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

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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, *Thiomicrorhabdus 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, *Thiosulfativibrio 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.

Keywords: Sulfur-oxidizing bacteria; chemolithoautotroph; *Thiomicrorhabdus*; brackish lake; novel genus.

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 1016). 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

59 Strains AkT22^T and aks77^T were enriched and isolated from samples of eelgrass and
60 sediment, respectively. The samples were collected at a site (43.05N, 144.89E), in Lake
61 Akkeshi, a brackish lake in Japan. The sample of eelgrass was inoculated into a
62 bicarbonate-buffured low-salt defined medium, which comprised (l^{-1}): 2.5 g $Na_2S_2O_3 \cdot$
63 $5H_2O$, 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,
64 1 ml trace element solution, 1 ml selenite-tungstate solution, 1 ml vitamin mixture
65 solution, 30 ml $NaHCO_3$ solution. The medium and respective stock solutions were
66 prepared as described previously (Kojima et al. 2016). The strain was isolated in pure
67 culture by repeated serial dilution and agar shake dilution. The agar shake tubes did not
68 contain oxygen scavenger, and headspace was filled with air. The resulting pure culture
69 was designated as strain AkT22^T. The sediment sample was inoculated into a medium
70 used in a previous study (Kojima & Fukui 2016), which contained 5 g $Na_2S_2O_3 \cdot 5H_2O$,
71 20 g NaCl, 3 g $MgCl_2 \cdot 6H_2O$, and 0.3 g $MgSO_4 \cdot 7H_2O$. The other components were same
72 as the medium used for isolation of AkT22^T. After four times transfer to medium of the
73 same composition (0.4% v/v), the medium was changed to the medium used for isolation
74 of AkT22^T, with which strain aks77^T was isolated by repeated serial dilution. The
75 enrichment and isolation of both strains were performed at 22°C. Purity was routinely
76 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 *Thiomicrorhabdus*, *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 caldicuralii* 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 6.6–8.1with 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 $Na_2S_2O_3$ and $NaNO_3$ (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 NH_4Cl in the basal medium with $NaNO_3$ (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 Dsicussion

Phylogeny based on the 16S rRNA gene

149 The phylogenetic positions of the novel isolates were identified by analyzing their 16S
150 rRNA genes sequences. Among cultured bacterial strains, *Thiomicrorhabdus aquaedulcis*
151 HaS4^T showed the highest sequence identities to strains AkT22^T (94%) and aks77^T (95%).
152 Only for strain AkT22^T, there were some environmental clones which showed sequence
153 identity higher than that of *T. aquaedulcis* HaS4^T. The clones of high identity (99%) were
154 reported from a terrestrial sulfidic spring (Headd & Engel 2014). The sequence identity
155 between strains AkT22^T and aks77^T was 93%. Phylogenetic tree constructed with the
156 maximum-likelihood method is shown in Fig. 1. Almost identical branching patterns were
157 observed in trees constructed with methods of neighbor-joining and minimum evolution
158 (Figs. S1 and S2).
159
160 **Phenotypic characteristics**
161 Basic characteristics of strains AkT22^T and aks77^T are summarized in Table 1 and
162 respective species descriptions. Cells of the strains were rod-shaped, motile, Gram-stain-
163 negative and oxidase-negative. Electron microscopic images of the cells are shown in
164 Figure S3 (TEM) and S4 (SEM). Strain AkT22^T was catalase-negative, whereas strain
165 aks77^T was catalase-positive. The strains grew at 5°C or higher temperatures, with
166 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 gl^{-1}), sulfide (2 mM)
169 and hydrogen gas (air/H_2 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 NaCI 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

185 AkT22^T and aks77^T, $C_{16:1}$, $C_{18:1}$ and $C_{16:0}$, are known to be dominant in *Thiomicrorhabdus*
186 species (Boden et al, 2017a, 2017b; Kojima & Fukui 2019; Liu et al. 2020). In contrast,
187 $C_{10:0}$ 3-OH has not been detected as major fatty acid in *Thiomicrorhabdus* or related
188 genera (Boden et al, 2017). A previous study reported that it accounted 5% in total fatty
189 acids of *T. indica* 13-15A^T (Liu et al. 2020).
190
191 **Genomic characteristics**
192 The complete genomes of strains AkT22^T and aks77^T were successfully reconstructed
193 as circular chromosomes, with size of 2,645,427 bp and 2,722,826 bp, respectively. Their
194 G + C contents were 43.2% and 45.5%. Basic characteristics of the genomes are
195 summarized in Table S1. With reference genome of *T. aquaedulcis* HaS4^T, orthoANI
196 values were calculated for all combinations of three strains, resulting in 70–71%. In the
197 genomes of strains AkT22^T and aks77^T, 2373 and 2501 protein-coding sequences were
198 predicted, respectively. With these sequences, values of POCP were calculated to be as
199 follows: AkT22^T-aks77^T, 62.7%; AkT22^T-HaS4^T, 68.1%; aks77^T-HaS4^T, 64.0%. Those
200 of AAI were 60.9% (AkT22^T-aks77^T), 63.8% (AkT22^T-HaS4^T) and 63.6% (aks77^T-
201 HaS4^T). In the phylogenetic tree based on the ribosomal proteins, strain AkT22^T was
202 located in a position isolated from *Thiomicrorhabdus* 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 *Piscirickettsiacea* (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 *Thiomicrorhabdus* 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 &

