220819

Thiomicrorhabdus immobilis sp. nov., a mesophilic sulfur-oxidizing bacterium isolated from sediment of a brackish lake in northern Japan

Hisaya Kojima[1*], Jun Mochizuki[1,2], Mamoru Kanda[1,2], Tomohiro Watanabe[1] and Manabu Fukui[1]

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

2. Graduate School of Environmental Science, Hokkaido University, Kita-10, Nishi-5, Kita-ku, Sapporo 060-0810, Japan

---

*Corresponding author

Tel/fax number: +81 11 706 5460

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

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

Abstract

A novel sulfur-oxidizing bacterium, strain Am19^T, was isolated from sediment of a brackish lake. Strain Am19^T grew chemolithoautotrophically on inorganic sulfur compounds, and heterotrophic growth was not observed. Cells were rod-shaped with length of 1.1–3.0 μm and diameter of 0.5–0.8 μm. Growth was observed at 5–37°C with an optimum growth temperature of 30°C. The pH range for growth was 5.6–8.5 with an optimum pH of 6.6–7.0. Major fatty acids were summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) and $C_{16:0}$. The sole respiratory quinone was ubiquinone-8. The complete genome of strain Am19^T is composed of a circular chromosome with length of 2.5 Mbp and G + C content of 42.7 mol%. Phylogenetic analysis based on genomic data indicated that strain Am19^T belongs to the genus *Thiomicrorhabdus* but is distinct from any existing species. Analysis of the 16S rRNA gene supported creation of a new species to accommodate strain Am19^T. On the basis of genomic and phenotypic characteristics, strain Am19^T (= NBRC 114602^T = BCRC 81336^T) is proposed as the type strain of a new species, with name of *Thiomicrorhabdus immobilis* sp. nov.

Introduction

The genus *Thiomicrorhabdus* was established in 2017, within the family *Piscirickettsiaceae* (Boden et al., 2017a). According to the List of Prokaryotic Names with Standing in Nomenclature (LPSN) (Parte et al., 2020), there are 11 species with validly published names in this genus, at the time of writing (15 August 2022). They are all obligate chemolithoautotrophs which oxidize sulfur compounds. They require oxygen as electron acceptor for growth, and rely on sulfur compounds as electron donor. As exceptional cases, anaerobic growth with nitrite was reported in *T. sediminis* (Liu et al., 2021), and hydrogen-dependent growth was observed in *T. hydrogeniphila* and *T. heinhorstiae* (Watsuji et al., 2016; Boden et al. 2017b; Updegraff et al. 2022). As common chemotaxonomic feature, all species examined share three major fatty acids, $C_{16:1}$, $C_{18:1}$ and $C_{16:0}$. It was suggested that fourth-dominant fatty acid may work as diagnostic character among species in this genus (Boden et al. 2017b).

In this study, a novel aerobic and mesophilic autotroph was isolated and characterized, as a representative of a new species in the genus *Thiomicrorhabdus*.

