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2 ***Thiosulfativibrio zosteræ* gen. nov., sp. nov., and *Thiosulfatimonas***
3 ***sediminis* gen. nov., sp. nov.**

4

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

18

19 Abstract

20 Aerobic, Gram-stain-negative, obligately chemolithoautotrophic thiosulfate-oxidizing
21 bacteria, strains AkT22^T and aks77^T were isolated from a brackish lake in Japan. Strains
22 AkT22^T and aks77^T were isolated from samples of eelgrass and sediment, respectively.
23 Growth on sulfide, tetrathionate, elemental sulfur, and organic substrates was not
24 observed for both strains. Growth of the strains was observed at 5°C or higher temperature,
25 with optimum growth at 22°C. Strain AkT22^T grew at a pH range of 5.8–8.0, with
26 optimum growth at pH 6.7–7.8. Strain aks77^T grew at a pH range of 5.8–8.5, with
27 optimum growth at pH 7.0–7.9. Major cellular fatty acids (>10% of total) of strain
28 AkT22^T were C_{16:1}, C_{18:1}, and C_{16:0}. The sole respiratory quinone was ubiquinone-8 in
29 both strains. The genome of strain AkT22^T consisted of a circular chromosome, with size
30 of approximately 2.6 Mbp and G + C content of 43.2%. Those values of the genome of
31 strain aks77^T were *ca.* 2.7 Mbp and 45.5%, respectively. Among cultured bacteria,
32 *Thiomicrothabodus aquaedulcis* HaS4^T showed the highest sequence identities of the 16S
33 rRNA gene, to strains AkT22^T (94%) and aks77^T (95%). On the basis of these results,
34 *Thiosulfatovibrio zosteriae* gen. nov., sp. nov. and *Thiosulfatimonas sediminis* gen. nov.,
35 sp. nov. are proposed, with type strains of AkT22^T (= BCRC 81184^T = NBRC 114012^T =
36 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

41 **Introduction**

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

56

57 **Materials and methods**

58 **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-buffred low-salt defined medium, which comprised (l⁻¹): 2.5 g Na₂S₂O₃ ·
63 5H₂O, 0.2 g MgCl₂ · 6H₂O, 0.1 g CaCl₂ · 2H₂O, 0.1 g NH₄Cl, 0.1 g KH₂PO₄, 0.1 g KCl,
64 1 ml trace element solution, 1 ml selenite-tungstate solution, 1 ml vitamin mixture
65 solution, 30 ml NaHCO₃ 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 Na₂S₂O₃ · 5H₂O,
71 20 g NaCl, 3 g MgCl₂ · 6H₂O, and 0.3 g MgSO₄ · 7H₂O. 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 *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 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**

95 For phenotypic characterization of the strains, a medium of the following composition
96 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
97 $CaCl_2 \cdot 2H_2O$, 0.1 g NH_4Cl , 0.1 g KH_2PO_4 , 0.1 g KCl , 1 ml trace element solution, 1 ml
98 selenite-tungstate solution, 30 ml $NaHCO_3$ solution. Culturing experiments were
99 performed at 22°C without shaking unless otherwise specified. The Gram-staining test
100 was conducted with a kit (Fluka). Morphology of the cells were observed with phase-
101 contrast light microscopy, transmission electron microscopy (TEM) and electron
102 microscopy (SEM). Oxidase activity was tested using an oxidase test reagent
103 (bioMérieux). Catalase activity was assessed by pouring 3% H_2O_2 solution onto a pellet
104 of cells. For chemotaxonomic characterization, strains AkT22^T and aks77^T were grown
105 in the basal medium supplemented with the vitamin solution (1 ml l^{-1}). Cellular fatty acid
106 profile of each strain was analyzed using the Sherlock Microbial Identification System
107 Version 6.0 (MIDI) with database TSBA6. Respiratory quinones and polar lipids were
108 analyzed as described previously (Bligh & Dyer 1959; Minnikin et al. 1979). Effects of
109 temperature on growth were examined by culturing strains at 0, 5, 8, 13, 15, 18, 22, 25,
110 28, 30, 32, 37 and 45°C. Effects of salt concentration on growth was examined by
111 culturing the strains in the basal medium supplemented with various concentration of
112 $NaCl$, ranging from 0 to 12% (w/v) at 1.0% intervals. To examine effects of pH on growth,

