Supporting Information

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SI Results and Discussion

Genome Annotation and Analysis of Sulturimonas gotlandica str. GD1. In pairwise BLASTP comparisons of predicted proteins against the National Center for Biotechnology Information nonredundant protein database, *Sulfurimonas gotlandica* str. GD1 proteins demonstrated the greatest similarities to proteins of other *Epsilonproteobacteria*. Of the genes, 53.7% exhibited closest similarities to proteins encoded by *Sulfurimonas denitrificans* DSM 1251 (1), confirming results from the 16S rDNA analysis (2). Homologs to proteins from other members of the *Epsilonproteobacteria* include the deep-sea vent strains *Sulfurovum* sp. NBC37-1 (6.2% best matches) (3), *Nitratiruptor* sp. SB155-2 (1.3%) (3), *Nautilia profundicola* AmH (0.6%) (4), the pathogen *Acrobacter butzleri* RM4018 (5.5%) (5), *Sulfurospirillum deleyianum* DSM 6946 (3.1%) isolated from freshwater sediment (6), and *Wolinella succinogenes* DSM 1740/ATCC 29543, isolated from bovine rumen fluid (7).

Approximately 74% of all predicted *S. gotlandica* str. GD1 protein-coding genes could be assigned functions based on similarity searches. The Sox-dependent sulfur oxidation pathway consisted of the Sox multienzyme complex, arranged in two sox gene clusters containing *soxXYZAB* and *soxZYCD*, sulfite:cytochrome *c* oxidoreductase (*sor*), and sulfide:quinone oxidoreductase (*sqr*), similar to that described in several deep-sea vent *Epsilonproteobacteria* (8). Multiple copies of the *sqr* gene were found distributed within the genome (Table S3). Despite the fact that genes encoding fumarate reductases (*frd*A, *frd*B) were present in the genome, growth with alcohol was not detected and the utilization of fumarate as an electron acceptor was not observed.

SI Materials and Methods

Environmental Sampling. All samples were collected in free-flow bottles attached to a conductivity, temperature, and depth-rosette. Cell numbers of *Sulfurimonas* subgroup GD17 were determined in samples collected from different locations in the central Baltic Sea, sampled between 2005 and 2009, using catalyzed reporter deposition (CARD)-FISH, as described previously (9).

Growth Conditions. Growth experiments were conducted in the dark at 15 °C at pressures of up to 2.5 bar and replicated up to 10 times. All growth experiments were conducted in anoxic artificial brackish water medium (ABW) modified according to Bruns et al. (10). ABW for standard growth conditions contained 95 mM NaCl, 11.2 mM $MgCl_2 \times 6H_2O$, 2.3 mM $CaCl_2 \times 2\ H_2O$, 2 mM KCl, 6.4 mM Na_2SO_4, 2–5 mM $NaHCO_3$, 192 μM KBr, 92 μM H_3BO_3, 34 μM $SrCl_2$, 92 μM NH_4Cl, 9 μM KH_2PO_4, 16 μM NaF, and 1–10 mM Hepes (pH 7.3). KNO_3 and $Na_2S_2O_3$ in the concentration range of 1–10 mM served as substrates for chemolithotrophic denitrification. Resazurin was added as a redox indicator. The medium was prepared and amended with supplements, as described previously (10).

To detect false-positive growth induced by potential contaminants, negative controls containing only electron donors or acceptors were used. As an additional control for the quantification of substrate turnover, sterile media without inoculi were treated and analyzed identical to the cultures. To determine the optimal growth temperature, strain GD1 was grown in ABW with 5 mM nitrate and thiosulfate over a temperature range of 4–40 °C. To examine oxygen sensitivity, GD1 growth was measured in media of varying oxygen concentration (Fig. S2*A*). Aerobic ABW was prepared and the oxygen content was measured with an optode (POF-PSt3; PreSens) (11). Anaerobic ABW was mixed with appropriate amounts of aerobic ABW to achieve different oxygen concentrations.

Cell growth was determined by DAPI staining and flow cytometry (12), either at the starting date and after 7 d of incubation, or daily in quantitative time courses. Using 5 mM nitrate as an electron acceptor, different compounds were tested for the ability to serve as electron donors: sulfite (1 mM), sulfide (10 μM, 20 μM, 100 μM), elemental sulfur in suspension (1 mM), and hydrogen. ABW was bubbled with forming gas containing 95% nitrogen and 5% hydrogen for several hours before inoculation to test hydrogen as a potential electron donor.

Nitrite (0.6 mM, 2 mM) and fumarate (100 μM) were tested as possible electron acceptors in ABW containing 5 mM thiosulfate. Further thiosulfate (5 mM) or elemental sulfur in suspension (1 mM) were added as sole supplements. Different organic compounds were tested as possible electron donors with 5 mM nitrate serving as an electron acceptor: yeast extract (10 $\mu g \times l^{-1}$), formate (100 μM), peptone (10 $\mu g \times l^{-1}$), pyruvate (100 μM), acetate (100 μM), alcohol mix (100 μM) (butanol, ethanol, methanol, propanol), and fumarate (100 μM).

