**Sulfurimonas paralvinellae** sp. nov., a novel mesophilic, hydrogen- and sulfur-oxidizing chemolithoautotroph within the *Epsilonproteobacteria* isolated from a deep-sea hydrothermal vent polychaete nest, reclassification of *Thiomicrospira denitrificans* as *Sulfurimonas denitrificans* comb. nov. and emended description of the genus *Sulfurimonas*

Ken Takai, Masae Suzuki, Satoshi Nakagawa, Masayuki Miyazaki, Yohey Suzuki, Fumio Inagaki and Koki Horikoshi

A novel mesophilic bacterium, strain GO25\(^T\), was isolated from a nest of hydrothermal vent polychaetes, *Paralvinella* sp., at the Iheya North field in the Mid-Okinawa Trough. Cells were motile short rods with a single polar flagellum. Growth was observed between 4 and 35 °C (optimum 30 °C; 13–16 h doubling time) and between pH 5.4 and 8.6 (optimum pH 6.1). The isolate was a facultatively anaerobic chemolithoautotroph capable of growth using molecular hydrogen, elemental sulfur or thiosulfate as the sole energy source, carbon dioxide as the sole carbon source, ammonium or nitrate as the sole nitrogen source and elemental sulfur, thiosulfate or yeast extract as the sole sulfur source. Strain GO25\(^T\) represents the first deep-sea epsilonproteobacterium capable of growth by both hydrogen and sulfur oxidation. Nitrate or molecular oxygen (up to 10 % partial pressure) could serve as the sole electron acceptor to support growth. Metabolic products of nitrate reduction shifted in response to the electron donor provided. The G+C content of genomic DNA was 37.6 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the novel isolate belonged to the genus *Sulfurimonas* and was most closely related to *Sulfurimonas autotrophica* OK10\(^T\) (96.3 % sequence similarity). DNA–DNA hybridization demonstrated that the novel isolate could be differentiated genotypically from *Sulfurimonas autotrophica* OK10\(^T\). On the basis of the physiological and molecular properties of the novel isolate, the name *Sulfurimonas paralvinellae* sp. nov. is proposed, with strain GO25\(^T\) (=JCM 13212\(^T\) = DSM 17229\(^T\)) as the type strain. *Thiomicrospira denitrificans* DSM 1251\(^T\) (=ATCC 33889\(^T\)) is phylogenetically associated with *Sulfurimonas autotrophica* OK10\(^T\) and *Sulfurimonas paralvinellae* GO25\(^T\). Based on the phylogenetic relationship between *Thiomicrospira denitrificans* DSM 1251\(^T\), *Sulfurimonas autotrophica* OK10\(^T\) and *Sulfurimonas paralvinellae* GO25\(^T\), we propose the reclassification of *Thiomicrospira denitrificans* as *Sulfurimonas denitrificans* comb. nov. (type strain DSM 1251\(^T\) = ATCC 33889\(^T\)). In addition, an emended description of the genus *Sulfurimonas* is proposed.

An increasing number of previously uncultivated members of the *Epsilonproteobacteria* from deep-sea hydrothermal environments have been isolated and characterized recently (Alain et al., 2002; Campbell et al., 2001; Inagaki et al., 2003, 2004; Miroshnichenko et al., 2002, 2004; Nakagawa et al., 2005a, b, c; Takai et al., 2003, 2004a, c, 2005a; Voordockers et al., 2005). All the deep-sea epsilonproteobacterial strains

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GO25\(^T\) is AB252048.

An electron micrograph of a cell of *Sulfurimonas paralvinellae* GO25\(^T\) and graphs showing the effects of temperature, pH and NaCl concentration on the growth of the strain are available as supplementary figures in IJSEM Online.
described so far can be classified into the following groups with respect to their energy yielding process: (i) sulfur-oxidizing, (ii) hydrogen-oxidizing and (iii) both hydrogen- and formate-oxidizing. *Sulfurimonas autotrophica* OK10<sup>T</sup> (Inagaki et al., 2003) and *Sulfurovum lithothrophicum* 42BKT<sup>T</sup> (Inagaki et al., 2004) are sulfur-oxidizing chemolithoautotrophs that utilize reduced sulfur compounds such as thiosulfate and elemental sulfur (S<sub>0</sub>). The hydrogen-oxidizing group consists of *Hydrogenimonas thermophila* EP1-55-1%<sup>T</sup> (Takai et al., 2004c), *Thiotherea micantiosi* BKB25T<sup>S</sup>-Y<sup>T</sup> (Nakagawa et al., 2005a), *Nitrifractor salsuginis* E9317-1<sup>T</sup> (Nakagawa et al., 2005b), *Nitratiruptor tergarcus* M155-1<sup>T</sup> (Nakagawa et al., 2005b), *Lebetimonas acidiphila* PD55-1<sup>T</sup> (Takai et al., 2005a), *Caminibacter mediatlanticus* TB-2<sup>T</sup> (Voordendeckers et al., 2005) and *Caminibacter profundus* CR<sup>T</sup> (Miroshnichenko et al., 2004). For *Nautilia lithothrophicus* 525<sup>T</sup> (Miroshnichenko et al., 2002) and *Caminibacter hydrophilus* AM1116<sup>T</sup> (Alain et al., 2002), formate can serve as the sole energy source in addition to molecular hydrogen. Several as yet undescribed strains potentially belonging to the genus *Sulfurospirillum*, isolated from nests of the tube-dwelling polychaete *Alvinella pompejana*, also represent the hydrogen- and formate-oxidizing group (Campbell et al., 2001). Although previously described deep-sea members of the *Epsilonproteobacteria* gain energy by either hydrogen- or sulfur-oxidation, Nakagawa et al. (2005c) demonstrated that many of the epsilonproteobacterial isolates within group B and group F from a variety of deep-sea hydrothermal habitats have versatile energy metabolisms capable of using both molecular hydrogen and reduced sulfur compounds as energy sources.