221 Whitman 2018; Watanabe et al. 2020). The AAI values among the three strains were lower
222 than 65%, suggesting that they can be placed in different genera. Accordingly,
223 phylogenetic analysis based on the 16S rRNA gene raised a doubt about affiliation of
224 strain aks77^T to the genus *Thiomicrorhabdus* (Fig. 1). It is also questionable to classify
225 strain AkT22^T in this genus, as indicated by the phylogenetic analysis of the ribosomal
226 proteins (Fig. 2). To draw conclusions about genus-level classification supported by more
227 comprehensive analysis, the whole genomes of novel isolates and *T. aquaedulcis* HaS4^T
228 were analyzed by using the GTDB-Tk, which classifies bacterial genomes based on
229 phylogeny of 120 marker genes and ANI (Chaumeil et al. 2020). As a result, these strains
230 were classified into three different genera. *T. aquaedulcis* HaS4^T was classified in the
231 genus *Thiomicrorhabdus*, along with other members of the genus included in the GTDB
232 release 89. On the other hand, strains aks77^T and strain AkT22^T were classified as sole
233 representatives of novel genera, respectively. In this situation, creation of two new genera
234 must be the most reasonable and practical way to determine taxonomic positions of strains
235 AkT22^T and aks77^T. Based on these results, *Thiosulfativibrio zosterae* gen. nov., sp. nov.
236 and *Thiosulfatimonas sediminis sediminis* gen. nov., sp. nov. are proposed here, with the
237 type strains of AkT22^T and aks77^T, respectively.
238

Description of *Thiosulfativibrio* gen. nov.

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

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*.

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

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

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

257 Growth is observed at pH 5.8–8.0, with an optimum range of 6.7–7.8. Grows in the
258 presence of 0–5% (w/v) NaCl. Ammonium is required as a nitrogen source. The G+C
259 content of genomic DNA is 43.2 %. Major cellular fatty acids are summed feature 3 ($C_{16:1}$
260 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
261 strain AkT22^T (= BCRC 81184 = NBRC 114012^T = DSM 109948^T) was isolated from
262 leaf of eelgrass (*Zostera marina*) collected in a brackish lake in Japan (Lake Akkeshi).
263 The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and complete
264 genome sequence of strain AkT22^T are LC510548 and AP021888, respectively
265
266 **Description of *Thiosulfatimonas* gen. nov.**
267 *Thiosulfatimonas* (Thi.o.sul.fa.ti.mo'nas. N.L. masc. n. *thiosulfas*, *-atis* thiosulfate; Gr.
268 fem. n. *monas* a unit, monad; N.L. fem. n. *Thiosulfatimonas* thiosulfate-oxidizing unit).
269 This genus is circumscribed on the basis of whole-genome-based phylogeny. Cells are
270 motile and Gram-stain-negative. Grow chemolithoautotrophically by the oxidation of
271 thiosulfate. Respiratory quinone is ubiquinone-8. The type species is *Thiosulfativibrio*
272 *zosterae*.
273 The type species is *Thiosulfatimonas sediminis*.
274

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

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

Cells are motile, rod-shaped, 1.4–2.8 µm in length and 0.6–0.9 µm in width. Oxidase-negative and catalase-positive. Chemolithoautotrophic growth occurs with oxidation of thiosulfate. Sulfide, tetrathionate, elemental sulfur and hydrogen gas are not utilized as electron donor for autotrophic growth. Heterotrophic growth is not observed on lactate, acetate, formate, fumarate, glucose, maltose, fructose, *N*-acetyl-D-glucosamin, sucrose 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 0–6% (w/v) NaCl. Nitrate and ammonium are utilized as a nitrogen source. The G+C content of genomic DNA is 45.5% (genome). 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), $C_{10:0}$ 3-OH and $C_{16:0}$.