We also tested for chemoorganoheterotrophic oxygen respiration by using between 8 μM and 423 μM O_2 as electron acceptor; glucose, at concentrations of 10 μM to 11 mM, and peptone, between 2 ng $\times$ l^{-1} and 2 g $\times$ l^{-1} served, respectively, as electron donor and carbon source. Here, oxygen concentrations were adjusted by adding sterile air to anoxic medium according to Henry's law: $c_{aq} = K_H p_{gas}$ (c_{aq} = oxygen concentration in the medium; $K_H = 1.28 \times 10^{-3}$ mole $\times$ l^{-1} $\times$ bar^{-1}; p_{gas} = partial pressure of oxygen in the headspace of the culture flask).

Chemical Analysis. Thiosulfate, sulfite and hydrogen sulfide were quantified by HPLC-analysis using 3-(bromomethyl)-2,5,6-trimethyl-1*H*,7*H*-pyrazolo[1,2-*a*]pyrazole-1,7-dione [also known as (mono)bromobimane] derivatization, as previously described (13). Samples were diluted 1:100 before derivatization and derivatized preparations were frozen at −80 or −20 °C until processed further. HPLC-analysis equipment from Merck was used, including a LiChrosphere 60RP select B column (125 × 4 mm, 5 μm). A flow rate of 1 mL $\times$ min^{-1} was used to pass the mobile phase [0.25% (vol/vol) acetic acid, pH 3.5, (eluent A) and HPLC grade methanol (eluent B)] over the column using the following gradient: 0 min: 0% B; 0.5 min: 0% B; 1 min: 8% B; 4.5 min: 10% B; 7 min: 32% B; 11 min: 32% B; 18 min: 50% B; 22 min: 100% B; 24 min: 100% B; 25 min: 0% B; 30 min: 0% B. After a test run, derivatized samples were diluted to a final thiosulfate concentration of less than 20 μM. The optimal linear relationship between the integrated detector signal and thiosulfate concentration is observed under these conditions. Sulfate concentration was determined turbidometrically by barium precipitation, modified as previously described (14). Here, to avoid the formation and precipitation of zero-valent sulfur from thiosulfate, samples were not acidified by citric acid. Nitrate and nitrite concentrations were determined colorimetrically using the Spongy Cadmium method (15). Oxygen contamination in all chemical analyses was minimized by extensive N_2 use. For nutrient analysis, cells were removed by centrifugation in N_2-flushed centrifugation tubes.

Chemotaxis. Experiments were conducted in an anaerobic chamber to maintain anoxic conditions (COY Laboratory Products). Bacteria were pregrown in ABW with 1 mM thiosulfate and nitrate for 7–10 d. The bacterial suspension was diluted to 10^5 to 10^6 cells mL^{-1} in nitrate-free medium and filled in 5-mL glass vacutainer

(Becton Dickinson; VACUTAINER Systems). Capillaries were filled by capillary action with ABW containing both, thiosulfate (1 mM) and nitrate (1 mM), or only thiosulfate (1 mM) as control, sealed with plasticine, and inserted into the vacutainer. After the incubation the capillaries were carefully removed, wiped with tissue paper, depleted (washed two times with sterile MQ water), filtered on black polycarbonate filters (pore size 0.2 μm; diameter 25 mm; Whatman), and stained with DAPI. Filters were examined with an epifluorescence microscope (Axioskop 2 mot plus; Zeiss), and equipped with a 100 Plan Apochromat oil objective lens (Zeiss). The relative response was determined as a ratio of the mean *Sulfurimonas* str. GD1 cell density in attractant-containing capillaries and the mean cell number in control capillaries (16).

Genome Sequencing, Annotation, and Analysis. Potential signal peptides and transmembrane helices were predicted using SignalP Ver. 2.0 (17) and TMHMM Ver. 2.0 (18), respectively, and transfer RNA genes were identified using tRNAScan-SE (19). In addition, conserved functional domains were identified using InterProScan (20) and HMMer on Pfam (21), TIGRfam (22), and PantherDB (23). Proteins involved in sensing and signal transduction were identified searching for Pfams EAL, GGDEF, Response_reg, either one of PAS, PAS_2, PAS_3, PAS_4 or PAS_6, and either one of H-kinase_dim, HD, HWE_HK, HisKA_2, HisKA_3 or DUF2222 using hmmpfam with an e-value cutoff of 1×10^{-4}. Those of metabolic relevance were identified searching for Pfams APS_kinase, ATP-sulfurylase, PAPS_reduct in the absence of TIGR02055, SoxZ, NapD, NIR_SIR, together with NIR_SIR_ferr, Succ_DH_flav_C, PF02967, Oxidored_q1_N, Molybtopterin, CCP_MauG, either one of NiFeSe_Hases or Ni_Hydr_CYTB, TIGRFAMS TIGR02055 and PantherDB's SQR using an expected value (e-value) cutoff of 1×10^{-4}. E-values for Pfam's NrFD and NosD (0.01) were adjusted in a way that results matched published data. Additionally, homologs of SorA and FCC from *Ralstonia metallidurans* CH34, NrfA and NrfH from *W. succinogenes* DSMZ 1740 (e-value $< 1 \times 10^{-30}$), and SoxC from *Thermus thermophilus* HB27 (e-value < E-35) were identified using BLAST (24). E-values in BLAST searches for homologs of NorB (e-value $< 1 \times 10^{-9}$) and MQR ($<1 \times 10^{-4}$) from *Pseudomonas aeruginosa*, NorC from *S. denitrificans*, the Cyt b subunit of the Cyt b/c1 complex and subunit I of the Cyt c oxidase from *Helicobacter pylori* as well as subunit II of the Cyt bd oxidase complex from *P. aeruginosa* ($<1 \times 10^{-10}$) were again adjusted to match published data.

