Sulfurimonas gotlandica sp. nov., a chemoautotrophic and psychrotolerant epsilonproteobacterium isolated from a pelagic redoxcline, and an emended description of the genus Sulfurimonas

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A psychro- and aerotolerant bacterium was isolated from the sulfidic water of a pelagic redox zone of the central Baltic Sea. The slightly curved rod- or spiral-shaped cells were motile by one polar flagellum or two bipolar flagella. Growth was chemolithoautotrophic, with nitrate or nitrite as electron acceptor and either a variety of sulfur species of different oxidation states or hydrogen as electron donor. Although the bacterium was able to utilize organic substances such as acetate, pyruvate, peptone and yeast extract for growth, these compounds yielded considerably lower cell numbers than obtained with reduced sulfur or hydrogen; in addition, bicarbonate supplementation was necessary. The cells also had an absolute requirement for NaCl. Optimal growth occurred at 15 °C and at pH 6.6–8.0. The predominant fatty acid of this organism was 16 : 1 ω7c, with 3-OH 14 : 0, 16 : 0, 16 : 1 ω5c + t and 18 : 1 ω7c present in smaller amounts. The DNA G+C content was 33.6 mol%. As determined in 16S rRNA gene sequence phylogeny analysis, the isolate belongs to the genus Sulfurimonas, within the class Epsilonproteobacteria, with 93.7 to 94.2 % similarity to the other species of the genus Sulfurimonas, Sulfurimonas autotrophica, Sulfurimonas paralvinellae and Sulfurimonas denitrificans. However, the distinct physiological and genotypic differences from these previously described taxa support the description of a novel species, Sulfurimonas gotlandica sp. nov. The type strain is GD1T (=DSM 19862T =JCM 16533T). Our results also justify an emended description of the genus Sulfurimonas.

Deep-sea vents are among the most productive marine systems on Earth. The discovery of these primarily chemoautotrophic environments, in 1977, has been followed by an appreciation of the remarkable physiological and phylogenetic diversity of their endosymbiotic and often thermophilic inhabitants, most commonly species of the class Epsilonproteobacteria. Moreover, deep-sea vent chemoautotrophs are thought to be representatives of the earliest biological communities on Earth (see the review by Nakagawa & Takai, 2008). Indeed, many epsilonproteobacteria are globally ubiquitous in oxygen-deficient and sulfide-rich marine and terrestrial ecosystems, which accommodate their predominantly auto- to mixotrophic lifestyles (Campbell et al., 2006). A number of studies have verified the significant role of epsilonproteobacteria in biogeochemical cycles, particularly those which are sulfur-dependent, as is the case in deep-sea hydrothermal fields (Nakagawa et al., 2005; Campbell et al., 2006), sulfidic cave springs (Engel et al., 2004) and autotrophic episymbiotic associations (Suzuki et al., 2006). In the suboxic to sulfidic transition zones of aquatic pelagic redox zones, high dark CO2 fixation rates, mainly due to the activities of epsilonproteobacterial chemolithoautotrophs, have been determined, for instance,
in the Black Sea and the Baltic Sea (Grote et al., 2008; Glaubitz et al., 2010; Jost et al., 2008).

The Baltic Sea is among the largest brackish basins of the world, with periodically anoxic conditions in its bottom waters. In the region known as the Baltic Proper there are a number of such areas, including the Gotland Deep, where at depths below 50–60 m a stable halocline separates the water column into an upper oxygenated layer and underlying oxygen-deficient and anoxic/sulfidic layers (Lepland & Stevens, 1998; Neretin et al., 2003), in which high dark CO₂ fixation rates have been reported (Jost et al., 2010).

