatty acids. This may be associated with the higher temperature range for growth of strain I78T.

Nucleic acid analyses

Genomic DNA of strain I78T was prepared as described by Marmur & Doty (1962). The DNA G+C content was determined by direct analysis of deoxyribonucleotides by HPLC (Tamaoka & Komagata, 1984). The G+C content of the genomic DNA of strain I78T was found to be 43.8 mol%, which is similar to those of Thiomicrospira crunogena strain TH-55T (44.2 mol%) (Jannasch et al., 1985) and Thiomicrospira kueneniai strain JB-A1T (42.4 mol%) (Brinkhoff et al., 1999a) (Table 1).

The 16S rRNA gene sequence was amplified by the PCR using primers Bac 27F and 1492R (DeLong, 1992; Lane, 1985) as described previously (Takai et al., 2001). The nearly complete sequence (1478 bp) of the 16S rRNA gene from strain I78T was directly sequenced by both strands using the dideoxynucleotide chain-termination method with a DNA sequencer model 3100 (Perkin Elmer/Applied Biosystems). The rRNA gene sequence was analysed using the gapped-BLAST search algorithm (Altschul et al., 1997; Benson et al., 1998) and was found to be most closely related to the sequences of Thiomicrospira sp. strain L-12 (Ruby & Jannasch, 1982) and Thiomicrospira crunogena strain TH-55T (Jannasch et al., 1985) (Fig. 3). Bootstrap analysis was performed to provide confidence estimates for phylogenetic tree topologies. The phylogenetic tree indicated that strain I78T was most closely related to Thiomicrospira sp. strain L-12 (Ruby & Jannasch, 1982) and Thiomicrospira crunogena strain TH-55T (Jannasch et al., 1985) (Fig. 3).

DNA–DNA hybridization was carried out at 42 °C for 3 h and was measured fluorometrically using photobiotin according to the method of Ezaki et al. (1989) among the genomic DNA of strain I78T, Thiomicrospira sp. strain L-12 and Thiomicrospira crunogena strain TH-55T. Mean hybridization values were 24.0% between strain I78T and Thiomicrospira crunogena strain TH-55T and 37.3% between strain I78T and Thiomicrospira sp. strain L-12, indicating that strain I78T could be genotypically differentiated from both these strains.

Comparison with related species

Strain I78T was isolated from an STR-ISCS device deployed in a hydrothermal diffusing flow of a deep-sea hydrothermal fumarole at a depth of 2922 m at the TOTO caldera in the Mariana Arc. This is the first Thiomicrospira strain obtained from the Western Pacific region. Phylogenetic analysis
indicated that strain I78\(^T\) is most closely related to *Thiomicrospira crunogena* strain TH-55\(^T\), which was isolated from the outer tube of ventimifera tube worms in the deep-sea hydrothermal vent of the EPR (Jannasch et al., 1985), and *Thiomicrospira* sp. strain L-12, which was isolated from a hydrothermal mussel colony on the Galapagos Rift (Ruby & Jannasch, 1982). However, many of the physiological characteristics of strain I78\(^T\) are different from those of deep-sea hydrothermal vent *Thiomicrospira* strains and even from other previously described species such as *Thiomicrospira kueneniai* strain JB-A1\(^T\) (Brinkhoff et al., 1999a) and *Hydrogenovibrio marinus* strain MH-110\(^T\) (Nishihara et al., 1989, 1991) obtained from coastal environments (Table 1). Strain I78\(^T\) is highly thermophilic, growing optimally at 35–40 °C and up to 55 °C, much higher than the optimum and highest temperatures for growth of any previously described members of the genus *Thiomicrospira* and of *Hydrogenovibrio marinus* strain MH-110\(^T\) (Nishihara et al., 1989, 1991) (Table 1). In comparison with phylogenetically related *Thiomicrospira* sp. strain L-12 (Ruby & Jannasch, 1982) and *Thiomicrospira crunogena* strain TH-55\(^T\) from deep-sea hydrothermal environments, strain I78\(^T\) has a lower optimal pH for growth (pH 6-0, Table 1). Another distinctive feature is its ability to use molecular nitrogen as the nitrogen source (Table 1). These are distinctive physiological features that clearly differentiate strain I78\(^T\) from previously described deep-sea hydrothermal vent *Thiomicrospira* strains. In addition, DNA hybridization analysis clearly reveals that the novel isolate can be genetically differentiated from *Thiomicrospira* sp. strain L-12 (Ruby & Jannasch, 1982) and *Thiomicrospira crunogena* strain TH-55\(^T\) at the species level. On the basis of these physiological and genetic properties, we suggest that strain I78\(^T\) is representative of a novel species of the genus *Thiomicrospira*, for which the name *Thiomicrospira thermophila* sp. nov. is proposed.

