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ORIGINAL PAPER

Sulfurimonas aquatica sp. nov., a sulfur-oxidizing bacterium isolated from water of a brackish lake

Hisaya Kojima[1] · Yukako Kato[1] · Tomohiro Watanabe[1] · Manabu Fukui[1]

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Abstract

A novel chemolithoautotrophic bacterium, strain H1576^T, was isolated from water of a brackish lake. Strain H1576^T grew aerobically on inorganic sulfur compounds. Hydrogen gas did not support autotrophic growth, and heterotrophic growth was not observed. Cells were rod shaped, motile, 1.5–2.7 µm in length and 0.6–0.7 µm in width. Growth was observed at 3–22 °C with an optimum growth temperature of 13–15 °C. The pH range for growth was 6.0–7.4 with an optimum pH of 6.6–6.8. Major fatty acids were summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$). The complete genome of strain H1576^T consists of a circular chromosome and a plasmid, with total length of 2.8 Mbp and G+C content of 46.4 mol%. Phylogenetic analyses indicated that strain H1576^T belongs to the genus *Sulfurimonas* but distinct from representatives of existing species. On the basis of genomic and phenotypic characteristics, a new species named *Sulfurimonas aquatica* sp. nov. is proposed with the type strain of strain H1576^T (= BCRC 81254^T = JCM 35004^T).

Keywords Sulfur-oxidizing bacteria · Chemolithoautotroph · Sulfurimonas · Brackish lake

Introduction

According to the List of Prokaryotic Names with Standing in Nomenclature, LPSN (Parte et al. 2020), the genus *Sulfurimonas* belongs to the family *Helicobacteraceae* and currently includes eight species with validly published names (as of 26 July 2022). They grow chemolithoautotrophically by oxidizing inorganic sulfur compounds, with oxygen as electron acceptor. In some species, anaerobic growth and H_2 gas oxidation are observed. As chemotaxonomic feature, they share major fatty acids of $C_{16:1}$, $C_{18:1}$ and $C_{16:0}$. Besides these eight species, three other species and two *Candidatus* species have been proposed in this genus, on the basis of genomic and phenotypic characterizations of isolated strains (Table 1).

As reviewed previously (Han and Perner 2015), members of the genus *Sulfurimonas* have been repeatedly detected by 16S rRNA gene sequence analysis, in various ecosystems represented by hydrothermal vents, marine sediments and water columns. In addition, *Sulfurimonas* is known to be a dominating bacterial genus in some engineered microbial systems, as shown in recent studies employing 16S rRNA gene amplicon sequencing (Fu et al. 2020; Wu et al. 2020; Haosagul et al. 2021). With the same approach, a dominance of *Sulfurimonas* species at specific water depths of a stratified brackish lake was recently reported (Watanabe et al. 2022). This shallow eutrophic lake, Lake Harutori in Japan, is characterized by steep chemocline and high concentration of sulfide in bottom water (Kubo et al. 2014; Watanabe et al. 2022). In this study, a novel sulfur-oxidizing autotroph was isolated from anoxic water of Lake Harutori, and characterized as a representative of a new species in the genus *Sulfurimonas*.

Materials and methods

Sampling of water from Lake Harutori was conducted on 16 Feb 2016. A sample of anoxic bottom water was collected from 5 m depth, at a site where previous studies were conducted (Kubo et al. 2014; Watanabe et al. 2022). A portion of the sample (0.3 ml) was inoculated into 30 ml of a

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✉ Hisaya Kojima
kojimah@lowtem.hokudai.ac.jp

[1] The Institute of Low Temperature Science, Hokkaido University, Kita-19, Nishi-8, Kita-ku, Sapporo 060-0819, Japan

Table 1 Differential properties of strain H1576^T and strains representing *Sulfurimonas* species