Materials and methods

57 Enrichment and isolation

58 Strains Am19^T was isolated from a sediment collected at a site (43.05N, 144.89E), in
59 Lake Akkeshi, a brackish lake in Japan. The sediment was inoculated into a bicarbonate-
60 buffered enrichment medium. To prepare the medium, the following constituents (g l^{-1})
61 were dissolved in distilled water and sterilized by autoclaving: $Na_2S_2O_3 \cdot 5H_2O$ (2.5),
62 NaCl (20), $MgCl_2 \cdot 6H_2O$ (0.2), $CaCl_2 \cdot 2H_2O$ (0.1), NH_4Cl (0.1), KH_2PO_4 (0.1), KCl
63 (0.1). After cooling down to room temperature, the following solutions were aseptically
64 added to the main body of medium (ml/l): trace element solution (1), selenite-tungstate
65 solution (1), vitamin mixture solution (1) and 1M $NaHCO_3$ solution (30). The trace
66 element solution contained 0.3% HCl and following constituents (mg l^{-1}): $FeSO_4 \cdot 7H_2O$
67 (2100), H_3BO_3 (30), $MnCl_2 \cdot 4H_2O$ (100), $CoCl_2 \cdot 6H_2O$ (190), $NiCl_2 \cdot 6H_2O$ (24), $CuCl_2 \cdot$
68 $2H_2O$ (2), $ZnSO_4 \cdot 7H_2O$ (144) and $Na_2MoO_4 \cdot 2H_2O$ (36). The selenite-tungstate solution
69 consists of the followings (mg l^{-1}): NaOH (400), $Na_2SeO_3 \cdot 5H_2O$ (6) and $Na_2WO_4 \cdot 2H_2O$
70 (8). The vitamin mixture solution contained the followings (mg l^{-1}): biotin (20), folic acid
71 (20), pyridoxine hydrochloride (100), thiamine hydrochloride (50), riboflavin (50), niacin
72 (50), hemicalcium D-(+)-pantothenate (50), *p*-aminobenzoic acid (50), *α*-lipoic acid (50)
73 and cyanocobalamin (1). The complete medium was adjusted to pH 7.0-7.2 using
74 hydrochloric acid. After some serial subcultures into the same enrichment medium,

cultures were switched to a medium hereafter termed 'basal medium', which was prepared in the same manner but with the following modifications. It contained $MgSO_4 \cdot 7H_2O$ (0.3 g l^{-1}), and concentrations of $Na_2S_2O_3 \cdot 5H_2O$ and $MgCl_2 \cdot 6H_2O$ were increased to 5 and 3 g l^{-1}, respectively. From the enrichment culture grown on the basal medium, strain Am19^T was isolated with the dilution-to-extinction approach. A grown culture was subjected to serial 10-fold dilutions in the basal medium. The resulting positive culture of the highest dilution was subjected to the same procedure again, to obtain pure culture of strain Am19^T. The enrichment and isolation were performed at 22°C. Purity of culture was routinely checked by microscopy and sequencing of the 16S rRNA gene fragments.

Phenotypic characterization

In experiments for phenotypic characterizations, strain Am19^T was cultured at 30°C in the basal medium unless otherwise specified. Cell morphology was observed with phase-contrast light microscopy. Gram staining was performed using the Gram-Color kit (Merck). Activities of catalase and oxidase were assessed as described previously (Kojima & Fukui 2016).

Effect of temperature on growth was examined by attempting cultivation at 0, 5, 8, 10, 13, 15, 18, 22, 25, 28, 30, 32, 37 and 45°C in the basal medium. Effect of salt

93 concentration on growth was examined with modified versions of the basal medium,
94 which contained no $MgCl_2$ and varying concentrations of NaCl, ranging from 0 to 1,400
95 mM at 100 mM intervals.

96 For test of pH effect on growth, media of various pH prepared as below. From the basal
97 medium, $MgCl_2$ was excluded and bicarbonate concentration was reduced to 1 g l^{-1}.
98 Depending on the pH to be tested, the modified media were buffered with 20 mM of MES,
99 MOPS or TAPS. All ingredients were mixed and then sterilized by filtration after pH
100 adjustment with NaOH or HCl. The pH tested and buffering reagents were as follows;
101 pH 3.3, 3.7, 4.2, 5.0, 5.1, 5.6, 6.1, 6.4, 6.6, 6.8, 7.0 and 7.2 with MES; pH 6.4, 7.0, 7.2,
102 7.4, 7.6, 7.8, 7.9, 8.5 and 8.8 with MOPS; pH 8.1, 8.2, 8.5, 8.7 and 9.1 with TAPS.