The physiological properties of *Thiomicrospira thermophila* described in this study may provide a key to understanding the potential ecological niches of *Thiomicrospira thermophila* in the deep-sea hydrothermal field of the TOTO caldera. *Thiomicrospira thermophila* was obtained from an STR-ISCS device deployed in a hydrothermal diffusing flow with a maximum temperature of 78 °C and a pH of 5-3. The substratum trapping a viable population of *Thiomicrospira thermophila* was exposed to a temperature of about 70 °C for at least 2 days. *Thiomicrospira thermophila* was probably entrained by the diffusing flow from the sub-seafloor and survived in the substratum. The relative thermophilicity, thermostolerance and acidophilicity of *Thiomicrospira thermophila* among the genus *Thiomicrospira* and its potential for nitrogen fixation are consistent with its occurrence in the sub-seafloor hydrothermal mixing zones with oxygenated sea water. Because the TOTO caldera hydrothermal system is characterized by strongly acidified fluids enriched with volatile volcanic gas components, the sub-seafloor mixing zones would provide microaerobic, acidic, mesophilic to thermophilic microbial habitats with abundant reduced sulfur compounds and molecular nitrogen provided from the volcanic gas input. Indeed, successful cultivation of *Thiomicrospira thermophila* was only via the ISCS, not from any other samples such as the ambient sea waters, hydrothermal plumes, chimney structures and rocks (data not shown). Localization of the viable population and the physiology of *Thiomicrospira thermophila* will point to the existence of novel sub-seafloor habitats beneath the TOTO caldera deep-sea hydrothermal field.

**Description of *Thiomicrospira thermophila* sp. nov.**

*Thiomicrospira thermophila* (ther.mo’phi.la. Gr. fem. n. thermе heat; Gr. adj. philos loving; N.L. fem. adj. thermophila heat-loving).

Cells occur singly, as Gram-negative, motile, straight to curved rods with a polar flagellum, with a mean length of 0.8–1.5 μm and a diameter of approximately 0.4–0.7 μm. Cells are microaerobic, tolerating up to 10% O₂ in the gas phase. The temperature range for growth is 15–55 °C (optimum 35–40 °C). The pH range for growth is 5–0–8–0 (optimum pH 6.0). NaCl in the concentration range 3–70 g l\(^{-1}\) is an absolute growth requirement; optimum growth occurs at 12–20 g l\(^{-1}\). Chemolithoautotrophic growth occurs with reduced sulfur compounds such as thiosulfate, elemental sulfur and sulfide as electron donors and molecular oxygen as an electron acceptor. Heterotrophic growth is possible with 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) CMC, 0.1% (w/v) Casamino acids, 5 mM glycerol, 5 mM fumarate, 5 mM malate, 5 mM citrate, 5 mM glycine, 5 mM alanine, 5 mM leucine, 5 mM isoleucine, 5 mM valine, 5 mM lysine, 5 mM cysteine, 5 mM methionine, 5 mM arginine, 5 mM histidine, 5 mM asparagine, 5 mM glutamine, 5 mM aspartate, 5 mM glutamate, 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 or 0.02% (w/v) trehalose as the sole carbon source. Thiosulfate is oxidized to sulfate and elemental sulfur during growth. Nitrate, ammonium, organic nitrogen compounds and molecular nitrogen are utilized as nitrogen sources. Vitamins, selenium and tungsten are not required for growth. The major cellular fatty acids are C₁十四_0 (7.4%), C₁十四_0 (16.3%), C₁十四_1 (37.2%), anteiso-C₁十四_0 (4.0%), C₁十八_0 (21.3%) and C₁十八_1 (13.8%). The DNA G+C content is 43.8 mol% (by HPLC). Isolated from an in situ colonization device deployed in the hydrothermal diffusing flow (maximally 78 °C) at the TOTO caldera in the Mariana Arc, Western Pacific.

The type strain is I78\(^T\) (=JCM 12397\(^T\)=DSM 16397\(^T\)).

**Acknowledgements**

We would like to thank Mr Katsuyuki Uematsu for assistance in preparing electron micrographs. We are very grateful to the R/V...
References