The *S. gotlandica* str. GD1 genome was compared with the genomes of the following *Epsilonproteobacteria* (http://www.ncbi.nlm.nih.gov): *Caminibacter mediatlanticus* TB-2 (NZ_ABCJ00000000), *N. profundicola* AmH (NC_012115) (4), *Sulfurovum* sp. NBC37-1 (NC_009663) (3), *S. deleyianum* DSM 6946 (NC_013512) (6); *S. denitrificans* DSM 1251^{T} (NC_007575) (1), *Sulfurimonas autotrophica* DSM 16294 (NC_014506) (25), *A. butzleri* RM4018 (NC_009850) (5), and *Nitratiruptor* sp. SB155-2 (NC_009662) (3).

Phylogenetic Analysis. Alignment and phylogenetic analyses of the *S. gotlandica* str. GD1 16S rRNA sequence were performed using the ARB software package (26). Sequences for analysis were reduced to unambiguously alignable positions using group-specific filters. For phylogenetic analyses, three different trees were calculated using the neighbor-joining, parsimony and maximum-likelihood (Phyml) algorithms based on nearly full-length 16S rRNA sequences (> 1,400 bp). Eventually, shorter sequences were inserted into the reconstructed tree without changing the topology. For neighbor-joining, the Jukes-Cantor-Correction was applied.

Biogeographic Distribution of *Epsilon-* and *Gammaproteobacteria* in Hypoxic Systems. The biogeographic distribution of 16S rRNA sequences of the genera *Sulfurimonas* and *Arcobacter* as well as those of the gammaproteobacterial sulfur oxidizer SUP05 (27) were determined using the SILVA 106 database (1200/900) (28). SILVA 106 was supplemented by 1424 *Sulfurimonas*, 1840 *Arcobacter* and 27710 unclassified gammaproteobacterial sequences downloaded from the Ribosomal Database Project (RDP) database (RDP Release 10, Update 27, Aug 9, 2011) (29). Dataset options in RDP were "Type+Non Type, Uncultured +Isolates, Size: >1200 <1200, Quality: Good." Search terms within the field "isolation_source" in SILVA were: OMZ, Saanich, Nitinat, Black Sea, upwelling, oxygen minimum zone, Cariaco, Arabia, Urania, Venezuela, Eastern Tropical, Framvaren, Mariager, Chile, Peru, redox, chemocline, *anoxic* *water*, *sulfidic* *water*, deoxygen, suboxic, Namibia, transient. In some cases, all *Sulfurimonas* and *Arcobacter* sequences were also checked manually for other potential habitats not covered by the above terms. If positive, the gammaproteobacterial sequences were specifically proven for their occurrence in these habitats. Hypoxic systems were further classified into marine sulfidic redoxclines and marine oxygen minimum zones. The occurrences of members of the *Sulfurimonas* and *Arcobacter* genera and SUP05 are given in Table S5, as well as the references for the published sequences.