113 the strains were cultured at 20 different pH values respectively. The medium for pH test
114 was prepared as described previously (Kojima et al. 2016), but vitamins were omitted.
115 The tested pH range and buffering reagents for strain AkT22^T were as follows; pH 5.7–
116 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;
117 pH 8.7–9.5 with CHES. Those for strain aks77^T were as follows; pH 5.7–7.0 with MES;
118 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
119 with CHES. Utilization of electron donors was tested in the basal medium supplemented
120 with one of the substances listed later. Anaerobic growth of the strains was tested in the
121 presence of Na₂S₂O₃ and NaNO₃ (10 mM each). Heterotrophic growth in complex liquid
122 media was tested for Reasoner's 2A broth (R2A) broth (Daigo), one-tenth-strength R2A,
123 nutrient broth (Difco), LB broth Miller (Merck) and tryptone soya broth (Oxoid).
124 Utilization of nitrate as nitrogen source was tested by replacing NH₄Cl in the basal
125 medium with NaNO₃ (0.2 g l⁻¹).

126

127 **Genomic characterization**

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

131 sequenced using PacBio RS II platform. Assembly was performed using
132 RS_HGAP_Assembly.3 to generate a linear contig with average coverage of 349-fold,
133 which were manually converted to a circular chromosome. For the resulting genome
134 sequences, values of the average nucleotide identity (ANI) were calculated based on
135 OrthoANIu algorithm (Yoon et al. 2017), by using ANI calculator available in
136 EzBioCloud. The genome sequences were annotated with DFAST (Tanizawa et al. 2013).
137 Based on the annotations, percentage of conserved proteins (POCP) values were
138 calculated as described previously (Qin et al. 2014). Two-way average amino acid identity
139 (AAI) scores were calculated by using an online tool, AAI calculator from the Kostas lab
140 (<http://enve-omics.ce.gatech.edu/>). Phylogenetic analysis based on the 53 ribosomal
141 proteins was performed as described previously (Jolley et al. 2012; Kojima & Fukui 2019).
142 Whole genome-based phylogenetic analysis was conducted with the Genome Taxonomy
143 Database (GTDB) (Parks et al. 2018). For the strains Akt22^T and aks77^T, their taxonomic
144 assignments in GTDB (release 89) were identified by using GTDB-Tk (Chaumeil et al.
145 2020).

146

147 **Results and Discussion**

148 **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, *Thiomicroorhabdus 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 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

185 AkT22^T and aks77^T, C_{16:1}, C_{18:1} and C_{16:0}, are known to be dominant in *Thiomicrothabodus*
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 *Thiomicrothabodus* 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 *Thiomicrothabodus* species and formed a cluster with

203 *Hydrogenovibrio* species (Fig. 2). In the genomes of strains AkT22^T and aks77^T, genes
204 involved in thiosulfate oxidation (*soxXYZABCD*) were identified. They both have the *sqr*
205 gene and lack the *dsrAB*, *aprBA* and *sat* genes. This presence-absence pattern of the sulfur
206 oxidation genes is conserved in sulfur oxidizers of the family *Piscirickettsiaceae*
207 (Watanabe et al., 2019). In the genome of AkT22^T, the *cbbL* and *cbbM* genes encoding
208 two forms of ribulose-1,5-bisphosphate carboxylase/oxygenase (form I and form II
209 RuBisCO) were identified, as is the case with *Thiomicrothabodus* species (Boden et al,
210 2017a, 2017b). On the other hand, strain aks77 turned out to lack the *cbbM* gene encoding
211 form II RuBisCO (Table S1).

212

213 **Taxonomic assignment of the novel isolates**

214 The low values of the 16S rRNA gene sequence identity and ANI indicated that strains
215 AkT22^T and aks77^T respectively represent two novel species. These strains must be
216 described as type strains of independent species, but their genus-level classification would
217 be controvertible. The POCP values among AkT22^T, aks77^T and *T. aquaedulcis* HaS4^T
218 were greater than 50%, proposed as threshold for genus-level delineation (Qin et al. 2020).
219 However, POCP values greater than 50% have been observed between many
220 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 *Thiomicrohabdus* (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 *Thiomicrohabdus*, 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, *Thiosulfat vibrio zosteræ* 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

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.

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 *zosteriae*.