103 Utilization of growth substrates was tested with the basal medium, by replacing
104 thiosulfate with one of the followings (mM); tetrathionate (20), sulfite (5), sulfide (2),
105 acetate (5), formate (5), succinate (2.5), fumarate (5), *iso*-butyrate (2.5), methanol (5),
106 ethanol (2.5), D-(+)-glucose (2.5), D-(-)-fructose (5), D-(+)-xylose (2.5), D-(+)-maltose
107 (5), L-(+)-arabinose (5), D-(+)-cellobiose (1). Utilization of elemental sulfur was tested
108 by adding flowers of sulfur (approximately 0.5 g l^{-1}, which is equivalent to 1.9 mmol
109 *cyclo*-octasulfur, the major component of flowers-of-sulfur) to the thiosulfate-free basal
110 medium. The sulfur was moistened and sterilized as described previously (Widdel &

Pfennig, 1992). Chemolithoautotrophic growth on hydrogen was tested by filling the headspace of the culturing containers with 1:1 mixture of air and H_2 gas, at a total pressure of 2 atm. Heterotrophic growth was also tested in complex liquid media listed below; Reasoner's 2A broth (R2A) broth neat and at 1:10 dilution; nutrient broth; lysogeny broth and tryptic soy broth. Utilization of electron acceptor was tested under anoxia, with the basal medium supplemented with nitrate (20 mM) or nitrite (5 mM).

Chemotaxonomic characterization

For analyses of cellular fatty acids and respiratory quinone, the basal medium was simplified by omitting $MgCl_2$. Strain Am19^T was grown in the simplified basal medium at 30°C. The fatty acid profile was obtained with the Sherlock Microbial Identification System (MIDI) version 6.0 (database; TSBA6). Respiratory quinones were extracted as described previously (Bligh & Dyer, 1959), and then analyzed with HPLC.

Genomic characterization

Whole genome sequencing was performed using the platforms of Illumina NextSeq and Nanopore GridION, to obtain short and long reads respectively. The resulting reads from the platforms were assembled by using Unicycler (Ver 0.4.7). The reconstructed genome sequence was annotated with DFAST (Tanizawa et al., 2018).

A genome-based taxonomic classification was made with reference to the Genome Taxonomy Database (GTDB) (Parks et al., 2018). Taxonomic position in GTDB release 95 was determined by analyzing the genome of strain Am19^T with GTDB-Tk (Chaumeil et al., 2020).

To estimate genome relatedness between Am19^T and its close relatives, values of average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) were calculated, by using tools provided by Kostas lab (http://enve-omics.ce.gatech.edu/) and DSMZ, respectively. In the calculation of dDDH with Genome-to-Genome Distance Calculator (GGDC), the formula 2 was applied (Meier-Kolthoff et al., 2013).

Phylogenetic analysis based on the 16S rRNA gene

Phylogenetic analysis of the 16S rRNA gene was conducted using MEGA version 11 (Tamura et al., 2021). The gene sequence of strain Am19^T was aligned with reference sequences, using the MUSCLE algorithm. The reference sequences were obtained from LPSN (accessed on 15 August 2022), as those of type strains representing species with validly published names. They include all species in the genera *Thiomicrorhabdus*, *Thiomicrospira*, *Hydrogenovibrio*, *Thiosulfativibrio*, *Thiosulfatimonas*, *Galenea* and *Sulfurivirga* (as an outgroup). The sequences collected from LPSN were checked for

quality, and those including ambiguous bases were replaced with the 16S rRNA gene sequences extracted from genomes, when they were publicly available. With the resulting alignment, the best substitution model was selected by the model selection tool in MEGA. The selection was conducted with default settings, to identify the best model with the lowest Bayesian Information Criterion (BIC) score. Phylogenic tree was constructed with the selected model by excluding positions with gaps. An alternative phylogenetic tree was constructed with a model with the lowest corrected Akaike Information Criterion (AIC_C) value, to confirm that resulting tree topology is identical to that of the model selected by BIC.

