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ORIGINAL PAPER

Sulfurimonas sediminis sp. nov., a novel hydrogen- and sulfur-oxidizing chemolithoautotroph isolated from a hydrothermal vent at the Longqi system, southwestern Indian ocean

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Abstract A novel marine hydrogen- and sulfur-oxidizing bacterium, designated strain S2-6 ^{T}, was isolated from the deep-sea sediment samples at the Longqi hydrothermal system, southwestern Indian Ocean. Cells were Gram-stain-negative, motile, short rods with a single polar flagellum. Growth was observed at 10–45 °C (optimum 33 °C), pH 5.0–8.0 (optimum pH 7.0) and 1.5 to 6.0% (w/v) NaCl with an optimum at 3.0% (w/v). The isolate was an obligate chemolithoautotroph capable of growth using thiosulfate, tetrathionate, elemental sulfur or sodium sulfide as the energy source, and oxygen or nitrate as the sole electron acceptor. When hydrogen was used as the energy source, strain S2-6 ^{T} could respire oxygen, nitrate or element sulfur. The major cellular fatty acids of strain S2-6 ^{T} were summed feature 3 ($C_{16:1}\omega 7c$ and/or $C_{16:1}\omega 6c$), $C_{16:0}$ and summed feature 8 ($C_{18:1}\omega 7c$ and/or $C_{18:1}\omega 6c$). The total size of its genome was 2,320,257 bp and the genomic DNA G + C content was 37.3 mol%. Phylogenetic analysis based on 16S rRNA gene sequences and core genes showed that the novel isolate belonged to the genus *Sulfurimonas* and was most closely related to *Sulfurimonas paralvinellae* GO25^{T} (96.8% sequence identity) and *Sulfurimonas autotrophica* OK10^{T} (95.8% sequence identity). The average nucleotide identity and DNA-DNA hybridization values between strain S2-6 ^{T} and *S. paralvinellae* GO25^{T} and *S. autotrophica* OK10^{T} were 74.6%-81.2% and 19.1%-24.6%, respectively. Based on the polyphase taxonomical data, strain S2-6 ^{T} represents a novel species of the genus *Sulfurimonas*, for which the name *Sulfurimonas sediminis* sp. nov. is proposed, with the type strain S2-6 ^{T} (= MCCC 1A14513^{T} = KCTC 15854 ^{T}).

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Keywords *Sulfurimonas sediminis* · Hydrothermal vent · Sulfur-oxidation · Polyphasic taxonomy

Abbreviations
MCCC Marine Culture Collection of China

KCTC Korean Collection for Type Cultures
ANI Average nucleotide identity
DDH DNA-DNA hybridization

Introduction

Deep-sea hydrothermal vent environments are characterized by the steep gradients of physical and chemical parameters in the mixing zones between hot vent fluids and cold deep-sea water (Dick 2019). Chemosynthetic bacteria and archaea inhabited there utilize chemical energy to fix inorganic carbon into organic carbon for microbial growth, and constitute the foundation of vent ecosystems (McNichol et al. 2018; Nadine et al. 2018). Chemolithoautotrophs are an important component of the microbial community in the deep-sea hydrothermal vent, and play critical roles in deep-sea carbon, nitrogen and sulfur cycling (Cao et al. 2014; Ding et al. 2017).

The genus *Sulfurimonas* was first proposed by Inagaki et al. (2003) and belonged to the class *Campylobacteria* (formerly *Epsilonproteobacteria*) within the phylum of Epsilonbacteraeota (formerly Proteobacteria) (Inagaki et al. 2003; Waite et al. 2017, 2018). Members of the genus *Sulfurimonas* are ubiquitous in diverse habitats, including deep-sea hydrothermal vents, marine sediments, pelagic water column redoxclines and terrestrial soils (Han and Perner 2015). They are characterized as small sulfur-oxidizing bacteria, which are capable of growing chemoautotrophically with reduced sulfur compounds, such as sulfide, thiosulfate and elemental sulfur (Han and Perner 2015). At the time of writing, the genus contains eight validly published species with *Sulfurimonas autotrophica* as type species and three Candidatus species including *Ca.* Sulfurimonas hongkongensis AST-10, *Ca.* Sulfurimonas marisnigri SoZ1 and *Ca.* Sulfurimonas baltica GD2. Among them, *S. autotrophica* OK10^T, *Sulfurimonas denitrificans* DSM1251^T, *Ca.* S. hongkongensis AST-10, *Sulfurimonas xiamensis* 1-1N^T and *Sulfurimonas lithotrophica* GYSZ_1^T were isolated from coastal or deep-sea marine sediments. *Sulfurimonas paralvinellae* GO25^T was isolated from a hydrothermal polychaete nest, *Sulfurimonas indica* NW8N^T was isolated from a hydrothermal sulfide chimney, *Sulfurimonas crateris* SN118^T was isolated from a terrestrial mud volcano, and *Sulfurimonas gotlandica* GD1^T, *Ca.* S. baltica GD2 and *Ca.* S. marisnigri SoZ1 were isolated from the pelagic redoxcline of the Baltic Sea and Black Sea (Hoor 1975; Takai et al. 2006; Labrenz et al. 2013; Cai et al. 2014; Ratnikova et al. 2019; Hu et al. 2020; Wang et al. 2020b; Henkel et al. 2020). In this study, we report a novel strain, designated S2-6 ^{T}, isolated from the deep-sea sediment samples at the Longqi hydrothermal system, southwestern Indian Ocean. Comparative 16S rRNA gene sequence analysis and physiological properties indicated that strain S2-6 ^{T} belonged to the genus *Sulfurimonas.* The aim of the present work is to determine the exact taxonomic position of strain S2-6 ^{T} by using a polyphasic approach.

