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

Correspondence

Ken Takai

kent@jamstec.go.jp

Subground Animalcule Retrieval (SUGAR) Program, Japan Agency for Marine-Earth Science and Technology, 2-15 Natsushima-cho, Yokosuka 237-0061, Japan

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.

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.

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; Voordeckers *et al.*, 2005). All the deep-sea epsilonproteobacterial strains

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^T (Inagaki *et al.*, 2003) and *Sulfurovum lithotrophicum* 42BKT^T (Inagaki *et al.*, 2004) are sulfur-oxidizing chemolitho-autotrophs that utilize reduced sulfur compounds such as thiosulfate and elemental sulfur (S⁰). The hydrogen-oxidizing group consists of *Hydrogenimonas thermophila* EP1-55-1^T (Takai *et al.*, 2004c), *Thioreductor micantisoli* BKB25Ts-Y^T (Nakagawa *et al.*, 2005a), *Nitratiraptor salsuginis* E9I37-1^T (Nakagawa *et al.*, 2005b), *Nitratiraptor tergarcus* MI55-1^T (Nakagawa *et al.*, 2005b), *Lebetimonas acidiphila* Pd55^T (Takai *et al.*, 2005a), *Caminibacter mediatlanticus* TB-2^T (Voordeckers *et al.*, 2005) and *Caminibacter profundus* CR^T (Miroshnichenko *et al.*, 2004). For *Nautilia lithotrophica* 525^T (Miroshnichenko *et al.*, 2002) and *Caminibacter hydrogophilus* AM1116^T (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^T (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 Iheya 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^T, but its energy metabolism is considerably different from that of *Sulfurimonas autotrophica* OK10^T (Takai *et al.*, 2003; Nakagawa *et al.*, 2005c). Strain GO25^T 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^T 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^T 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^T was obtained as a pure culture using the dilution-to-extinction technique (Takai & Horikoshi, 2000). MMJHS medium contained 1 g of each of NaHCO₃, Na₂S₂O₃·5H₂O and NaNO₃, 30 g S⁰ 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^T was routinely cultivated with MMJHS (thiosulfate-minus) medium, which was MMJHS medium without thiosulfate, with a gas phase of 80 % H₂ and 20 % CO₂ (200 kPa) (Takai *et al.*, 2003).

Sulfurimonas autotrophica OK10^T (Inagaki *et al.*, 2003) was isolated by our laboratory and *Thiomicrospira denitrificans* DSM 1251^T (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^T 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^T are similar to those of the previously described members of the *Epsilonproteobacteria* (Table 1).

Growth characteristics

Strain GO25^T was routinely cultivated in MMJHS (thiosulfate-minus) medium. To prepare the medium, all the components other than the vitamin solution, elemental sulfur and NaHCO₃ 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, NaHCO₃ and elemental sulfur were added to the medium under gas purging of 80 % H₂ and 20 % CO₂ 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 % H₂ and 20 %

Table 1. Comparison of characteristics of *Sulfurimonas paralvinellae* GO25^T sp. nov. and related type strains

Taxa: 1, *Sulfurimonas paralvinellae* GO25^T sp. nov. (data from this study; Suzuki *et al.*, 2005; Takai *et al.*, 2005b); 2, *Sulfurimonas autotrophica* OK10^T (Inagaki *et al.*, 2003; Takai *et al.*, 2005b); 3, *Sulfurimonas (Thiomicrospira) denitrificans* DSM 1251^T (Timmer-ten Hoor, 1975; Kuenen *et al.*, 1991; Brinkhoff *et al.*, 2005; Hügler *et al.*, 2005); 4, *Nitratifractor salsuginis* E9I37-1^T (Nakagawa *et al.*, 2005b; Takai *et al.*, 2005b). ND, Not determined. All four strains grow under microaerobic conditions. All strains possess key genes and enzymes for the reductive TCA cycle.

