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ORIGINAL PAPER

Sulfurimicrobium lacus gen. nov., sp. nov., a sulfur oxidizer isolated from lake water, and review of the family *Sulfuricellaceae* to show that it is not a later synonym of *Gallionellaceae*

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Abstract

A facultatively anaerobic sulfur-oxidizing bacterium, strain skT11^T, was isolated from anoxic lake water of a stratified freshwater lake. As electron donor for chemolithoautotrophic growth, strain skT11^T oxidized thiosulfate, tetrathionate, and elemental sulfur under nitrate-reducing conditions. Oxygen-dependent growth was observed under microoxic conditions, but not under fully oxygenated conditions. Growth was observed at a temperature range of 5–37 °C, with optimum growth at 28 °C. Strain skT11^T grew at a pH range of 5.1–7.5, with optimum growth at pH 6.5–6.9. Heterotrophic growth was not observed. Major components in the cellular fatty acid profile were $C_{16:1}$ and $C_{16:0}$. The complete genome of strain skT11^T consisted of a circular chromosome with a size of 3.8 Mbp and G + C content of 60.2 mol%. Phylogenetic analysis based on the 16S rRNA gene sequences indicated that the strain skT11^T is related to sulfur-oxidizing bacteria of the genera *Sulfuricella*, *Sulfurirhabdus,* and *Sulfuriferula*, with sequence identities of 95.4% or lower. The analysis also indicated that these three genera should be excluded from the family *Gallionellaceae*, as members of another family. On the basis of its genomic and phenotypic properties, strain skT11^T (= DSM 110711^T = NBRC 114323^T) is proposed as the type strain of a new species in a new genus, *Sulfurimicrobium lacus* gen. nov., sp. nov. In addition, emended descriptions of the families *Gallionellaceae* and *Sulfuricellaceae* are proposed to declare that *Sulfuricellaceae* is not a later synonym of *Gallionellaceae*.

Keywords Sulfur-oxidizing bacteria · Chemolithoautotroph · *Sulfuricellaceae*; *gallionellaceae* · Novel genus

Introduction

The order *Nitrosomonadales* originally encompassed three families, *Nitrosomonadaceae*, *Gallionellaceae,* and *Spirillaceae* (Garrity et al. 2005). A restructuring of the order was made by Boden et al. 2017, along with reclassification of some taxa at levels ranging from class to genus (Boden et al. 2017). As a result, the order *Nitrosomonadales* currently includes *Methylophilaceae*, *Thiobacillaceae,* and *Sterolibacteriaceae*, in addition to the original three families. They also emended the description of the family *Gallionellaceae*, to encompass the genera *Sulfuricella*, *Sulfurirhabdus,* and *Sulfuriferula* (Boden et al. 2017). These three genera formerly constituted the family *Sulfuricellaceae* (Watanabe et al. 2014, 2015), which is currently regarded as a later synonym of the family *Gallionellaceae*. The families *Sulfuricellaceae* and *Gallionellaceae* were judged to be the same family, on the basis of analysis of the 16S rRNA gene sequences (Boden et al. 2017). The 16S rRNA gene analysis, however, included only one sequence as representative of

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the family *Gallionellaceae*. The sole sequence representing *Gallionellaceae* was that of *Ferriphaselus amnicola*, a stalk-forming iron-oxidizing bacterium (Kato et al. 2014). After the reclassification in 2017, the genus *Ferrigenium* was added to the family *Gallionellaceae* (Khalifa et al. 2018). This genus is represented by *Ferrigenium kumadai*, an iron-oxidizing bacterium which does not form stalks during iron-dependent growth (Khalifa et al. 2018).

In the genera which once belonged to the family *Sulfuricellaceae*, there are six species with validly published names. *Sulfuricella denitrificans* and *Sulfurirhabdus autotrophica* represent monospecific genera (Kojima and Fukui 2010; Watanabe et al. 2016a). The genus *Sulfuriferula* encompasses *Sulfuriferula multivorans*, *Sulfuriferula plumbiphila*, *Sulfuriferula thiophila,* and *Sulfuriferula nivalis* (Watanabe et al. 2015, 2016b; Kojima et al. 2020). All these species grow chemolithoautotrophically by oxidizing inorganic sulfur compounds. As an alternative electron donor, *Sulfuriferula plumbiphila* can use hydrogen, and *Sulfuriferula multivorans* can grow heterotrophically. Among these species, considerable variations in sulfur oxidation pathways have been identified (Watanabe et al. 2019). In the present study, a novel sulfur-oxidizing bacterium related to these bacteria was isolated and characterized. In addition, the taxonomic status of *Sulfuricellaceae* was reassessed to indicate that it is not a later synonym of *Gallionellaceae*.