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

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

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

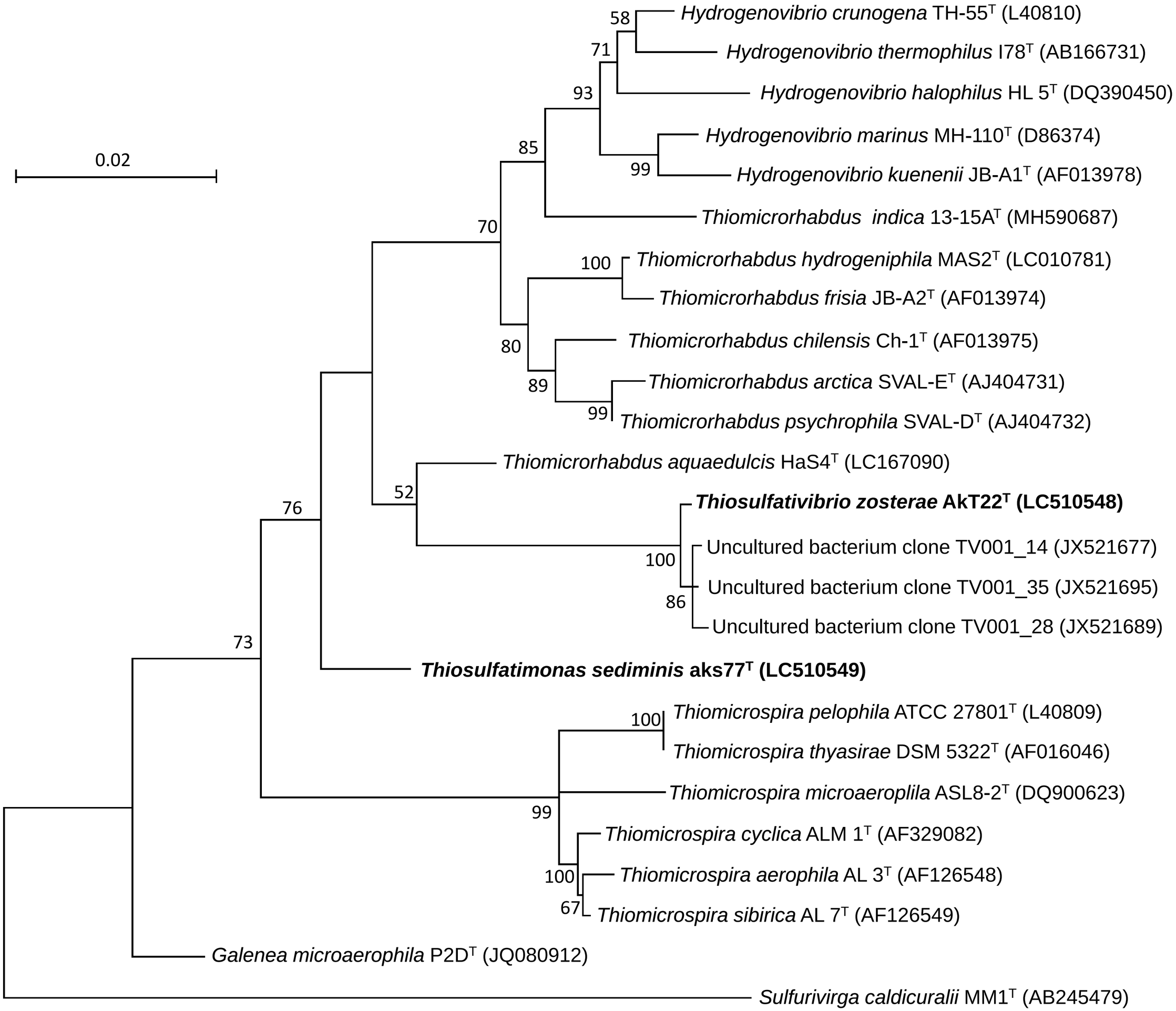
Characteristic	1	2	3
Cell size (length/width, μm)	1.5–3.0 / 0.5–1.1	1.4–2.8 / 0.6–0.9	1.6–2.5 / 0.7–0.9
Catalase activity	-	+	-
Growth on tetrathionate	-	-	+
Growth on elemental sulfur	-	-	+
Growth on sulfide	-	-	+
Utilization of nitrate as nitrogen source	-	+	+
Optimal temperature for growth (range)	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

383

384 Table 2. Cellular fatty acids profiles of strains AkT22^T, aks77^T and *T. aquaedulcis*
385 HaS4^T.
386 Strains: 1, AkT22^T (this study); 2, aks77^T (this study); 3, *T. aquaedulcis* HaS4^T
387 (Kojima& Fukui 2019). Summed feature 2 contains $C_{12:0}$ aldehyde, unknown 10.928,
388 $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I. Summed feature 3 contains $C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c.
389 Summed feature 8 contains $C_{18:1}$ω7c and/or $C_{18:1}\omega$6c.
390

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

391 Figure legends
392
393 Fig. 1 Phylogenetic positions of strains AkT22^{T} and aks77^{T}, based on the 16S rRNA
394 gene sequence analysis. The tree was obtained with maximum likelihood approach,
395 based on Kimura 2-parameter model with gamma distribution and invariant sites. All
396 positions containing gaps and missing data were eliminated (1250 positions in the final
397 dataset). Bar represents substitutions per site. Numbers on nodes represent percentage
398 values of 1000 bootstrap resampling (values larger than 50 are shown).
399
400 Fig. 2 Phylogenetic tree based on the 53 ribosomal proteins encoded in the genomes.
401 This unrooted was obtained with maximum likelihood approach. Evolutionary distances
402 were calculated using Jones-Taylor-Thornton model, with among-site rate variation
403 modeled with a gamma distribution and invariant sites. All positions containing gaps
404 and missing data were eliminated (6663 amino acid positions in the final dataset). Bar
405 represents substitutions per site. Numbers on nodes represent percentage values of 500
406 bootstrap resampling. Accession numbers of the genomes in NCBI database are shown
407 in parentheses.


