Sulfurivirga caldicuralii gen. nov., sp. nov., a novel microaerobic, thermophilic, thiosulfate-oxidizing chemolithoautotroph, isolated from a shallow marine hydrothermal system occurring in a coral reef, Japan

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Novel thermophilic bacteria, designated strains VW1 and MM1^T, were isolated from hydrothermal fluid and microbial mat samples, respectively, collected from a shallow marine hydrothermal system (water depth 22 m) occurring in coral reefs off Taketomi Island, Okinawa, Japan. Cells of the two novel strains were motile rods with a single polar flagellum in the exponential growth phase. In a medium that included elemental sulfur, cells of the two strains became non-motile with oval to spherical cell shapes. For both strains, growth occurred at between 30 and 60 °C (optimum temperature of 50–55 °C; 60–80 min doubling time) and between pH 5.5 and 7.1 (optimum pH 6.0). The isolates were microaerobic chemolithoautotrophs capable of using thiosulfate or tetrathionate as the sole energy source, O₂ as the sole electron acceptor and CO₂ as the sole carbon source. Organic substrates, such as yeast extract and tryptone, inhibited growth of both strains. The G + C contents of genomic DNA were 51.3 and 49.5 mol% for strains VW1 and MM1^T, respectively. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the two strains were closely related to each other (99.9 % sequence similarity) and were distantly related to other previously described genera within the *Gammaproteobacteria*. The novel isolates could also be differentiated from other gammaproteobacterial genera on the basis of their physiological properties. It is suggested that the novel isolates represent the type species of a new genus, for which the name *Sulfurivirga caldicuralii* gen. nov., sp. nov. (type strain MM1^T = JCM 13439^T = DSM 17737^T) is proposed.

The occurrence of thermophilic, sulfur-oxidizing, chemolithoautotrophic bacteria is at present confined to a relatively narrow spectrum of taxa such as the *Aquificae*, *Betaproteobacteria*, *Gammaproteobacteria* and *Epsilonproteobacteria* (Garrity *et al.*, 2005a, b, c; Reysenbach, 2001). Isolates from marine environments are further restricted to within the *Aquificae* and *Epsilonproteobacteria* (Götz *et al.*, 2002; Huber *et al.*, 1992; Nakagawa *et al.*, 2004, 2005;

Nakagawa & Takai, 2006; Reysenbach *et al.*, 2000; Takai *et al.*, 2003a). Several strains of thermophilic sulfur-oxidizing chemolithoautotrophs are known within the *Gammaproteobacteria* such as *Acidithiobacillus caldus* KU^T (Hallberg & Lindström, 1994; Kelly & Wood, 2000, 2005) and *Thermithiobacillus tepidarius* DSM 3134^T (Kelly & Wood, 2000; Wood & Kelly, 1985), both of which were isolated from terrestrial, acidic sulfide springs and metal sulfide ore biota. In contrast, a great diversity of mesophilic sulfur-oxidizing members of the *Gammaproteobacteria* have been found in a variety of marine environments, such as deep-sea and shallow marine hydrothermal systems and oceanic and coastal oxic–anoxic interfaces of sediments and water columns (Brinkhoff *et al.*, 2005; Dando *et al.*, 1998; Imhoff, 2005a, b; Jørgensen *et al.*, 2005; Kuenen, 2005; Kuenen & Dubinina, 2005; Nishihara *et al.*, 1991; Strohl, 2005; Schulz *et al.*, 1999; Schulz & Jørgensen, 2005; Takai *et al.*, 2004).

Abbreviation: ORP, oxidation–reduction potential.

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of strains MM1^T and VW1 are AB245479 and AB245480, respectively.

Graphs showing the effects of temperature, pH and NaCl concentration on the growth of strains MM1^T and VW1 in MMJS medium are available as a supplementary figure in IJSEM Online.

