Title	Thiosulfatovibrio zosteraegen. nov., sp. nov., and Thiosulfatimonas sediminis gen. nov., sp. nov.
Author(s)	Mochizuki, Jun; Kojima, Hisaya; Fukui, Manabu
Citation	Archives of Microbiology, 203, 951-957 https://doi.org/10.1007/s00203-020-02090-9
Issue Date	2020-10-21
Doc URL	http://hdl.handle.net/2115/82984
Rights	This is a post-peer-review, pre-copyedit version of an article published in Archives of Microbiology. The final authenticated version is available online at: http://dx.doi.org/10.1007/s00203-020-02090-9
Type	article (author version)
File Information	Archives of Microbiology s00203-020-02090-9.pdf

[Instructions for use](#)

***Thiosulfativibrio zosterae* gen. nov., sp. nov., and *Thiosulfatimonas sediminis* gen. nov., sp. nov.**

Jun Mochizuki^{1,2}, Hisaya Kojima^{*1} and Manabu Fukui¹

¹ The Institute of Low Temperature Science, Hokkaido University, Sapporo, Japan

² Graduate School of Environmental Science, Hokkaido University, Sapporo, Japan

---

*Corresponding author.

E-mail: kojimah@pop.lowtem.hokudai.ac.jp

Phone: +81-11-706-5460

Fax: +81-11-706-5460

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains AkT22^T and aks77^T are LC510548 and LC510549, respectively. The numbers for their complete genomes are AP021888 (AkT22^T) and AP021889 (aks77^T).

Abstract

Aerobic, Gram-stain-negative, obligately chemolithoautotrophic thiosulfate-oxidizing bacteria, strains AkT22^T and aks77^T were isolated from a brackish lake in Japan. Strains AkT22^T and aks77^T were isolated from samples of eelgrass and sediment, respectively. Growth on sulfide, tetrathionate, elemental sulfur, and organic substrates was not observed for both strains. Growth of the strains was observed at 5°C or higher temperature, with optimum growth at 22°C. Strain AkT22^T grew at a pH range of 5.8–8.0, with optimum growth at pH 6.7–7.8. Strain aks77^T grew at a pH range of 5.8–8.5, with optimum growth at pH 7.0–7.9. Major cellular fatty acids (>10% of total) of strain AkT22^T were C_{16:1}, C_{18:1}, and C_{16:0}. The sole respiratory quinone was ubiquinone-8 in both strains. The genome of strain AkT22^T consisted of a circular chromosome, with size of approximately 2.6 Mbp and G + C content of 43.2%. Those values of the genome of strain aks77^T were *ca.* 2.7 Mbp and 45.5%, respectively. Among cultured bacteria, *Thiomicrothrix aquaedulcis* HaS4^T showed the highest sequence identities of the 16S rRNA gene, to strains AkT22^T (94%) and aks77^T (95%). On the basis of these results, *Thiosulfatovibrio zosterae* gen. nov., sp. nov. and *Thiosulfatimonas sediminis* gen. nov., sp. nov. are proposed, with type strains of AkT22^T (= BCRC 81184^T = NBRC 114012^T = DSM 109948^T) and aks77^T (= BCRC 81183^T = NBRC 114013^T), respectively.

37

38   Keywords: Sulfur-oxidizing   bacteria;   chemolithoautotroph;   *Thiomicrothrix*;
39   brackish lake; novel genus.

40

Introduction

The genus *Thiomicrorhabdus* in the family *Piscirickettsiaceae* was originally established with four species, *Thiomicrorhabdus frisia*, *Thiomicrorhabdus chilensis*, *Thiomicrorhabdus arctica* and *Thiomicrorhabdus psychrophila* (Boden et al. 2017a). Immediately after that, *Thiomicrorhabdus hydrogeniphila* was added to the genus as a result of reclassification (Boden et al. 2017b). These five species were originally described as *Thiomicrospira* species (Brinkhoff et al., 1999a, 1999b; Knittel et al. 2005; Watsuji et al. 2016). In the genus *Thiomicrorhabdus*, the first non-marine species was described as *Thiomicrorhabdus aquaedulcis* (Kojima & Fuki 2019), and the most recently described species is *Thiomicrorhabdus indica* (Liu et al. 2020). Consequently, there are seven *Thiomicrorhabdus* species with validly published names at present. They are obligately chemolithoautotrophic bacteria which oxidize inorganic sulfur compounds. They all use thiosulfate, elemental sulfur, sulfide as electron donor for their aerobic growth. In the present study, two novel isolates related to *Thiomicrorhabdus* were isolated and characterized.