0.02
58
71
93
85
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100
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100
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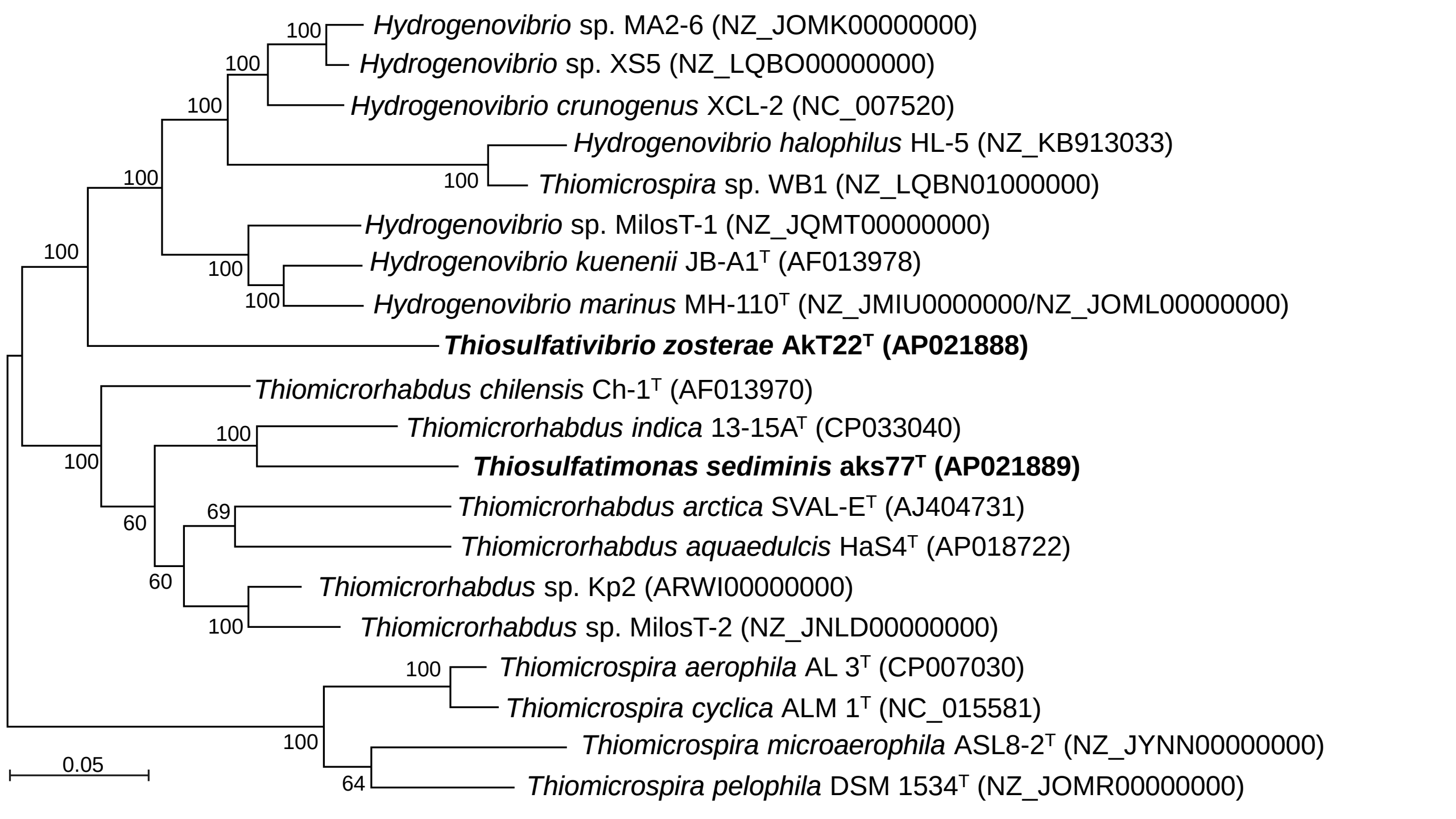
100
67
86
Hydrogenovibrio crunogena TH-55T (L40810)
Hydrogenovibrio thermophilus I78T (AB166731)
Hydrogenovibrio halophilus HL 5T (DQ390450)
Hydrogenovibrio marinus MH-110T (D86374)
Hydrogenovibrio kuenenii JB-A1T (AF013978)
Thiomicrorhabdus indica 13-15AT (MH590687)
Thiomicrorhabdus hydrogeniphila MAS2T (LC010781)
Thiomicrorhabdus frisia JB-A2T (AF013974)
Thiomicrorhabdus chilensis Ch-1T (AF013975)
Thiomicrorhabdus arctica SVAL-ET (AJ404731)
Thiomicrorhabdus psychrophila SVAL-DT (AJ404732)
Thiomicrorhabdus aquaedulcis HaS4T (LC167090)
Thiosulfativibrio zosterae AkT22T (LC510548)
Uncultured bacterium clone TV001_14 (JX521677)
Uncultured bacterium clone TV001_35 (JX521695)
Uncultured bacterium clone TV001_28 (JX521689)
Thiosulfatimonas sediminis aks77T (LC510549)
Thiomicrospira pelophila ATCC 27801T (L40809)
Thiomicrospira thyasirae DSM 5322T (AF016046)
Thiomicrospira microaeroplila ASL8-2T (DQ900623)
Thiomicrospira cyclica ALM 1T (AF329082)
Thiomicrospira aerophila AL 3T (AF126548)
Thiomicrospira sibirica AL 7T (AF126549)
Galenea microaerophila P2DT (JQ080912)
Sulfurivirga caldicuralii MM1T (AB245479)