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Optimum temperature for growth (°C)	13–15	23–26	30	22	15	30	30	33	33	30	33	33	20	15
Growth at 5°C or lower	+	–	+	–	+	+	–	+	+	–	+	–	+	+
Growth at 35°C or higher	–	+	+	–	–	+	+	+	+	+	+	+	–	–
Optimum pH for growth	6.6–6.8	6.5	6.1	7.0	6.7–8.0	8.0	7.0	6.5	5.5	7.0–7.5	6.0–6.5	7.0	7.5–8.0	7.0–7.5
Growth at 8.5 or higher pH	–	+	+	NR	–	+	–	+	–	–	–	–	–	–
Growth at 5.5 or lower pH	–	+	+	NR	–	–	+	+	+	–	–	+	–	–
Growth by H_2 oxidation	–	–	+	–	+	–	+	+	+	+	+	+	+	+
Nitrate respiration	–	–	+	+	+	+	+	+	–	+	+	+	+	+
Nitrite respiration	–	–	–	+	+	+	–	–	–	–	–	–	–	–
dDDH with strain H1576^T (%)	100	19.4	18.5	19.1	20.1	18.2	21.1	18.6	18.9	19.0	18.7	19.1	20.2	20.4
ANI with strain H1576^T (%)	100	73.5	72.2	71.9	73.5	71.3	72.2	71.7	72.9	72.4	72.2	72.5	73.3	72.7

Strains: 1, H1576^T; 2, *S. autotrophica* OK10^T (Inagaki et al. 2003); 3, *S. paralvinellae* GO25^T (Takai et al. 2006); 4, *S. denitrificans* DSM 1251^T (Timmer-Ten Hoor 1975); 5, *S. gotlandica* GD1^T (Labrenz et al. 2013); 6, *S. crateris* SN118^T (Ratnikova et al. 2020); 7, *S. xiamenensis* 1-IN^T (Wang et al., 2020); 8, *S. lithotrophica* GYSG_1^T (Wang et al. 2020); 9, *S. indica* NW8N^T (Hu et al. 2021); 10, '*S. hongkongensis*' AST-10^T (Cai et al. 2014); 11, '*S. hydrogeniphila*' NW10^T (Wang et al. 2021a); 12, '*S. sediminis*' S2-6^T (Wang et al. 2021b); 13, *Ca.* S. marisnigri SoZ1 (Henkel et al. 2021); 14, *Ca.* S. baltica GD2 (Henkel et al. 2021). NR, not reported

medium for aerobic thiosulfate oxidizers. The medium (hereafter referred to as basal medium) was prepared as described below. First, the following salts (g l^{-1}) were dissolved in distilled water and then sterilized by autoclaving: NaCl (20), $Na_2S_2O_3{\cdot}5H_2O$ (5), $MgCl_2{\cdot}6H_2O$ (3), $MgSO_4{\cdot}7H_2O$ (0.3), $CaCl_2{\cdot}2H_2O$ (0.1), NH_4Cl (0.1), KH_2PO_4 (0.1) and KCl (0.1). To the autoclaved and cooled salt solution, the following stock solutions (ml l^{-1}) were aseptically added: trace element solution (1), selenite-tungstate solution (1), vitamin mixture solution (1) and 1 M $NaHCO_3$ solution (30). The vitamin mixture solution consisted of the followings (mg l^{-1}): biotin (20), folic acid (20), pyridoxine–HCl (100), thiamine–HCl$\cdot 2H_2O$ (50), riboflavin (50), nicotinic acid (50), calcium D(+) pantothenate (50), 4-Aminobenzoic acid (50), lipoic acid (50) and cyanocobalamine (1). The other stock solutions were prepared as described previously (Widdel and Bak 1992). Finally, pH of the medium was adjusted to 7.0–7.2 with HCl. From the enrichment culture established, pure culture of strain H1576^T was obtained by repeated serial dilution with the basal medium. The enrichment and isolation were performed at 15 °C in the dark.

Phenotypic characteristics of strain H1576^T were investigate by culturing the strain at 15 °C in the basal medium, unless otherwise specified. Cell morphology was observed with phase-contrast light microscopy, and Gram stain test was conducted with a kit (Fluka). Cellular fatty acid profile was obtained with the Sherlock Microbial Identification System (MIDI) version 6.0 (database; TSBA6).