Results

Physiological and chemotaxonomic characteristics

Cells of strain Am19^T were Gram-stain-negative, rod-shaped, non-motile, 0.5–0.8 μm in diameter and 1.1–3.0 μm in length. They were catalase-negative and oxidase-positive. Strain Am19^T grew at 5–37°C with optimum growth at 30°C. Its growth was observed at pH range of 5.6–8.5, with optimum growth at pH 6.6–7.0 (Table 1). In the test of salt concentration, growth was observed in the presence of 1,300 mM or lower concentrations of NaCl. Strain Am19^T did not require NaCl for growth.

167 Strain Am19^T grew chemolithotrophically on thiosulfate, tetrathionate, sulfide
168 and elemental sulfur. Hydrogen gas did not serve as electron donor for
169 chemolithotrophic growth. None of the tested organic substrate supported growth of the
170 strain. No heterotrophic growth was observed in the complex media tested. In the
171 presence of thiosulfate, anaerobic growth was not observed in the medium containing
172 nitrate or nitrate as sole electron acceptor.

173 The full fatty acid profile of strain Am19^T is shown in Table S1. Major
174 components in the profile were summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; 46%),
175 summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$; 33%) and $C_{16:0}$ (14%). The fourth-
176 dominant fatty acid, next to the major fatty acids mentioned above, was previously
177 suggested to be a diagnostic character among *Thiomicrorhabdus* species (Boden et al.,
178 2017b). In strain Am19^T, it was $C_{17:1}$ ($C_{17:1}\omega6c$; 1.3% and $C_{17:1}\omega8c$; 0.7%), unlike
179 known *Thiomicrorhabdus* species (Table 1).

180 In the analysis of respiratory quinones, only ubiquinone-8 was detected.

181

182 Genomic characteristics and phylogenetic position

183 The complete genome of strain Am19^T was reconstructed from reads of

184 DNBSEQ and GridION. The sequencing coverage was 825.8-fold. The genome consists
185 of a single circular chromosome with size of 2,539,444 bp and G+C content of 42.7
186 mol%. In the genome, 2251 protein-coding sequences, 9 rRNA genes and 46 tRNA
187 genes were predicted.

188 The reconstructed genome was analyzed with GTDB-tk, for phylogenetic
189 analysis based on 120 conserved proteins. As a result, strain Am19^T was classified into
190 the genus *Thiomicrorhabdus*. On the other hand, the analysis also indicated that strain
191 Am19^T does not belong to any existing species with validly published name. The
192 genome-based phylogenetic tree obtained with GTDB-tk is shown in Fig S1.

193 Three copies of the 16S rRNA gene were identified in the genome, and they
194 have an identical sequence. The sequence indicated high identities to the 16S rRNA
195 gene sequences of *Thiomicrorhabdus* species. Among species with validly published
196 names, *T. frisia* indicated the highest identity of 97.7% (Table 1). Phylogenetic tree
197 based on the 16S rRNA gene sequences is shown in Fig. 1. In the tree, monophyly of
198 the genus *Thiomicrorhabdus* was not supported. However, strain Am19^T belonged to a
199 clade exclusively consisting of *Thiomicrorhabdus* species. This clade also included *T.*
200 *frisia*, the type species of the genus. These results indicated that strain Am19^T should be
201 classified in the genus *Thiomicrorhabdus*.

202 The values of dDDH and ANI between strain Am19^T and genome-sequenced
203 strains in the genus *Thiomicrorhabdus* are shown in Table 2. The values are lower than
204 thresholds for species delineation, 70% and 95–96% for dDDH and ANI, respectively
205 (Richter & Rosselló-Móra, 2009; Meier-Kolthoff et al., 2013).

206

207 **Conclusions**

208 The genomic characteristics of strain Am19^T, including the 16S rRNA gene
209 sequence, indicated that this novel isolate is close relative of the *Thiomicrorhabdus*
210 species but should not be classified into any existing species. Between strain Am19^T and
211 type strains of existing species, dDDH and ANI values were lower than species
212 delineation thresholds (Table 2). Within the genus, strain Am19^T and existing species are
213 differentiated by combinations of phenotypic characteristics (Table 1). On the basis of
214 these genomic and phenotypic characteristics, strain Am19^T is proposed to be assigned to
215 a novel species of the genus *Thiomicrorhabdus*, with the name *Thiomicrorhabdus*
216 *immobilis* sp. nov.