100
100
100
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100
100
60
60
100
100
100
64
0.05
Hydrogenovibrio sp. MA2-6 (NZ_JOMK00000000)
Hydrogenovibrio sp. XS5 (NZ_LQBO00000000)
Hydrogenovibrio crunogenus XCL-2 (NC_007520)
Hydrogenovibrio halophilus HL-5 (NZ_KB913033)
Thiomicrospira sp. WB1 (NZ_LQBN01000000)
Hydrogenovibrio sp. MilosT-1 (NZ_JQMT00000000)
Hydrogenovibrio kuenenii JB-A1^{T} (AF013978)
Hydrogenovibrio marinus MH-110^{T} (NZ_JMIU0000000/NZ_JOML00000000)
***Thiosulfativibrio zosterae* AkT22^{T} (AP021888)**
Thiomicrorhabdus chilensis Ch-1^{T} (AF013970)
Thiomicrorhabdus indica 13-15A^{T} (CP033040)
***Thiosulfatimonas sediminis* aks77^{T} (AP021889)**
Thiomicrorhabdus arctica SVAL-E^{T} (AJ404731)
Thiomicrorhabdus aquaedulcis HaS4^{T} (AP018722)
Thiomicrorhabdus sp. Kp2 (ARWI00000000)
Thiomicrorhabdus sp. MilosT-2 (NZ_JNLD00000000)
Thiomicrospira aerophila AL 3^{T} (CP007030)
Thiomicrospira cyclica ALM 1^{T} (NC_015581)
Thiomicrospira microaerophila ASL8-2^{T} (NZ_JYNN00000000)
Thiomicrospira pelophila DSM 1534^{T} (NZ_JOMR00000000)

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

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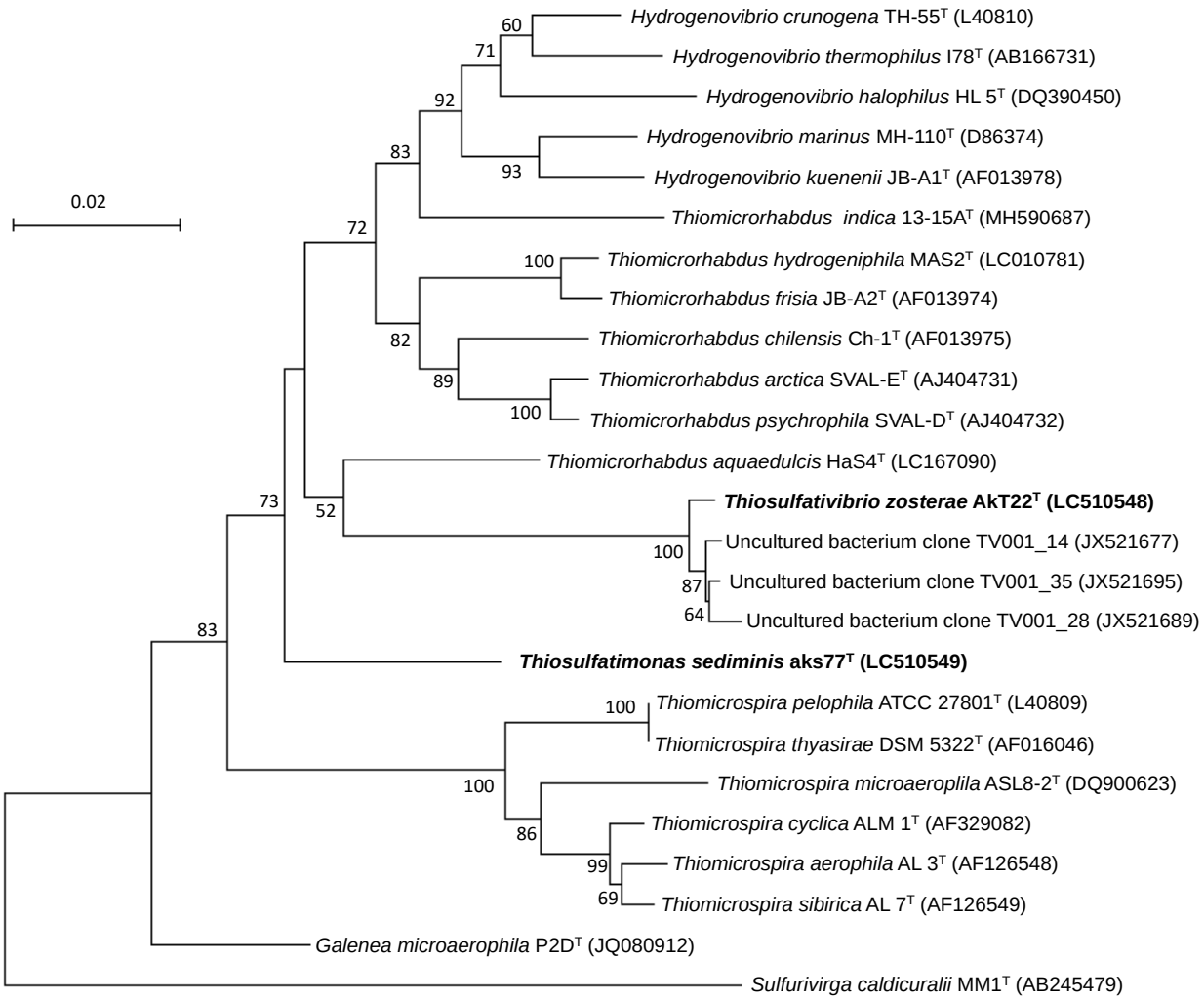