Materials and methods

Bacterial isolation and culture condition

The sediment sample was collected from the Longqi hydrothermal system in the southwestern Indian Ocean (49°64′E, 37°78′S; Site 49I-SWIR-S11TVG04) in January 2018. For isolation, 1 g of each sediment sample was transferred into 50 ml serum bottles containing 10 ml MMJS (Takai et al. 2006) medium under a gas phase mixture of 78% N_2/18% CO_2/2% O_2 (200 kPa) and incubated at 28 °C according to the previous description (Jiang et al. 2017). MMJS medium consisted of (per litre of distilled, deionized water) 30.0 g NaCl, 0.14 g K_2HPO_4, 0.14 g $CaCl_2 \cdot 2H_2O$, 0.25 g NH_4Cl, 4.18 g $MgCl \cdot 6H_2O$, 0.33 g KCl, 0.5 mg $NiCl \cdot 6H_2O$, 0.5 mg $Na_2SeO_3 \cdot 5H_2O$, 0.01 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 10 mM $Na_2S_2O_3 \cdot 5H_2O$, 1 g $NaHCO_3$, 10 ml trace mineral solution and 1 ml vitamin solution (Balch et al. 1979). After incubation for 7 days, growth of motile and rod-shaped cells in MMJS medium was observed. The well-grown culture was further purified using the dilution-to-extinction method with the same medium (Takai and Horikoshi 2000). After repeating the dilution-to-extinction for three times from the well-grown culture at the maximum dilution series (10^{-7}), a sulfur-oxidizing bacterium, designated strain S2-6 ^{T}, was obtained. The purity of the isolate was verified by microscopic observation, cultivation on heterotrophic media and determination of the 16S rRNA gene sequence.

DNA extraction, genomic and phylogenetic analyses

The genomic DNA of strain S2-6 ^{T} was extracted according to a previously described method (Jiang et al. 2010), and the 16S rRNA gene was amplified by PCR primers described previously (Liu and Shao 2005). The 16S rRNA gene sequence was identified using global alignment algorithm implemented at the EzBioCloud server (https://www.ezbiocloud.net/; (Yoon et al. 2017)). Phylogenetic trees were performed using MEGA software package version 6.0 (Tamura et al. 2013) using neighbor-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and minimum evolution (Rzhetsky and Nei 1992) methods after multiple alignments of the data by CLUSTAL_W. Evolutionary distances were calculated using Kimura's two-parameter model (Kimura 1980) and bootstrap values were determined based on 1000 replications (Felsenstein 1985).

The complete genome sequence of strain S2-6 ^{T} was sequenced by Tianjin Biochip Corporation (Tianjin, PR China), using the single molecule real-time (SMRT) technology on the Pacific Biosciences (PacBio) sequencing platform. The sequenced reads were filtered, and high quality paired-end reads were assembled to construct a circular genome using SOAPdonovo software (Luo et al. 2012). The G + C content of the chromosomal DNA was determined according to the genome sequence. tRNAscan-SE (Lowe and Chan 2016) and rRNAmmer (Lagesen et al. 2007) were used to predict the tRNA and rRNA contained in the genome. Gene prediction by using Glimmer v3.02 (Delcher et al. 2007). The average nucleotide identity (ANI) values of strain S2-6 ^{T} and the relatives were estimated using OrthoANI computation (Yoon et al. 2017). Digital DNA-DNA hybridization (DDH) estimates were calculated on the GGDC website (https://ggdc.dsmz.de/). The phylogenomic tree was constructed based on an up-to-date 92 bacterial core gene sets by UBCG version 3.0 (Na et al. 2018).