273 The type species is *Thiosulfatimonas sediminis*.

274

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} ω 7c and/or C_{16:1} ω 6c), summed feature 8 (C_{18:1} ω 7c and/or
287 C_{18:1} ω 6c), 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

293 **CONFLICTS OF INTEREST**

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

295

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378
379

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}ω6c.
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

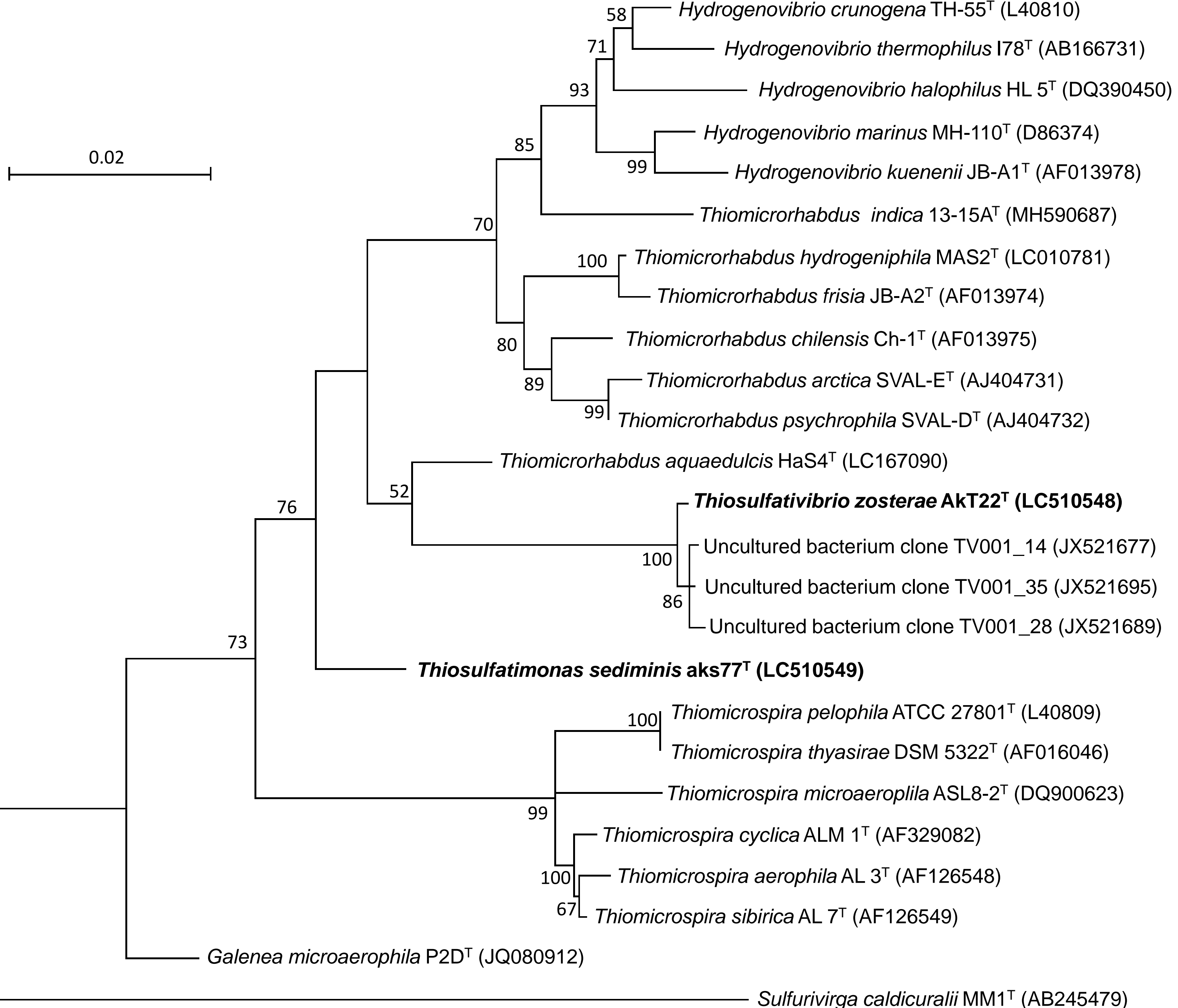
403 modeled with a gamma distribution and invariant sites. All positions containing gaps