Materials and methods

Isolation of novel bacterial strain

Strain skT11^T was isolated from an enrichment culture growing on thiosulfate disproportionation, described in a previous study (Watanabe et al. 2016c). The enrichment culture was established from anoxic lake water of a stratified freshwater lake, Lake Mizugaki in Japan (35° 51′ 35" N, 138° 29′ 58" E), and maintained by periodical transfer. From the enrichment culture, a small portion was transferred to the medium S5 (Kojima et al. 2017), supplemented with $NaNO_3$ (10 mM). As described previously, the medium S5 was buffered with 30 mM bicarbonate and contained 10 mM thiosulfate as the sole electron donor. The culture was incubated at 28 °C under anoxic condition. The resulting nitrate-reducing culture was further transferred to the same medium for three times. After that, pure culture of strain skT11^T was obtained with repeated agar shake dilution using the same medium, by picking up white colonies (approx. 0.5 mm in diameter) appeared after 1–2-week incubation. Purity of the culture was culture was routinely checked by microscopy and sequencing of PCR-amplified fragments of the 16S rRNA gene.

Analysis of the 16S rRNA gene sequences

For strain skT11^T, nearly full length of the 16S rRNA gene sequence was determined as described previously (Kojima et al. 2015). The obtained sequence was subjected to BLASTN search against the public database nr at the NCBI to identify its close relatives. Based on the results obtained, a custom database was created with genomes of the type strains in the genera *Sulfuricella*, *Sulfurirhabdus,* and *Sulfuriferula*. The 16S rRNA gene sequence identities were obtained by BLASTN search against the custom database, for strain skT11^T and representatives of the family *Gallionellaceae*. Further phylogenetic analysis was conducted with the program MEGA version X (Kumar et al. 2018). The sequence of skT11^T was aligned with reference sequences, using the MUSCLE algorithm. The references were selected from type strains of species with validly published names in the order *Nitrosomonadales*. Additionally, sequences of *Gallionella ferruginea* (lacking a pure culture of the type strain) and its close relatives (without validly published names) were also included in the analysis for a close inspection of the family *Gallionellaceae*. Based on the resulting alignment, models for genetic distance calculation were evaluated using the model selection tool in MEGA X. With the selected models, genetic distances were calculated by excluding positions with gaps.

Phenotypic characterization

For phenotypic characterization, the medium used for the isolation (S5 supplemented with nitrate) was used as basal medium. Unless otherwise specified, headspace of the culturing bottles was filled with mixture of N_2 and CO_2 (80:20; *v/v*), and the cultures were incubated at 28 °C in the dark. Tests of Gram-stain, catalase activity, and oxidase activity were performed as described previously (Kojima et al. 2015).

Utilization of electron donors was tested under nitrate-reducing conditions, by excluding thiosulfate from the basal medium. Oxygen-dependent growth was tested with S5 medium without nitrate, under different oxygen concentrations in the headspace, 0% (as negative control), 1%, 2%, and 20% *v/v*. The ability for thiosulfate disproportionation was assessed by inoculating the isolate to the medium used for the original enrichment culture (Watanabe et al. 2016c). Temperature dependence of growth was tested by culturing at 0, 5, 8, 10, 13, 15, 18, 22, 25, 28, 30, 32, 35, 37, 42, 45, and 50 °C. Effect of NaCl on growth was examined with the basal medium supplemented with different concentrations of NaCl (0, 10, 50, 100, 250, and 500 mM). Effects of pH on growth were examined