Strain GO25<sup>T</sup> (previously designated strain GO25-1) was isolated from a nest of a deep-sea hydrothermal vent polychaete (*Paralvinella* sp.) at a sulfide mound called North Big Chimney (NBC) (Takai et al., 2004b) at the Ihey North field in the Mid-Okinawa Trough (Takai et al., 2003). Preliminary phylogenetic and physiological characterizations have suggested that this strain is phylogenetically related to *Sulfurimonas autotrophica* OK10<sup>T</sup>, but its energy metabolism is considerably different from that of *Sulfurimonas autotrophica* OK10<sup>T</sup> (Takai et al., 2003; Nakagawa et al., 2005c). Strain GO25<sup>T</sup> can be characterized by its ability to utilize both molecular hydrogen and reduced sulfur compounds as the sole energy source (Nakagawa et al., 2005c). This type of energy metabolism might be a key not only to elucidate genetic and biochemical aspects of epsilonproteobacterial energy metabolism, but also to understand the ecophysiological roles of these bacteria, which dominate a variety of deep-sea hydrothermal environments. The taxonomic and physiological properties of strain GO25<sup>T</sup> are reported in this study. The aim of this study was to establish the basis for further comparative genetic and biochemical investigations and for *in situ* ecophysiological surveys.

**Sample collection, enrichment and purification**

Strain GO25<sup>T</sup> was isolated from a polychaete nest colonizing NBC as previously described (Nakagawa et al., 2005c; Takai et al., 2003, 2004b). After successful enrichment with MMJHS medium, as described by Takai et al. (2003), strain GO25<sup>T</sup> was obtained as a pure culture using the dilution-to-extinction technique (Takai & Horikoshi, 2000). MMJHS medium contained 1 g of each of NaHCO<sub>3</sub>, Na<sub>2</sub>SO<sub>3</sub>, H<sub>2</sub>O and NaNO<sub>3</sub>, 30 g S<sub>0</sub> and 10 ml vitamin solution (Balch et al., 1979) per litre of MJ synthetic seawater (Takai et al., 2003). The purity was confirmed routinely by microscopic observation and by repeated partial sequencing of the 16S rRNA gene using several primers (Lane, 1991). Strain GO25<sup>T</sup> was routinely cultivated with MMJHS (thiosulfate-minus) medium, which was MMJHS medium without thiosulfate, with a gas phase of 80 % H<sub>2</sub> and 20 % CO<sub>2</sub> (200 kPa) (Takai et al., 2003).

*Sulfurimonas autotrophica* OK10<sup>T</sup> (Inagaki et al., 2003) was isolated by our laboratory and *Thiomicrospira denitrificans* DSM 1251<sup>T</sup> (Timmer-ten Hoor, 1975) was purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen. These strains were routinely cultivated at their optimal conditions as previously described (Inagaki et al., 2003; Timmer-ten Hoor, 1975).

**Morphology**

Cells were observed under a phase-contrast microscope (BX51; Olympus) with the SPOT RT Slider CCD camera system (Diagnostic Instruments). Gram-staining was performed by using a Gram-stain kit (Wako). Transmission electron microscopy of negatively stained cells was carried out as described by Zillig et al. (1990). Cells grown in MMJHS (thiosulfate-minus) medium at 30 °C that were in the mid-exponential phase of growth were used for microscopic observation. Cells of strain GO25<sup>T</sup> were Gram-negative short rods, which were about 0.6–0.8 μm in width, 1.5–2.5 μm in length and motile with a polar flagellum (see Supplementary Fig. S1 in IJSEM Online). Spore formation was not observed under any culture conditions. These morphological features of strain GO25<sup>T</sup> are similar to those of the previously described members of the *Epsilonproteobacteria* (Table 1).