Fig S1. Neighbor-joining tree based on the 16S rRNA gene sequences. The tree was obtained with Kimura 2-parameter model with gamma distribution. All positions containing gaps and missing data were eliminated and there were a total of 1250 positions in the final dataset. Bar represents substitutions per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

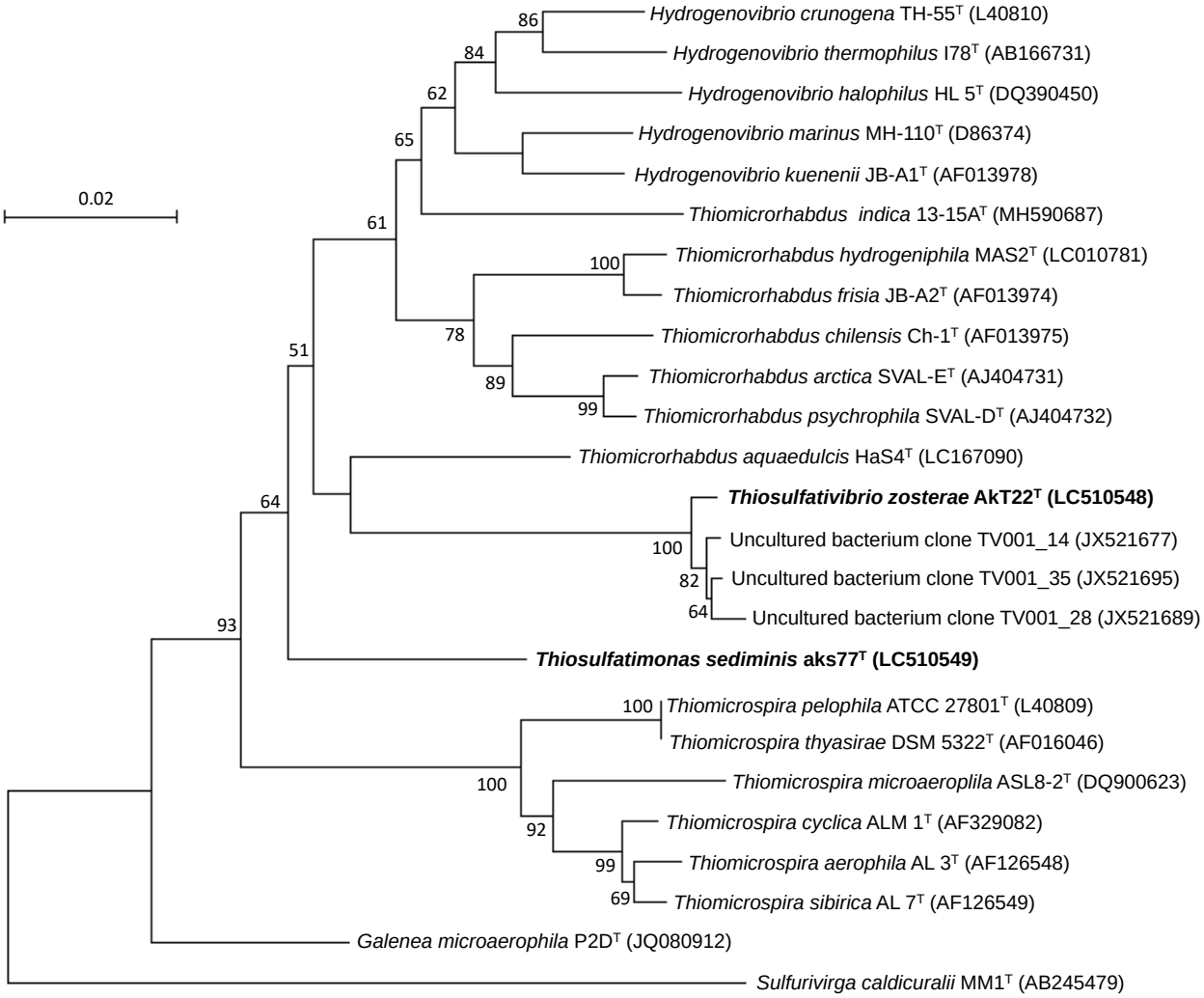

Fig S2. Minimum evolution tree based on the 16S rRNA gene sequences. The tree was obtained with Kimura 2-parameter model with gamma distribution. All positions containing gaps and missing data were eliminated and there were a total of 1250 positions in the final dataset. Bar represents substitutions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