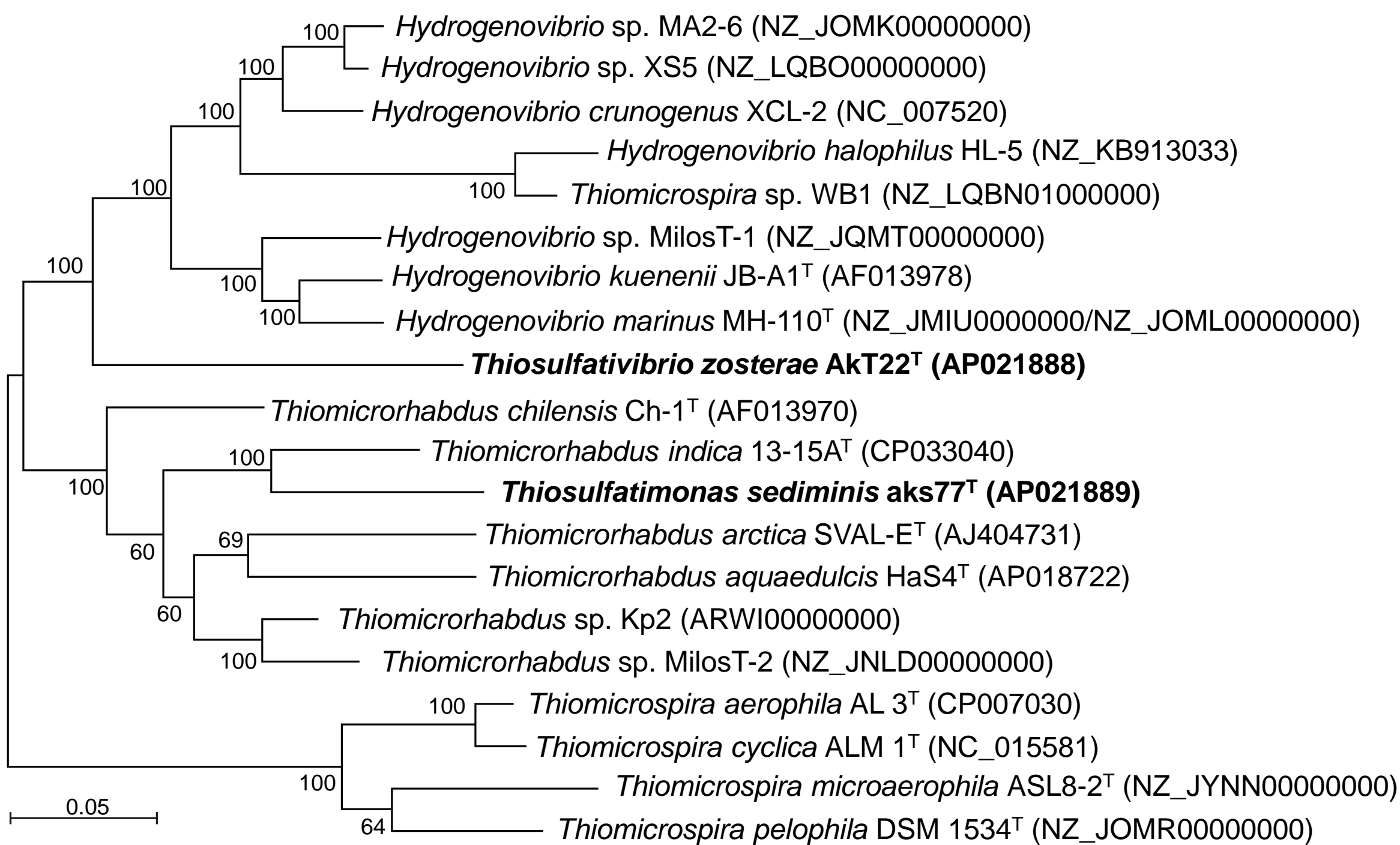
404 and missing data were eliminated (6663 amino acid positions in the final dataset). Bar

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.