Morphology, physiology and chemotaxonomic analysis

The cell morphology of strain S2-6 ^{T} was observed with the cells cultured in MMJS liquid medium at 28 °C for 1 day with a transmission electron microscopy (Model JEM-1230; JEOL). The Gram-stain test was conducted with a Gram staining kit (Hangzhou Tianhe Microorganism Reagent Co.). Growth characteristics were determined by direct cell counting using a phase contrast microscope (Eclipse 80i, Nikon, Japan) according to the previous method (Jiang et al. 2017). All experiments described below were conducted in triplicate. The growth temperature was determined under various temperatures for 1 day (15, 20, 25, 28, 30, 33, 35, 37, 40, 45, 50 and 60 °C) or 4 days (4 and 10 °C) to observe its growth. The growth salinity range was examined by adjusting the concentrations of 0 to 9.0% (w/v) NaCl, at 0.5% (w/v) intervals. The pH range of growth was tested in MMJS medium by adjusting pH with different buffers, including 10 mM acetate/acetic acid buffer (pH 3.0–5.5), MES (pH 5.0–6.5), PIPES (pH 6.5–7.0), HEPES (pH 7.0–8.0), Tris and CAPSO (pH 8.0 and above). Oxygen sensitivity was examined using MMJS basal medium without nitrate with different O_2 concentrations gradients (0%, 1%, 2%, 4%, 6%, 8%, 10% at 200 kPa and 20% at 100 kPa) in the headspace gas. In the case of oxygen absence, 10 mM nitrate was added as a potential electron acceptor.

The ability for sulfur oxidation was tested in the MMJS medium using various sulfur compounds other than thiosulfate as the sole energy source, including sulfite (5 mM), tetrathionate (5 mM), thiocyanate (5 mM), elemental sulfur (1% w/v) or sodium sulfide (50, 100, 200, 400, 800 μM, 1, 2 mM) under a gas phase of 76% N_2/20% CO_2/4% O_2 (200 kPa). Molecular hydrogen was also examined in MMJH medium in the absence of thiosulfate under a gas phase of 76% H_2 /20% CO_2 /4% O_2 (200 kPa). In an attempt to determine the utilization of electron acceptors, sulfate (5 mM), sulfite (5 mM), elemental sulfur (1%, w/v), nitrate (10 mM) and nitrite (1 or 5 mM) were tested with H_2 as the sole electron donor under 80% H_2 /20% CO_2 (200 kPa) and ferric citrate (20 mM), ferrihydrite (20 mM), manganese(IV) oxide (200 μM), nitrate (10 mM) and nitrite (1 or 5 mM) were tested with thiosulfate as the sole electron donor under 80% N_2/ 20% CO_2 (200 kPa). Utilization of inorganic nitrogen source, such as ammonium chloride (5 mM), sodium nitrate (5 mM), sodium nitrite (5 mM) or molecular nitrogen (N_2), which was added to MMJHS medium lacking all nitrogen source under a gas phase of 76% H_2 /20% CO_2 /4% O_2 (200 kPa), was examined. The potential nutrients required for growth such as

selenite, tungstate and vitamins were also examined with MMJS medium with and without the specified nutrients.

Heterotrophic growth was examined in a MMJS medium without $NaHCO_3$ under a gas phase of 96% N_2 /4% O_2 (200 kPa), containing the following organic carbon sources: 0.1% (w/v) peptone, yeast extract, tryptone, starch, casein and casamino acids, 5 mM acetate, formate, citrate, tartrate, succinate, propionate and pyruvate, 5 mM each of 20 amino acids, 0.02% (w/v) sucrose, galactose, glucose, lactose, fructose, maltose and trehalose. Utilization of these organic compounds as alternative energy sources was also examined in MMJ medium in the absence of thiosulfate under a gas phase of 76% N_2 /20% CO_2 / 4% O_2 (200 kPa).

The cellular fatty acid composition was analyzed from cells grown in MMJS medium at 33 °C for 24 h. Fatty acids were saponified, extracted, and methylated using the standard protocol of the Microbial Identification System (MIDI, Sherlock Microbial Identification System, version 6.0B). The fatty acids were analyzed by gas chromatography (GC, Agilent Technologies 6850) and identified by using the TSBA 6.0 database of the Microbial Identification System (Frolov et al. 2017).

CRISPRs and genomic islands

Identification of the CRISPR arrays were analyzed by using the CRISPRCas Finder webserver, with default parameters (https://crispr.i2bc.paris-saclay.fr/) (Grissa et al. 2007). Presence of genomic islands was investigated by using the IslandViewer4 webserver (http://www.pathogenomics.sfu.ca/islandviewer/) (Bertelli et al. 2017).