under nitrate-reducing conditions, with a method modified from that previously described (Kojima et al. 2015). Briefly, the composition of the medium was changed as follows (l^{-1}): 2.5 g $Na_2S_2O_3.5H_2O$, 0.85 g $NaNO_3$, 0.3 g $MgSO_4.7H_2O$, 0.1 g $CaCl_2$. $2H_2O$, 0.1 g NH_4Cl, 0.1 g KH_2PO_4, and 0.1 g KCl. It also contained solutions of trace elements, selenite-tungstate, and vitamin mixture as same in the original medium. As a buffering agent, MES or MOPS was used at a final concentration of 20 mM, as follows; pH 3.4, 4.2, 4.4, 4.7, 5.1, 5.5, 5.8, 6.0, and 6.5 with MES; pH 6.4, 6.6, 6.8, 6.9, 7.2, 7.5, 7.7, 8.1, 8.3, and 8.6 with MOPS. The ingredients were mixed and then sterilized by filtration, after pH adjustment with HCl or NaOH. The sterilized media were dispensed in closed culture bottles whose headspace was filled with N_2 gas.

For fatty acid analysis, cells were grown at 28 °C in a modified version of the basal medium, which contained increased thiosulfate and nitrate (20 mM each). The cellular fatty acid profile was analyzed using the Sherlock Microbial Identification System Version 6.0 (MIDI) with database TSBA6.

Genomic analysis

The genome sequencing was performed using the Illumina NextSeq and Nanopore GridION platforms. A hybrid assembly was performed using Unicycler (Ver 0.4.7), to generate a circular contig with coverage of 861-fold. The genome sequence was annotated with DFAST (Tanizawa et al. 2017), and genes involved in sulfur oxidation were identified as described previously (Watanabe et al. 2019).

A genome-based taxonomic classification of the strain skT11^T was conducted with the Genome Taxonomy Database (GTDB), based on 120 proteins coded in the genome (Parks et al. 2018). Taxonomic position of the strain skT11^T in the GTDB (release 89) was identified using GTDB-Tk (Chaumeil et al. 2020).

Results and discussion

Basic characteristics of the novel isolate

The fundamental characteristics of skT11^T are summarized in Table 1 and the species description. Cells of strain skT11^T were motile, rod-shaped (Fig. S1), and

Table 1 Differential properties of strain skT11^T and its phylogenetic neighbors

Characteristic	1	2	3	4	5	6	7
Cell size (length/width, μm)	1.1–3.7 / 0.5–0.7	0.8–2.0 / 0.4–0.6	1.4–4.6 / 0.4–0.7	1.0–2.2 / 0.3–0.5	1.2–3.5 / 0.15–0.8	1.2–4.0 / 0.5–0.7	1.0–3.5 / 0.4–0.7
Catalase activity	–	–	–	+	+	+	–
Oxidase activity	+	+	+	+	–	+	–
Growth on sulfide	–	–	–	–	+	–	–
Growth on hydrogen	–	–	–	–	+	–	–
Heterotrophic growth	–	–	–	+	–	–	– –
Anaerobic growth	+	+	–	+	–	–	–
Growth under atmospheric oxygen tension	–	+	+	+	+	+	+
Optimal temperature for growth (range)	28 (5–37)	22 (0–28)	15–22 (0–32)	22–25 (8–32)	21–34 (4–41)	32 (5–34)	18 (5–28)
Optimal pH for growth (range)	6.5–6.9 (5.1–7.5)	7.5–8.0 (6.0–9.0)	6.1–6.3 (5.2–8.1)	6.4–7.0 (5.3–8.6)	6.3–7.0 (4.0–7.8)	5.9–6.2 (4.6–8.1)	6.1–7.1 (4.3–7.4)
Third most abundant fatty acid	$C_{18:1}$	$C_{18:1}$	$C_{10:0}$ 3-OH	$C_{12:0}$	$C_{12:0}$	$C_{12:0}$	$C_{12:0}$
G + C content (mol%, genome)	60.2	56.1	46.3	56.9	59.1	52.6	47.8
Core sulfur oxidation pathway	Sox–Dsr–Soe	Sox–Dsr–Soe	Sox–Dsr–Soe Sox-Hdr-Soe	Sox–Dsr–Soe Sox-Hdr-Soe	Sox–Hdr–Soe	Sox–Hdr–Soe	Sox–Hdr–Soe
Lineage of Apr	I, II	II	II	II	I, II	n/a*	n/a*