In stimulation experiments (Labrenz et al., 2005; Brettar et al., 2006), quantitative 16S rRNA PCR (Labrenz et al., 2004), catalysed reporter deposition–fluorescence in situ hybridization (CARD-FISH; Grote et al., 2007) and micro-aautoradiography (MICRO)-CARD-FISH (Grote et al., 2008) analyses, as well as 16S rRNA stable isotope probing (RNA-SIP; Glaubitz et al., 2009), the epsilonproteobacterial ‘Uncultured Helicobacteraceae G138eps1/GD17’ subgroup was shown to account for up to 30 % of the total cell numbers in pelagic redox zones of the central Baltic Sea. The abundance of these bacteria highlights the importance of chemolithoautotrophic denitrification, which was convincingly demonstrated to be the major N-loss process in water columns with a sulfide–nitrate interface (Brettar & Rheinheimer, 1991; Hannig et al., 2007; Jensen et al., 2009), catalysed by the GD17 group as potential key organisms for this process. According to its 16S rRNA phylogeny, the ‘Uncultured Helicobacteraceae G138eps1/GD17’ subgroup belongs to the genus Sulfurimonas, which comprises mesophilic, facultatively anaerobic, chemolithoautotrophic species originating from deep-sea hydrothermal and marine sulfidic environments (Takai et al., 2006). In previous work (Grote et al., 2012) we described the isolation of strain Gotland Deep 1 (GD1T), a close phylogenetic relative (16S rRNA similarity of 95.7 %) and thus representative of the Baltic Sulfurimonas ‘Uncultured Helicobacteraceae G138eps1/GD17’ subgroup. Selected genomic and physiological data suggested an ecological role for GD1T, especially with respect to its sulfide detoxification ability (Grote et al., 2012). Here, we expand on previous work by presenting the taxonomic characteristics of GD1T. Our results form the basis of an emended description of the genus Sulfurimonas.

Strain GD1T was isolated from a pelagic redox zone of the Gotland Deep in the central Baltic Sea during a research cruise on board the RV Alkor in May 2005 (57° 19.2’ N 20° 03’ E). Water was collected in a free-flow bottle attached to a CTD-rosette from a depth of 215 m. The in situ temperature was 6 °C, the salinity 13 practical salinity units (PSU), and the sulfide concentration 11 μM. Directly on board, 100 μM KNO₃ and 100 μM Na₂S₂O₃ were added to the water samples, which were then incubated in the dark at 10 °C under anoxic conditions. For further isolation and cultivation in the laboratory, a modified version of artificial brackish water medium (ABW) (Bruns et al., 2002) was used, consisting of 95 mM NaCl, 11.2 mM MgCl₂·6H₂O, 2.3 mM CaCl₂·2H₂O, 2.0 mM KCl, 6.4 mM Na₂SO₄, 192 μM KBr, 92 μM H₂BO₃, 34 μM SrCl₂, 92 μM NH₄Cl, 9 μM KH₂PO₄ and 16 μM NaF, buffered with 10 mM HEPES (pH 7.3). For anaerobic cultivation, the medium was boiled, bubbled with N₂ for 30 min, and then autoclaved under anoxic conditions. Subsequently, anoxic and sterile-filtered 0.1 % (v/v) of the trace element solution SL10 (Widdel et al., 1983), 0.2 % (v/v) of a vitaprotein solution (Balch et al., 1979), 0.02 % (v/v) of a selenite–tungstate solution (Widdel & Bak, 1992), and 2–5 mM NaHCO₃ were added. The standard medium ABW + nitrate + thiosulfate (ABW + NS) was prepared by the variable addition of 10 mM KNO₃ and 10 mM Na₂S₂O₃, with the final concentration depending on the experiment. A pure culture was acquired by the dilution to extinction method and was cryopreserved at −80 °C in glycerol for long-term storage.

Morphological, physiological, and metabolic characteristics were, for the most part, analysed as described earlier (Grote et al., 2012). For these analyses, strain GD1T was cultivated in triplicate for 7–10 days at 15 °C in the dark. Growth was usually measured by counting 4’,6’-diamidino-2-phenylindol (DAPI) stained cells, observed using epifluorescence microscopy, or by flow cytometric determinations of SYBR-Green I (Molecular Probes) stained cells (Labrenz et al., 2007) at the end of the experiment. Sulfurimonas denitrificans DSM 1251T was used as the reference strain in the cultivation experiments.