Results and discussion

Morphology

Cells of strain S2-6 ^{T} were Gram-stain-negative, rod-shaped (0.5–1.0 × 0.8–2.0 μm) and motile with a polar flagellum (Fig. 1). Morphological features of strain S2-6 ^{T} were similar to those of *S. autotrophica* OK10^T, *S. paralvinellae* GO25^T and *S. indica* NW8N^T (Table 1).

Phylogenetic and phylogenomic analyses

Comparison of the 16S rRNA gene sequences obtained from PCR amplification showed that strain S2-6 ^{T} is most closely related to *S. paralvinellae* GO25^T (96.8% sequence similarities), *S. autotrophica* OK10^T (95.8%) and *S. indica* NW8N^T (95.1%). Phylogenetic tree based on the neighbour-joining method indicated that strain S2-6 ^{T} formed a distinct cluster with *S. paralvinellae* GO25^T, *S. autotrophica* OK10^T and *S. indica* NW8N^T (Fig. 2). This topology was confirmed by the phylogenetic trees reconstructed with the maximum-likelihood and minimum evolution methods (Fig. S1; S2), which indicated that strain S2-6 ^{T} belonged to the genus *Sulfurimonas* and represent a separate species.

Pairwise ANI values between strain S2-6 ^{T} and its closest relative organism, *S. paralvinellae* GO25^T, was 77.8%. The DDH values between strains S2-6 ^{T} and *S. paralvinellae* GO25^T was 21.1%. The ANI and DDH values between strains S2-6 ^{T} and *S. autotrophica* OK10^T were 81.2% and 24.6%, respectively. All these values are much lower than the threshold criterion for prokaryotic species delineation, which is 95–96% for ANI and 70% for DDH (Chun et al. 2018), indicating that strain S2-6 ^{T} represent a novel species in genus *Sulfurimonas*. The phylogenetic tree based on the 92 core gene sequences indicated that strain S2-6 ^{T} formed a branch with strains *S. autotrophica* OK10^T, *S. paralvinellae* GO25^T and *S. indica* NW8N^T (Fig. 3), which supported that strain S2-6 ^{T} should be assigned to one novel species of the genus *Sulfurimonas*. This result was in agreement with the result of 16S rRNA gene phylogeny.

Physiological characteristics

Growth tests revealed that strain S2-6 ^{T} can grow at 10–45 °C (optimum 33 °C), pH 5.0–8.0 (optimum pH 7.0) and 1.5–6.0% (w/v) NaCl with an optimum at 3.0% (w/v). In addition, strain S2-6 ^{T} grew over a range of 0–20% O_2 and the optimum growth occurred at 4% O_2. Under optimal conditions, the cell doubling time of strain S2-6 ^{T} was 5.3 h (Table 1).

The chemoautotrophic growth tests showed that strain S2-6 ^{T} could grow with thiosulfate, tetrathionate, elemental sulfur or sodium sulfide as the sole energy source, and oxygen or nitrate as the sole electron acceptor. Molecular hydrogen could also be

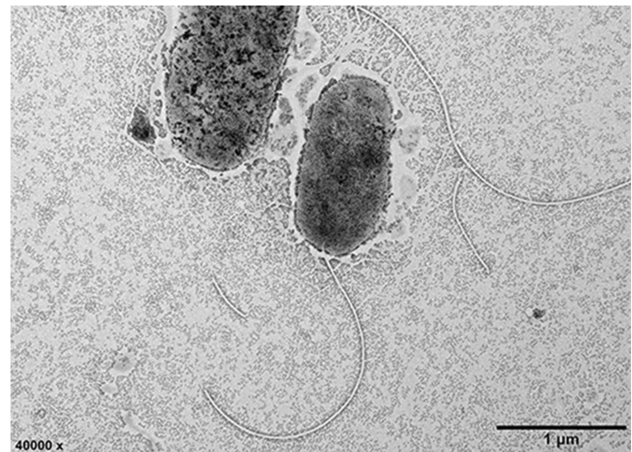

Fig. 1 Transmission electron micrograph of cells of strain S2-6 ^{T}. Bar, 1.0 µm

Table 1 Comparison of characteristic among *Sulfurimonas sediminis* sp. nov. S2-6 ^{T} and closely related strains within the genus *Sulfurimonas*