Most of the marine, sulfur-oxidizing gammaproteobacteria belong to the order *Thiotrichales* within the families *Thiotrichaceae* and *Piscirickettsiaceae*. Many marine, sulfur-oxidizing, chemolithoautotrophic members of genera such as *Achromatium*, *Beggiatoa*, *Thiobacterium*, *Thiomargarita*, *Thioploca* and *Thiospira* within the family *Thiotrichaceae* are pure-culture-resistant, non-motile bacteria, often forming filamentous or mat-like macrostructures (Dando *et al.*, 1998; Jørgensen *et al.*, 2005; Kuenen, 2005; Kuenen & Dubinina, 2005; Strohl, 2005; Schulz *et al.*, 1999; Schulz & Jørgensen, 2005). Members of the genus *Thiomicrospira*, of the family *Piscirickettsiaceae*, have frequently been isolated from various marine habitats, including deep-sea hydrothermal environments (Brinkhoff *et al.*, 2005; Knittel *et al.*, 2005; Takai *et al.*, 2004). However, none of the thermophilic, sulfur-oxidizing, chemolithoautotrophic members of the *Gammaproteobacteria* have been reported from hydrothermal environments, even though a thermotolerant species, *Thiomicrospira thermophila* I78^T, was isolated from hydrothermal fluids at >70 °C (Takai *et al.*, 2004).

A shallow, submarine hydrothermal system has been discovered in barrier reefs situated in the inter-islands of the Yaeyama Islands (between the Ishigaki and Taketomi Islands) (Oomori, 1987). Hydrothermal ventings are located at depths of 10 to 22 m and several representative vent sites are characterized by high temperature fluid effluents and vigorous gas bubbleings composed mainly of methane and molecular nitrogen. Although numerous shallow marine hydrothermal systems are known in the world and some of these systems have been microbiologically investigated (Kalanetra *et al.*, 2004; Rusch & Amend, 2004; Sievert *et al.*, 1999, 2000; Takai & Sako, 1999), only a few of these systems have been identified that are associated with coral reef formations. Examples of such systems have been found in Ambitle Island, Papua New Guinea (Pichler *et al.*, 1999) and in Bahia Concepcion, Baja California Peninsula (Prol-Ledesma *et al.*, 2004) and their endemic microbial communities and components remain poorly explored.

A series of geochemical-geomicrobiological investigations has been conducted in a shallow marine hydrothermal system that occurs in the coral reefs off Taketomi Island, Japan. During the investigation, hydrothermal fluid and filamentous microbial community samples were collected from the main vent site in the coral reef breccia seafloor. Analysis of 16S rRNA gene sequences has suggested that the filamentous microbial components are closely related to *Thioploca*-like filamentous sulfur-oxidizing bacteria found in White Point, California (Kalanetra *et al.*, 2004). In this study, thermophilic sulfur-oxidizing, chemolithoautotrophic bacteria were isolated from hydrothermal fluid and microbial mat samples. This paper describes a taxonomic study of two novel representative bacterial strains, MM1^T and VW1, and proposes a new genus, *Sulfurivirga*.

Sample collection, enrichment and purification

Hydrothermal fluid and microbial mat samples from the Taketomi shallow marine hydrothermal system (24° 20' 9" N 124° 06' 10" E) were obtained by SCUBA diving. Physico-chemical factors at the sampling points were measured using a portable water quality analyser (U-20XD; Horiba). The effluent hydrothermal fluid [51.9 °C, 3.3 % salinity, pH 6.56, 1 g dissolved oxygen l⁻¹, oxidation-reduction potential (ORP) -220 mV at the vent orifice] was collected by 50 ml sterilized syringes from the main vent at a depth of 22 m. The microbial mats, developed on dead coral skeleton, were collected from a gas bubbling site (41.1 °C, 3.3 % salinity, pH 6.67, 0.4 g dissolved oxygen l⁻¹, ORP -340 mV on the seafloor) at depth of 13 m. Immediately after recovery of the samples on the boat, the hydrothermal fluid was transferred anaerobically into vials under N₂ gas purging. Vials, with or without the addition of 0.05 % (w/v) sodium sulfide, were tightly sealed with butyl rubber caps under a gas phase of 100 % N₂ (100 kPa). Microbial mat samples (0.1 g wet weight) were suspended with 20 ml sterilized MJ synthetic seawater (Takai *et al.*, 1999) in the presence or absence of 0.05 % (w/v) sodium sulfide in glass vials tightly sealed with a butyl rubber cap under a gas phase of 100 % N₂ (100 kPa). These subsamples were transferred to the laboratory under atmospheric temperatures 24 h prior to the experiments. The subsamples were used to inoculate a series of media, including MMJHS medium (Takai *et al.*, 2003a), under a gas phase of 80 % H₂, 19 % CO₂ and 1 % O₂ (200 kPa) and cultures were incubated at 55 °C in a dry oven.