Isolate GD1T is a motile, Gram-reaction-negative, slightly curved or spirilla-shaped bacterium typically with one polar flagellum (Fig. 1a, b), but in some cases two flagella at opposite poles (Fig. 1c). Cell width was rather constant (mean=0.66 μm, SD=0.083 μm, n=112) whereas cell length, i.e. from pole to pole, was variable (mean=2.1 μm, SD=0.54 μm, n=112). The cells had a positive chemotactic response to nitrate (Grote et al., 2012). Under optimal conditions in ABW + NS medium the cell doubling time of strain GD1T was 13 h. Cells in older cultures tended to form aggregates. Growth at temperatures in the range of 4–40 °C was investigated, with highest cell numbers obtained between 4 and 20 °C and optimal growth at 15 °C (Grote et al., 2012). Thus, isolate GD1T is the first psychrotolerant species within the genus Sulfurimonas, in which all member species at the time of writing are mesophilic (Table 1).

To obtain media with different pH values, the pH of a 20 ml subsample from the anoxic ABW + NS was adjusted to the pH 6.0, 6.5, 6.7, 6.9, 7.1, 7.5, 8.0, 8.4 and 9.0 by the addition of the appropriate amount of 0.1M HCl. For the experimental setup, the corresponding amount of 1 M HCl was added to the media preparations, which were then inoculated. After 14 days of incubation, the pH was measured. At an initial pH of 6.5–8.4, it remained constant (±0.02) throughout the experiment whereas below and above this range it decreased by about 0.18–0.25 pH units. Optimal growth occurred over a wide pH range (6.7–8.0)
Fig. 1. Cell morphology of spirilla-shaped cells of strain GD1$^T$ cultivated on ABW + NS medium. (a) Fluorescence microscopy of 4′,6′-diamidino-2-phenylindol (DAPI) stained cells. (b) Transmission electron microscopy of a bacterium with one flagellum and (c) of a bacterium with two flagella (indicated by arrows), both negatively stained with phosphotungstic acid.

Table 1. Differential characteristics between strain GD1$^T$ and species of the genus Sulfurimonas

| Characteristic                  | 1                      | 2                      | 3                      | 4                      |
|--------------------------------|------------------------|------------------------|------------------------|------------------------|
| **Morphology**                 |                        |                        |                        |                        |
| Cell shape                     | Curved rods to spirilla-like | Rods to spirilla-like | Rods                   | Rods                   |
| Motility                       | +                      | –                      | +                      | +                      |
| Anaerobic growth               | +                      | +                      | +                      | –                      |
| Growth                         | 13                     | 12                     | 13–16                  | 1.4                    |
| Doubling time under optimal conditions (h) | 15                     | 22                     | 30                     | 23–26                  |
| **Temperature dependence**     |                        |                        |                        |                        |
| Temperature range (°C)         | 4–20                   | 10–30                  | 4–35                   | 10–40                  |
| Temperature optimum (°C)       | 22                     | 30                     | 30                     | 23–26                  |
| Temperature range (°C)         | 6.7–8.0                | 6.7–8.0                | 6.1                    | 6.5                    |
| pH range                       | 10–<20                 | 0.5                    | 10                     | 15                     |
| pH optimum                     | 15                     | 7                      | 6.1                    | 6.5                    |
| NaCl requirement               | –                      | +                      | +                      | +                      |
| **Inorganic electron donors**  |                        |                        |                        |                        |
| H$_2$, HS$^-$, S$_0$, S$_2$O$_3^{2-}$ | HS$^-$, S$_2$O$_3^{2-}$ | H$_2$, S$_0$, S$_2$O$_3^{2-}$ | –                      | –                      |
| **Organic electron donors**    | Formate, acetate, yeast extract, pyruvate, amino acid mix | Formate, fumarate, yeast extract, alcohol mix | –                      | –                      |
| **Electron acceptors**         |                        |                        |                        |                        |
| Fatty acids (mol%)             | 0.9                    | 0.4                    | 5                      | 8.4                    |
| 14:0                           | 2.5                    | 7                      |                         |                         |
| 16:1o7c                        | 66.0                   | 67.9                   | 22$^\dagger$           | 45.2$^\ddagger$        |
| 16:1o5c+t                      | 1.3                    | 2.0                    | 25                     | 37.1                   |
| 16:0                           | 15.5                   | 15.3                   | 4                      |                         |
| 18:0                           | 13.1                   | 12.1                   | 37$^\dagger$           |                         |
| 18:1o7c                        | 36                     | 37.6                   |                         | 35.2                   |
| DNA G+C content (mol%)         | 33.6$^\|$              | 36                     |                         |                         |