Characteristic	1	2	3	4
Origin	Hydrothermal sediment, southwestern Indian Ocean	Polychaete nest, deep-sea hydrotherma	Hydrothermal sediment, Mid-Okinawa Trough	Hydrothermal chimneys, Northwest Indian Ocean
Shape	Rods	Rods	Rods	Rods
Motility	+	+	+	+
Doubling time under optimal	5	13–16	1.4	9
Temperature range (Optimal T) (°C)	10–45 (33)	4–35 (30)	10–40 (23–26)	4–40 (33)
pH range (Optimal pH)	5.0–8.0 (7.0)	5.4–8.6 (6.1)	5.0–9.0 (6.5)	4.5–7.5 (5.5)
NaCl requirement	+	+	+	+
Maximum O_2 concentration (%)	20	10	15	20
Energy sources	H_2, S^o, $S_2O_3{}^{2,-}$ HS^-, $S_4O_6{}^{2-}$	H_2, S^o, $S_2O_3{}^{2-}$	S^o, $S_2O_3{}^{2-}$	H_2, S^o, $S_2O_3{}^{2,-}$ HS^-
Organic electron donors	–	–	–	–
Electron acceptors	S^o, $NO_3{}^-$, O_2	S^o, $NO_3{}^-$, O_2	O_2	O_2
G + C content (mol%)	37.3	37.6	35.2	36.9

Taxa: 1, *Sulfurimonas sediminis* sp. nov. S2-6 ^{T} (data from this study); 2, *Sulfurimonas paralvinellae* GO25^{T} (Takai et al. 2006); 3, *Sulfurimonas autotrophica* OK10^{T} (Inagaki et al. 2003); 4, *Sulfurimonas indica* NW8N^{T} (Hu et al. 2020); + , positive; -, negative

used as an energy source. When hydrogen was used as the sole energy source, strain S2-6 ^{T} could grow with oxygen, nitrate or element sulfur as electron acceptors. The end product of nitrate reduction was N_2, and nitrite or ammonia did not accumulate; the product of sulfur compounds oxidation was sulfate; the product of elemental sulfur reduction was hydrogen sulfide. Inorganic nitrogen sources tests showed strain S2-6 ^{T} could grow with ammonium as the nitrogen sources, but not use sodium nitrate, sodium nitrite and molecular nitrogen. Selenium, tungsten and vitamins supplementation were not required for growth.