Materials and methods

Isolation of novel strains

Strains AkT22^T and aks77^T were enriched and isolated from samples of eelgrass and sediment, respectively. The samples were collected at a site (43.05N, 144.89E), in Lake Akkeshi, a brackish lake in Japan. The sample of eelgrass was inoculated into a bicarbonate-buffured low-salt defined medium, which comprised (l⁻¹): 2.5 g Na₂S₂O₃ • 5H₂O, 0.2 g MgCl₂ • 6H₂O, 0.1 g CaCl₂ • 2H₂O, 0.1 g NH₄Cl, 0.1 g KH₂PO₄, 0.1 g KCl, 1 ml trace element solution, 1 ml selenite-tungstate solution, 1 ml vitamin mixture solution, 30 ml NaHCO₃ solution. The medium and respective stock solutions were prepared as described previously (Kojima et al. 2016). The strain was isolated in pure culture by repeated serial dilution and agar shake dilution. The agar shake tubes did not contain oxygen scavenger, and headspace was filled with air. The resulting pure culture was designated as strain AkT22^T. The sediment sample was inoculated into a medium used in a previous study (Kojima & Fukui 2016), which contained 5 g Na₂S₂O₃ • 5H₂O, 20 g NaCl, 3 g MgCl₂ • 6H₂O, and 0.3 g MgSO₄ • 7H₂O. The other components were same as the medium used for isolation of AkT22^T. After four times transfer to medium of the same composition (0.4% v/v), the medium was changed to the medium used for isolation of AkT22^T, with which strain aks77^T was isolated by repeated serial dilution. The enrichment and isolation of both strains were performed at 22°C. Purity was routinely checked by microscopy and sequencing of the 16S rRNA gene fragments.

77

78 **Analysis of the 16S rRNA gene sequences**

79 The 16S rRNA gene fragments of the novel strains were amplified by PCR using the
80 primer pair 27F and 1492R (Lane 1991) and then directly sequenced. The resulting
81 sequences were subjected to the Megablast search at NCBI against the nucleotide
82 collection (nr/nt) database, to identify their close relatives. Further phylogenetic analyses
83 were conducted using the program MEGA version X (Kumar et al. 2018). The 16S rRNA
84 gene sequences of the novel isolates were aligned with reference sequences identified by
85 the database search described above, using the MUSCLE algorithm (Edgar 2004). The
86 references included type strains of species with validly published names in the genera
87 *Thiomicrothabodus*, *Hydrogenovibrio*, *Thiomicrospira* and *Galenea*, as well as uncultured
88 bacteria which showed high sequence identities (>95%) to strain AkT22^T or strain aks77^T.
89 As an outgroup, *Sulfurivirga caldicurarii* MM1^T was also included in the analysis. The
90 model selection tool in MEGA X was used to find out the best model for calculation of
91 genetic distances, which gave the lowest Bayesian Information Criterion (BIC) score. All
92 positions with gaps were excluded from the calculation.

93

94 **Phenotypic characterization**

For phenotypic characterization of the strains, a medium of the following composition was used as the basal medium (l^{-1}): 5 g $Na_2S_2O_3 \cdot 5H_2O$, 0.5 g $MgSO_4 \cdot 6H_2O$, 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, 30 ml $NaHCO_3$ solution. Culturing experiments were performed at 22°C without shaking unless otherwise specified. The Gram-staining test was conducted with a kit (Fluka). Morphology of the cells were observed with phase-contrast light microscopy, transmission electron microscopy (TEM) and electron microscopy (SEM). Oxidase activity was tested using an oxidase test reagent (bioMérieux). Catalase activity was assessed by pouring 3% H_2O_2 solution onto a pellet of cells. For chemotaxonomic characterization, strains AkT22^T and aks77^T were grown in the basal medium supplemented with the vitamin solution (1 ml l^{-1}). Cellular fatty acid profile of each strain was analyzed using the Sherlock Microbial Identification System Version 6.0 (MIDI) with database TSBA6. Respiratory quinones and polar lipids were analyzed as described previously (Bligh & Dyer 1959; Minnikin et al. 1979). Effects of temperature on growth were examined by culturing strains at 0, 5, 8, 13, 15, 18, 22, 25, 28, 30, 32, 37 and 45°C. Effects of salt concentration on growth was examined by culturing the strains in the basal medium supplemented with various concentration of NaCl, ranging from 0 to 12% (w/v) at 1.0% intervals. To examine effects of pH on growth,