**Growth characteristics**

Strain GO25<sup>T</sup> was routinely cultivated in MMJHS (thiosulfate-minus) medium. To prepare the medium, all the components other than the vitamin solution, elemental sulfur and NaHCO<sub>3</sub> were dissolved in distilled deionized water and the pH was adjusted to around 6.0 with HCl before autoclaving. After autoclaving, concentrated solutions of vitamins, NaHCO<sub>3</sub> and elemental sulfur were added to the medium under gas purging of 80 % H<sub>2</sub> and 20 % CO<sub>2</sub> and the pH was readjusted to 6.0 with HCl unless otherwise noted. These solutions were separately sterilized by filtration and the elemental sulfur was sterilized by autoclaving at 95 °C for 3 h three times. The medium was dispensed at 20 % of the total bottle (Schott Glaswerke) or tube (Iwaki Glass) volume and containers were tightly sealed with a butyl rubber stopper under a gas phase of 80 % H<sub>2</sub> and 20 %
CO2 at 200 kPa. All experiments described below were conducted in duplicate.

Growth of strain GO25T was measured by direct cell counting after staining with 4',6-diamidino-2-phenylindole (DAPI) (Porter & Feig, 1980) using a phase-contrast microscope (BX51; Olympus). The cultures were grown in 100 ml glass bottles (Schott Glaswerke) each containing 20 ml medium, with shaking (100 r.p.m.) in a temperature-controlled dry oven. With MMJHS (thiosulfate-minus) medium, strain GO25T grew over a temperature range of about 4–35°C, showing optimal growth at 30°C. The generation time at 30°C, pH 6·1, was about 13–16 h (see Supplementary Fig. S2a in IJSEM Online). When the effect of pH on growth was tested at 30°C, the pH of the MMJHS (thiosulfate-minus) medium was adjusted to various values with 10 mM acetate/acetic acid buffer (pH 3–5), MES (pH 5–6), PIPES (pH 6–7), HEPES (pH 7–7·5) or Tris (pH 8–9·5) at room temperature (Supplementary Fig. S2b). Growth occurred between pH 5·4–8·6, with optimum growth at about pH 6·5–7 (Supplementary Fig. S2b). No growth was observed at pH 4·9 or 8·2. The pH of the medium was found to be stable during growth. When grown in MMJHS (thiosulfate-minus) medium with variable concentrations of NaCl, strain GO25T grew over an NaCl concentration range of 12 to 50 g l–1, with optimum growth at 30 g l–1 at 30°C and pH 6·1 (Supplementary Fig. S2c). In general, the effects of temperature, pH and NaCl concentration on the growth of strain GO25T are similar to those observed for Sulfurimonas autotrophica OK10T (Table 1). However, growth of strain GO25T is much slower than that of Sulfurimonas autotrophica OK10T

Table 1. Comparison of characteristics of Sulfurimonas paralvinellae GO25T sp. nov. and related type strains

<table>
<thead>
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<th>Characteristic</th>
<th>1</th>
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<td>Maximum O2 concentration (%)</td>
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<td>Sulfite oxidoreductase activity</td>
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<td>–</td>
<td>–</td>
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<td>Major fatty acids</td>
<td>C18:1 (37 %), C16:1 (45 %), C16:0 (25 %), C16:1 (22%)</td>
<td>C16:0 (37 %), C18:1 (9 %)</td>
<td>C18:1 (42 %), C16:1 (31 %), C16:0 (24 %)</td>
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<td>DNA G+C content (mol%)</td>
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<td>35·2</td>
<td>36</td>
<td>35·5</td>
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*Cells of Thiomicrospira denitrificans DSM 1251T have previously been reported to be spiral, but in this study straight to slightly curved, short rods were observed in the exponential growth phase.
and the ability to grow at 4 °C is a novel physiological feature of strain GO25T compared with all other previously described deep-sea epsilonproteobacteria (Table 1).

Heterotrophic growth of strain GO25T was tested in MMJHS (thiosulfate-minus) medium without NaHCO3 under a gas phase of 100 % H2 (200 kPa), containing each of the following potential organic carbon sources: 0-1 % (w/v) yeast extract, 0-1 % (w/v) tryptone, 0-1 % (w/v) Casamino acids, 5 mM formate, 5 mM acetate, 5 mM glycerol, 0-025 % (v/v) methanol, 0-05 % (v/v) ethanol, 0-1 % (v/v) 2-propanol, 5 mM citrate, 5 mM L-tartrate, 5 mM fumarate, 5 mM succinate, 5 mM propionate, 5 mM L-malate, 5 mM L-lactate, 5 mM oxalate, 5 mM pyruvate, 5 mM of each of 20 L-amino acids, 0-1 % (w/v) D-glucose, 0-1 % (w/v) D-galactose, 0-1 % (w/v) sucrose, 0-1 % (w/v) D-fructose, 0-1 % (w/v) D-lactose, 0-1 % (w/v) D-maltose and 0-1 % (w/v) starch. Strain GO25T was not able to grow heterotrophically using H2 and S0 as potential energy sources and nitrate as an electron acceptor. Utilization of these organic compounds as an alternative energy source instead of H2 and S0 was also examined in MMJHS (thiosulfate-minus) medium containing each of the organic compounds described above under a gas phase of 80 % N2 and 20 % CO2 (200 kPa). Under these conditions, none of the organic compounds sustained the growth of strain GO25T as the energy source.