Characteristic	1	2	3	4
Morphology:				
Cell shape	Rod	Rod	Rod*	Rod
Motility	+	+	—	—
Growth:				
Temperature range (°C)	4–35	10–40	ND	30–40
Temperature optimum (°C)	30	23–26	22	37
Doubling time under optimal conditions (h)	13–16	1·4	12	2·5
pH range	5·4–8·6	5·0–9·0	ND	5·6–7·6
pH optimum	6·1	6·5	7	7
NaCl requirement	+	+	—	+
Maximum O ₂ concentration (%)	10	15	0·5	0·6
Anaerobic growth	+	—	+	+
Sulfur sources	S ⁰ , S ₂ O ₃ ²⁻ , yeast extract	ND	ND	S ⁰
Energy metabolism:				
Electron donors	H ₂ , S ⁰ , S ₂ O ₃ ²⁻	S ⁰ , S ₂ O ₃ ²⁻	HS ⁻ , S ₂ O ₃ ²⁻	H ₂
Electron acceptors	NO ₃ ⁻ , O ₂	O ₂	NO ₃ ⁻ , NO ₂ ⁻ , O ₂	NO ₃ ⁻ , O ₂
Products of nitrate reduction	NO ₂ ⁻ , N ₂ O, N ₂	None	N ₂	N ₂
Hydrogenase activity	+	—	ND	+
Hydrogenase genes	+	—	ND	+
Sulfite oxidoreductase activity	+	+	ND	—
Major fatty acids	C _{18:1} (37 %), C _{16:0} (25 %), C _{16:1} (22 %)	C _{16:1} (45 %), C _{16:0} (37 %), C _{18:1} (9 %)	ND	C _{18:1} (42 %), C _{16:1} (31 %), C _{16:0} (24 %)
DNA G+C content (mol%)	37·6	35·2	36	35·5

*Cells of *Thiomicrospira denitrificans* DSM 1251^T have previously been reported to be spiral, but in this study straight to slightly curved, short rods were observed in the exponential growth phase.

CO₂ at 200 kPa. All experiments described below were conducted in duplicate.

Growth of strain GO25^T 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 GO25^T 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 and 8·6, with optimum growth at about pH 6·1 (Supplementary Fig. S2b). No growth was observed at pH 4·9 or 9·2. 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 GO25^T 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 GO25^T are similar to those observed for *Sulfurimonas autotrophica* OK10^T (Table 1). However, growth of strain GO25^T is much slower than that of *Sulfurimonas autotrophica* OK10^T

and the ability to grow at 4 °C is a novel physiological feature of strain GO25^T compared with all other previously described deep-sea epsilonproteobacteria (Table 1).

Heterotrophic growth of strain GO25^T 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 GO25^T 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 GO25^T as the energy source.

In an attempt to determine electron acceptors for growth of strain GO25^T, each of the potential electron acceptors, such as sulfite (2 mM and 10 mM), thiosulfate (10 mM), tetrathionate (10 mM), nitrate (10 mM), nitrite (1 mM and 5 mM), ferric citrate (20 mM), selenate (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 GO25^T 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 GO25^T, potential sources such as sulfate (5 mM), sulfite (2 mM), thiosulfate (5 mM), S⁰ (1 %, w/v), sodium sulfide (2 mM), cysteine 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 GO25^T 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 GO25^T 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 GO25^T 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 GO25^T.

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, NaNO₂, N₂, NaNO₃ or yeast extract) was also examined. Selenium, tungsten and vitamins were not required for growth. Isolate GO25^T utilized ammonium, nitrate or yeast extract as a nitrogen source, but could not utilize nitrite or molecular nitrogen.

Isolate GO25^T 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* E9I37-1^T (Nakagawa *et al.*, 2005b). However, S⁰ is not absolutely required and no oxidation of S⁰ occurred during the growth of *Nitratifactor salsuginis* E9I37-1^T (Nakagawa *et al.*, 2005b).

Since strain GO25^T 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 GO25^T 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, sulfite 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 GO25^T 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

Fig. 1. Time-courses of the oxidation of H₂ and S⁰ and the reduction of nitrate during growth of strain GO25^T. (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.