the strains were cultured at 20 different pH values respectively. The medium for pH test was prepared as described previously (Kojima et al. 2016), but vitamins were omitted. The tested pH range and buffering reagents for strain AkT22^T were as follows; pH 5.7–7.0 with MES; pH 6.7–7.3 with PIPES; pH 7.1–7.9 with MOPS; pH 7.5–8.4 with Tricine; pH 8.7–9.5 with CHES. Those for strain aks77^T were as follows; pH 5.7–7.0 with MES; pH 7.3–7.8 with PIPES; pH 6.6–8.1 with MOPS; pH 7.5–8.5 with Tricine; pH 8.7–9.8 with CHES. Utilization of electron donors was tested in the basal medium supplemented with one of the substances listed later. Anaerobic growth of the strains was tested in the presence of Na₂S₂O₃ and NaNO₃ (10 mM each). Heterotrophic growth in complex liquid media was tested for Reasoner's 2A broth (R2A) broth (Daigo), one-tenth-strength R2A, nutrient broth (Difco), LB broth Miller (Merck) and tryptone soya broth (Oxoid). Utilization of nitrate as nitrogen source was tested by replacing NH₄Cl in the basal medium with NaNO₃ (0.2 g l⁻¹).

Genomic characterization

The genome of strain AkT22^T was sequenced using the Illumina NextSeq and Nanopore GridION platforms. Hybrid assembly was performed using Unicycler (Ver 0.4.7), to generate a circular contig with coverage of 300-fold. The genome of strain aks77^T was

sequenced using PacBio RS II platform. Assembly was performed using RS_HGAP_Assembly.3 to generate a linear contig with average coverage of 349-fold, which were manually converted to a circular chromosome. For the resulting genome sequences, values of the average nucleotide identity (ANI) were calculated based on OrthoANIu algorithm (Yoon et al. 2017), by using ANI calculator available in EzBioCloud. The genome sequences were annotated with DFAST (Tanizawa et al. 2013). Based on the annotations, percentage of conserved proteins (POCP) values were calculated as described previously (Qin et al. 2014). Two-way average amino acid identity (AAI) scores were calculated by using an online tool, AAI calculator from the Kostas lab (<http://enve-omics.ce.gatech.edu/>). Phylogenetic analysis based on the 53 ribosomal proteins was performed as described previously (Jolley et al. 2012; Kojima & Fukui 2019). Whole genome-based phylogenetic analysis was conducted with the Genome Taxonomy Database (GTDB) (Parks et al. 2018). For the strains Akt22^T and aks77^T, their taxonomic assignments in GTDB (release 89) were identified by using GTDB-Tk (Chaumeil et al. 2020).

Results and Discussion

Phylogeny based on the 16S rRNA gene

The phylogenetic positions of the novel isolates were identified by analyzing their 16S rRNA genes sequences. Among cultured bacterial strains, *Thiomicrospira aquaedulcis* HaS4^T showed the highest sequence identities to strains AkT22^T (94%) and aks77^T (95%). Only for strain AkT22^T, there were some environmental clones which showed sequence identity higher than that of *T. aquaedulcis* HaS4^T. The clones of high identity (99%) were reported from a terrestrial sulfidic spring (Headd & Engel 2014). The sequence identity between strains AkT22^T and aks77^T was 93%. Phylogenetic tree constructed with the maximum-likelihood method is shown in Fig. 1. Almost identical branching patterns were observed in trees constructed with methods of neighbor-joining and minimum evolution (Figs. S1 and S2).

Phenotypic characteristics

Basic characteristics of strains AkT22^T and aks77^T are summarized in Table 1 and respective species descriptions. Cells of the strains were rod-shaped, motile, Gram-stain-negative and oxidase-negative. Electron microscopic images of the cells are shown in Figure S3 (TEM) and S4 (SEM). Strain AkT22^T was catalase-negative, whereas strain aks77^T was catalase-positive. The strains grew at 5°C or higher temperatures, with optimal growth at 22°C. The upper limit of growth temperature of strain AkT22^T was

167 slightly higher than that of aks77^T. The strains grew chemolithotrophically on thiosulfate.
 168 They did not grow on tetrathionate (10 mM), elemental sulfur (0.5 g l⁻¹), sulfide (2 mM)
 169 and hydrogen gas (air/H₂ 80:20 v/v; 125 kPa in total pressure). The following organic
 170 substrates did not support growth of the strains: lactate (10 mM), acetate (10 mM),
 171 formate (10 mM), fumarate (5 mM), glucose (5 mM), maltose (5 mM), fructose (5 mM),
 172 *N*-acetyl-D-glucosamin (2 mM), sucrose (2 mM) and cellobiose (1 mM). No
 173 heterotrophic growth was observed in the complex media tested. Strains AkT22^T and
 174 aks77^T did not grow anaerobically, under nitrate-reducing conditions. In the medium
 175 containing nitrate as sole nitrogen source, strain AkT22^T did not grow but growth of strain
 176 aks77^T was observed. The strains exhibited optimum growth at NaCl concentrations of
 177 2% (w/v). The strains AkT22^T and aks77^T shared the ubiquinone-8 (UQ-8) as the sole
 178 respiratory quinone. Their polar lipid profiles are shown in Fig S5. The cellular fatty acid
 179 profiles of strains AkT22^T and aks77^T are shown in Table 2. The major cellular fatty acids
 180 (>10% of total) of strain AkT22^T were summed feature 3 (C_{16:1} ω7c and/or C_{16:1} ω6c;
 181 47.1%), summed feature 8 (C_{18:1} ω7c and/or C_{18:1} ω6c; 26.7%) and C_{16:0} (13.0%). They
 182 were also major components in the fatty acid profile of strain aks77^T, accounting for
 183 51.9%, 19.2% and 10.7%, respectively. In addition to these fatty acids, C_{10:0} 3-OH was
 184 abundantly detected in strain aks77^T (11.4%). The major fatty acids shared by strains