Growth of non-motile, oval to coccoid cells was observed in MMJHS medium including hydrothermal fluid or microbial mat after 2 days incubation at 55 °C. Pure cultures were obtained by using the dilution-to-extinction technique at 55 °C with the same medium as used for enrichment (Takai & Horikoshi, 2000). Cultures from the microbial mat and the hydrothermal fluid were designated strains MM1^T (= JCM 13439^T = DSM 17737^T) and VW1 (= JCM 13440), respectively. 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 microscope (BX51; Olympus) with a SPOT RT Slider CCD camera system (Diagnostic Instruments). Transmission electron microscopy of negatively stained cells and thin sections of cells was carried out as described by Zillig *et al.* (1990). Cells of the two novel strains, grown in MMJS medium (Takai *et al.*, 2004) under microaerobic conditions (1 % partial pressure of O₂) at 55 °C and in the mid-exponential phase of growth, were used for electron microscopic observations (JEM-1210; JEOL). When the two strains were grown in a medium containing elemental sulfur (S⁰), such as MMJHS medium (Takai *et al.*, 2003a) under microaerobic conditions, cells were non-motile, oval to coccoid shapes.

However, when grown in MMJS medium (Takai *et al.*, 2004) under microaerobic conditions with only thiosulfate and not S^0 , cells of both strains were always short rods (Fig. 1). In the exponential growth phase in MMJS medium, cells of the two novel strains were Gram-negative, slightly curved rods, about 0.3–0.6 μm wide, 1–3 μm in long and motile with a polar flagellum (Fig. 1). No spore formation was observed for either of the two novel strains in any of the growth conditions examined. The morphological features of strains MM1^T and VW1 were similar to those of members of the genus *Thiomicrospira* (Brinkhoff *et al.*, 2005; Takai *et al.*, 2004) (Table 1).

Growth characteristics

The two novel strains were routinely cultivated in MMJS medium under a gas phase of 80 % N_2 , 19 % CO_2 and 1 % O_2 (2 atmospheres) (Takai *et al.*, 2004). Growth was measured by direct cell counting after staining with DAPI

(Porter & Feig, 1980) using a phase-contrast microscope (BX51; Olympus). Cultures were grown in 100 ml glass bottles (Schott Glaswerke) with shaking (100 r.p.m.) in a temperature-controlled dry incubator. Experiments were performed in duplicate. With MMJS medium, the two novel strains grew over a temperature range of 30 to 60 °C, showing optimal growth at 50–55 °C. The generation time at 55 °C and pH 6.0 was about 60–80 min (see Supplementary Fig. S1a in IJSEM Online). The effect of pH on growth was tested at 55 °C using MMJS medium adjusted to various pH values using 30 mM of each of acetate/acetic acid buffer (pH 4–5), MES (pH 5–6), PIPES (pH 6–7), HEPES (pH 7–7.5) and Tris (pH 8–9.5) at room temperature (see Supplementary Fig. S1b in IJSEM Online). Growth of the two novel strains occurred at pH 5.5–7.1, 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. In MMJS medium, strains MM1^T and VW1 grew over a NaCl concentration range of between 12 and 44 g l⁻¹. Optimum growth was observed at NaCl concentrations of 20 g l⁻¹ at 55 °C and pH 6.0 (see Supplementary Fig. S1c in IJSEM Online). The effects of temperature, pH and NaCl concentration were quite similar for strains MM1^T and VW1.