*Tested without bicarbonate supplementation.
†No differentiation in cis/trans.
‡Identified as 16:1cis.
§Potentially also 18:1o7c, which has a similar retention time to 18:1o9t.
$^\dagger$Based on genome analyses.
but no growth occurred at pH 6.0 and 8.4. The NaCl requirement was determined by cultivation in ABW + NS containing the following salt concentrations [NaCl (g l⁻¹)/MgCl₂·6H₂O (g l⁻¹)]: 0/0, 0/0.50, 2.50/0.38, 5.00/0.75, 7.50/1.13, 10.00/1.50, 12.50/1.88, 15.00/2.25, 17.50/2.63 and 20.00/3.00. The isolate had an absolute requirement for NaCl and grew best with between 10 and 20 g NaCl l⁻¹; the upper limit for growth was not further determined. No growth was observed in media without added NaCl, in contrast to Sulfurimonas denitrificans DSM 1251T, which grew equally well without NaCl and at all NaCl concentrations tested (Table 1).

To identify the electron donors sustaining chemooxidotrophic growth of isolate GD1ᵀ, ABW medium containing 5 mM nitrate was supplemented with sulfate (1 mM), sulfide (10 µM, 20 µM, 100 µM) or elemental sulfur (1 mM). Hydrogen utilization was assessed by bubbling ABW + NS with forming gas (N₂/H₂, 95:5) for several hours prior to inoculation and cultivation. Strain GD1ᵀ was able to use all of the tested electron donors as an energy source for growth although growth was inhibited by sulfate concentrations >20 µM (Grote et al., 2012). This observation is in accordance with in situ activities of chemooxidotrophic micro-organisms in pelagic Gotland Deep redox zones, where dark CO₂ fixation rates are significantly reduced at environmental sulfate concentrations >20 µM (Jost et al., 2010). As electron acceptors, nitrate (100 µM, 2 mM, 5 mM, 10 mM), nitrite (600 µM, 2 mM) (Grote et al., 2012), manganese(IV) oxide (200 µM), manganese(III) acetate dihydrate (2.4 mM), iron(III) chloride hexahydrate (5 mM), fumarate (100 µM) and oxygen (4 % saturation, approx. 12 µmol O₂ l⁻¹) were tested in ABW containing 5 mM thiosulfate. For the oxygen experiment, the oxygen content in fully oxygenated ABW + thiosulfate was measured with an optode (POF-PSt3; PreSens) and the content in fully oxygenated ABW confirmed with an optode (POF-PSt3; PreSens) and the content in fully oxygenated ABW + NS with oxygen saturations of 0.5, 3, 5, 10, 20, 30, 40 and 50 %. Compared to oxygen-free conditions, oxygen concentrations ≥ 20 % reduced or inhibited the growth of this strain whereas oxygen concentration ≤ 10 % had no such effect (Grote et al., 2012). Thus, the oxygen tolerance of strain GD1ᵀ is similar to that of aerobic Sulfurimonas autotrophica OK10ᵀ (Table 1). Based on our current knowledge, we consider strain GD1ᵀ to be an aerotolerant representative of the genus Sulfurimonas.