AkT22^T and aks77^T, C_{16:1}, C_{18:1} and C_{16:0}, are known to be dominant in *Thiomicrothabodus* species (Boden et al, 2017a, 2017b; Kojima & Fukui 2019; Liu et al. 2020). In contrast, C_{10:0} 3-OH has not been detected as major fatty acid in *Thiomicrothabodus* or related genera (Boden et al, 2017). A previous study reported that it accounted 5% in total fatty acids of *T. indica* 13-15A^T (Liu et al. 2020).

Genomic characteristics

The complete genomes of strains AkT22^T and aks77^T were successfully reconstructed as circular chromosomes, with size of 2,645,427 bp and 2,722,826 bp, respectively. Their G + C contents were 43.2% and 45.5%. Basic characteristics of the genomes are summarized in Table S1. With reference genome of *T. aquaedulcis* HaS4^T, orthoANI values were calculated for all combinations of three strains, resulting in 70–71%. In the genomes of strains AkT22^T and aks77^T, 2373 and 2501 protein-coding sequences were predicted, respectively. With these sequences, values of POCP were calculated to be as follows: AkT22^T-aks77^T, 62.7%; AkT22^T-HaS4^T, 68.1%; aks77^T-HaS4^T, 64.0%. Those of AAI were 60.9% (AkT22^T-aks77^T), 63.8% (AkT22^T-HaS4^T) and 63.6% (aks77^T-HaS4^T). In the phylogenetic tree based on the ribosomal proteins, strain AkT22^T was located in a position isolated from *Thiomicrothabodus* species and formed a cluster with

Hydrogenovibrio species (Fig. 2). In the genomes of strains AkT22^T and aks77^T, genes involved in thiosulfate oxidation (*soxXYZABCD*) were identified. They both have the *sqr* gene and lack the *dsrAB*, *aprBA* and *sat* genes. This presence-absence pattern of the sulfur oxidation genes is conserved in sulfur oxidizers of the family *Piscirickettsiaceae* (Watanabe et al., 2019). In the genome of AkT22^T, the *cbbL* and *cbbM* genes encoding two forms of ribulose-1,5-bisphosphate carboxylase/oxygenase (form I and form II RuBisCO) were identified, as is the case with *Thiomicrothrix* species (Boden et al., 2017a, 2017b). On the other hand, strain aks77 turned out to lack the *cbbM* gene encoding form II RuBisCO (Table S1).

Taxonomic assignment of the novel isolates

The low values of the 16S rRNA gene sequence identity and ANI indicated that strains AkT22^T and aks77^T respectively represent two novel species. These strains must be described as type strains of independent species, but their genus-level classification would be controvertible. The POCP values among AkT22^T, aks77^T and *T. aquaedulcis* HaS4^T were greater than 50%, proposed as threshold for genus-level delineation (Qin et al. 2020). However, POCP values greater than 50% have been observed between many combinations of strains from different genera, in various bacterial lineages (Wirth &

Whitman 2018; Watanabe et al. 2020). The AAI values among the three strains were lower than 65%, suggesting that they can be placed in different genera. Accordingly, phylogenetic analysis based on the 16S rRNA gene raised a doubt about affiliation of strain aks77^T to the genus *Thiomicrohabdus* (Fig. 1). It is also questionable to classify strain AkT22^T in this genus, as indicated by the phylogenetic analysis of the ribosomal proteins (Fig. 2). To draw conclusions about genus-level classification supported by more comprehensive analysis, the whole genomes of novel isolates and *T. aquaedulcis* HaS4^T were analyzed by using the GTDB-Tk, which classifies bacterial genomes based on phylogeny of 120 marker genes and ANI (Chaumeil et al. 2020). As a result, these strains were classified into three different genera. *T. aquaedulcis* HaS4^T was classified in the genus *Thiomicrohabdus*, along with other members of the genus included in the GTDB release 89. On the other hand, strains aks77^T and strain AkT22^T were classified as sole representatives of novel genera, respectively. In this situation, creation of two new genera must be the most reasonable and practical way to determine taxonomic positions of strains AkT22^T and aks77^T. Based on these results, *Thiosulfativibrio zosterae* gen. nov., sp. nov. and *Thiosulfatimonas sediminis sediminis* gen. nov., sp. nov. are proposed here, with the type strains of AkT22^T and aks77^T, respectively.