The effect of O_2 concentration in the gas phase on the growth of strains MM1^T and VW1 was tested with MMJS medium under the following gas mixtures: 80 % N_2 + 20 % CO_2 , 80 % N_2 + 19.8 % CO_2 + 0.2 % O_2 , 80 % N_2 + 19.5 % CO_2 + 0.5 % O_2 , 80 % N_2 + 19 % CO_2 + 1 % O_2 , 80 % N_2 + 18 % CO_2 + 2 % O_2 , 80 % N_2 + 17 % CO_2 + 3 % O_2 , 80 % N_2 + 15 % CO_2 + 5 % O_2 , 75 % N_2 + 15 % CO_2 + 10 % O_2 or 65 % N_2 + 15 % CO_2 + 20 % O_2 at 200 kPa. In the absence of oxygen as the electron acceptor, either 10 mM nitrate or 10 mM fumarate was added to MMJS medium as a potential alternative electron acceptor. The maximum cell yields of both strains were approx. $6\text{--}8 \times 10^8$ cells ml⁻¹ under a gas phase in the presence of 2–5 % O_2 , while slightly lower yields ($1\text{--}4 \times 10^8$ cells ml⁻¹) were seen with 0.2, 0.5, 1.0 and 10 % O_2 . No growth was observed in the absence of O_2 or with > 15 % O_2 in the gas phase. These results indicate that the two novel strains grow under microaerobic (up to 10 % O_2) conditions.

Heterotrophic growth was tested in MMJS medium without $NaHCO_3$ under a gas phase of 99 % N_2 and 1 % O_2 (200 kPa), containing the following 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) 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 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 or 0.02 % (w/v) trehalose. None of these organic carbon sources supported the growth of

Fig. 1. Electron micrographs of a negatively stained cell (a) and a thin-section (b) of *Sulfurivirga caldicuralii* MM1^T. Cells grown in MMJS medium under microaerobic conditions (1 % partial pressure of O_2) at 55 °C in the mid-exponential phase of growth were used for microscopic observations. Bars, 1.0 μm (a) and 0.5 μm (b).

Table 1. Comparison of properties among representative sulfur-oxidizing, chemolithoautotrophic genera within the *Gammaproteobacteria*

Strains: 1, *Sulfurivirga caldicuralii* MM1^T (data from this study); 2, *Thiomicrospira pelophila* DSM 1534^T (Kuenen & Veldkamp, 1972; Brinkhoff *et al.*, 2005); 3, *Thiomicrospira thermophila* I78^T (Takai *et al.*, 2004); 4, *Acidithiobacillus caldus* KU^T (Hallberg & Lindström, 1994; Kelly & Wood, 2000, 2005). –, Negative; +, positive; ND, not determined.

Characteristic	1	2	3	4
Source	Shallow marine hydrothermal system in coral reefs	Intertidal mud flat in the Dutch Wadden Sea	Deep-sea hydrothermal fluids in the Mariana Volcanic Arc	Unknown
Cell shape	Short curved rod (oval to coccoid with S ⁰)	Vibrio to spiral	Short curved rod	Short rod
Growth requirements:				
Temperature range (°C)	30–60	4–42	15–55	32–52
Optimum temperature (°C)	50–55	28–30	35–40	45
pH range	5.5–7.1	5.6–9.0	5.0–8.0	1.0–3.5
Optimum pH	6	7	6	2.0–2.5
NaCl concentration range (%)	1.2–4.4	0.3–7.2	0.3–7.0	ND
Optimum NaCl concentration (%)	2	2.8	1.2–2.0	0
O ₂ sensitivity	Microaerobic (up to 10 % O ₂)	Fully aerobic	Microaerobic (up to 10 % O ₂)	Fully aerobic
Organic carbon compounds utilized as carbon sources	–	–	+	+
Electron donor:				
Sulfide	–	+	+	+
S ⁰	–	+	+	+
DNA G+C content (mol%)	49.5–51.3	45.7	43.8	63.1–63.9

strains MM1^T or VW1. Utilization of these organic compounds as alternative energy sources was examined in MMJS medium in the absence of thiosulfate under a gas phase of 80 % N₂, 19 % CO₂ and 1 % O₂ (200 kPa). None of the organic compounds were able to sustain the growth of strains MM1^T or VW1. Growth enhancement of the two novel strains by organic compounds was tested with MMJS medium containing each of the above organic compounds as additional energy and/or carbon sources under a gas phase of 80 % N₂, 19 % CO₂ and 1 % O₂ (200 kPa). No growth enhancement was observed and complex organic substrates, such as yeast extract, peptone and tryptone, completely inhibited the growth of the two novel strains.