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 GO25^T, while H₂ resulted in a higher growth rate. These results strongly suggest that H₂ is a better energy source for faster growth of strain GO25^T 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 GO25^T to antibiotics (at 50 and 100 $\mu\text{g ml}^{-1}$) such as chloramphenicol, streptomycin, kanamycin, ampicillin and rifampicin was tested at 30 °C. Strain GO25^T was sensitive to all the antibiotics tested at a concentration of 50 $\mu\text{g ml}^{-1}$.

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 GO25^T were C_{18:1} (37 %), C_{16:0} (25 %), C_{16:1} (22 %), 3-OH C_{14:0} (7 %), C_{14:0} (5 %) and C_{18:0} (4 %) (Suzuki *et al.*, 2005). This composition was similar to that of *Nitratifactor salsuginis* E9I37-1^T (Nakagawa *et al.*, 2005b) rather than to that of *Sulfurimonas autotrophica* OK10^T (Inagaki *et al.*, 2003).

Nucleic acid analyses

Genomic DNA of strain GO25^T 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 GO25^T was 37.6 mol%, which is similar to those of *Sulfurimonas autotrophica* OK10^T (35.2 mol%) (Inagaki *et al.*, 2003), *Thiomicrospira denitrificans* DSM 1251^T (36.0 mol%) (Brinkhoff *et al.*, 2005; Kuenen *et al.*, 1991; Timmer-ten Hoor, 1975) and *Thioreductor micantisoli* BKB25Ts-Y^T (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 GO25^T 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 gapped BLAST 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* OK10^T (96.3 %) (Inagaki *et al.*, 2003) and *Thiomicrospira denitrificans* DSM 1251^T (93.1 %) (Brinkhoff *et al.*, 2005; Kuenen *et al.*, 1991; Timmer-ten Hoor, 1975). The 16S rRNA gene sequence of *Sulfurimonas autotrophica* OK10^T showed 93.3 % similarity to that of *Thiomicrospira denitrificans* DSM 1251^T. The sequence was manually aligned with a subset of 16S rRNA gene sequences

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^T formed a clade with an *Alviniconcha* sp. gill symbiont, deeply branched prior to the divergence of *Sulfurimonas autotrophica* OK10^T (Inagaki *et al.*, 2003) and *Thiomicrospira denitrificans* DSM 1251^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^T belonged to the genus *Sulfurimonas*.

DNA–DNA hybridization between genomic DNA of strain GO25^T and *Sulfurimonas autotrophica* OK10^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^T could be differentiated genotypically from *Sulfurimonas autotrophica* OK10^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^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 sulfate reductase and sulfite oxidoreductase) were examined as reported previously (Takai *et al.*, 2005b). Genes for key

enzymes of inorganic carbon fixation and H₂ oxidation were also determined as described previously (Takai *et al.*, 2005b). Strain GO25^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 H₂ and S⁰ oxidation (hydrogenase and sulfite oxidoreductase) (Takai *et al.*, 2005b). In addition, genes for the enzymes ATP-dependent citrate lyase (*aclB*), pyruvate:acceptor oxidoreductase (*porAB*), 2-oxoglutarate:acceptor oxidoreductase (*oorAB*) and hydrogenase (*hynSL*) were identified in the genome of strain GO25^T (Takai *et al.*, 2005b). These were clear enzyme and genetic signatures for the H₂- and S⁰-oxidizing chemolithoautotrophy of strain GO25^T.

Comparison with related species

Strain GO25^T is a mesophilic, facultatively anaerobic, strict chemolithoautotroph using H₂ 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 H₂ and reduced sulfur compounds as energy sources and only the second reported epsilonproteobacterium to do so, after *Sulfuricurvum kujiense* YK-1^T, isolated from an underground crude-oil storage cavity in Japan (Kodama & Watanabe, 2004). Phylogenetic analysis indicated that strain GO25^T is most closely related to *Sulfurimonas autotrophica* OK10^T, isolated from deep-sea hydrothermal sediments at Hatoma Knoll in the Okinawa Trough (Inagaki *et al.*, 2003; Takai *et al.*, 2003) (Fig. 2). However, many of the physiological characteristics of strain GO25^T are different from those of *Sulfurimonas autotrophica* OK10^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^T can be differentiated genotypically from *Sulfurimonas autotrophica* OK10^T at the species level.