*Not applicable

Strains: 1, skT11^T (this study); 2, *Sulfuricella denitrificans* skB26^T (Kojima & Fukui 2010; Watanabe et al. 2014); 3, *Sulfurirhabdus autotrophica* BiS0^T (Watanabe et al. 2016a; 2019); 4, *Sulfuriferula multivorans* TTN^T (Watanabe et al. 2015; 2019); 5, *Sulfuriferula plumbiphila* Gro7^T (Drobner et al. 1992; Kojima et al. 2020); 6, *Sulfuriferula thiophila* mst6^T (Watanabe et al. 2016b; 2019); 7, *Sulfuriferula nivalis* (Kojima et al. 2020). All strains are chemolithoautotrophs which oxidize thiosulfate and elemental sulfur. In all strains, the most and second most abundant fatty acids are $C_{16:1}$ and $C_{16:0}$, respectively. Strains 6 and 7 lack the *aprBA* genes

Gram-staining-negative. In the BLASTN analysis of the 16S rRNA gene sequence of strain skT11^T (1461 bp), the highest sequence identity of 95.4% to *Sulfuriferula multivorans* TTN^T was indicated, followed by 95.2% to *Sulfuricella denitrificans* skB26^T (Table S1). Phylogenetic relationships between strain skT11^T and these strains are shown in Fig. 1.

Chemolithoautotrophic growth of strain skT11^T under nitrate-reducing condition was supported by thiosulfate (10 and 20 mM), tetrathionate (10 mM), and elemental sulfur (0.5 g l^{-1}). Sulfide (2 mM) and hydrogen (H_2:N_2:CO_2, 5:4:1 *v/v/v*; 200 kPa total pressure) did not support autotrophic growth. The following organic substrates did not support growth of strain skT11^T: lactate (5 mM), acetate (5 mM), propionate (2.5 mM), fumarate (2.5 mM), malate (2.5 mM), butyrate (2.5 mM), isobutyrate (2.5 mM), benzoate (2.5 m M), methanol (5 mM), ethanol (2.5 mM), formate (5 mM), citrate (5 mM), and glucose (2.5 mM). In the medium without nitrate, growth of skT11^T was observed under microaerobic conditions (1–2% *v/v* O_2), but not under atmospheric oxygen tension (20%). Although strain skT11^T was isolated from the enrichment culture growing on thiosulfate disproportionation, it did not disproportionate thiosulfate. A negative effect of NaCl on growth was observed at 50 mM or higher concentrations, and strain skT11^T did not grow in the medium supplemented with 250 mM or 500 mM NaCl.

In the cellular fatty acid profile of strain skT11^T, summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; 51.7%) was the most dominant, followed by $C_{16:0}$ (26.2%) and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$; 11.8%). These three major components accounted for nearly 90% of the total. As minor components, $C_{10:0}$ 3-OH (3.4%), $C_{12:0}$ (2.2%), $C_{16:1}\omega5c$ (1.3%), and anteiso-$C_{17:1}$ $\omega9c$ (0.6%) were detected. The other fatty acids detected (< 0.5% of total) were $C_{18:1}\omega5c$, $C_{17:1}\omega5c$, $C_{18:1}\omega9c$, $C_{17:0}$, $C_{18:0}$ $C_{10:0}$, $C_{14:0}$, $C_{17:1}\omega6c$, iso-$C_{20:0}$, $C_{17:1}\omega8c$, and $C_{16:0}$ 3-OH. In fatty acid profiles of the related genera (*Sulfuricella*, *Sulfurirhabdus,* and *Sulfuriferula*), summed feature 3 and $C_{16:0}$ were detected as the most and second most dominant components [3, 4, 8, 9, 10].