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

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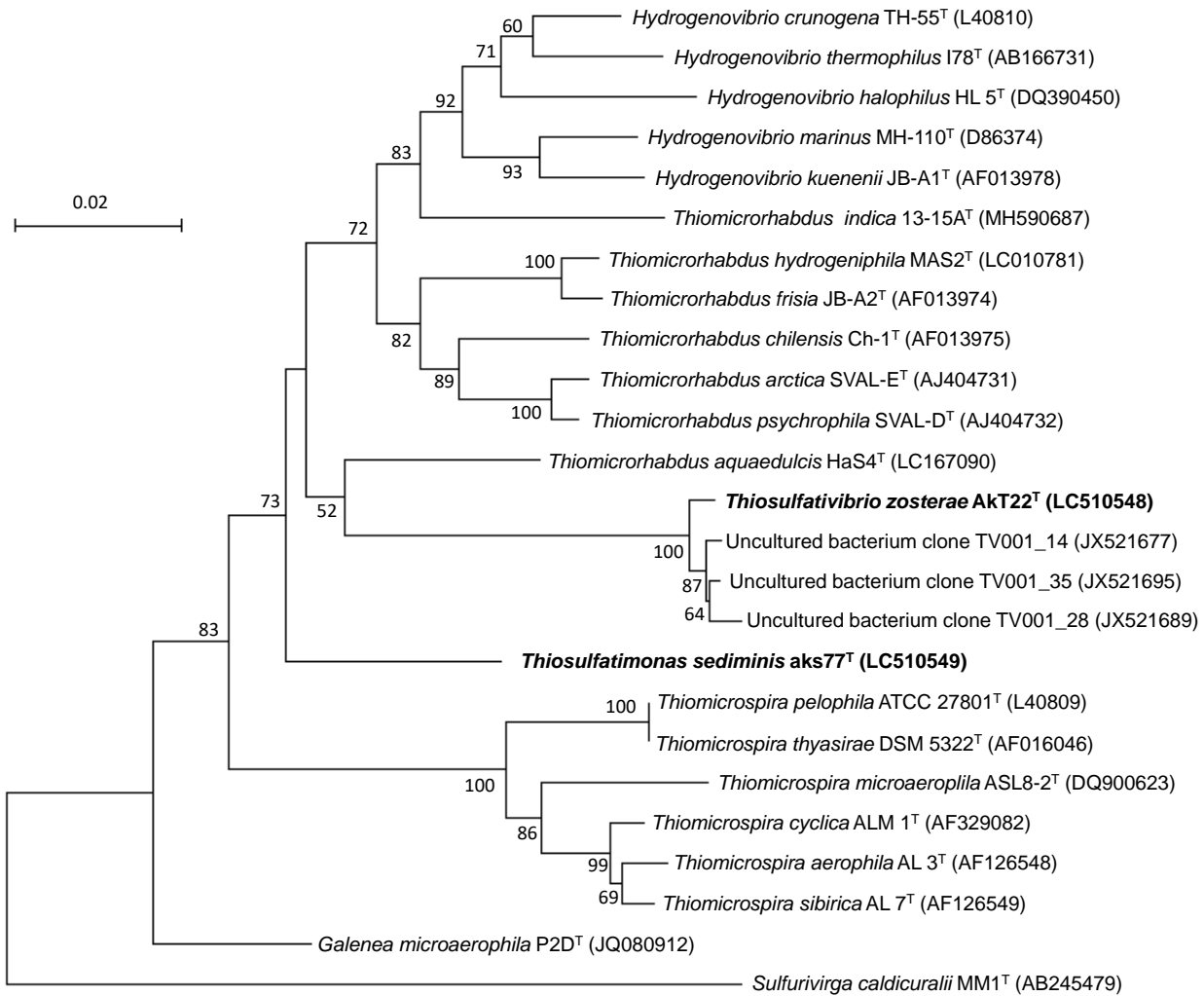


Fig S1. Neighbor-joining tree based on the 16S rRNA gene sequences. The tree was obtained with Kimura 2-parameter model with gamma distribution. All positions containing gaps and missing data were eliminated and there were a total of 1250 positions in the final dataset. Bar represents substitutions per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