Chemolithoautotrophic growth was directly confirmed in ABW + NS containing ¹⁴C-bicarbonate followed by a combination of fluorescence in situ hybridization and microautoradiography (MICRO-CARD-FISH) (Grote et al., 2012). As electron donor (in ABW + 5 mM KNO₃) alone or as electron donor and sole carbon source (in NaHCO₃-free ABW + 5 mM KNO₃) the following compounds were tested: (a) glucose (0.1 mM), (b) a mixture of lactate, malate, fumarate, succinate, glycerine and glucose (abbreviated as mix 4) (100 µM), (c) yeast extract (0.01 mg l⁻¹), (d) pyruvate (100 µM), (e) acetate (100 µM), (f) fumarate (100 µM), (g) alcohol mix (butanol, ethanol, methanol, propanol; 100 µM) (Grote et al., 2012) and (h) an amino acid mix (0.1 mM) consisting of (g l⁻¹): β-alanine 0.466, l-arginine 0.872, l-asparagine 0.750, l-cysteine 0.606, l-glutamine 0.730, l-glutamic acid 0.736, glycine 0.376, isoleucine 0.656, l-leucine 0.656, l-methionine 0.746, l-phenylalanine 0.826, l-serine 0.526, l-threonine 0.596, l-valine 0.586, l-proline 0.576, l-tryptophan 1.022, l-histidine 0.776, l-lysine 0.822, l-tyrosine 0.906 and l-asparagine 0.666.

In the presence of 2 mM NaHCO₃, the growth of isolate GD1ᵀ was promoted with formate, acetate, yeast extract, pyruvate and the amino acid mix as electron donors. However, maximal cell numbers were usually more than a magnitude less than those reached with thiosulfate/nitrate-containing medium, as shown in Fig. 2(a) for pyruvate, which was also used in radiotracer experiments aimed at confirming the capability of strain GD1ᵀ to use organics as electron donor. In those experiments, CO₂ production was measured following the addition of 16 kBq [²⁴⁻¹⁴C]pyruvate (specific activity 0.6 GBq mmol⁻¹) to cultures grown solely on pyruvate or on thiosulfate/pyruvate. After 24 h or 72 h of incubation, CO₂ was degassed by the acidification of cell-free medium and trapped in ethanalamine. In nitrate/pyruvate medium, the growth of strain GD1ᵀ was accompanied by elevated CO₂ production (Fig. 2b). The simultaneous incorporation of [²⁴⁻¹⁴C]pyruvate into GD1T cells was much less pronounced, but its uptake and contribution to biomass production were clearly determined in thiosulfate/nitrate/pyruvate medium, where total cell numbers were also higher than those reached in thiosulfate/nitrate medium (Fig. 2a), but the difference was not statistically significant (unpublished data). By contrast, in NaHCO₃-free medium strain GD1ᵀ was unable to use any of the organics offered simultaneously as electron donor and carbon source (Fig. 2a). It has long been
recognized that even heterotrophic bacteria may require CO₂ for growth (Dehority, 1971), e.g. in anaplerotic reactions (Alonso-Sáez et al., 2010). Similar findings were reported for Nitrobacter hamburgensis, which requires atmospheric CO₂ or the addition of sodium carbonate for mixotrophic growth (in the presence of NO₃⁻) on d-lactate (Starkenburg et al., 2008). The authors of that study suggested that CO₂ fixation served as a reductant sink necessary to maintain cellular redox balance. The physiological background for the growth of isolate GD1T on organics is thus far unclear. In other species of the genus Sulfurimonas, organic substance utilization is variable. For example, a similar experiment Sulfurimonas denitrificans was able to use formate, fumarate, yeast extract and the alcohol mix as electron donors (Table 1). The ability of this bacterium to oxidize formate was proposed in a genome analysis, which identified a formate dehydrogenase complex (Sievert et al., 2008). Homologues of genes involved in glycolysis and proteolysis are also present in the genome of strain GD1T (Grote et al., 2012), whereas Sulfurimonas autotrophica (Inagaki et al., 2003; but tested without bicarbonate supplementation to the organic medium) and Sulfurimonas paralvinellae (Takai et al., 2006) are unable to grow on organic compounds. In conclusion, although under specific circumstances organic compounds enhance the growth of some species of the genus Sulfurimonas, members of this genus characteristically grow chemolithoautotrophically.