Fig. 2. Phylogenetic tree of representative epsilonproteobacteria and environmental rRNA gene clones inferred from 16S 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 indicated where identical topology is given by the two trees, while only one number 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^T represents the type strain of a novel species of the genus *Sulfurimonas*, *Sulfurimonas paralvinellae* sp. nov.

Ecological implications

Strain GO25^T grows on both H₂ and reduced sulfur compounds as energy sources. However, in this study, it was demonstrated that H₂ provided a higher growth rate for strain GO25^T than reduced sulfur compounds and that H₂ 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^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 H₂ 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 H₂S is approximately two orders of magnitude higher than that of H₂ 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 H₂ as energy sources for strain GO25^T *in situ*. Considering the higher growth rate observed with H₂ compared with reduced sulfur compounds and the preferred utilization of H₂ coincident with more abundant reduced sulfur compounds shown in this study, strain GO25^T may be sustained primarily by smaller amounts of H₂ 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^T, in complex and diverse deep-sea hydrothermal vent habitats.

Reclassification of *Thiomicrospira denitrificans*

Thiomicrospira denitrificans DSM 1251^T (= ATCC 33889^T) (Brinkhoff *et al.*, 2005; Kuenen *et al.*, 1991; Timmer-ten Hoor, 1975) is phylogenetically associated with *Sulfurimonas autotrophica* OK10^T and strain GO25^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 *Thiomicrospira* (type species *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 *Gammaproteobacteria*. It has often been pointed out that *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 *T. denitrificans* DSM 1251^T, particularly the utilization pattern of electron donors and acceptors, are different from those of *Sulfurimonas autotrophica* OK10^T and/or strain GO25^T. However, differences in physiological properties have also been found between *Sulfurimonas autotrophica* OK10^T and strain GO25^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 *Epsilonproteobacteria*. The 16S rRNA gene sequence of *T. denitrificans* DSM 1251^T has 93.3 and 93.1 % similarity with those of *Sulfurimonas autotrophica* OK10^T and strain GO25^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 *T. denitrificans* DSM 1251^T, *Sulfurimonas autotrophica* OK10^T and strain GO25^T, as indicated in Fig. 2, suggests that *T. denitrificans* DSM 1251^T should be recognized as a member of the genus *Sulfurimonas*. Therefore, primarily based on the phylogenetic relationship, we propose the reclassification of *Thiomicrospira denitrificans* as *Sulfurimonas denitrificans* comb. nov.

Emended description of the genus *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, S⁰, thiosulfate and H₂ as electron donors and with nitrate, nitrite and O₂ as electron acceptors, using CO₂ as a carbon source. Potential ecological niches are deep-sea hydrothermal environments and marine sulfidic environments. The type species is *Sulfurimonas autotrophica* (Inagaki *et al.*, 2003).

Description of *Sulfurimonas paralvinellae* sp. nov.

Sulfurimonas paralvinellae (pa.ral.vi.nell'ae. N.L. gen. n. *paralvinellae* of *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 µm long and approximately 0.6–0.8 µm 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 6.1. 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, S⁰ 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 C_{18:1} (37 %), C_{16:0} (25 %), C_{16:1} (22 %), 3-OH C_{14:0} (7 %), C_{14:0} (5 %) and C_{18:0} (4 %). The DNA G+C content is 37.6 mol% (by HPLC).

The type strain, strain GO25^T (=JCM 13212^T=DSM 17229^T), 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.

Sulfurimonas denitrificans (de.ni.tri'fi.cans. N.L. v. *denitrifico* to denitrify; N.L. part. adj. *denitrificans* denitrifying).

Basonym: *Thiomicrospira denitrificans* Timmer-ten Hoor 1975 (Approved Lists 1980).