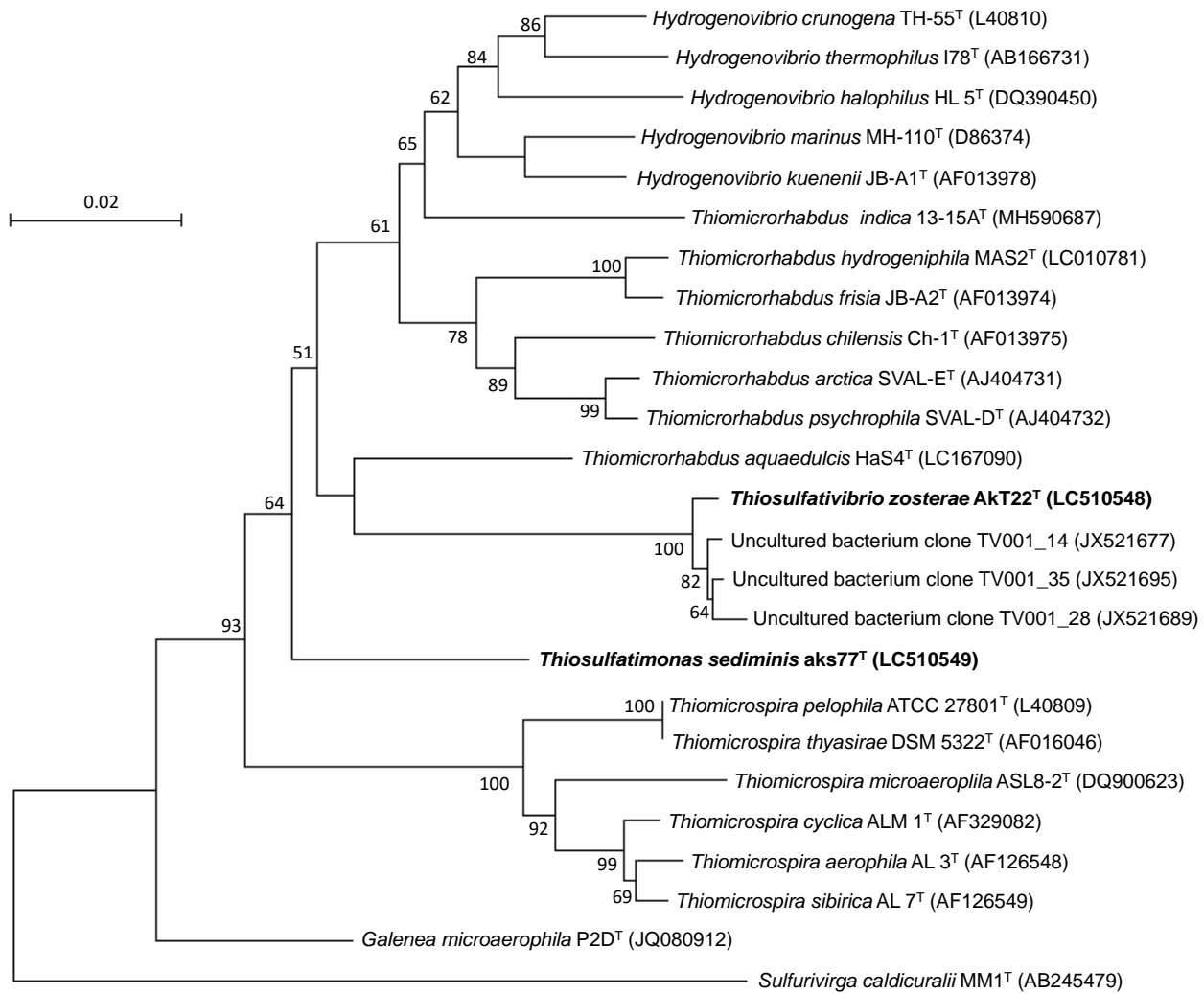


Fig S2. Minimum evolution tree based on the 16S rRNA gene sequences. The tree was obtained with Kimura 2-parameter model with gamma distribution. All positions containing gaps and missing data were eliminated and there were a total of 1250 positions in the final dataset. Bar represents substitutions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