239   **Description of *Thiosulfativibrio* gen. nov.**

240   *Thiosulfativibrio* (Thi.o.sul.fa.ti.vi'bri.o. N.L. masc. n. *thiosulfas*, *-atis* thiosulfate; N.L.
241   masc. n. *Vibrio* a bacterial genus; N.L. masc. n. *Thiosulfativibrio* thiosulfate-oxidizing
242   vibrio).

243   This genus is circumscribed on the basis of whole-genome-based phylogeny. Cells are
244   motile and Gram-stain-negative. Grow chemolithoautotrophically by the oxidation of
245   thiosulfate. Respiratory quinone is ubiquinone-8.

246   The type species is *Thiosulfativibrio zosterae*.

247

248   **Description of *Thiosulfativibrio zosterae* gen. nov. sp. nov.**

249   *Thiosulfativibrio zosterae* (zos'te.rae. N.L. gen. n. *zosterae* of the botanical genus
250   *Zostera*).

251   Cells are motile, rod-shaped, 1.5–3.0 µm in length and 0.5–1.1 µm in width. Oxidase-
252   negative and catalase-negative. Chemolithoautotrophic growth occurs with oxidation of
253   thiosulfate. Sulfide, tetrathionate, elemental sulfur and hydrogen gas are not utilized as
254   electron donor for autotrophic growth. Heterotrophic growth is not observed on lactate,
255   acetate, formate, fumarate, glucose, maltose, fructose, *N*-acetyl-D-glucosamin, sucrose
256   and cellobiose. Growth occurs at temperatures 5–37°C, with optimum growth at 22°C.

Growth is observed at pH 5.8–8.0, with an optimum range of 6.7–7.8. Grows in the presence of 0–5% (w/v) NaCl. Ammonium is required as a nitrogen source. The G+C content of genomic DNA is 43.2 %. Major cellular fatty acids are summed feature 3 (C_{16:1} ω7c and/or C_{16:1} ω6c), summed feature 8 (C_{18:1} ω7c and/or C_{18:1} ω6c) and C_{16:0}. The type strain AkT22^T (= BCRC 81184 = NBRC 114012^T = DSM 109948^T) was isolated from leaf of eelgrass (*Zostera marina*) collected in a brackish lake in Japan (Lake Akkeshi). The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and complete genome sequence of strain AkT22^T are LC510548 and AP021888, respectively

Description of *Thiosulfatimonas* gen. nov.

Thiosulfatimonas (Thi.o.sul.fa.ti.mo'nas. N.L. masc. n. *thiosulfas*, -*atis* thiosulfate; Gr. fem. n. *monas* a unit, monad; N.L. fem. n. *Thiosulfatimonas* thiosulfate-oxidizing unit).

This genus is circumscribed on the basis of whole-genome-based phylogeny. Cells are motile and Gram-stain-negative. Grow chemolithoautotrophically by the oxidation of thiosulfate. Respiratory quinone is ubiquinone-8. The type species is *Thiosulfativibrio zosterae*.

The type species is *Thiosulfatimonas sediminis*.

275   **Description of *Thiosulfatimonas sediminis* gen. nov. sp. nov.**

276   *Thiosulfatimonas sediminis* (se.di'mi.nis. L. gen. n. *sediminis* of a sediment)

277   Cells are motile, rod-shaped, 1.4–2.8 µm in length and 0.6–0.9 µm in width. Oxidase-
278   negative and catalase-positive. Chemolithoautotrophic growth occurs with oxidation of
279   thiosulfate. Sulfide, tetrathionate, elemental sulfur and hydrogen gas are not utilized as
280   electron donor for autotrophic growth. Heterotrophic growth is not observed on lactate,
281   acetate, formate, fumarate, glucose, maltose, fructose, *N*-acetyl-D-glucosamin, sucrose
282   and cellobiose. Growth occurs at temperatures 5–37°C, with optimum growth at 22°C.
283   Growth is observed at pH 5.8–8.0, with an optimum range of 6.7–7.8. Grows in the
284   presence of 0–6% (w/v) NaCl. Nitrate and ammonium are utilized as a nitrogen source.
285   The G+C content of genomic DNA is 45.5% (genome). Major cellular fatty acids are
286   summed feature 3 (C_{16:1} ω7c and/or C_{16:1} ω6c), summed feature 8 (C_{18:1} ω7c and/or
287   C_{18:1} ω6c), C_{10:0} 3-OH and C_{16:0}.