In an attempt to determine electron acceptors for growth of strain GO25T, each of the potential electron acceptors, such as sulfite (2 mM and 10 mM), thiosulfate (10 mM), tetraphionate (10 mM), nitrate (10 mM) and nitrite (1 mM and 5 mM), ferric citrate (20 mM), selenite (5 mM), arsenate (5 mM), fumarate (10 mM) and O2 (0-5, 1, 2, 3, 5, 10, 15 and 20 % partial pressure) was tested with H2 and S0 as the electron donors. Nitrate and O2 (0-5-10 % partial pressure) supported growth as the sole electron acceptor while nitrate provided a better cell yield than the optimal concentrations of O2 (0-5 and 1 % partial pressure).

Strain GO25T did not grow in the absence of S0 with MMJHS (thiosulfate-minus) medium under a gas phase of 80 % H2 and 20 % CO2 at 200 kPa. This might have two different explanations: (i) H2 does not serve as the energy source and S0 is absolutely required as the sole energy source or (ii) H2 serves as the energy source but S0 is absolutely required as the sulfur source. In an attempt to determine a sulfur source for growth of strain GO25T, potential sources such as sulfate (5 mM), sulfite (2 mM), thiosulfate (5 mM), S0 (1 %, w/v), sodium sulfide (2 mM), cystine hydrochloride (2 mM) and yeast extract (0-1 % w/v) were examined in MMJHS (thiosulfate-minus) medium in which sulfur compounds were removed and replaced with the chloride salts under a gas phase of 80 % H2 and 20 % CO2 at 200 kPa. Strain GO25T grew using H2 as the energy source only when thiosulfate, S0 or yeast extract was provided as the sole sulfur source. No growth occurred when strain GO25T was transferred directly from the culture grown in MMJHS (thiosulfate-minus) medium under a gas phase of 80 % H2 and 20 % CO2 at 200 kPa to H2-free MMJHS (thiosulfate-minus) medium under a gas phase of 80 % N2 and 20 % CO2 at 200 kPa. However, after acclimatization of strain GO25T to the medium with decreasing partial pressures of H2, the strain was eventually able to grow on S0 or thiosulfate as the sole electron donor using nitrate or O2 as an electron acceptor. Thus, H2, S0 or thiosulfate can serve as the sole electron donor for growth of strain GO25T.

Potential trace nutrients required for growth, such as selenite, tungstate and vitamins, were examined in MMJHS (thiosulfate-minus) medium in the absence of the tested compounds and the nitrogen source for growth (NH4Cl, NaNO3, N2, NaNO3 or yeast extract) was also examined. Selenium, tungsten and vitamins were not required for growth. Isolate GO25T utilized ammonium, nitrate or yeast extract as a nitrogen source, but could not utilize nitrite or molecular nitrogen.

Isolate GO25T is the first deep-sea epsilonproteobacterium capable of utilizing both H2 and reduced sulfur compounds as energy sources (Table 1). The possible requirement of S0 as a sulfur source for growth is reported for Nitratifactor salsuginis E9I37-1T (Nakagawa et al., 2005b). However, S0 is not absolutely required and no oxidation of S0 occurred during the growth of Nitratifactor salsuginis E9I37-1T (Nakagawa et al., 2005b).