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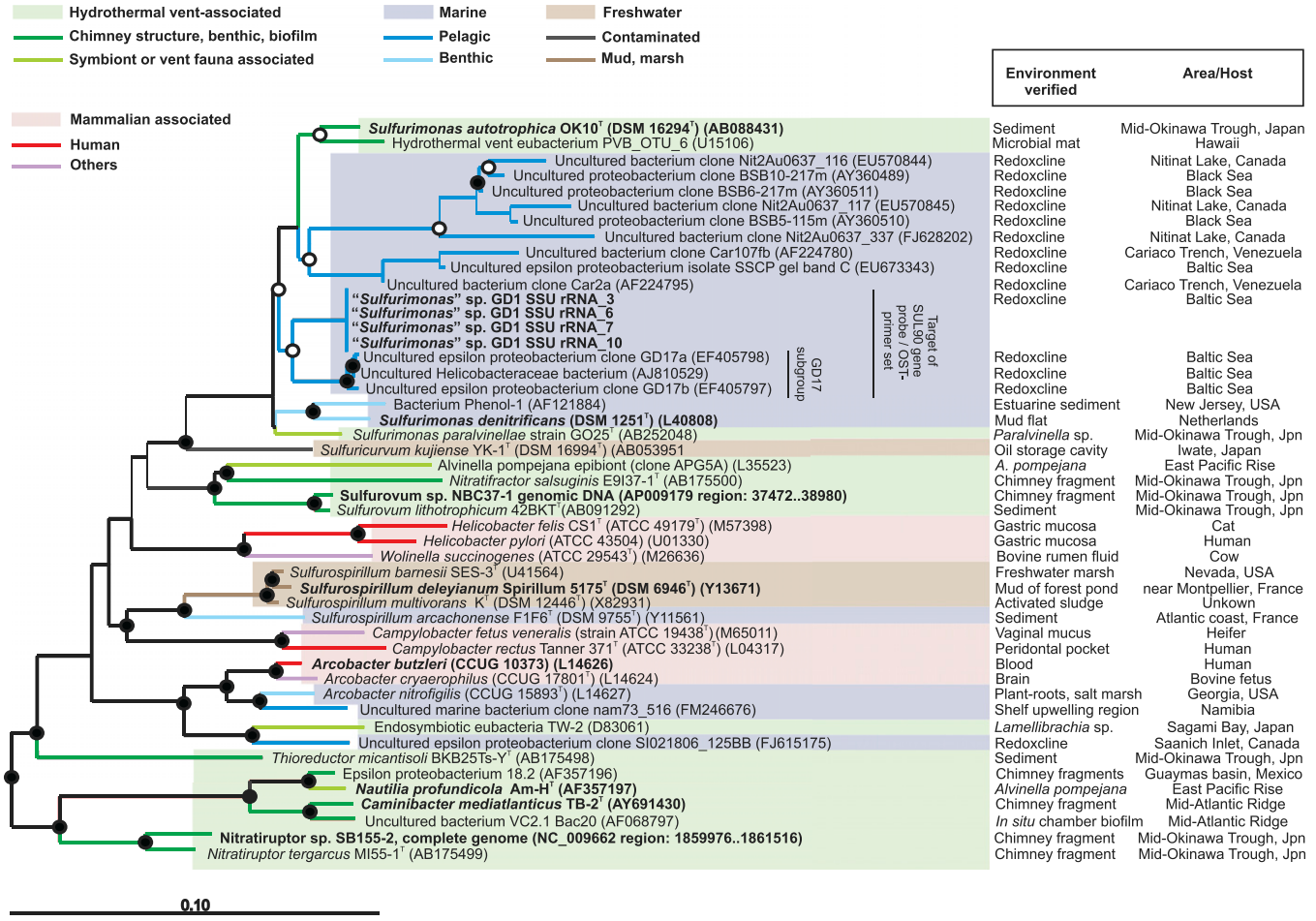

Fig. S1. Phylogenetic analysis of *S. gotlandica* str. GD1. Unrooted tree showing the phylogenetic relationships of isolate *S. gotlandica* str. GD1, which is calculated based on the four GD1 16S rRNA operons, and the closely related members of the *Epsilonproteobacteria*. The evolutionary distance dendrogram was reconstructed using the Jukes-Cantor correction and neighbor-joining. Branching points supported by neighbor-joining, maximum likelihood, and maximum parsimony algorithms are marked by a filled circle. Branching points supported by two algorithms are marked by an open circle. Genome-sequenced bacteria included in comparative genomics of this study are marked in bold. Target organisms of the SUL90 gene probe (1) and the OST primer set (2) are indicated. The origins of the isolates or clones are indicated by color codes of the background, the rectangles and branches, as well as descriptions on the right, and demonstrate the metabolic versatility of the *Epsilonproteobacteria*. Strain deposition and nucleotide sequence accession numbers are given in brackets. Bar, 1 estimated substitution per 100 bp.

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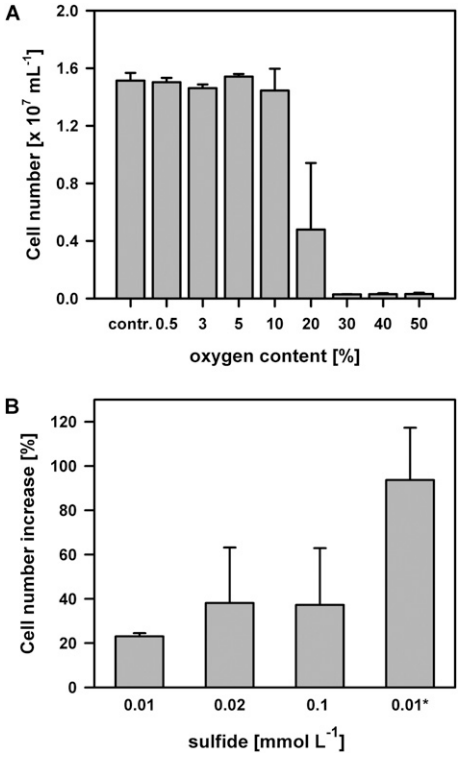

Fig. S2. Laboratory batch experiments with *S. gotlandica* str. GD1. (*A*) Oxygen sensitivity during incubation with nitrate (5 mM), thiosulfate (5 mM), and different oxygen concentrations. Cell numbers after 7 d of incubation are given. Anoxic ABW was used in the controls. Error bars indicate the SD of three replicates. (*B*) Growth with 5 mM nitrate and different concentrations of sulfide expressed as percent cell increase after an incubation time of 7 d. "0.01*" Means that a concentration of 0.01 mM sulfide was added every second day. Error bars indicate the SD of three replicates. For every formed GD1-cell, 23 fmol sulfide were oxidized. During exponential growth, chemolithotrophic denitrification with sulfide as electron donor was determined to occur according to the following equation: $6HS^- + 7NO_3^- + 4H^+ \rightarrow 4SO_4^{2-} + 2S_0 + 3.5N_2 + e^- + 5H_2O$; during this turnover, one molecule of CO_2 was fixed.