288   The type strain aks77^T (= BCRC 81183^T = NBRC 114013^T) was isolated from sediment
289   of a brackish lake in Japan (Lake Akkeshi). The GenBank/EMBL/DDBJ accession
290   numbers for the 16S rRNA gene and complete genome sequence of strain aks77^T are
291   LC510549 and AP021889, respectively

292

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

ACKNOWLEDGMENTS

We thank A. Shinohara and K. Umezawa, Hokkaido University, for their technical assistance.

FUNDING INFORMATION

This work received no specific grant from any funding agency

REFERENCES

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can

J Biochem Physiol 37:911–917.

Boden R, Scott KM, Williams J, Russel S, Antonen K *et al.* (2017a) An evaluation of

Thiomicrospira, *Hydrogenovibrio* and *Thioalkalimicrobium*: reclassification of four

species of *Thiomicrospira* to each *Thiomicrobium* gen. nov. and *Hydrogenovibrio*,

and reclassification of all four species of *Thioalkalimicrobium* to *Thiomicrospira* Int

J Syst Evol Microbiol 67:1140–1151

311 Boden R, Scott KM, Rae AW, Hutt LP (2017b) Reclassification of *Thiomicrospira*
 312 *hydrogeniphila* (Watsuji et al. 2016) to *Thiomicrorhabdus hydrogenophila* comb. nov.,
 313 with emended description of *Thiomicrorhabdus* (Boden et al., 2017) Int J Syst Evol
 314 Microbiol 67:4205–4209

315 Brinkhoff T, Muyzer G, Wirsén CO, Kuever J. (1999a) *Thiomicrospira kuenenii* sp. nov.
 316 and *Thiomicrospira frisia* sp. nov., two mesophilic obligately chemolithoautotrophic
 317 sulfur-oxidizing bacteria isolated from an intertidal mud flat. Int J Syst Bacteriol
 318 49:385–392

319 Brinkhoff T, Muyzer G, Wirsén CO, Kuever J (1999b) *Thiomicrospira chilensis* sp. nov.,
 320 a mesophilic obligately chemolithoautotrophic sulfur-oxidizing bacterium isolated
 321 from a Thioploca mat. Int J Syst Bacteriol 49:875–879.

322 Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH (2020) GTDB-Tk: a toolkit to classify
 323 genomes with the Genome Taxonomy Database. Bioinformatics 36:1925–1927

324 Edgar RC (2004) Muscle: multiple sequence alignment with high accuracy and high
 325 throughput. Nucleic Acids Res 32:1792–1797

326 Headd B, Engel AS (2014) Biogeographic congruency among bacterial communities
 327 from terrestrial sulfidic springs Front Microbiol 5:473

328 Jolley KA, Bliss CM, Bennett JS, Bratcher HB, Brehony C *et al.* (2012) Ribosomal

329 multilocus sequence typing: universal characterization of bacteria from domain to
 330 strain. *Microbiology* 158:1005–1015

331 Kojima H, Watanabe T, Fukui M (2016) *Sulfuricaulis limicola* gen. nov., sp. nov., a sulfur
 332 oxidizer isolated from a lake. *Int J Syst Evol Microbiol* 66:266–270

333 Kojima H, Fukui M (2016) *Sulfuriflexus mobilis* gen. nov., sp. nov., a sulfur-oxidizing
 334 bacterium isolated from a brackish lake sediment. *Int J Syst Evol Microbiol* 66: 3515-
 335 3518

336 Kojima H, Fukui M (2019) *Thiomicrothrix aquaedulcis* sp. nov., a sulfur-oxidizing
 337 bacterium isolated from lake water *Int J Syst Evol Microbiol* 69:2849–2853

338 Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary
 339 Genetics Analysis across computing platforms. *Mol Biol Evol* 35:1547–1549

340 Knittel K, Kuever J, Meyerdierks A, Meinke R, Amann R et al. (2005) *Thiomicrospira*
 341 *arctica* sp. nov. and *Thiomicrospira psychrophila* sp. nov., psychrophilic, obligately
 342 chemolithoautotrophic, sulfur-oxidizing bacteria isolated from marine Arctic
 343 sediments. *Int J Syst Evol Microbiol* 55:781–786

344 Lane DJ (1991) 16S/23S rRNA sequencing. In Stackebrandt E, Goodfellow M. (editors)
 345 *Nucleic Acid Techniques in Bacterial Systematics* New York: John Wiley and Sons;
 346 pp 115–175