To determine upper and lower limits of temperature for growth, strain H1576^T was inoculated into the basal medium and incubated at 0, 3, 5, 8, 13, 15, 18, 22, 25, 28, 30 and 32 °C. Effect of NaCl concentration on growth was examined by using media modified from the basal medium, with lowered concentration of $MgCl_2{\cdot}6H_2O$ (0.2 g l^{-1}) and varying concentrations of NaCl (0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0% and 6.0 w/v). Effect of pH on growth was tested with media of various pH which were prepared as below. The media commonly contained the following constituents (l^{-1}): 20 g NaCl, 5 g $Na_2S_2O_3{\cdot}5H_2O$, 1 g $NaHCO_3$, 0.2 g $MgCl_2{\cdot}6H_2O$, 0.1 g $CaCl_2{\cdot}2H_2O$, 0.1 g NH_4Cl, 0.1 g KH_2PO_4, 0.1 g KCl, 1 ml trace element solution, 1 ml selenite-tungstate solution and 1 ml vitamin mixture solution. Each medium of varying pH contained one of buffering reagents listed below (at a final concentration of 20 mM), along with NaOH for pH adjustment. Tested pH and buffering reagents were as follows; pH 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.7, 6.8, 6.9, 7.1, 7.2, 7.4 and 7.7 with MES; pH 6.6, 6.9 and 7.2 with PIPES; pH 7.0, 7.2, 7.3 and 7.6 with MOPS. All ingredients were mixed and then sterilized by filtration.

Utilization of electron donors was tested with the basal medium, by replacing thiosulfate with one of the followings (mM); sulfide (2), pyruvate (5), lactate (5), acetate (5), propionate (2.5), succinate (2.5), fumarate (2.5), malate (2.5),

butyrate (2.5), benzoate (2.5), isobutyrate (2.5), methanol (5), ethanol (2.5), formate (5), citrate (5), glucose (2.5), xylose (2.5), phenol (2), *m*-cresol (1). As insoluble substrates, elemental sulfur (0.5 g l^{-1}) and hydrogen gas (air/H_2; 2: 1, v/v; 150 kPa total pressure) were also tested with the thiosulfate-free basal medium. Utilization of electron acceptors was tested with the basal medium supplemented with nitrite (2 mM) or nitrate (5, 10 mM), under atmosphere of N_2 and CO_2 (80% and 20% in volume, respectively).

The novel isolate was subjected to whole genome sequencing, with the PacBio RS II platform. From linear contigs obtained, circular chromosome and plasmid were manually reconstructed based on sequence alignment. The resulting complete genome sequence was subjected to comparative analysis with the closest relatives, by the TYGS web server (https://tygs.dsmz.de). In the TYGS, the Type (Strain) Genome Server, relatives of the subjected genome were automatically identified for subsequent genome-based phylogenetic analysis and calculation of digital DNA-DNA hybridization (dDDH) values (Meier-Kolthoff and Göker 2019). Phylogenetic analysis was also conducted with the 16S rRNA gene identified in the genome, by using MEGA version 11 (Tamura et al. 2021). The reference sequences of *Sulfurimonas* species were retrieved from LPSN (accessed on 06 July 2022). The sequences of strain H1576^T and references were aligned with the MUSCLE algorithm. As an outgroup, *Sulfuricurvum kujiense* YK-1^T was included in the alignment. The best substitution model with the lowest Bayesian Information Criterion score was selected by the model selection tool in MEGA. Phylogenic tree was constructed with the selected model by excluding positions with gaps. Values of average nucleotide identity (ANI) between strain H1576^T and type strains of *Sulfurimonas* species were computed by ANI calculator available in the EzBioCloud, based on the OrthoANIu algorithm (Yoon et al. 2017).

Results

Cells of the novel isolate, strain H1576^T, were Gram stain negative, motile, rod shaped, 1.5–2.7 µm in width, 0.6–0.7 µm in length. The strain grew at 3–22 °C with optimum growth at 13–15 °C. At 15 °C, growth was observed at pH range of 6.0–7.4, with optimum growth at pH of 6.6–6.8. Growth was observed in the presence of 2–5% (w/v) NaCl. The cellular fatty acid profile of strain H1576^T is shown in Table S1. In the profile, summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\ \omega6c$) and $C_{16:0}$ were predominant, accounting for 65.5% and 21.9%, respectively.

Chemolithoautotrophic growth of strain H1576^T was supported by thiosulfate, sulfide and elemental sulfur, but not by H_2 gas. None of the tested organic substrate supported aerobic growth of the strain. As sole electron acceptor for thiosulfate oxidation, nitrate and nitrite did not support anaerobic growth of strain H1576^T.