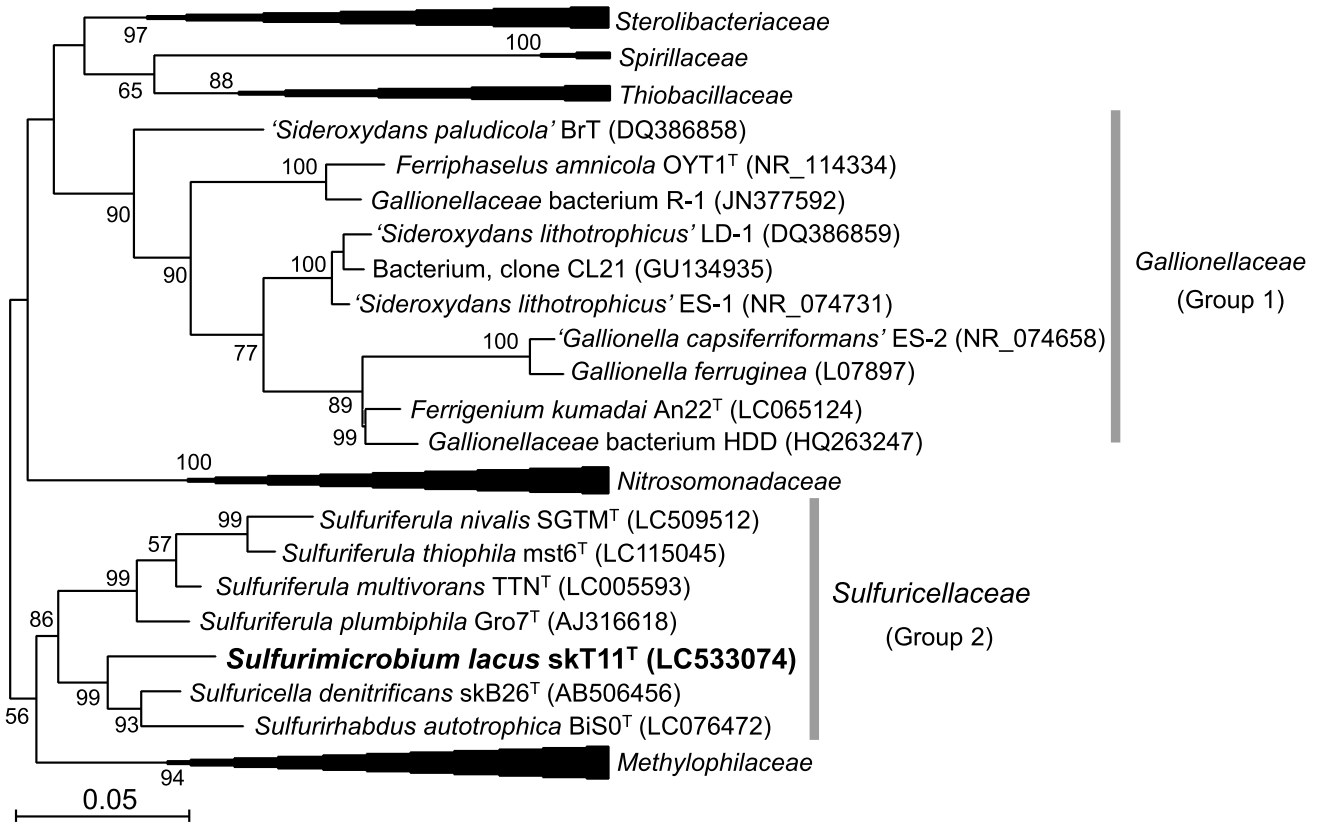

Fig. 1 Phylogenetic position of strain skT11^T within the order *Nitrosomonadales*. This unrooted phylogenetic tree was constructed with the maximum likelihood method, based on Kimura 2-parameter model with gamma distribution and invariant sites. All positions containing gaps were eliminated and there were a total of 1279 positions in the final dataset. Bar represents substitutions per site. Numbers on nodes represent percentage values of 1000 bootstrap resampling (values lower than 50 are not shown). Names of all strains included in the analysis are shown in full version of the tree provided as Figure S1