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

Acknowledgements

We would like to thank Dr Katsuyuki Uematsu for assistance in preparing electron micrographs. We are very grateful to the R/V *Natsushima* and the *Shinkai 2000* operation teams for helping us to collect deep-sea hydrothermal vent samples.

References

- Alain, K., Querellou, J., Lesongeur, F., Pignet, P., Crassous, P., Raguene, G., Cuff, V. & Cambon-Bonavita, M. A. (2002). *Camini-bacter hydrogeniphilus* gen. nov., sp. nov., a novel thermophilic, hydrogen-oxidizing bacterium isolated from an East Pacific Rise hydrothermal vent. *Int J Syst Evol Microbiol* **52**, 1317–1323.
- Allen, S. E., Grimshaw, H. M., Parkinson, J. A. & Quarmby, C. (1974). Inorganic constituents: nitrogen. In *Chemical Analysis of Ecological Materials*, pp. 184–206. Edited by S. E. Allen. London: Blackwell Scientific.
- Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- Balch, W. E., Fox, G. E., Magrum, L. J., Woese, C. R. & Wolfe, R. S. (1979). Methanogens: reevaluation of a unique biological group. *Microbiol Rev* **43**, 260–296.
- Benson, D. A., Boguski, M. S., Lipman, D. J., Ostell, J. & Ouellette, B. F. F. (1998). GenBank. *Nucleic Acids Res* **26**, 1–7.
- Brinkhoff, T., Kuever, J., Muyzer, G. & Jannasch, H. W. (2005). Genus VI. *Thiomicrospira*. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 193–199. Edited by D. J. Brenner, N. R. Krieg & J. T. Staley. New York: Springer.
- Campbell, B. J., Jeannot, C., Kostka, J. E., Luther, G. W., III & Cary, S. C. (2001). Growth and phylogenetic properties of novel bacteria belonging to the epsilon subdivision of the *Proteobacteria* enriched from *Alvinella pompejana* and deep-sea hydrothermal vents. *Appl Environ Microbiol* **67**, 4566–4572.
- DeLong, E. F. (1992). Archaea in coastal marine environments. *Proc Natl Acad Sci U S A* **89**, 5685–5689.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Gamo, T. (1995). Wide variation of chemical characteristics of submarine hydrothermal fluids due to secondary modification process after high temperature water-rock interaction: a review. In *Biogeochemical Processes and Ocean Flux in the Western Pacific*, pp. 425–451. Edited by H. Sakai & Y. Nozaki. Tokyo: Terra Scientific Publishing.
- Gillis, M., Vandamme, P., De Vos, P., Swings, J. & Kerster, K. (2001). Polyphasic taxonomy. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 43–48. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. London: Springer.
- Hügler, M., Wirsén, C. O., Fuchs, G., Taylor, C. D. & Sievert, S. M. (2005). Evidence for autotrophic CO₂ fixation via the reductive tricarboxylic acid cycle by members of the ϵ subdivision of *Proteobacteria*. *J Bacteriol* **187**, 3020–3027.
- Inagaki, F., Takai, K., Kobayashi, H., Nealson, K. H. & Horikoshi, K. (2003). *Sulfurimonas autotrophica* gen. nov., sp. nov., a novel sulfur-oxidizing ϵ -proteobacterium isolated from hydrothermal sediments in the Mid-Okinawa Trough. *Int J Syst Evol Microbiol* **53**, 1801–1805.
- Inagaki, F., Takai, K., Nealson, K. H. & Horikoshi, K. (2004). *Sulfurovum lithotrophicum* gen. nov., sp. nov., a novel sulfur-oxidizing chemolithoautotroph within the ϵ -*Proteobacteria* isolated from Okinawa Trough hydrothermal sediments. *Int J Syst Evol Microbiol* **54**, 1477–1482.
- Kodama, Y. & Watanabe, K. (2004). *Sulfuricurvum kujense* gen. nov., sp. nov., a facultatively anaerobic, chemolithoautotrophic, sulfur-oxidizing bacterium isolated from an underground crude-oil storage cavity. *Int J Syst Evol Microbiol* **54**, 2297–2300.
- Kuenen, J. G. & Veldkamp, H. (1972). *Thiomicrospira pelophila*, gen. n., sp. n., a new obligately chemolithotrophic colourless sulfur bacterium. *Antonie van Leeuwenhoek* **38**, 241–256.
- Kuenen, J. G., Robertson, L. A. & Tuovinen, O. H. (1991). The genera *Thiobacillus*, *Thiomicrospira*, and *Thiosphaera*. In *The Prokaryotes*, 2nd edn, pp. 2638–2657. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–176. Edited by E. Stackbrandt & M. Goodfellow. New York: Wiley.
- Ludwig, W., Strunk, O., Westram, R. & 28 other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Miroshnichenko, M. L., Kostrikina, N. A., L'Haridon, S., Jeannot, C., Hippe, H., Stackebrandt, E. & Bonch-Osmolovskaya, E. A. (2002). *Nautilia lithotrophica* gen. nov., sp. nov., a thermophilic sulfur-reducing epsilon-proteobacterium isolated from a deep-sea hydrothermal vent. *Int J Syst Evol Microbiol* **52**, 1299–1304.