217

218 **Description of *Thiomicrorhabdus immobilis* sp. nov.**

219 *Thiomicrorhabdus immobilis* (im.mo'bi.lis. L. fem. adj. *immobilis*, immobile, immovable,

pertaining to a feature of the type strain).

Cells are rod-shaped, 1.1–3.0 μm in length and 0.5–0.8 μm in diameter. Strictly aerobic. Gram-stain-negative. Catalase-negative and oxidase-positive. Chemolithoautotrophic growth occurs with thiosulfate, tetrathionate, elemental sulfur and sulfide. Acetate, formate, succinate, fumarate, *iso*-butyrate, methanol, ethanol, D-(+)-glucose, D-(-)-fructose, D-(+)-xylose, D-(+)-maltose, L-(+)-arabinose and D-(+)-cellobiose are not utilized as growth substrates. Growth is observed at temperature range from 5°C to 37°C, with an optimum growth at 30°C. The pH range for growth is 5.6–8.5, and the optimum pH was 6.6–7.0. The G+C content of genomic DNA of type strain is 42.7 mol% (genome).

The type strain Am19^T (= NBRC 114602^T = BCRC 81336^T) was isolated from sediment of a brackish lake in Japan (Lake Akkeshi).

The GenBank/EMBL/DDBJ accession numbers for the complete of strain Am19^T is AP024202.

References

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 *Thiomicrorhabdus* gen. nov. and *Hydrogenovibrio*,

239 and reclassification of all four species of *Thioalkalimicrobium* to *Thiomicrospira* Int
240 J Syst Evol Microbiol 67:1140–1151. https://doi.org/10.1099/ijsem.0.001855
241 Boden R, Scott KM, Rae AW, Hutt LP (2017b) Reclassification of *Thiomicrospira*
242 *hydrogeniphila* (Watsuji et al. 2016) to *Thiomicrorhabdus hydrogenophila* comb. nov.,
243 with emended description of *Thiomicrorhabdus* (Boden et al., 2017) Int J Syst Evol
244 Microbiol 67:4205–4209. https://doi.org/10.1099/ijsem.0.002279
245 Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can
246 J Biochem Physiol 37:911–917. https://doi.org/10.1139/o59-099
247 Brinkhoff T, Muyzer G, Wirsen CO, Kuever J. (1999a) *Thiomicrospira kuenenii* sp. nov.
248 and *Thiomicrospira frisia* sp. nov., two mesophilic obligately chemolithoautotrophic
249 sulfur-oxidizing bacteria isolated from an intertidal mud flat. Int J Syst Bacteriol
250 49:385–392. https://doi.org/10.1099/00207713-49-2-385
251 Brinkhoff T, Muyzer G, Wirsen CO, Kuever J (1999b) *Thiomicrospira chilensis* sp. nov.,
252 a mesophilic obligately chemolithoautotrophic sulfur-oxidizing bacterium isolated
253 from a *Thioploca* mat. Int J Syst Bacteriol 49:875–879.
254 https://doi.org/10.1099/00207713-49-2-875
255 Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH (2020) GTDB-Tk: a toolkit to classify
256 genomes with the genome taxonomy database. Bioinformatics 36:1925–1927.

https://doi.org/10.1093/bioinformatics/btz848

Kojima H, Fukui M (2016) *Sulfuriflexus mobilis* gen. nov., sp. nov., a sulfur-oxidizing bacterium isolated from a brackish lake sediment. Int J Syst Evol Microbiol 66:3515–3518. https://doi.org/10.1099/ijsem.0.001227

Kojima H, Fukui M (2019) *Thiomicrorhabdus aquaedulcis* sp. nov., a sulfur-oxidizing bacterium isolated from lake water Int J Syst Evol Microbiol 69:2849–2853. https://doi.org/10.1099/ijsem.0.003567