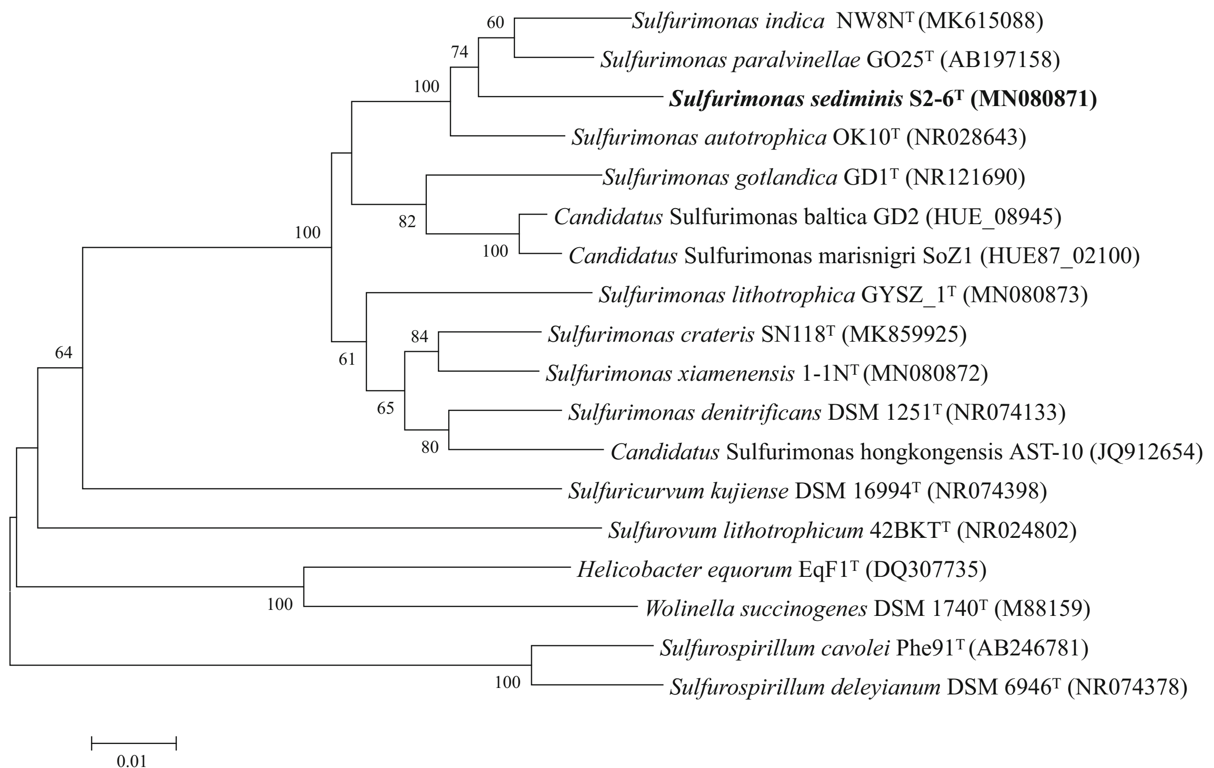

Fig. 2 Neighbor-joining tree based on 16S rRNA gene sequences showing the the relationship between strain *Sulfurimonas sediminis* S2-6 ^{T} and other members within the genus *Sulfurimonas.* Bootstrap values based on 1000 replicates are shown at branch nodes. Branch node values below 50% are not shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Bar = 0.01 substitutions per nucleotide position

Heterotrophic growth tests showed that none of the organic compounds sustained the growth of strain S2-6 ^{T} as the carbon or energy source. These results confirmed that strain S2-6 ^{T} is a typical chemolithoautotroph.

The major cellular fatty acids of strain S2-6 ^{T} were summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), $C_{16:0}$ and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) (Table 1), which were similar to those of *S. paralvinellae* GO25^{T}, *S. indica* NW8N^{T} and *S. xiamenensis* 1-1N^{T} and distinctly different from those of *S. autotrophica* OK10^{T} and *Ca.* S. hongkongensis AST-10. The significant differences between strains S2-6 ^{T} and *S. paralvinellae* GO25^{T} were that the fatty acid $C_{16:1}$ $\omega5c$ was observed at 1.3% in strain S2-6 ^{T}, but it was not found in strain *S. paralvinellae* GO25^{T}. In addition, the fatty acid $C_{18:0}$ was detected at 1.5% in strain S2-6 ^{T}, while it was only trace level of 0.7% in strain *S. paralvinellae* GO25^{T}.

Genome annotation and analysis

The complete genome of strain S2-6 ^{T} consisted of a single circular chromosome with a total length of 2,320,257 bp long with a G + C content of 37.3 mol%. No plasmids were detected. Genome contained 2,445 predicted genes including 2,344 protein-coding genes, 59 rRNAs (four 5S rRNA, four 16S rRNA, and four 23S rRNA), 44 tRNAs, and 3 ncRNAs. Among the all protein-coding gene sequences, 1,755 genes were mapped over to 20 functional categories of COG database, while the remaining 589 genes could not be assigned to any categories. As shown in Fig. S3, the major categories were energy production and conversion (8.43%); replication, recombination and repair (8.21%); translation, ribosomal structure and biogenesis (7.75%); amino acid transport and metabolism (7.58%); cell wall/membrane/envelope biogenesis (6.61%); function unknown (6.04%); coenzyme transport and