- Miroshnichenko, M. L., L'Haridon, S., Schumann, P., Spring, S., Bonch-Osmolovskaya, E. A., Jeannot, C. & Stackebrandt, E. (2004). *Caminibacter profundus* sp. nov., a novel thermophile of Nautiliales ord. nov. within the class 'Epsilonproteobacteria', isolated from a deep-sea hydrothermal vent. *Int J Syst Evol Microbiol* **54**, 41–45.
- Muyzer, G., Teske, A., Wirsén, C. O. & Jannasch, H. W. (1995). Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. *Arch Microbiol* **164**, 165–172.
- Nakagawa, S., Inagaki, F., Takai, K., Horikoshi, K. & Sako, Y. (2005a). *Thioreductor micantisoli* gen. nov., sp. nov., a novel mesophilic, sulfur-reducing chemolithoautotroph within the ϵ -Proteobacteria isolated from the hydrothermal sediments in the Mid-Okinawa Trough. *Int J Syst Evol Microbiol* **55**, 599–605.
- Nakagawa, S., Takai, K., Inagaki, F., Horikoshi, K. & Sako, Y. (2005b). *Nitratiruptor tergarcus* gen. nov., sp. nov. and *Nitratifractor salsuginis* gen. nov., sp. nov., nitrate-reducing chemolithoautotrophs of the ϵ -Proteobacteria isolated from a deep-sea hydrothermal system in the Mid-Okinawa Trough. *Int J Syst Evol Microbiol* **55**, 925–933.
- Nakagawa, S., Takai, K., Inagaki, F., Hirayama, H., Nunoura, T., Horikoshi, K. & Sako, Y. (2005c). Distribution, phylogenetic diversity and physiological characteristics of epsilon-Proteobacteria in a deep-sea hydrothermal field. *Environ Microbiol* **7**, 1619–1632.
- Porter, K. G. & Feig, Y. S. (1980). The use of DAPI for identifying and counting aquatic microflora. *Limnol Oceanogr* **25**, 943–948.
- Schmidt, H. A., Strimmer, K., Vingron, M. & von Haeseler, A. (2002). TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. *Bioinformatics* **18**, 502–504.
- Suzuki, Y., Sasaki, T., Suzuki, M., Nogi, Y., Miwa, T., Takai, K., Nealson, K. H. & Horikoshi, K. (2005). Novel chemoautotrophic endosymbiosis between a member of the epsilonproteobacteria and the hydrothermal-vent gastropod *Alviniconcha* aff. *hessleri* (Gastropoda: Provannidae) from the Indian Ocean. *Appl Environ Microbiol* **71**, 5440–5450.
- Takai, K. & Horikoshi, K. (2000). *Thermosiphon japonicus* sp. nov., an extremely thermophilic bacterium isolated from a deep-sea hydrothermal vent in Japan. *Extremophiles* **4**, 9–17.
- Takai, K., Komatsu, T. & Horikoshi, K. (2001). *Hydrogenobacter subterraneus* sp. nov., an extremely thermophilic, heterotrophic bacterium unable to grow on hydrogen gas, from deep subsurface geothermal water. *Int J Syst Evol Microbiol* **51**, 1425–1435.
- Takai, K., Inagaki, F., Nakagawa, S., Hirayama, H., Nunoura, T., Sako, Y., Nealson, K. H. & Horikoshi, K. (2003). Isolation and phylogenetic diversity of members of previously uncultivated ϵ -Proteobacteria in deep-sea hydrothermal fields. *FEMS Microbiol Lett* **218**, 167–174.