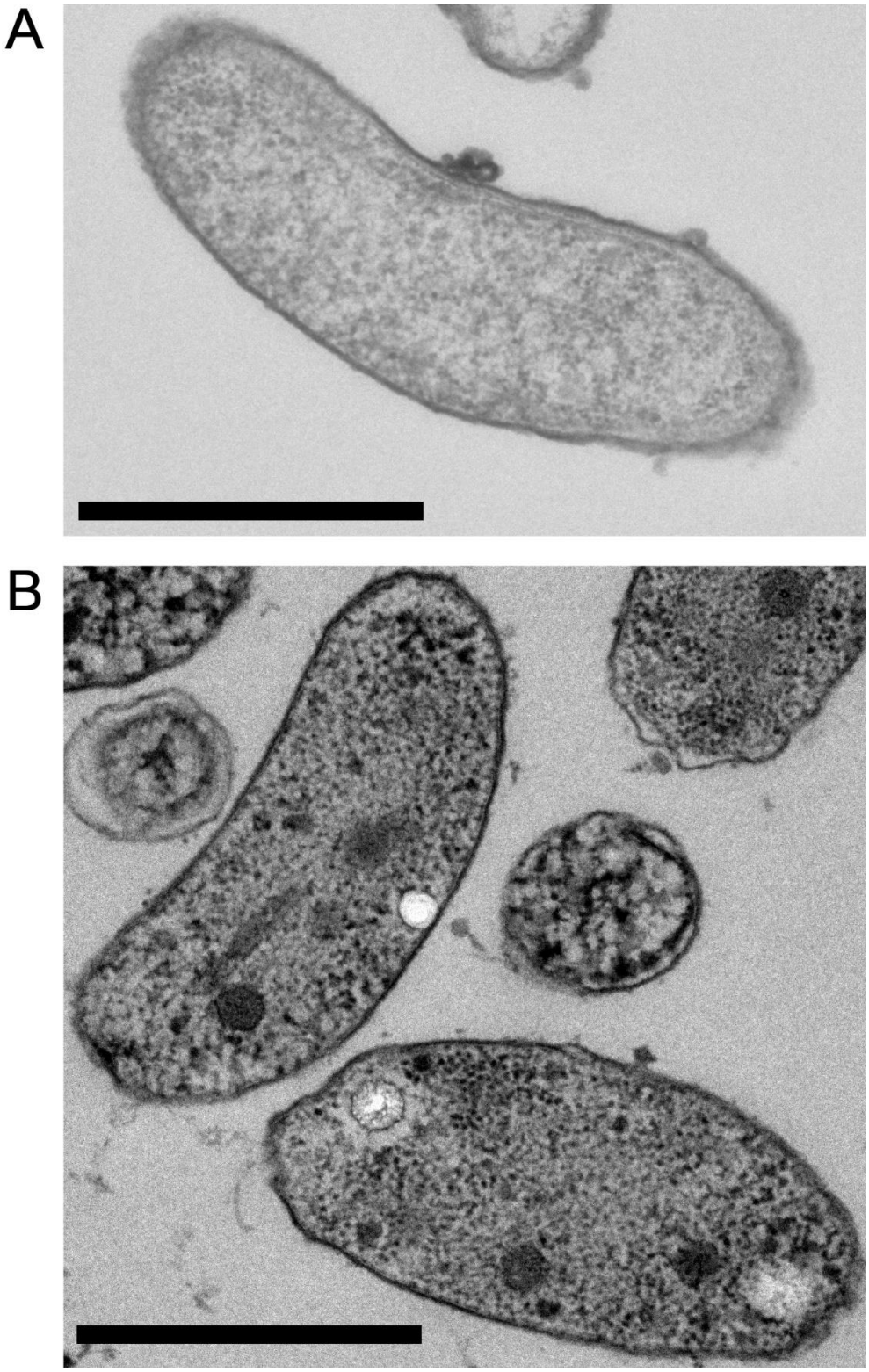

Fig. S3. Transmission electron micrographs of strain AkT22^{T} (A) and strain aks77^{T} (B). Bar, 1 μm. Ultra-thin sections (70 nm) were stained with uranyl acetate.