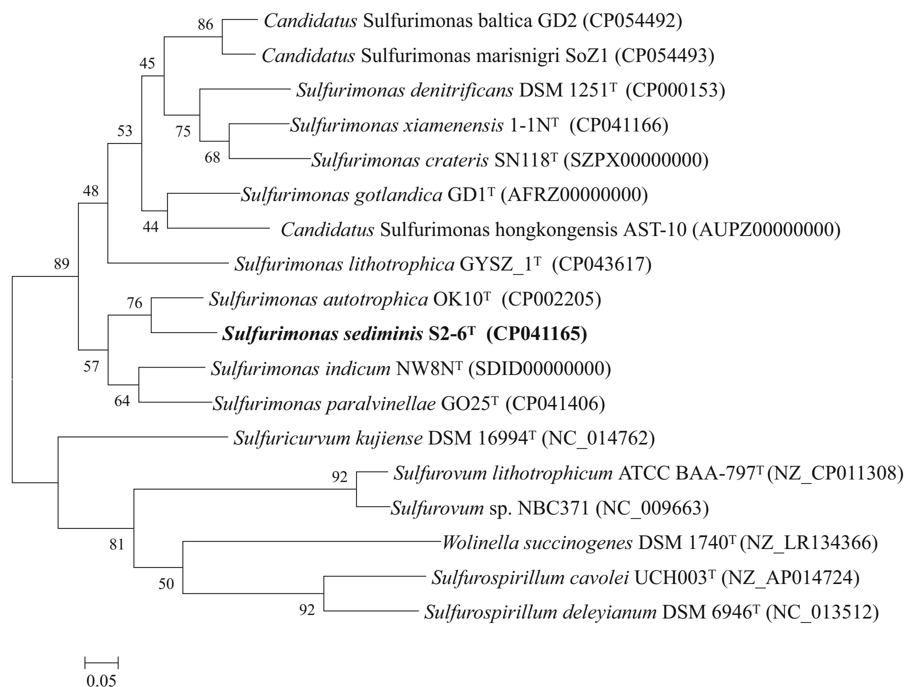

Fig. 3 Phylogenetic tree inferred using UBCGs showing the position of *Sulfurimonas sediminis* S2-6 ^{T} and closely related taxa within the genus *Sulfurimonas* using the maximum-likelihood algorithm. The node is labeled with Gene Support Index (GSI) values. The accession numbers of the genomes are shown in parentheses. Bar, 0.05 substitutions per position

metabolism (5.87%); signal transduction mechanisms (5.75%); posttranslational modification, protein turnover, chaperones (5.07%) and inorganic ion transport and metabolism (5.01%).

No CRISPR loci were found, while 13 genomic islands (GI) of a total length of 173.8 kb were detected (Table 2). The size of the 13 putative islands ranged from 3,460 bp (GI 7) to 28,629 bp (GI 6). The largest GI 6 contained 44 genes, whereas the smallest GI 7 had 7 genes. The vast majority of the genes located on the genomic islands encode proteins annotated as hypothetical proteins. In these 13 GIs, 269 CDS were identified, including CDS encoding mobile element protein, transcriptional regulator, ribosomal proteins, ABC transport family systems, glycosyltransferase protein family, DNA polymerase, type I (II) restriction enzyme, two-component system, carbohydrate metabolism, thioredoxin reductase, iron-sulfur assembly protein, oxidoreductases for metabolism and so on. Among these GIs, seven contain mobile genetic elements, such as integrase and transposase genes, suggesting that these GIs can self-mobilize and could also support potential active horizontal gene transfer in the strain. Meanwhile, one of genomic islands carried genes encoding assimilatory sulfate reduction pathway including sulfate adenylyltransferase (Sat and CysDN) and adenylylsulfate kinase (CysC) (Table 2). The detection of sulfate reducing genes on GI indicates the contribution of mobile elements in the adaptation of bacteria to the environment and in active participation in the sulfur cycle.

The whole-genome characteristics were generally accordant with the main metabolic features experimentally demonstrated in strain S2-6 ^{T}. The genome of strain S2-6 ^{T} possessed all genes essential for carbon fixation via the reductive citric acid cycle (rTCA), such as ATP-dependent citrate lyase (*aclAB*, EC 2.3.3.8), 2-oxoglutarate:ferredoxin oxidoreductase (*oorABCD*, EC 1.2.7.3) and pyruvate:ferredoxin oxidoreductase (*porABC*D, EC 1.2.7.1) but not for the Calvin-Benson cycle. Strain S2-6 ^{T} could grow with hydrogen as an energy source. Genomic analysis revealed that strain S2-6 ^{T} contained a complete gene cluster encoding group 1, group 2 and group 4 [NiFe]-Hydrogenases. The ability to use oxygen as a terminal electron acceptor can be ensured by the presence of a gene cluster of cytochrome c oxidases cbb_3-type that encode the FixNOQP proteins. Notably, the genome contained all genes required for the complete reduction of nitrate to N_2 including nitrate reductases (*napAGHBFLD*), nitrite reductases (*nirS*, EC:1.7.2.1),

nitric oxide reductases (*norBC*, EC:1.7.2.5) and nitrous oxide reductases (*nosZ*, EC:1.7.2.4).

As for sulfur metabolism, the genome of strain S2-6 ^{T} contained homologs for genes encoding s*oxA-ZYXBCD*, all of which are required for assembling a fully functional complex that oxidizes reduced sulfur compounds. In addition, the *sox* genes in strain S2-6 ^{T} were present in two clusters, s*oxXYZAB* (*soxX*, EC:2.8.5.2; *soxA*, EC:2.8.5.2; *soxB*, EC:3.1.6.20) and s*oxZYCD* (*soxD*, EC:1.8.2.6). The genes encoding sulfide: quinone oxidoreductase (*sqr*, EC:1.8.5.4), participating in oxidizing sulfide to elemental sulfur, was also found in the genome. In addition, homologs of genes encoding known enzyme systems of reduced sulfur compounds oxidation such as sulfur oxygenase/re-ductase (Sor, EC:1.8.2.1) was present in genome of strain S2-6 ^{T}. No genes involved in dissimilatory sulfate reduction, such as adenylylsulfate reductase (*aprAB*, EC:1.8.99.2) and sulfite reductase (*dsrAB*, EC:1.8.99.5), and assimilatory sulfate reduction, such as phosphoadenosine phosphosulfate reductase (*cysH*, EC:1.8.4.10) and sulfite reductase (*cysI*, EC:1.8.1.2), were detected in this bacterium. In addition, strain S2-6 ^{T} can grow with elemental sulfur as the elector acceptor, which was consistent with the observe of polysulfide reductase (*psr*) genes found in the genome (Wang et al. 2020a).