In an attempt to discover other potential electron donors for autotrophic growth, sulfide (0.25, 0.5, 1, 2 or 5 mM), sulfite (1 or 5 mM), elemental sulfur (1 % w/v), cysteine hydrochloride (0.25, 0.5, 1, 2 or 5 mM) and tetrathionate (5 mM) were tested as the sole electron donor in MMJS medium without thiosulfate with a gas phase of 80 % N₂, 19 % CO₂ and 1 % O₂ (200 kPa). Molecular hydrogen was also tested in MMJS medium with a gas phase of 80 % H₂, 19 % CO₂ and 1 % O₂ (200 kPa). The provision of tetrathionate gave a significantly lower maximum cell yield than found for thiosulfate ($2\text{--}4 \times 10^7$ cells ml⁻¹ for strains MM1^T and VW1), while the other potential electron donors were unable to support the growth of the two strains. To test the utilization of electron acceptors, nitrate (10 mM), nitrite (1 mM or 5 mM), ferric citrate (20 mM), ferrihydrite (20 mM), selenate (5 mM), arsenate (5 mM) and fumarate (10 mM) were tested with MMJS medium under 80 % N₂ and 20 % CO₂ (200 kPa). Of these compounds, only O₂ served as an electron acceptor for the growth of strains MM1^T and VW1. To determine which nutrients, such as selenite, tungstate and vitamins, were required for growth, MMJS medium with and without the specified nutrients was tested. The nitrogen source (NH₄Cl, NaNO₂, N₂, NaNO₃) for growth was also examined with MMJS medium. The two novel strains utilized only ammonium as the nitrogen source. Selenium, tungsten and vitamins were not required for growth. These results indicate that strains MM1^T and VW1 are strict chemolithoautotrophs, utilizing thiosulfate or tetrathionate as the sole electron donors, O₂ (up to 10 %; v/v) as the sole electron acceptor and CO₂ as the sole carbon source.

The time-course for the oxidation of thiosulfate and concomitant growth of the two novel strains were examined with MMJS medium in which all the sulfate salts were replaced by chloride salts under a gas phase of 80 % N₂, 19 % CO₂ and 1 % O₂ (200 kPa) (Fig. 2). Concentrations of thiosulfate, sulfite and sulfate were analysed by ion chromatography using a Shim-pack IC column (Shimadzu) and the production of elemental sulfur during growth was monitored as previously described (Takai *et al.*, 2001). Consumption of thiosulfate and the production of sulfate and S⁰ occurred during the growth of strains MM1^T and VW1 (Fig. 2). Production of sulfite was not observed

Fig. 2. Time-course for the oxidation of thiosulfate, the production of sulfate and S⁰ and concomitant growth of *Sulfurivirga caldicuralii* MM1^T (a) or *S. caldicuralii* VW1 (b). ○, Cell count; ●, S⁰; ■, S₂O₃²⁻; △, SO₄²⁻.

during the growth of strains MM1^T and VW1. No oxidation of thiosulfate or production of either S⁰ or sulfate was found with control (non-inoculated) medium. Thus, strains MM1^T and VW1 were found to be respiratory thiosulfate-oxidizing, oxygen-reducing chemolithoautotrophs.

The two novel strains were found to be sensitive to a variety of antibiotics (50 µg ml⁻¹), including chloramphenicol, streptomycin, kanamycin, ampicillin, vancomycin and rifampicin.