Since strain GO25T utilizes S0 as both an energy and sulfur source, it is very interesting that the strain is able to grow with the oxidation of H2 and/or S0. The time-course of the oxidation of H2 and S0 and the concomitant reduction of nitrate during growth of strain GO25T was examined with MMJHS (thiosulfate-minus) medium in the absence of NH4Cl under a varying partial pressure of H2. The experiment was conducted at 30 °C and pH 6-1 using 100 ml bottles. The concentrations of H2, N2 and N2O in the gas phase during growth were measured using a gas chromatograph (Micro GC CP2002; GL Sciences) and anions such as chloride, nitrate, nitrite, thiosulfate, sulfate and sulfate were determined by HPLC using a Shim-pack IC column (Shimadzu). Production of ammonium ions was also checked by using Nessler’s reagent (Allen et al., 1974). When strain GO25T was grown with excess amounts of both H2 (80 % and 20 %, 2 atm., corresponding to 82 and 20-5 mM) and S0 (1 % w/v, corresponding to 300 mM), nitrate was reduced to nitrite and N2O (Fig. 1a). During growth, H2 was consumed predominantly and the oxidation of S0 to thiosulfate and sulfate remained at a low level (Fig. 1a). For growth with H2-limited or -free media, the oxidation of S0 was significantly increased (Fig. 1b, c). Under H2-limited (1 % and 0-2 %, 2 atm., corresponding to 1 and 0-2 mM) conditions, S0 was oxidized to thiosulfate and sulfate and nitrate was reduced to nitrite, N2O and N2 (Fig. 1b). However, during growth in the absence of H2, only sulfate and N2 were detected as the products of S0 oxidation and nitrate reduction, respectively (Fig. 1c). In all cases, no ammonification was detected and the pH of the medium was stable at pH 6-1 during growth. Based on final
cell yields and the consumption of the electron donors, in the cases of growth under H2-abundant and H2-free conditions, both H2 and S0 gave a similar efficiency (1.4 × 10^7 cells μmol^-1) for cellular yield of strain GO25T, while H2 resulted in a higher growth rate. These results strongly suggest that H2 is a better energy source for faster growth of strain GO25T than S0 in in vitro experiments and that oxidation of different electron donors is coupled with different extents of nitrate reduction. The biochemical and molecular mechanism of the electron donor-dependent shift in reduction of the electron acceptor (switching of nitrate reduction pathways by different electron donors) should be further investigated.

Sensitivity of strain GO25T to antibiotics (at 50 and 100 μg ml^-1) such as chloramphenicol, streptomycin, kanamycin, ampicillin and rifampicin was tested at 30°C. Strain GO25T was sensitive to all the antibiotics tested at a concentration of 50 μg ml^-1.

**Fatty acid analysis**

Cellular fatty acid composition was analysed from cells grown in MMJHS (thiosulfate-minus) medium at 30°C in the late-exponential growth phase as reported previously (Suzuki et al., 2005). The major cellular fatty acids of strain GO25T were C18:1 (37%), C16:0 (25%), C16:1 (22%), 3-OH C14:0 (7%), C14:0 (5%) and C18:0 (4%) (Suzuki et al., 2005). This composition was similar to that of *Nitratifactor salsuginis* E9I37-1T (Nakagawa et al., 2005b) rather than to that of *Sulfurimonas autotrophica* OK10T (Inagaki et al., 2003).

**Nucleic acid analyses**

Genomic DNA of strain GO25T was prepared as described by Marmur & Doty (1962). The DNA G+C content was determined by direct analysis of deoxyribonucleotides on HPLC (Tamaoka & Komagata, 1984). The DNA G+C content of strain GO25T was 37.6 mol%, which is similar to those of *Sulfurimonas autotrophica* OK10T (35.2 mol%) (Inagaki et al., 2003), *Thiomicrospira denitrificans* DSM 1251T (36.0 mol%) (Brinkhoff et al., 2005; Kuenen et al., 1991; Timmer-ten Hoor, 1975) and *Thioreductor micantisoli* BKB25Ts-YT (37.2 mol%) (Nakagawa et al., 2005a).

The 16S rRNA gene was amplified by PCR using primers Bac 27F and 1492R (DeLong, 1992; Lane, 1991) as described previously (Takai et al., 2001). The nearly complete sequence (1445 bp) of the 16S rRNA gene from strain GO25T was directly sequenced in both strands using the dideoxynucleotide chain-termination method with a DNA sequencer (Model 3100; Perkin Elmer). The 16S rRNA gene sequence was subjected to a gappedBLAST search (Altschul et al., 1997; Benson et al., 1998) and was found to be most closely related to the sequences of a gill symbiont of the deep-sea vent gastropod *Alviniconcha* sp. (97.4% sequence similarity) from the Manus Basin (Urakawa et al., 2005), *Sulfurimonas autotrophica* OK10T (96.3%) (Inagaki et al., 2003) and *Thiomicrospira denitrificans* DSM 1251T (93.1%) (Brinkhoff et al., 2005; Kuenen et al., 1991; Timmer-ten Hoor, 1975). The 16S rRNA gene sequence of *Sulfurimonas autotrophica* OK10T showed 93.3% similarity to that of *Thiomicrospira denitrificans* DSM 1251T. The sequence was manually aligned with a subset of 16S rRNA gene sequences.