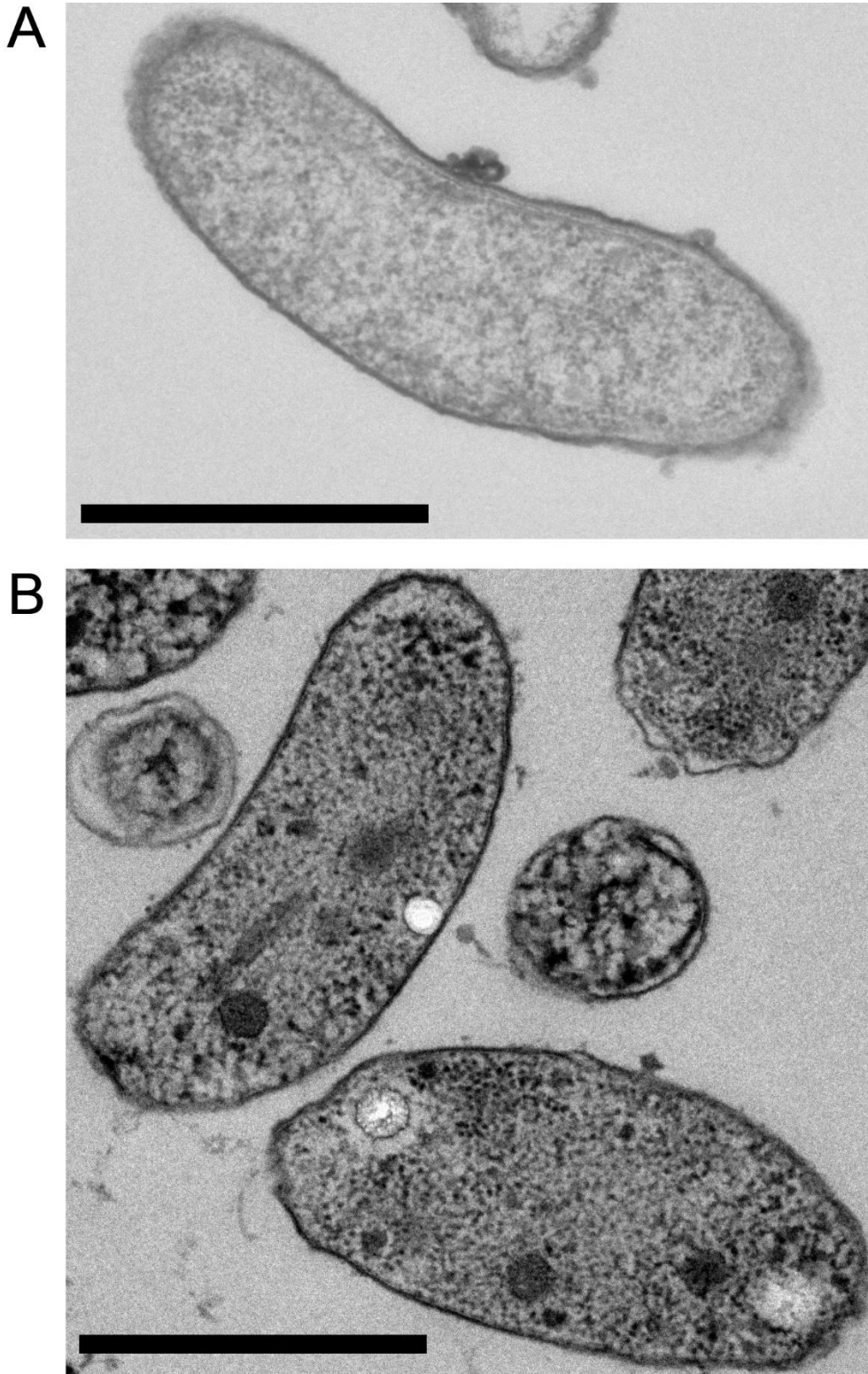


Fig. S3. Transmission electron micrographs of strain AkT22^T (A) and strain aks77^T (B). Bar, 1 μm . Ultra-thin sections (70 nm) were stained with uranyl acetate.