Fatty acid analysis

The cellular fatty acid contents for the two novel strains were analysed from cells grown in MMJS medium at 55 °C in the late exponential growth phase. Lyophilized cells (20 mg) were placed in a Teflon-lined, screw-capped tube containing 3 ml anhydrous methanolic HCl and heated at 100 °C for 3 h. The extraction and analysis of fatty acid methyl esters was as described previously (Takai *et al.*, 2003b). The major cellular fatty acids for strain MM1^T were C_{18:1} (42.9%),

C_{16:0} (27.0%), C_{18:0} (21.1%), 3-OH C_{14:0} (4.8%), C_{16:1} (2.3%) and C_{18:2} (1.2%). For strain VW1, major cellular fatty acids were C_{18:1} (48.1%), C_{16:0} (24.7%), C_{18:0} (18.7%), 3-OH C_{14:0} (3.2%), C_{16:1} (1.4%) and C_{18:2} (1.1%). Fatty acids C_{19:0} and C_{20:1} were detected as minor components in both strains. The cellular fatty acid content was generally similar between the two novel strains.

Nucleic acid analyses

Genomic DNAs of the two novel strains were prepared as described by Marmur & Doty (1962). The G + C content of DNA was determined by direct analysis of deoxyribonucleotides by HPLC (Tamaoka & Komagata, 1984). The DNA G + C contents were found to be 49.5 ± 2.5 mol% for strain MM1^T and 51.3 ± 2.3 mol% for strain VW1. These values were slightly higher than those previously determined for members of the genus *Thiomicrospira* (Brinkhoff *et al.*, 2005; Takai *et al.*, 2004) (Table 1).

The 16S rRNA gene was amplified by PCR using primers Bac 27F and 1492R (DeLong, 1992; Lane, 1991) as previously described (Takai *et al.*, 2001). The nearly complete sequences (1457 bp and 1456 bp) of the 16S rRNA genes from strains MM1^T and VW1 were directly sequenced by both strands using the dideoxynucleotide chain-termination method with a DNA sequencer (model 3100; Perkin Elmer/Applied Biosystems). The 16S rRNA gene sequences of the two novel strains were found to be almost identical (99.9% similarity) and to be most closely related

(90.6%) to the sequences of a gill endosymbiont from a deep-sea hydrothermal vent gastropod (*Alviniconcha hessleri*) obtained from the Mariana Trough (Suzuki *et al.*, 2005). Strains MM1^T and VW1 showed only a distant relationship (<90% sequence similarity) with any strains of currently described gammaproteobacterial genera. The nearly complete sequences were manually aligned according to secondary structures using ARB (Ludwig *et al.*, 2004). Phylogenetic analyses were restricted to unambiguously aligned nucleotide positions. Evolutionary distance matrix analysis (using the Jukes and 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 (Schmidt *et al.*, 2002). Bootstrap analysis was conducted to provide confidence estimates for the phylogenetic tree topologies. The resulting phylogenetic tree indicated that the two novel strains were phylogenetically associated with members of the genus *Thiomicrospira*, but represented a novel lineage, possibly within the family *Piscirickettsiaceae* of the *Gammaproteobacteria* (Fig. 3).

Comparison with related genera

Strains VW1 and MM1^T were isolated from hydrothermal fluid and adjacent *Thioplaca*-like microbial mats, respectively, in a shallow marine hydrothermal system occurring in the inter-islands coral reef off Taketomi Island, Japan. These novel strains represent the first neutrophilic, thermophilic, sulfur-oxidizing chemolithoautotrophs within the

Fig. 3. Phylogenetic tree of representative sulfur-oxidizing, chemolithoautotrophic genera and symbionts within the *Gammaproteobacteria* including *Sulfurivirga caldicurallii* MM1^T and VW1. The tree was inferred from 16S rRNA gene sequences using the neighbour-joining method on 1051 homologous sequence positions for each sequence. The number(s) at nodes represent the bootstrap value (percentages of 1000 replications). Two numbers are indicated if identical topology was given by both trees (neighbour-joining tree/maximum-likelihood tree); a single number represents the bootstrap value calculated by the neighbour-joining method. Bar, 2 substitutions per 100 nucleotides.