- Takai, K., Gamo, T., Tsunogai, U., Nakayama, N., Hirayama, H., Nealson, K. H. & Horikoshi, K. (2004a). Geochemical and microbiological evidence for a hydrogen-based, hyperthermophilic subsurface lithoautotrophic microbial ecosystem (HyperSLiME) beneath an active deep-sea hydrothermal field. *Extremophiles* **8**, 269–282.
- Takai, K., Oida, H., Suzuki, Y., Hirayama, H., Nakagawa, S., Nunoura, T., Inagaki, F., Nealson, K. H. & Horikoshi, K. (2004b). Spatial distribution of marine crenarchaeota group I in the vicinity of deep-sea hydrothermal systems. *Appl Environ Microbiol* **70**, 2404–2413.
- Takai, K., Nealson, K. H. & Horikoshi, K. (2004c). *Hydrogenimonas thermophila* gen. nov., sp. nov., a novel thermophilic, hydrogen-oxidizing chemolithoautotroph within the ϵ -Proteobacteria, isolated from a black smoker in a Central Indian Ridge hydrothermal field. *Int J Syst Evol Microbiol* **54**, 25–32.
- Takai, K., Hirayama, H., Nakagawa, T., Suzuki, Y., Nealson, K. H. & Horikoshi, K. (2004d). *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. *Int J Syst Evol Microbiol* **54**, 2325–2333.
- Takai, K., Hirayama, H., Nakagawa, T., Suzuki, Y., Nealson, K. H. & Horikoshi, K. (2005a). *Lebetimonas acidiphila* gen. nov., sp. nov., a novel thermophilic, acidophilic, hydrogen-oxidizing chemolithoautotroph within the 'Epsilonproteobacteria', isolated from a deep-sea hydrothermal fumarole in the Mariana Arc. *Int J Syst Evol Microbiol* **55**, 183–189.
- Takai, K., Campbell, B. J., Cary, S. C. & 8 other authors (2005b). Enzymatic and genetic characterization of carbon and energy metabolisms by deep-sea hydrothermal chemolithoautotrophic isolates of *Epsilonproteobacteria*. *Appl Environ Microbiol* **71**, 7310–7320.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Timmer-ten Hoor, A. (1975). A new type of thiosulphate oxidizing, nitrate reducing microorganisms: *Thiomicrospira denitrificans* sp. nov. *Neth J Sea Res* **9**, 344–350.
- Urakawa, H., Dubilier, N., Fujiwara, Y., Cunningham, D. E., Kojima, S. & Stahl, D. A. (2005). Hydrothermal vent gastropods from the same family (Provannidae) harbour ϵ - and γ -proteobacterial endosymbionts. *Environ Microbiol* **7**, 750–754.
- Voordeckers, J. W., Starovoytov, V. & Vetriani, C. (2005). *Caminibacter mediatlanticus* sp. nov., a thermophilic, chemolithoautotrophic, nitrate-ammonifying bacterium isolated from a deep-sea hydrothermal vent on the Mid Atlantic Ridge. *Int J Syst Evol Microbiol* **55**, 773–779.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Zillig, W., Holz, I., Janekovic, D. & 7 other authors (1990). *Hyperthermus butylicus*, a hyperthermophilic sulfur-reducing archaeobacterium that ferments peptides. *J Bacteriol* **172**, 3959–3965.