**Fig. 1.** Time-courses of the oxidation of H2 and S0 and the reduction of nitrate during growth of strain GO25T. (a) Redox reactions during growth with excess amounts of both H2 (20-5 mM) and S0 (300 mM) in the medium. (b) Redox reactions during growth under H2-limited (1 mM) conditions. (c) Redox reactions during growth in H2-free MMJHS (thiosulfate-minus) medium under a gas phase of 80% N2 and 20% CO2 at 200 kPa. Filled circles indicate the cell number during the growth.
from GenBank according to secondary structure using ARB (Ludwig et al., 2004). Phylogenetic analyses were restricted to unambiguously aligned nucleotide positions. Evolutionary distance matrix analysis (using the Jukes–Cantor correlation method) and neighbour-joining analysis were performed using the PHYLIP package (http://evolution.genetics. washington.edu/phylip.html). Maximum-likelihood analysis was performed using TREE-PUZZLE software (Schmidt et al., 2002). Bootstrap analysis was performed to provide confidence estimates for the phylogenetic tree topologies. The phylogenetic tree indicated that strain GO25\textsuperscript{T} formed a clade with an Alvinicroncha sp. gill symbiont, deeply branched prior to the divergence of Sulfurimonas autotrophica OK10\textsuperscript{T} (Inagaki et al., 2003) and Thiomicrospira denitrificans DSM 1251\textsuperscript{T} (Brinkhoff et al., 2005; Kuenen et al., 1991; Timmer-ten Hoor, 1975) (Fig. 2). Both the sequence similarity analysis and phylogenetic analysis suggested that strain GO25\textsuperscript{T} belonged to the genus Sulfurimonas.

DNA–DNA hybridization between genomic DNA of strain GO25\textsuperscript{T} and Sulfurimonas autotrophica OK10\textsuperscript{T} was carried out at 42 °C for 3 h and was measured fluorometrically using photobiotin according to the method of Ezaki et al. (1989). The mean hybridization value was 32.4\%, indicating that strain GO25\textsuperscript{T} could be differentiated genotypically from Sulfurimonas autotrophica OK10\textsuperscript{T}, according to the definition of a species based on DNA–DNA relatedness (Wayne et al., 1987).

**Enzyme and genetic features of energy and carbon metabolisms**

Potential key enzyme activities for chemolithoautotrophic growth of strain GO25\textsuperscript{T} (ATP-dependent citrate lyase, pyruvate:acceptor oxidoreductase, 2-oxoglutarate:acceptor oxidoreductase, isocitrate dehydrogenase, phosphoenolpyruvate carboxylase, pyruvate carboxylase, ribulose-1,5-bisphosphate carboxylase/oxygenase, hydrogenase, thiosulfate-oxidizing enzymes, adenosine 5\'-phosphate carboxylase/oxygenase, hydrogenase, ribulose-1,5-bisphosphate carboxylase/oxygenase, hydrogenase, thiosulfate-oxidizing enzymes, adenosine 5\'-phosphate carboxylase/oxygenase, hydrogenase) were identified from the genome of strain GO25\textsuperscript{T} (Takai et al., 2005b). These genes for the enzymes of inorganic carbon fixation and H\textsubscript{2} oxidation were also determined as described previously (Takai et al., 2005b). Strain GO25\textsuperscript{T} had key enzyme activities for a reductive TCA cycle (ATP-dependent citrate lyase, pyruvate : acceptor oxidoreductase, 2-oxoglutarate : acceptor oxidoreductase and isocitrate dehydrogenase) but not for the Calvin–Benson cycle and possessed enzymes involved in H\textsubscript{2} and S\textsuperscript{0} oxidation (hydrogenase and sulfite oxidoreductase) (Takai et al., 2005b). In addition, genes for the enzymes ATP-dependent citrate lyase (\textit{acdB}), pyruvate:acceptor oxidoreductase (\textit{porAB}), 2-oxoglutarate:acceptor oxidoreductase (\textit{oorAB}) and hydrogenase (\textit{hynSL}) were identified in the genome of strain GO25\textsuperscript{T} (Takai et al., 2005b). These were clear enzyme and genetic signatures for the H\textsubscript{2}- and S\textsuperscript{0}-oxidizing chemolithoautotrophy of strain GO25\textsuperscript{T}.

**Comparison with related species**

Strain GO25\textsuperscript{T} is a mesophilic, facultatively anaerobic, strict chemolithoautotroph using H\textsubscript{2} or reduced sulfur compounds as the sole energy source. This strain is the first described strain of deep-sea epsilonproteobacteria that is capable of utilizing both H\textsubscript{2} and reduced sulfur compounds as energy sources and only the second reported epsilonproteobacterium to do so, after Sulfuricurvum kuijense YK-1\textsuperscript{T}, isolated from an underground crude-oil storage cavity in Japan (Kodama & Watanabe, 2004). Phylogenetic analysis indicated that strain GO25\textsuperscript{T} is most closely related to Sulfurimonas autotrophica OK10\textsuperscript{T}, isolated from deep-sea hydrothermal sediments at Hatoma Knoll in the Okinawa Trough (Inagaki et al., 2003; Takai et al., 2005) (Fig. 2).