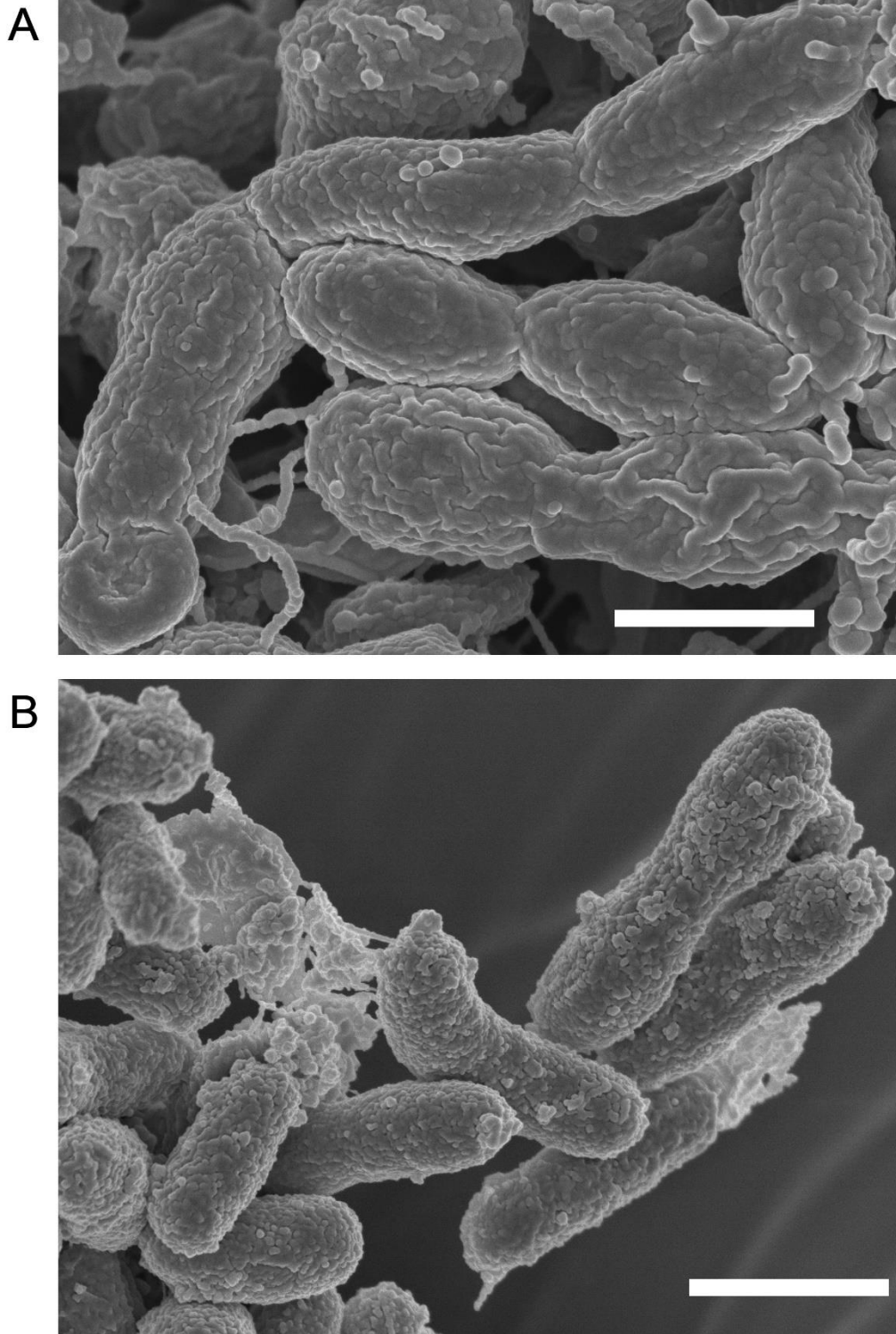


Fig S4. Scanning electron micrographs of strain AkT22^T (A) and strain aks77^T (B). Bar, 1 µm. Cells were coated with a layer of osmium (30 nm).

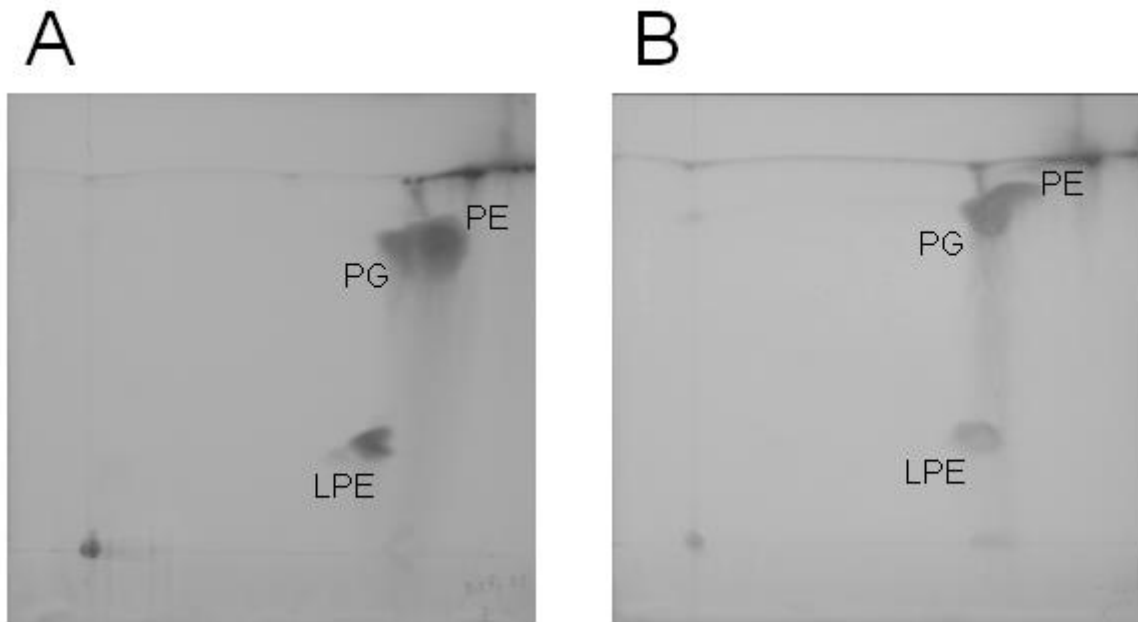


Fig S5. Polar lipid profiles of strain AkT22^T (A) and strain aks77^T (B). PG, Phosphatidylglycerol; PE, phosphatidylethanolamine; LPE, Lyso-phosphatidylethanolamine.

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
<i>cbbL</i> gene	2	2
<i>cbbM</i> gene	1	0
CRISPR loci	0	1