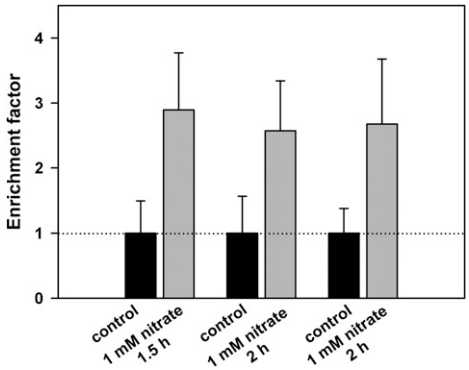

Fig. S3. Quantitative analysis of the chemotactic response of *S. gotlandica* str. GD1 toward nitrate containing capillaries expressed as relative response. Values of > 2 indicate significant chemotactic activity. The incubation time was 1.2–2 h. Negative control capillaries contained no nitrate. The capillary assay was performed three times. Error bars indicate the SD of three to four replicates for each assay.

Table S1. Summary of general genome features in *S. gotlandica* str. GD1

Feature	Value
Size (bp)	2,952,682
G+C content (%)	33.59
Total no. of coding sequences	2,879
CDS density (gene/kb)	0.975
Average gene length (bp)	955
Coding percentage (%)	93.2
Genes with function prediction	2,124
(Conserved) hypothetical genes	756
No. of rRNA operons	4
No. of tRNA genes	47

CDS, coding sequences.

Table S2. Electron donors and acceptors supporting growth of *S. gotlandica* str. GD1

Presumed metabolic pathway	e-Donor	e-Acceptor	Growth
Chemoautotrophic denitrification	Sulfite	Nitrate	+++
	Sulfide	Nitrate	+
	Sulfur	Nitrate	+++
	Hydrogen	Nitrate	+++
	Thiosulfate	Nitrate	+++
	Thiosulfate	Nitrite (0.6 mM)	+++
	Thiosulfate	Fumarate	—
Heterotrophic denitrification	Formate	Nitrate	+++
	Fumarate	Nitrate	—
	Alcohol mix	Nitrate	—
	Acetate	Nitrate	+
	Yeast	Nitrate	++
	Peptone	Nitrate	+++
	Pyruvate	Nitrate	+++
Unknown	Thiosulfate	Thiosulfate	+++
	Sulfur	Sulfur	++

Experiments were conducted in ABW (15 °C) and cell numbers were determined after 7 d of incubation. Maximum cell increase is scored as +++; cell increase of > 100% is scored as ++; > 50% is scored as +; no increase is scored as —. All experiments were conducted in three replicates.