Taxonomic conclusion

Characteristics of S2-6 ^{T} and species of the genus *Sulfurimonas* with validly published names are summarized in Table 1. There are many similar features between strain S2-6 ^{T} and the closely relatives of genus *Sulfurimonas* including Gram-stain negative and rod-shaped, with chemotaxonomic characteristics such as summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), $C_{16:0}$ and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) as the major fatty acid. The DNA G + C content of strain S2-6 ^{T} was 37.3 mol% and falls within the range (33.2–38.8 mol%) reported for members of the genus *Sulfurimonas*. Strain S2-6 ^{T} fell within the cluster comprising the genus *Sulfurimonas* and was most closely related to *S. paralvinellae* GO25^{T} (96.8% sequence identity) and *S. autotrophica* OK10^{T} (95.8% sequence identity). Strain S2-6 ^{T} exhibited many phenotypic similarities to *S. paralvinellae* GO25^{T}, including morphology and the ability to grow under atmospheric concentrations of oxygen. However, there were many differences between strain S2-6 ^{T} and *S. paralvinellae* GO25^{T} in some aspects (Table 1). The utilization patterns of electron donors are significantly different because that strain S2-6 ^{T} can use sulfide and tetrathionate as electron donors, whereas strain *S. paralvinellae* GO25^{T} can not. Strain S2-6 ^{T} can grow well even at 20% (v/v) oxygen concentrations in the headspace gas, while strain *S. paralvinellae* GO25^{T} only grow up to 10% partial pressure of O_2. In addition, strain *S. paralvinellae* GO25^{T} is a cold-tolerant bacterium and can grow well at 4 °C, while strain S2-6 ^{T} can not. The ANI and DDH values are below the species threshold. These data clearly indicate that strain S2-6 ^{T} can be differentiated from *S. paralvinellae* GO25^{T} at the species level. In conclusion, strain S2-6 ^{T} should represent a novel species of the genus *Sulfurimonas*, for which the name *Sulfurimonas sediminis* sp. nov. is proposed.

Description of Sulfurimonas sediminis sp. nov.

Sulfurimonas sediminis (se. di'mi. nis, L. gen. n. sediminis of sediment).

Cells are Gram-stain-negative, rod shaped (0.8–2.0 µm long and 0.5–1.0 µm in width) and motile by a polar flagellum. Facultatively anaerobic and microaerophilic. Growth occurs at 10–45 °C (optimum 33 °C), pH 5.0–8.0 (optimum pH 7.0), and 1.5–6.0% (w/v) NaCl (optimum 3.0% (w/v)). Obligate chemolithoautotrophic growth occurs with H_2, S^{o}, thiosulfate, tetrathionate and sulfide as electron donors, and nitrate, O_2 or S^{o} as the sole electron acceptor. The isolate was an obligate chemolithoautotroph capable of growth using thiosulfate, tetrathionate, elemental sulfur or sodium sulfide as the sole energy source, and oxygen or nitrate as the sole electron acceptor. When hydrogen was used as the energy source, strain S2-6 ^{T} could respire oxygen, nitrate or element sulfur. Organic substrates are not utilized as carbon sources and energy sources. Ammonium is utilized as nitrogen source. Vitamins, selenium and tungsten are not required for growth. Major cellular fatty acids are summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), $C_{16:0}$ and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$).

The type strain, S2-6 ^{T} (= MCCC 1A14513^{T} = KCTC 15854 ^{T}) was isolated from the deep-sea

sediment samples at the Longqi hydrothermal system, southwestern Indian Ocean. The DNA G + C content of the type strain is 37.3 mol%. The GenBank accession number for the 16S rRNA gene sequence is MN080871 and the complete genome accession number is CP041235.

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Author contributions LJ and SY supervised the project. SW, XL and SX carried out the experiments. SW, QL and LJ analyzed the data. SW, SY and ZS wrote and revised the manuscript. All authors approved the final manuscript.

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Data availability statement The GenBank accession numbers for the 16S rRNA gene sequence and complete genome sequence of *Sulfurimonas sediminis* S2-6 ^{T} are MN080871 and CP041235, respectively.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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