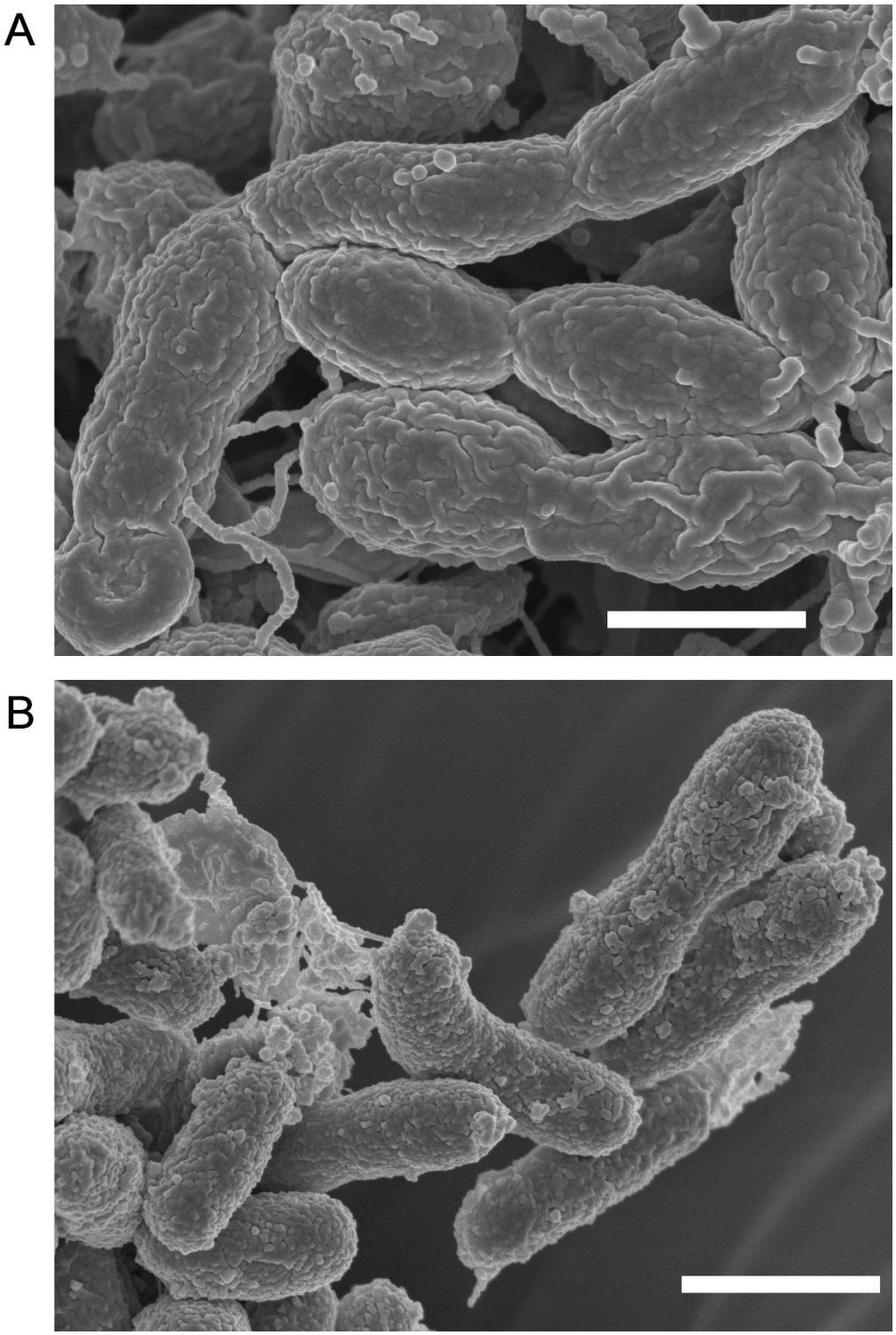

Fig S4. Scanning electron micrographs of strain AkT22^{T} (A) and strain aks77^{T} (B). Bar, 1 μm. Cells were coated with a layer of osmium (30 nm).

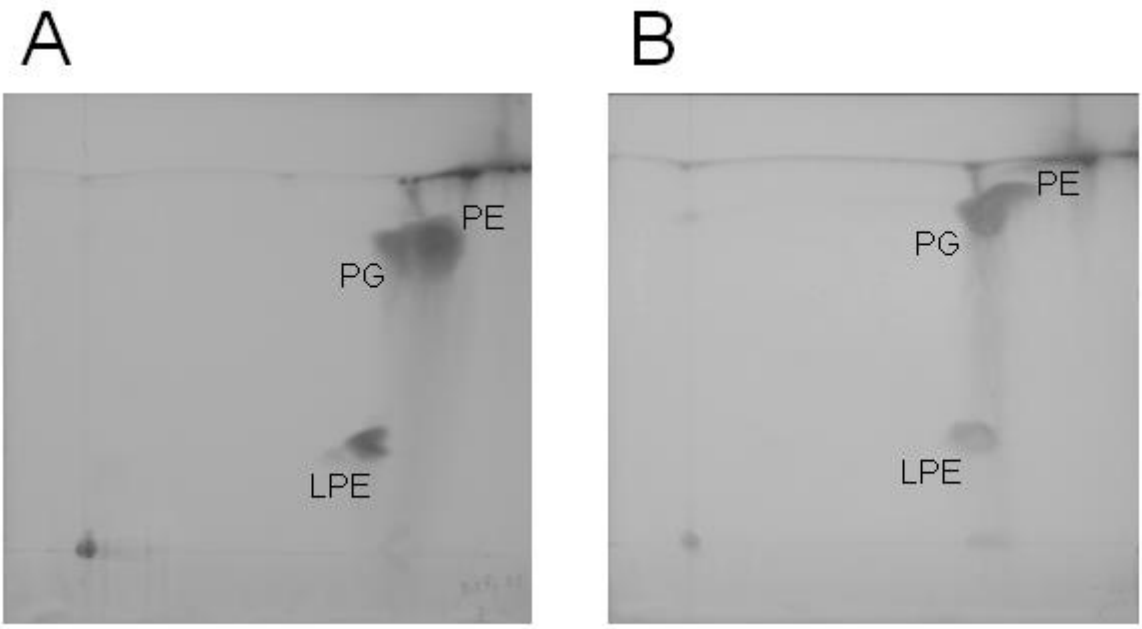

Fig S5. Polar lipid profiles of strain AkT22^T (A) and strain aks77^T (B). PG, Phosphatidylglycerol; PE, phosphatidylethanolamine; LPE, Lyso-phosphatidylethanolamine.

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
cbbL gene	2	2
cbbM gene	1	0
CRISPR loci	0	1