Total fatty acids and phospholipid-derived fatty acids were extracted as described by Sasser (1990) and Boschker (2004), respectively, and analysed by gas chromatography with a flame-ionization detector on a non-polar HP-5ms column (Agilent). The dominant cellular fatty acid of strain GD1T was 16:1ω7c, with 3-OH 14:0, 16:0, 16:1ω5c + t, and 18:1ω7c detected in lower amounts. This fatty acid profile is comparable to those of other species of the genus Sulfurimonas but most similar to that of Sulfurimonas denitrificans (Table 1). This may reflect the fact that strain GD1T and Sulfurimonas denitrificans were cultivated on ABW ± NS under identical conditions. However, a high percentage of C16:0 and one or both of the monounsaturated C16 and C18 fatty acids has also been described in other members of the class Epsilonproteobacteria, such as Nitratirigator salsuginis and Sulfurovum lithotrophicum (Suzuki et al., 2005). Accordingly, this combination may be a general characteristic of these epsilonproteobacteria.

The DNA guanine-plus-cytosine (G+C) content of strain GD1T was determined to be 33.6 mol%, as calculated by analysis of the whole genome (Grote et al., 2012).

To establish the closest relatives of strain GD1T based on 16S rRNA sequencing, preliminary searches in the EMBL Data Library were performed with the program FASTA (Pearson & Lipman, 1988). Closely related sequences were retrieved from GenBank and aligned and analysed with the newly determined sequence, within the program ARB (Ludwig et al., 2004). Sequences for analysis were reduced to unambiguously alignable positions using group-specific filters. For phylogenetic analyses, three different trees were calculated using the neighbour-joining, parsimony and maximum-likelihood (Phyml) algorithms based on nearly

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**Fig. 2.** Impact of pyruvate on the growth of isolate GD1T. Error bars indicate the standard deviation of three independent replicates for each assay. (a) Growth on media with different substrate combinations: 1, NaHCO₃, S₂O₃²⁻, NO₃⁻; 2, NaHCO₃, S₂O₃²⁻, NO₃⁻, pyruvate; 3, NaHCO₃, NO₃⁻, pyruvate; 4, NO₃⁻, pyruvate; 5, ABW without further supplements. The relative enrichment factor describes the increase of cell numbers after 7 days of incubation compared to the initial cell numbers after inoculation at day 0 (6.1×10⁶ ml⁻¹). (b) ¹⁴CO₂ production and [¹⁴C]pyruvate incorporation after 24 h and 72 h of incubation. Media: 1, NaHCO₃, S₂O₃²⁻, NO₃⁻, [¹⁴C]pyruvate; 2, NaHCO₃, NO₃⁻, [¹⁴C]pyruvate. P, pyruvate incorporation; CO₂, CO₂ production.
full-length 16S rRNA sequences (approx. 1400 bp). For neighbour-joining, the Jukes–Cantor-correction was applied. Shorter sequences were gradually inserted into the constructed tree without changing the topology. Sequence searches of the EMBL database (latest: 2013-05-14) revealed that our isolate is related to the epsilon class of the phylum Proteobacteria (data not shown). In a pairwise analysis, it displayed highest (93.7–94.2 %) 16S rRNA gene sequence similarity to species of the genus Sulfurimonas and to the Baltic ‘Uncultured Helicobacteraceae G138eps1/GD17’ subgroup (95.7 %). Lower levels of relatedness (≤ 91 % sequence similarity) were determined for the other examined species belonging to the epsilon class of the phylum Proteobacteria.