The reconstructed genome of strain H1576^T consists of a circular chromosome and a plasmid, with length of 2.76 Mbp and 81.9 kbp, respectively. The G+C contents of the chromosome and plasmid are 34.8% and 32.8%, respectively. By analyzing the genome with the TYGS platform, it was revealed that the closest relatives of strain H1576^T are *Sulfurimonas* species. Genome-based phylogenetic analysis by the TYGS indicated that strain H1576^T belongs to the genus *Sulfurimonas*, but not to any known species (Fig. S1). The calculated values of dDDH and ANI indicated strain H1576^T should not be affiliated to any *Sulfurimonas* species previously proposed (Table 1). Phylogenetic analysis was also conducted with the 16S rRNA gene identified in the genome. The generated phylogenetic tree indicated that strain H1576^T is phylogenetically distinct from all type strains of the genus (Fig. 1). The genome of H1576^T has been incorporated in the genome taxonomy database (GTDB), which provides genome-based taxonomy framework on the basis of conserved proteins (Parks et al. 2018). In the latest release of the GTDB (07-RS207), strain H1576^T is classified into a *Sulfurimonas* species which encompasses no other organisms. All these analyses

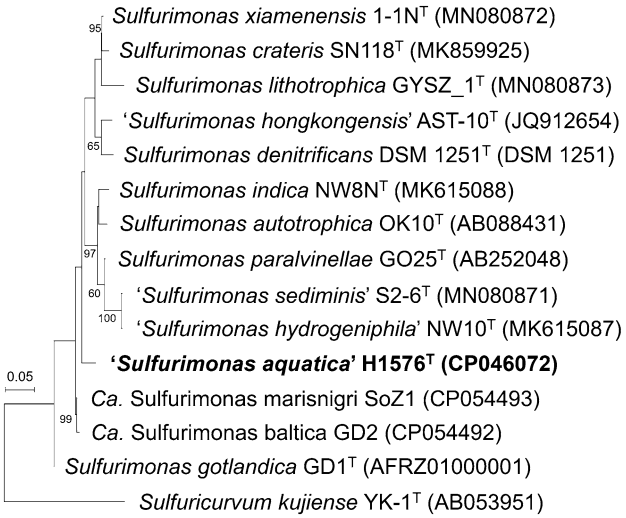

Fig. 1 Phylogenetic position of strain H1576^T within the genus *Sulfurimonas*, based on the 16S rRNA gene sequences. This maximum likelihood tree was constructed based on the Kimura 2-parameter model. All positions containing gaps and missing data were eliminated, leaving 1099 positions in the final dataset. A discrete gamma distribution was used to model differences in evolutionary rates among sites (5 categories, parameter = 0.3206). The rate variation model allowed for some sites to be invariable (68.83% sites). Bar, substitutions per site. Numbers on nodes represent percentage values of 1000 bootstrap resampling

consistently indicate that strain H1576^T is representative of a new species in the genus *Sulfurimonas*.

Conclusion

The genomic analyses of different approaches consistently indicated that strain H1576^T should be classified into a new species of the genus *Sulfurimonas*. Within the genus, strain H1576^T is differentiated from the type strains of the other species by a unique combination of phenotypic characteristics (Table 1). On the basis of these results, H1576^T is proposed to be assigned to a new species, with the name *Sulfurimonas aquatica* sp. nov.

Description of *Sulfurimonas aquatica* sp. nov

Sulfurimonas aquatica (a.qua'ti.ca. L. fem. adj. *aquatica*, aquatic)

Cells are rod shaped, motile, 1.5–2.7 µm in length and 0.6–0.7 µm in width. Gram stain negative. Grows chemolithoautotrophically by oxidizing thiosulfate, sulfide and elemental sulfur. Hydrogen gas is not used as electron donor. Aerobic. Nitrate and nitrite do not support anaerobic growth when thiosulfate is provided as the sole electron donor. Grows at 3–22 °C with an optimum growth at 13–15 °C. The pH range for growth is 6.0–7.4, with an optimum pH range of 6.6–6.8. Grows with 2–5% NaCl (optimum 2–3%). Predominant fatty acid is $C_{16:1}$. G+C content of genomic DNA of the type strain is 34.7 mol%.

The type strain H1576^T (= BCRC 81254^T = JCM 35004^T) was isolated from water of a brackish lake in Japan.

The GenBank/EMBL/DDBJ accession numbers for the chromosome and plasmid of type strain are CP046072 and CP046073, respectively.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00203-022-03167-3.

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Author contributions MF conducted water sampling. HK, YK and TW performed experiments for phenotypic characterization. HK isolated the strain, conducted genome analysis, wrote manuscript and prepared figures. All authors reviewed the manuscript.

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Declarations

Conflict of interest The authors declare no competing interests.

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