Gammaproteobacteria isolated from marine environments (Table 1). Since the morphological, physiological and chemotaxonomic characteristics of the two strains were very similar and the 16S rRNA gene sequences of the strains were almost identical, it is suggested that strains VW1 and MM1^T are representatives of the same species. Sequence similarity analysis indicated that the two novel strains had a distant relationship to previously described genera within the *Gammaproteobacteria*. Phylogenetic analysis indicated that the two novel strains were associated with members of the genus *Thiomicrospira*, but represented a novel lineage, possibly within the family *Piscirickettsiaceae* of the *Gammaproteobacteria* (Fig. 3). The family *Piscirickettsiaceae*, one of the three families within the order *Thiotrichales*, contains mesophilic hydrogen- or sulfur-oxidizing chemolithoautotrophs such as members of the genera *Thiomicrospira* and *Thioalkalimicrobium*. Apart from thermophily, the physiological characteristics of the two novel strains are generally comparable with members of the genus *Thiomicrospira* (Table 1). However, phylogenetic relationships clearly differentiate the novel isolates from members of the genus *Thiomicrospira*. On the basis of the physiological and molecular properties of the two novel strains, we propose the new genus, *Sulfurivirga*. The type species of the genus *Sulfurivirga* is proposed as *Sulfurivirga caldicuralii*, with strain MM1^T (=JCM 13439^T=DSM 17737^T) as the type strain.

Description of *Sulfurivirga* gen. nov.

Sulfurivirga (Sul.fur.i.vir'ga. L. neut. n. *sulfur* sulfur; L. n. *virga* rod; N.L. fem. n. *Sulfurivirga* sulfur-oxidizing rod).

Motile rods with a polar flagellum. Gram-negative. Microaerobic. Thermophilic and neutrophilic. Chemolithoautotrophic. Able to utilize thiosulfate as an electron donor and molecular oxygen as an electron acceptor. NaCl is absolutely required for growth. DNA G + C content is about 50 mol%. Major cellular fatty acids are C_{16:0}, C_{18:0} and C_{18:1}. On the basis of 16S rRNA gene sequence analysis, the genus *Sulfurivirga* is related to the genus *Thiomicrospira* within the *Gammaproteobacteria*. The type species is *Sulfurivirga caldicuralii*.

Description of *Sulfurivirga caldicuralii* sp. nov.

Sulfurivirga caldicuralii (cal.di.cu.ra'li.i. L. adj. *caldus* hot; L. n. *curalium* coral; N.L. gen. n. *caldicuralii* of a hot coral, as the type strain was isolated from a shallow marine hydrothermal system associated with coral reef formation).

Displays the following properties in addition to those given in the genus description. Cells occur singly, as motile, straight to curved rods with a polar flagellum. Cells have a mean length of 1–3 µm and a mean width of 0.3–0.6 µm in the exponential growth phase. Cells become oval to spherical in a medium containing S⁰. Microaerobic, tolerating up to 10 % O₂ in the gas phase. Temperature range for growth is 30 to 60 °C, with an optimum of 50–55 °C. The pH range for

growth is 5.5–7.1 (optimum growth at pH 6.0). NaCl in the concentration range 12–44 g l⁻¹ is an absolute growth requirement; optimum growth occurs at 20 g NaCl l⁻¹. Strictly chemolithoautotrophic with thiosulfate or tetrathionate as an electron donor and O₂ as an electron acceptor. Thiosulfate is oxidized to sulfate and S⁰ during growth. Ammonium is utilized as a sole nitrogen source. Vitamins, selenium and tungsten are not required for growth. The major cellular fatty acids are C_{18:1} (42.9 %), C_{16:0} (27.0 %), C_{18:0} (21.1 %), 3-OH C_{14:0} (4.8 %), C_{16:1} (2.3 %) and C_{18:2} (1.2 %).

The type strain, MM1^T (=JCM 13439^T=DSM 17737^T), was isolated from a shallow marine hydrothermal system occurring in coral reefs off Taketomi Island, Japan.

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