Table S3. Key enzymes identified in *S. gotlandica* str. GD1

Gene name	Function/protein	Locus tag
soxXYZAB	Oxidation of reduced sulfur compounds	
soxX	Sulfur oxidation protein	SMGD1_0062
soxY	Sulfur oxidation protein	SMGD1_0061
soxZ	Sulfur oxidation protein	SMGD1_0060
soxA	Diheme cytochrome	SMGD1_0059
soxB	Sulfate thiol esterase	SMGD1_0058
SoxZYCD		
soxZ	Sulfur oxidation protein*	SMGD1_1162
soxY	Sulfur oxidation protein	SMGD1_1163
soxC	Sulfur oxidation protein	SMGD1_1165
soxD	Sulfur oxidation protein	SMGD1_1164
soxF	Sulfide dehydrogenase	SMGD1_1158
soxH	Putative sulfur oxidation protein	SMGD1_1160
sorA	Sulfite oxidoreductase	SMGD1_1131
sorB	Sulfite oxidoreductase	SMGD1_1132
sqr1	Sulfide-quinone oxidoreductase	SMGD1_1402
sqr2	Sulfide-quinone reductase	SMGD1_2084
sqr3	Sulfide-quinone oxidoreductase	SMGD1_1224
sqr4	Sulfide-quinone oxidoreductase	SMGD1_1167
sqr5	Putative sulfide-quinone oxidoreductase	SMGD1_1602
Sir	Sulfite reductase [ferredoxin] 2	SMGD1_2501
	Probable inorganic disproportionation	
psrC	Polysulfide reductase, subunit C	SMGD1_0714
psrA	Polysulfide reductase, subunit A	SMGD1_0713
psrB	Polysulfide reductase, subunit B	SMGD1_0715
cysN	Sulfate adenylyltransferase	SMGD1_0549
sir	Sulfite reductase	SMGD1_2501
cysC	Adenylyl-sulfate kinase	SMGD1_0502
cysC2	Adenylyl-sulfate kinase	SMGD1_0501
cysN2	Sulfate adenylyltransferase, large subunit	SMGD1_2502
cysD2	Sulfate adenylyltransferase, small subunit	SMGD1_2503
cysH	Phosphoadenosine phosphosulfate reductase	SMGD1_2505
cysD1	Sulfate adenylyltransferase, small subunit	SMGD1_0499
cysN1	Sulfate adenylyltransferase, large subunit	SMGD1_0500
	Rhodanese-related sulfurtransferase	SMGD1_0564
napAGHBFLD	Reduction of nitrate	
napA	Nitrate reductase	SMGD1_0589
napG	Nitrate reductase	SMGD1_0590
napH	Nitrate reductase	SMGD1_0591
napB	Nitrate reductase	SMGD1_0592
napF	Nitrate reductase†	SMGD1_0593
napL	Nitrate reductase	SMGD1_0594
napD	Nitrate reductase	SMGD1_0595
nirS	Cytochrome cd1 nitrite reductase	SMGD1_1412
nirF	Nitrite reductase	SMGD1_1409
Nir	Ferrodoxin nitrite/sulfite reductase	SMGD1_0402
nir	Ferredoxin–nitrite reductase	SMGD1_2457
norB	Nitric oxide reductase	SMGD1_1414
norC	Nitric oxide reductase	SMGD1_1415
narB	Nitrate reductase	SMGD1_0403
narK/nasA	Nitrate transporter	SMGD1_1409
nosZGC$_1$C$_2$H	Reduction of nitrous oxide	
nosZ	Nitrous oxide reductase	SMGD1_2343
nosG	Nitrous oxide reductase	SMGD1_2340
nosC1	Nitrous oxide reductase	SMGD1_2339
nosC2	Nitrous oxide reductase	SMGD1_2338
nosH	Nitrous oxide reductase	SMGD1_2337
nosD	Nitrous oxidase accessory protein	SMGD1_2331
	CO_2 fixation via the reductive citric acid cycle	
oorD	2-Oxoglutarate:ferredoxin oxidoreductase	SMGD1_2658
oorA	2-Oxoglutarate:ferredoxin oxidoreductase	SMGD1_2659
oorB	2-Oxoglutarate:ferredoxin oxidoreductase	SMGD1_2660
oorC	2-Oxoglutarate:ferredoxin oxidoreductase	SMGD1_2661

Table S3. Cont.

Gene name	Function/protein	Locus tag
icd	Isocitrate dehydrogenase	SMGD1_2649
acly	ATP citrate lyase (α subunit)	SMGD1_1941
acly	ATP citrate lyase (β subunit)	SMGD1_1940
PorC	Pyruvate:ferredoxin oxidoreductase (γ subunit)	SMGD1_1531
porD	Pyruvate:ferredoxin oxidoreductase (δ subunit)	SMGD1_1532
porA	Pyruvate:ferredoxin oxidoreductase (α subunit)	SMGD1_1533
porB	Pyruvate:ferredoxin oxidoreductase (β subunit)	SMGD1_1534
	Fumarate reduction	
frdA	Fumarate reductase	SMGD1_0558
frdB	Fumarate reductase	SMGD1_0557
frdC	Fumarate reductase, cytochrome *b* subunit	SMGD1_0559
sdhA	Succinate dehydrogenase/fumarate reductase	SMGD1_2779
sdhB	Succinate dehydrogenase/fumarate reductase	SMGD1_2780
sdhC	Succinate dehydrogenase/fumarate reductase	SMGD1_2781
sdhA	Succinate dehydrogenase/fumarate reductase	SMGD1_1297
sdhB	Succinate dehydrogenase/fumarate reductase	SMGD1_1298
mqo	Malate:quinone oxidoreductase	SMGD1_2188
	Protection against oxidative stress	
sodB	Superoxide dismutase	SMGD1_2634
ccp2	Cytochrome *c* peroxidase	SMGD1_1422
ccp3	Cytochrome *c* peroxidase	SMGD1_2021
ccp1	Cytochrome *c* peroxidase	SMGD1_1001
ccpA	Cytochrome c551 peroxidase	SMGD1_1295
tsaA	Antioxidant, AhpC/Tsa family protein	SMGD1_1750
tpx	Probable thiol peroxidase	SMGD1_2073
trx	Thioredoxin	SMGD1_1242
trx	Thioredoxin	SMGD1_1640
trxB	Thioredoxin-disulfide reductase	SMGD1_1638
	Selected chemotaxis genes	
cheB2	Chemotaxis response regulator methylesterase	SMGD1_0384
cheR	MCP methyltransferase	SMGD1_0382
cheY	Chemotaxis protein	SMGD1_0379
cheA	Chemotaxis protein	SMGD1_0378
chew	Adapter chemotaxis protein	SMGD1_0377
cheA	Chemotaxis protein	SMGD1_0604
cheY	Chemotaxis protein	SMGD1_0605
Chew	Adapter chemotaxis protein	SMGD1_0603
cheY like	Response regulator receiver domain protein	SMGD1_2098
cheW like	Adapter chemotaxis protein	SMGD1_2099
mcp	Methyl-accepting chemotaxis protein	SMGD1_2100
cheA	Chemotaxis protein histidine kinase	SMGD1_2102
cheR	MCP methyltransferase	SMGD1_2103
cheD	Chemoreceptor glutamine deamidase	SMGD1_2105
cheB2	Chemotaxis response regulator methylesterase	SMGD1_2106
	Oxidation of hydrogen by Ni/Fe hydrogenases	
	Cytoplasmic hydrogenases (group 2)	
hupS	Hydrogenase, small subunit	SMGD1_2237
hupV	Hydrogenase, large subunit	SMGD1_2238
	Membrane bound hydrogenases (group 1)	
hydB	Hydrogenase, large subunit	SMGD1_2232
hydC	Hydrogenase, cytochrome *b* subunit	SMGD1_2233
hydD	Hydrogenase, expression/formation protease	SMGD1_2234
hydA	Hydrogenase, small subunit	SMGD1_2239
hydB	Hydrogenase, large subunit	SMGD1_2240
hydC	Hydrogenase, cytochrome *b* subunit	SMGD1_2241
hydD	Hydrogenase, maturation protease	SMGD1_2242
hydE	Hydrogenase related protein	SMGD1_2243
hypF	Hydrogenase maturation protein	SMGD1_2244
hypB	Hydrogenase accessory protein	SMGD1_2247
hypC	Hydrogenase expression/formation protein	SMGD1_2248
hypD	Hydrogenase expression/formation protein	SMGD1_2249
hypE	Hydrogenase expression/formation protein	SMGD1_2256