347 Liu X, Jiang L, Hu Q, Lyu J, Shao Z (2020) *Thiomicrothrix indica* sp. nov., an
 348 obligately chemolithoautotrophic, sulfur-oxidizing bacterium isolated from a deep-
 349 sea hydrothermal vent environment. *Int J Syst Evol Microbiol* 2020;70: 234–239
 350 Minnikin DE, Collins MD, Goodfellow M (1979) Fatty acid and polar lipid composition
 351 in the classification of *Cellulomonas*, *Oerskovia* and related taxa. *J Appl Bacteriol*
 352 47:87–95.
 353 Parks DH, Chuvochina M, Waite DW, Rinke C, Skarszewski A *et al.* (2018) A
 354 standardized bacterial taxonomy based on genome phylogeny substantially revises the
 355 tree of life. *Nature Biotech* 3:996–1004
 356 Qin QL, Xie BB, Zhang XY, Chen XL, Zhou BC *et al.* (2014). A proposed genus
 357 boundary for the prokaryotes based on genomic insights. *J Bacteriol* 196:2210–2215
 358 Tanizawa Y, Fujisawa T, Nakamura Y (2017) DFAST: a flexible prokaryotic genome
 359 annotation pipeline for faster genome publication. *Bioinformatics* 35:1037–1039. doi:
 360 10.1093/bioinformatics/btx713
 361 Watanabe T, Kojima H, Umezawa K, Hori C, Takasuka ET *et al.* (2019) Genomes of
 362 neutrophilic sulfur-oxidizing chemolithoautotrophs representing 9 proteobacterial
 363 species from 8 genera *Front Microbiol* 10:316.
 364 Watanabe M, Kojima H, Fukui M (2020) *Aerosticca soli* gen. nov., sp. nov., an aerobic

365   gammaproteobacterium isolated from crude oil-contaminated soil. Arch Microbiol
 366   202:1069–1076
 367   Watsuji TO, Hada E, Miyazaki M, Ichimura M, Takai K (2016) Thiomicrospira
 368   hydrogeniphila sp. nov., a novel aerobic, hydrogen-and sulfur-oxidizing
 369   chemolithoautotroph isolated from a seawater tank containing a block of beef tallow.
 370   Int J Syst Evol Microbiol 66:3688–3693
 371   Wirth JS, Whitman WB (2018) Phylogenomic analyses of a clade within the roseobacter
 372   group suggest taxonomic reassignments of species of the genera *Aestuariivita*,
 373   *Citricella*, *Loktanella*, *Nautella*, *Pelagibaca*, *Ruegeria*, *Thalassobius*,
 374   *Thiobacimonas* and *Tropicibacter*, and the proposal of six novel genera Int J Syst Evol
 375   Microbiol 68:2393–2411
 376   Yoon SH, Ha SM, Lim J, Kwon S, Chun J (2017) A large-scale evaluation of algorithms
 377   to calculate average nucleotide identity. Antonie van Leeuwenhoek 110:1281–1286
 378
 379

Table 1. Basic characteristics of strains AkT22^T, aks77^T and *T. aquaedulcis* HaS4^T.
 Strains: 1, AkT22^T (this study); 2, aks77^T (this study); 3, *T. aquaedulcis* HaS4^T (Kojima
 & Fukui 2019; Watanabe et al. 2019)

Characteristic	1	2	3
Cell size (length/width, μm)	1.5–3.0 / 0.5–1.1	1.4–2.8 / 0.6–0.9	1.6–2.5 / 0.7–0.9
Catalase activity	-	+	-
Growth on tetrathionate	-	-	+
Growth on elemental sulfur	-	-	+
Growth on sulfide	-	-	+
Utilization of nitrate as nitrogen source	-	+	+
Optimal temperature for growth (range)	22 (5-37)	22 (5-32)	22 (0-25)
Optimal pH for growth (range)	6.7-7.8 (5.8-8.0)	7.0-7.9 (5.8-8.5)	6.6-7.4 (6.2-8.8)
G + C content (%)	43.2	45.5	45.3
Isolation source	Eelgrass	Lake sediment	Lake water

Table 2. Cellular fatty acids profiles of strains AkT22^T, aks77^T and *T. aquaedulcis* HaS4^T.
 Strains: 1, AkT22^T (this study); 2, aks77^T (this study); 3, *T. aquaedulcis* HaS4^T (Kojima& Fukui 2019). Summed feature 2 contains C_{12:0} aldehyde, unknown 10.928, C_{14:0} 3-OH and/or iso-C_{16:1} I. Summed feature 3 contains C_{16:1} ω7c and/or C_{16:1} ω6c. Summed feature 8 contains C_{18:1}ω7c and/or C_{18:1}ω6c.