However, many of the physiological characteristics of strain GO25\textsuperscript{T} are different from those of \textit{Sulfurimonas autotrophica} OK10\textsuperscript{T} (Table 1). The utilization patterns of both electron donors and electron acceptors are different and the optimal growth conditions also differ between the two strains (Table 1). Major cellular fatty acid content is a chemotaxonomic feature that can differentiate the two strains. In addition, DNA–DNA hybridization analysis clearly indicates that strain GO25\textsuperscript{T} can be differentiated genotypically from \textit{Sulfurimonas autotrophica} OK10\textsuperscript{T} at the species level.

**Fig. 2.** Phylogenetic tree of representative epsilonproteobacteria and environmental \textit{rRNA} gene clones inferred from 16S \textit{rRNA} gene sequences using the neighbour-joining method on 1051 homologous sequence positions for each organism. Numbers at each node represent bootstrap values (as a percentage) determined from 1000 replicates (neighbour-joining tree/maximum-likelihood tree). Two numbers are represented where identical topology is given by the two trees, while only one tree represents the bootstrap value by the neighbour-joining treeing method. Bar, 2 substitutions per 100 nucleotides.
On the basis of these physiological and genetic properties, we propose that strain GO25\textsuperscript{T} represents the type strain of a novel species of the genus \textit{Sulfurimonas}, \textit{Sulfurimonas paralvinellae} sp. nov.

**Ecological implications**

Strain GO25\textsuperscript{T} grows on both \textit{H}_{2} and reduced sulfur compounds as energy sources. However, in this study, it was demonstrated that \textit{H}_{2} provided a higher growth rate for strain GO25\textsuperscript{T} than reduced sulfur compounds and that \textit{H}_{2} was predominantly utilized even though excess amounts of reduced sulfur compounds were also present. This is a very important clue to understanding the energy metabolism of strain GO25\textsuperscript{T} in situ in its habitat of polychaete nests. The polychaete colonies at NBC in the Iheya North field are situated adjacent to hydrothermal fluid ventings (Nakagawa et al., 2005c). Due to their close proximity to hydrothermal fluids, considerable amounts of both \textit{H}_{2} and reduced sulfur compounds are likely to be present in the habitat. However, according to the typical hydrothermal fluid chemistries in the Okinawa Trough, the concentration of \textit{H}_{2}\textit{S} is approximately two orders of magnitude higher than that of \textit{H}_{2} in the hydrothermal fluids (Gamo, 1995). In addition, it has been noted that the polychaete nests are covered with elemental sulfur particles, probably formed by chemical and microbiological processes. This implies that reduced sulfur compounds are always much more accessible and available than \textit{H}_{2} as energy sources for strain GO25\textsuperscript{T} in situ. Considering the higher growth rate observed with \textit{H}_{2} compared with reduced sulfur compounds and the preferred utilization of \textit{H}_{2} coincident with more abundant reduced sulfur compounds shown in this study, strain GO25\textsuperscript{T} may be sustained primarily by smaller amounts of \textit{H}_{2} as the energy source in situ. Although versatile energy metabolism using a variety of electron donor and acceptor species is a key feature of the deep-sea epsilonproteobacteria, it has been demonstrated that hydrogen-oxidizing chemolithoautotrophy is more common than sulfur-oxidizing forms among deep-sea epsilonproteobacterial isolates from various deep-sea hydrothermal vent niches (Nakagawa et al., 2005c). Further kinetic analyses of growth, using a variety of combinations and varying concentrations of electron donor and acceptor species, and determination of the molecular basis of biochemical characterizations will aid in the elucidation of the in situ energy metabolism of deep-sea epsilonproteobacteria showing versatile chemolithoautotrophy, such as strain GO25\textsuperscript{T}, in complex and diverse deep-sea hydrothermal vent habitats.