Table S3. Cont.

Gene name	Function/protein	Locus tag
hoxX	Hydrogenase maturation factor	SMGD1_2257
hypA	Hydrogenase expression/synthesis protein	SMGD1_2258
hydA	Hydrogenase, small subunit	SMGD1_2366
hydB	Hydrogenase, large subunit	SMGD1_2269
	Group 4 hydrogenases	
hyfD	Hydrogenase, type 4, subunit D	SMGD1_2372
hyfC	Hydrogenase, type 4, component C	SMGD1_2373
hyfI	Hydrogenase, type 3 or 4, small subunit	SMGD1_2374
echE	Hydrogenase, group 4	SMGD1_2383
echF	Hydrogenase, group 4	SMGD1_2384
	Oxidation of formate	
fdhA	Formate dehydrogenase, subunit alpha	SMGD1_0120
fdhB	Formate dehydrogenase, iron-sulfur subunit B	SMGD1_0121
fdhC	Formate dehydrogenase, gamma subunit	SMGD1_0122
fdhD	Formate dehydrogenase accessory protein	SMGD1_0123
	Other oxidoreductases	
nuoA	NADH dehydrogenase, subunit A	SMGD1_1681
nuoB	NADH dehydrogenase, subunit B	SMGD1_1682
nuoC	NADH dehydrogenase, subunit C	SMGD1_1683
nuoD	NADH dehydrogenase, subunit D	SMGD1_1684
nuoF	NADH dehydrogenase, subunit F	SMGD1_1685
nuoG	NADH dehydrogenase, subunit G	SMGD1_1686
nuoG2	NADH dehydrogenase, subunit G	SMGD1_1687
nuoG3	NADH dehydrogenase, subunit G truncated	SMGD1_1688
nuoH	NADH dehydrogenase, subunit H	SMGD1_1689
nuoI	NADH dehydrogenase, subunit I	SMGD1_1690
nuoJ	NADH dehydrogenase, subunit J	SMGD1_1691
nuoK	NADH dehydrogenase, subunit K	SMGD1_1692
nuoL	NADH dehydrogenase, subunit L	SMGD1_1693
nuoM	NADH dehydrogenase, subunit M	SMGD1_1694
nuoN	NADH dehydrogenase, subunit N	SMGD1_1695
ndh	NADH dehydrogenase	SMGD1_1716
	Molybdopterin oxidoreductase	SMGD1_2428
	Molybdopterin oxidoreductase, iron sulfur subunit	SMGD1_0405
ccoN	Cytochrome *c* oxidase, cbb3-type, subunit I	SMGD1_1513
ccoO	Cytochrome *c* oxidase, cbb3-type, subunit II	SMGD1_1514
ccoQ	Cytochrome *c* oxidase, cbb3-type, CcoQ subunit	SMGD1_1515
ccoP	Cytochrome *c* oxidase, cbb3-type, subunit III	SMGD1_1516
petA	Ubiquinol-cytochrome *c* reductase, iron-sulfur subunit	SMGD1_1545
petB	Ubiquinol cytochrome *c* oxidoreductase, cytochrome *b* subunit	SMGD1_1546
petC	Ubiquinol cytochrome *c* oxidoreductase, cytochrome c1 subunit	SMGD1_1547

*weak similarity.
[†]inter genic.