Genomic characters of the strain skT11^T

The genome of strain skT11^T consists of a circular chromosome with a size of 3,826,324 bp and G + C content of 60.2 mol%, respectively. In the complete genome, 3678 protein-coding sequences were predicted. It harbors two copies of the 16S rRNA gene with the identical sequence. As the genetic basis for sulfur oxidation, gene encoding proteins involved in the Sox–Dsr–Soe pathway were identified. This pathway is one of the three core pathways defined in a previous study (Watanabe et al. 2019), which revealed coexistence of the Sox–Dsr–Soe and Sox–Hdr–Soe pathways in *Sulfurirhabdus autotrophica* BiS0^T and *Sulfuriferula multivorans* TTN^T. The gene set for Sox–Hdr–Soe pathway, conserved in the genera *Sulfurirhabdus* and *Sulfuriferula*, was not identified in the genome of strain skT11^T (Table 1). Besides the genes involved in the core sulfur oxidation pathway, strain skT11^T has the *aprBA* genes encoding adenylylsulfate reductase (Apr). The *aprBA* genes in genomes of sulfur oxidizers are known to be divided into two groups, Apr lineage I and lineage II (Meyer and Kuver 2007; Watanabe et al. 2016c). In the genome of strain skT11^T, two copies of the *aprBA* genes were identified, and they belonged to the Apr lineage I and lineage II, respectively (Table 1).

Reassessment of the families *Gallionellaceae* and *Sulfuricellaceae*

Phylogenetic position of strain skT11^T within the order *Nitrosomonadales* was examined by constructing trees with three different methods, based on the 16S rRNA gene sequences (Fig. 1, Figs. S2–S4). Different branching patterns were obtained with different methods, but robust clusters corresponding to the families *Nitrosomonadaceae*, *Methylophilaceae*, *Spirillaceae*, *Thiobacillaceae,* and *Sterolibacteriaceae* were formed in all trees. On the other hand, none of the trees supported the monophyly of the family *Gallionellaceae*. All the trees indicated that members of the family belong to two distinct phylogenetic groups. One of the groups (hereafter referred to as group 1) included the genera *Gallionella*, *Ferriphaselus,* and *Ferrigenium.* The other group (hereafter referred to as group 2) consisted of the genera *Sulfuricella*, *Sulfurirhabdus,* and *Sulfuriferula*, along with strain skT11^T (Fig. 1). The phylogenetic dissociation of these two groups has also been observed in phylogenetic trees constructed in the previous studies (Watanabe et al. 2014, 2016c, 2019). These results suggest that the group 2 should be excluded from the family *Gallionellaceae* corresponding to the group 1. In the previous study which merged the *Gallionellaceae* and *Sulfuricellaceae*, sequence identity of 92.25% was used as a "guide" to define the families based on the 16S rRNA gene sequences (Boden et al. 2017). The previous analysis included *Ferriphaselus* as the solo representative of the *Gallionellaceae*, and the sequence identity between *Ferriphaselus amnicola* OYT1^T and *Sulfuricella denitrificans* skB26^T (> 92.5%) is greater than this guide value. However, those between *Ferriphaselus amnicola* OYT1^T and the other type strains belonging to the group 2 are lower than 92.2% (Table S1). *Ferrigenium kumadai* An22^T, the other type strain in the group 1, gave similar results in which identity greater than the guide value was observed only with *Sulfuricella denitrificans* skB26^T (Table S1). Although there is no isolated type strain of *Gallionella* species at present, the 16S rRNA gene sequences of *Gallionella* are available in the public database. They showed sequence identities lower than the guide value, to all type strains of the group 2 (Table S1). To obtain more evidence for the dissociation of two groups, some sequences closely related to the species of group 1 were additionally analyzed in the same manner. Even in the expanded analysis including uncultured bacteria, sequence identities greater than 92.25% were observed only in limited number of combinations across the two groups (Table S1). These results indicate that the group 2 should be regarded as independent family, and thus, *Sulfuricellaceae* is not later synonym of *Gallionellaceae*. Accordingly, description of *Gallionellaceae* should be emended to exclude the genera belonging to the group 2, i.e., *Sulfuricellaceae*.