**Reclassification of \textit{Thiomicrospira} denitrificans**

\textit{Thiomicrospira} denitrificans DSM 1251\textsuperscript{T} (= ATCC 33889\textsuperscript{T}) (Brinkhoff et al., 2005; Kuenen et al., 1991; Timmer-ten Hoor, 1975) is phylogenetically associated with \textit{Sulfurimonas autotrophica} OK10\textsuperscript{T} and strain GO25\textsuperscript{T} (Fig. 2). This bacterium was isolated from coastal marine sediments with an interface between oxygen and sulfide and is a mesophilic, neutrophilic, facultatively anaerobic, denitrifying chemolithoautotroph that uses sulfide or thiosulfate and nitrate or nitrite as the electron donor and acceptor (Brinkhoff et al., 2005; Kuenen et al., 1991; Timmer-ten Hoor, 1975). The genus \textit{Thiomicrospira} (type species \textit{Thiomicrospira pelophila}) was proposed for a group of sulfur-oxidizing micro-organisms (Kuenen & Veldkamp, 1972). Muyzer et al. (1995) suggested that this group belonged within the \textit{Gammaproteobacteria}. It has often been pointed out that \textit{T. denitrificans}, an epsilonproteobacterium, should be reclassified and renamed (Brinkhoff et al., 2005; Takai et al., 2004d). As shown in Table 1, many physiological properties of \textit{T. denitrificans} DSM 1251\textsuperscript{T}, particularly the utilization pattern of electron donors and acceptors, are different from those of \textit{Sulfurimonas autotrophica} OK10\textsuperscript{T} and/or strain GO25\textsuperscript{T}. However, differences in physiological properties have also been found between \textit{Sulfurimonas autotrophica} OK10\textsuperscript{T} and strain GO25\textsuperscript{T}. Nakagawa et al. (2005c) clearly demonstrated that the metabolic versatility of deep-sea epsilonproteobacteria is not relevant to their 16S rRNA gene phylogeny. Thus, differences in metabolic traits might not always be a good marker for the classification of taxa within the \textit{Epsilonproteobacteria}. The 16S rRNA gene sequence of \textit{T. denitrificans} DSM 1251\textsuperscript{T} has 93-3 and 93-1\% similarity with those of \textit{Sulfurimonas autotrophica} OK10\textsuperscript{T} and strain GO25\textsuperscript{T}, respectively; these values fall within the range of 16S rRNA gene sequence similarity recognized for differentiation at the genus level (90–96\%) (Gillis et al., 2001). However, the phylogenetic relationship among \textit{T. denitrificans} DSM 1251\textsuperscript{T}, \textit{Sulfurimonas autotrophica} OK10\textsuperscript{T} and strain GO25\textsuperscript{T}, as indicated in Fig. 2, suggests that \textit{T. denitrificans} DSM 1251\textsuperscript{T} should be recognized as a member of the genus \textit{Sulfurimonas}. Therefore, primarily based on the phylogenetic relationship, we propose the reclassification of \textit{Thiomicrospira} denitrificans as \textit{Sulfurimonas denitrificans} comb. nov.

**Emended description of the genus \textit{Sulfurimonas}**

Cells are Gram-negative and morphologically variable. Straight to slightly short rods, elongated rods and spiral in different growth phases and under different growth conditions. Mesophilic and facultatively anaerobic. Do not always require NaCl for growth. Growth occurs chemolithoautotrophically with sulfide, \textit{S}^{2}\text{-}, thiosulfate and \textit{H}_{2} as electron donors and with nitrate, nitrite and \textit{O}_{2} as electron acceptors, using \textit{CO}_{2} as a carbon source. Potential ecological niches are deep-sea hydrothermal environments and marine sulfidic environments. The type species is \textit{Sulfurimonas autotrophica} (Inagaki et al., 2003).

**Description of \textit{Sulfurimonas paralvinellae} sp. nov.**

\textit{Sulfurimonas paralvinellae} (pa.ral.vin.ell'ae. N.L. gen. n. \textit{paralvinellae} of \textit{Paralvinella}, a genus of annelid polychaetes from which the organism was first isolated).

Each cell is a motile rod with a polar flagellum, 1.5–2.5 \textmu m long and approximately 0.6–0.8 \textmu m wide. Cells occur...
singly. Gram-negative. Anaerobic to microaerobic (up to 10% partial pressure of O₂). Temperature range for growth is 4–35 °C, optimum is 30 °C. The pH range for growth is 5.4–8.6, with an optimum at pH 6. NaCl is required for growth at 12–50 g l⁻¹; optimum growth occurs at 30 g l⁻¹. Strictly chemolithoautotrophic growth occurs with H₂, S⁻ or thiosulfate as an electron donor and with nitrate or O₂ as an electron acceptor. Nitrate is reduced to nitrite, N₂O and N₂ during growth. Elemental sulfur, thiosulfate or yeast extract serves as a sulfur source for growth. Nitrate or ammonium is required as a nitrogen source. Vitamins, selenium and tungsten are not required for growth. The major cellular fatty acids are C₁₈ : 1 (37%), C₁₆ : 0 (25%), C₁₆ : 1 (22%), 3-OH C₁₄ : 0 (7%), C₁₄ : 0 (5%) and C₁₈ : 0 (4%). The DNA G+C content is 37.6 mol% (by HPLC).

The type strain, strain GO25ᵀ (≡ JCM 13212ᵀ = DSM 17229ᵀ), was isolated from a polychaete nest of Paralvinella sp. at the Iheya North Field in the Mid-Okinawa Trough.

**Description of Sulfurimonas denitrificans comb. nov.**


The description remains as given by Brinkhoff *et al.* (2005). The type strain is DSM 1251ᵀ (≡ ATCC 33889ᵀ).

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**References**