Fatty acids	1	2	3
C _{9:0} 3-OH	-	0.1	-
C _{10:0} 3-OH	2.0	11.4	0.6
C _{10:0}	0.1	1.4	-
C _{11:0}	0.1	0.1	-
C _{12:0} 3-OH	0.3	-	0.1
C _{12:0}	4.6	2.4	2.6
C _{13:0}	0.2	-	-
C _{14:0}	2.0	0.2	0.3
C _{15:1} ω6c	0.1	0.1	-
C _{16:1} ω5c	0.2	-	-
C _{16:0}	13.0	10.7	16.1
C _{17:1} ω8c	0.2	0.2	0.2
C _{17:1} ω6c	0.7	0.4	0.3
C _{17:0}	0.7	0.5	0.7
C _{18:1} ω9c	0.1	-	-
C _{18:1} ω5c	0.5	0.2	0.3
C _{18:0}	1.3	1.0	3.7
C _{18:1} ω7c 11-methyl	0.2	-	-
C _{20:1} ω7c	-	-	0.2
Summed feature 2	0.2	0.2	0.1
Summed feature 3	47.1	51.9	45.7
Summed feature 8	26.7	19.2	29.3

Figure legends

Fig. 1 Phylogenetic positions of strains AkT22^T and aks77^T, based on the 16S rRNA gene sequence analysis. The tree was obtained with maximum likelihood approach, based on Kimura 2-parameter model with gamma distribution and invariant sites. All positions containing gaps and missing data were eliminated (1250 positions in the final dataset). Bar represents substitutions per site. Numbers on nodes represent percentage values of 1000 bootstrap resampling (values larger than 50 are shown).

Fig. 2 Phylogenetic tree based on the 53 ribosomal proteins encoded in the genomes. This unrooted was obtained with maximum likelihood approach. Evolutionary distances were calculated using Jones-Taylor-Thornton model, with among-site rate variation modeled with a gamma distribution and invariant sites. All positions containing gaps and missing data were eliminated (6663 amino acid positions in the final dataset). Bar represents substitutions per site. Numbers on nodes represent percentage values of 500 bootstrap resampling. Accession numbers of the genomes in NCBI database are shown in parentheses.

Thiosulfativibrio zosterae gen. nov., sp. nov., and *Thiosulfatimonas sediminis* gen.
nov., sp. nov.

Jun Mochizuki^{1,2}, Hisaya Kojima^{*1} and Manabu Fukui¹

¹ The Institute of Low Temperature Science, Hokkaido University, Sapporo, Japan

² Graduate School of Environmental Science, Hokkaido University, Sapporo, Japan

*Corresponding author. E-mail: kojimah@pop.lowtem.hokudai.ac.jp

---

Fig S1. Neighbor-joining tree based on the 16S rRNA gene sequences. The tree was obtained with Kimura 2-parameter model with gamma distribution. All positions containing gaps and missing data were eliminated and there were a total of 1250 positions in the final dataset. Bar represents substitutions per site. Numbers on nodes represent percentage values of 1000 bootstrap resampling (values larger than 50 are shown).

Fig S2. Minimum evolution tree based on the 16S rRNA gene sequences. The tree was obtained with Kimura 2-parameter model with gamma distribution. All positions containing gaps and missing data were eliminated and there were a total of 1250 positions in the final dataset. Bar represents substitutions per site. Numbers on nodes represent percentage values of 1000 bootstrap resampling (values larger than 50 are shown).

Fig. S3. Transmission electron micrographs of strain AkT22^T (A) and strain aks77^T (B). Bar, 1 μ m. Ultra-thin sections (70 nm) were stained with uranyl acetate.

Fig S4. Scanning electron micrographs of strain AkT22^T (A) and strain aks77^T (B). Bar, 1 μm. Cells were coated with a layer of osmium (30 nm).

Fig S5. Polar lipid profiles of strain AkT22^T (A) and strain aks77^T (B). PG, Phosphatidylglycerol; PE, phosphatidylethanolamine; LPE, Lyso-phosphatidylethanolamine.

Table S1. Genomic characteristics of strain AkT22^T strain aks77^T.

	AkT22 ^T	aks77 ^T
Accession number	AP021888	AP021889
Genome size (bp)	2,645,427	2,722,826
G + C content (%)	43.22	45.49
Number of		
Protein coding genes	2373	2501
rRNA genes	9	12
tRNA genes	44	53
tmRNA genes	1	1
<i>cbbL</i> gene	2	2
<i>cbbM</i> gene	1	0
CRISPR loci	0	1