Taxonomic position of the strain skT11T within the family *Sulfuricellaceae*

The phylogenetic trees of 16S rRNA gene indicate that strain skT11^T is member of the family *Sulfuricellaceae,* but does not belong to any existing genera (Fig. 1, Figs. S2–S4). It was also shown that strain skT11^T is most closely related to *Sulfuricella* and *Sulfurirhabdus*. To draw conclusion about genus-level classification based on whole genomic data, the genome sequences of strain skT11^T and *Sulfurirhabdus autotrophica* BiS0^T were analyzed using the GTDB-Tk. As a result, these organisms and *Sulfuricella denitrificans* skB26^T (included in the GTDB release 89) were classified into three different genera. This means that a novel genus should be created to accommodate strain skT11^T. As shown in Table 1, strain skT11^T has some characteristics distinct from those of the type strains of species in the genera *Sulfuricella*, *Sulfurirhabdus,* and *Sulfuriferula*. On the basis of these results, strain skT11^T is proposed as the type strain of a novel species of a new genus in the family *Sulfuricellaceae*, with the name *Sulfurimicrobium lacus* gen. nov., sp. nov.

Description of *Sulfurimicrobium* gen. nov.

Sulfurimicrobium (Sul.fu.ri.mi.cro'bi.um. L. neut. n. *sulfur* sulfur; N.L. neut. n. *microbium*, a microbe; N.L. neut. n. *Sulfurimicrobium* sulfur-oxidizing microbe).

Grows by oxidation of inorganic sulfur compounds; microaerophilic and neutrophilic; Gram-stain-negative. Major cellular fatty acids are $C_{16:1}$ and $C_{16:0}$. Belongs to the family *Sulfuricellaceae*. The type species is *Sulfurimicrobium lacus*.

Description of *Sulfurimicrobium lacus* sp. nov.

Sulfurimicrobium lacus (la'cus. L. gen. n. *lacus*, of a lake).

In addition to the properties listed for the genus, cells are motile, rod-shaped, 1.1–3.7 µm long and 0.5–0.7 µm wide. Catalase-negative and oxidase-positive. Grows chemolithoautotrophically by oxidation of thiosulfate, tetrathionate, and elemental sulfur. Uses oxygen and nitrate as electron acceptor. Oxygen-dependent growth occurs only under microoxic conditions. Temperature range for growth is 5–37 °C, with an optimum of 28 °C. Growth occurs at pH 5.1–7.5, with an optimum of pH 6.5–6.9. G + C content of genomic DNA of the type strain is 60.2 mol%.

The type strain skT11^{T} (= DSM 110711 ^{T} = NBRC 114323 ^{T}) was isolated from anoxic lake water of a stratified freshwater lake in Japan. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and complete genome sequence of strain skT11^{T} are LC533074 and AP022853, respectively.

Emended description of *Sulfuricellaceae* (Watanabe et al. 2015)

Sulfuricellaceae (Sul.fu.ri.cel.la.ce'ae. N.L. fem. n. *Sulfuricella* type genus of the family; -aceae ending to denote family; N.L. fem. pl. n. *Sulfuricellaceae* the family of the genus *Sulfuricella*).

Delineation is primarily based on phylogenetic information from 16S rRNA gene sequences. Belongs to the order *Nitrosomonadales*. Encompasses Gram-stain-negative bacteria isolated from freshwater environments. At the time of writing, the family contains the genera *Sulfuricella*, *Sulfurirhabdus*, *Sulfuriferula,* and *Sulfurimicrobium* gen. nov. All members utilize inorganic sulfur compounds as their energy source. Uses oxygen or nitrate as terminal electron acceptors for respiration. The type genus is *Sulfuricella*.

Emended description of *Gallionellaceae* (Henrici and Johnson 1935 (Approved Lists 1980) emend. Boden et al. 2017)

Gallionellaceae (Gal.li.o.nel.la.ce'ae. N.L. fem. n. *Gallionella* type genus; -aceae suffix to denote family; N.L. fem. pl. n. *Gallionellaceae* the *Gallionella* family).

Delineation is primarily based on phylogenetic information from 16S rRNA gene sequences. Comprises iron-oxidizing bacteria of the genera *Gallionella*, *Ferriphaselus,,* and *Ferrigenium*. Microaerophilic and neutrophilic. Motile with one polar flagellum. Cells are curved-rod or reniform. Reduced sulfur or ferrous iron is used as electron donors for autotrophic and/or mixotrophic growth. Stalk formation is observed during the iron-dependent growth of some species. The type genus is *Gallionella*.

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Compliance with ethical standards

Conflicts of interest The authors declare that there are no conflicts of interest.

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