Table S4. Quantitative comparative genomic analysis of *S. gotlandica* str. GD1 with other *Epsilonproteobacteria*

	S. gotlandica str. GD1	*Sulfurimonas denitrificans* DSM1251	*Sulfurospirillum deleyianum* DSM6946	*Arcobacter butzleri* RM4018	*Sulfurimonas autotrophica* DSM16294	*Sulfurovum* sp. NBC37-1	*Nitratiruptor* sp. SB155-2	*Nautilia profundicola* AmH	*Caminibacter mediatlanticus* TB-2
Accession no.	AFRZ01000000	NC_007575	NC_013512	NC_009850	NC_014506	NC_009663	NC_009662	NC_012115	NZ_ABCJ00000000
Sensing and signal transduction domains									
PAS	60	23	14	16	22	11	9	9	5
MCP	26	13	27	29	9	0	8	11	6
EAL	7	0	8	2	2	1	2	5	0
GGDEF	66	23	16	14	29	6	12	19	10
GGDEF and EAL	40	15	13	9	21	12	20	11	9
Response_reg	108	54	48	45	38	36	20	18	15
Hist.Kin.	73	38	40	35	27	31	17	13	10
Sum	380	166	166	150	148	97	88	86	55
Domains related to energy generation by redox reactions									
APS pathway									
APS reductase	1	1	0	0	1	0	0	0	0
APS kinase	2	0	0	0	2	2	0	0	0
ATP sulfurylase	1	1	1	0	1	1	1	1	1
PAPS reductase	2	1	0	2	2	1	1	0	0
S metabolism									
sorAB	1	0	0	0	1	2	0	0	0
SQR	7	5	1	2	7	8	5	1	3
Sulfide-DH/soxF/Fcc	1	1	0	0	0	2	1	0	1
soxCD	1	1	0	1	1	1	0	0	0
soxYZ	2	2	0	1	2	2	2	0	0
PSR/NrfD	1	1	4	0	1	1	1	1	1
N metabolism									
NAP	1	1	1	1	1	1	1	1	1
cNOR/gNOR	1	2	0	1	3	2	1	0	0
NIR/SIR	3	2	0	2	2	2	2	0	1
nosD(nosZ/D)	1	2	0	0	2	2	1	0	0
NrfHA	0	0	2	1	0	0	0	0	0
Hydrogenases									
Type 1–4	6	4	3	5	1	4	2	4	7
Other oxidoreductases/dehydrogenases									
bc1-complex	1	1	2	1	1	1	1	0	0
cyt c oxidase	1	1	1	1	1	1	1	0	0
cyt bd quinol-oxidase-complex	0	0	1	1	0	0	1	1	1
cyt c peroxidase	4	2	1	3	3	3	4	2	2
SDH/fumarate reductase	3	2	1	1	2	3	2	3	3
QFR	1	0	1	1	0	1	0	0	0
MQR	1	1	1	1	0	1	0	0	0
NDH I	1	1	1	1	1	2	1	1	1
Molybdopterin-ox.-reductase	7	5	10	4	7	4	4	4	7
Sum	50	37	31	30	42	47	32	19	29

APS, adenylyl sulfate; EAL, EAL domain; GGDEF, GGDEF domain; Hist.Kin., Histidine kinase; MCP, methyl-accepting chemotaxis protein signaling domains; MQR, malate-quinone-reductase; PAS, PAS fold; NAP/NAR, nitrate reductase; NIR, nitrite reductase; NOR, nitric oxide reductase; QFR, Quinol-fumarate reductase; SDH, succinate dehydrogenase; SIR, sulfite reductase; SOX, sulfur oxidation multienzyme complex; SQR, sulfide quinone reductase.

Table S5. Biogeographic distribution of 16S rRNA sequences of the genera *Sulfurimonas* and *Arcobacter* and the gammaproteobacterial sulfur oxidizer SUP05 in marine hypoxic systems

	Epsilonproteobacteria		*Gammaroteobacteria*	
Habitat	*Sulfurimonas*	*Arcobacter*	SUP05	References
Marine sulfidic redoxclines				
Baltic Sea	+	+	+	Grote et al., 2007 (1); Brettar et al., 2006 (2); Labrenz et al., 2007 (3);
Black Sea	+	+	+	Vetriani et al., 2003 (4); Thamdrup et al., 2000 (5)
Cariaco Basin, Venezuela	+	+	(–)*	Madrid et al., 2001 (6); Lin et al., 2006 (7), Lin et al., 2007 (8)
Nitinat Lake, Canada	+	+	+	Schmidtova et al., 2009 (9)
Saanich Inlet, Canada	+	+	+	Walsh et al.,2009 (10); Zaikova et al., 2010 (11)
Marine oxygen minimum zone				
Namibian upwelling	–	+	+	Lavik et al., 2009 (12)
Arabian Sea	–	–	+	Fuchs et al., 2005 (13)
Eastern tropical South Pacific	–	–	+	Stevens and Ulloa, 2008 (14)

Analyses were done using the SILVA 106 database (1200/900) (15). SILVA 106 was supplemented by 1424 *Sulfurimonas*, 1840 *Arcobacter*, and 27710 unclassified gammaproteobacterial sequences downloaded from the Ribosomal Database Project (RDP) database (RDP Release 10, Update 27, Aug 9, 2011) (16). For further description see *SI Text*. +, detected; –, not detected.

*Gammaproteobacteria are present as determined by FISH (7) or T-RFLP analyses (8), but SUP05 was not detected in a 16S rRNA clone library (6).

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