An unrooted tree reconstructed using the neighbour-joining method showed the phylogenetic position of the novel bacterium, strain GD1T, amongst the members of the class Epsilonproteobacteria (Fig. 3). Treeing analyses confirmed it to be a member of the genus Sulfurimonas, forming a stable cluster with the ‘Uncultured Helicobacteraceae G138eps1/GD17’ subgroup. This cluster is specifically detected by the SUL90 16S rRNA gene probe, originally developed to be 100 % complementary to the G138eps1/GD17 target site (Grote et al., 2007).

There is no precise correlation between percentage 16S rRNA sequence divergence and species delineation, but it is generally recognized that divergence values ≥ 3 % are significant (Stackebrandt & Goebel, 1994). However, it is pertinent to note that the phylogenetic separateness of strain GD1T is strongly supported by phenotypic considerations. For instance, this novel bacterium is distinguishable from other species of the genus Sulfurimonas by its psychrotolerance and energy metabolism (Table 1). Additional characteristics useful in differentiating Baltic isolate GD1T from related organisms are shown in Table 1. Based on phenotypic and genetic evidence, we propose the classification of strain GD1T as a representative of a novel species of the genus Sulfurimonas: Sulfurimonas gotlandica sp. nov.

**Emended description of the genus Sulfurimonas**

The description is based on that by Takai et al. (2006). 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. Psychrotolerant to mesophilic and aerotolerant to facultatively anaerobic. Do not always require NaCl for growth. Optimal 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. Supplementation of bicarbonate can enable growth on organic substances, but yields much lower cell numbers compared to growth on reduced sulfur or hydrogen. Potential ecological niches are deep-sea hydrothermal environments and benthic or pelagic marine to brackish transition zones from oxic to anoxic/sulfidic environments. The type species is Sulfurimonas autotrophica (Inagaki et al. 2003).

**Description of Sulfurimonas gotlandica sp. nov.**

*Sulfurimonas gotlandica* (got.lan’di.ca. N.L. fem. adj. gotlandica pertaining to the Gotland Deep, the basin in the central Baltic Sea from which the organism was first isolated).

Gram-negative, slightly curved or spirilla-shaped cells. Motile by one polar flagellum or two flagella at opposite poles. Cells exhibit a positive chemotactic response to nitrate. Cell sizes are 0.66 ± 0.083 x 2.1 ± 0.54 µm. Cells have a tendency to aggregate at older stages. Psycho- and aerotolerant. The temperature range for growth is 4–20 °C. Optimal growth occurs at 15 °C and pH 6.7–8.0. The cells have an absolute requirement for NaCl. Chemolithoautotrophic growth occurs with H2, HS-, S0 and thiosulfate. Supplementation of bicarbonate can enable growth on formate, acetate, yeast extract, pyruvate or amino acid mix, but yields much lower cell numbers compared with growth on reduced sulfur or hydrogen. Sulfide concentrations of

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**Fig. 3.** Unrooted tree showing phylogenetic relationships of isolate GD1T and closely related members of the class Epsilonproteobacteria. The tree was reconstructed using the neighbour-joining method and was based on a comparison of approximately 1400 nt. Solid squares indicate that the corresponding nodes (or groups) were recovered in neighbour-joining, maximum-parsimony and maximum-likelihood methods. Branching points supported by two algorithms are marked by an open square. The following strains were used as an outgroup (not shown): *Antarctobacter heliothermus* EL-219T, *Sagitella stellata* E-37T, *Roseovarius tolerans* EL-172T, *Roseovarius nubinhibens* ISM T and *Roseovarius mucusos DFL-24T*. Bar, 1 substitution per 10 nt.
more than 20 μM inhibit, but up to 10% of oxygen in the medium does not influence growth. Dominant cellular fatty acid is 16:1ω7c, with 14:0, 16:0, 16:1ω5c+2, and 18:1ω7c present in smaller amounts.

The type strain is GDi1T (=DSM 19862T=JCM 16533T), isolated from water of a pelagic redox zone of the central Baltic Sea. The G+C content of the type strain is 33.6 mol%.

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