Knittel K, Kuever J, Meyerdierks A, Meinke R, Amann R et al. (2005) *Thiomicrospira arctica* sp. nov. and *Thiomicrospira psychrophila* sp. nov., psychrophilic, obligately chemolithoautotrophic, sulfur-oxidizing bacteria isolated from marine Arctic sediments. Int J Syst Evol Microbiol 55:781–786. https://doi.org/10.1099/ijs.0.63362-0

Liu X, Jiang L, Hu Q, Lyu J, Shao Z (2020) *Thiomicrorhabdus indica* sp. nov., an obligately chemolithoautotrophic, sulfur-oxidizing bacterium isolated from a deep-sea hydrothermal vent environment. Int J Syst Evol Microbiol 2020;70: 234–239. https://doi.org/10.1099/ijsem.0.003744

Liu X, Chen B, Lai Q, Shao Z, Jiang L (2021) *Thiomicrorhabdus sediminis* sp. nov. and *Thiomicrorhabdus xiamenensis* sp. nov., novel sulfur-oxidizing bacteria isolated from

coastal sediments and an emended description of the genus *Thiomicrorhabdus* Int J Syst Evol Microbiol 2021;71:004660. https://doi.org/10.1099/ijsem.0.004660

Meier-Kolthoff JP, Göker M, Klenk H-P (2014) Taxonomic use of DNA G+C content and DNA-DNA hybridization in the genomic age. Int J Syst Evol Microbiol 64:352–356. https://doi.org/10.1099/ijs.0.056994-0

Parks DH, Chuvochina M, Waite DW, Rinke C, Skarshewski A, Chaumeil PA, Hugenholtz P (2018) A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nat Biotechnol 36:996–1004. https://doi.org/10.1038/nbt.4229

Parte AC, Sardà Carbasse J, Meier-Kolthoff JP, Reimer LC, Göker M (2020) List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. Int J Syst Evol Microbiol 70:5607–5612. https://doi.org/10.1099/ijsem.0.004332

Richter M, Rosselló-Móra R (2009) Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci 106:19126–19131. https://doi.org/10.1073/pnas.0906412106

Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis version 11. Mol Biol Evol 38:3022–3027. https://doi.org/10.1093/molbev/msab120

Tanizawa Y, Fujisawa T, Nakamura Y (2018) DFAST: a flexible prokaryotic genome annotation pipeline for faster genome publication. Bioinformatics 34:1037–1039. https://doi.org/10.1093/bioinformatics/btx713

Updegraff T, Schiff-Clark G, Gossett H, Parsi S, Peterson R, Whittaker R et al. (2022) *Thiomicrorhabdus heinhorstiae* sp. nov. and *Thiomicrorhabdus cannonii* sp. nov.: novel sulphur-oxidizing chemolithoautotrophs isolated from the chemocline of Hospital Hole, an anchialine sinkhole in Spring Hill, Florida, USA. Int J Syst Evol Microbiol 72:005233. https://doi.org/10.1099/ijsem.0.005233

Watsuji TO, Hada E, Miyazaki M, Ichimura M, Takai K (2016) *Thiomicrospira hydrogeniphila* sp. nov., a novel aerobic, hydrogen-and sulfur-oxidizing chemolithoautotroph isolated from a seawater tank containing a block of beef tallow. Int J Syst Evol Microbiol 66:3688–3693. https://doi.org/10.1099/ijsem.0.001250

Widdel F, Pfennig N (1992) The genus *Desulfuromonas* and other gram-negative sulfur-reducing eubacteria. In: Balows, A., Trüper, M., Dworkin, M., Harder, W., Schleifer, K.-H. (Eds.), The Prokaryotes, second edition, volume IV, Springer, New York, pp. 3379–3389.

Statements and Declarations

The authors declare that no funds, grants, or other support were received during

311 the preparation of this manuscript. The authors have no relevant financial or non-financial

312 interests to disclose.

313

Figure legend

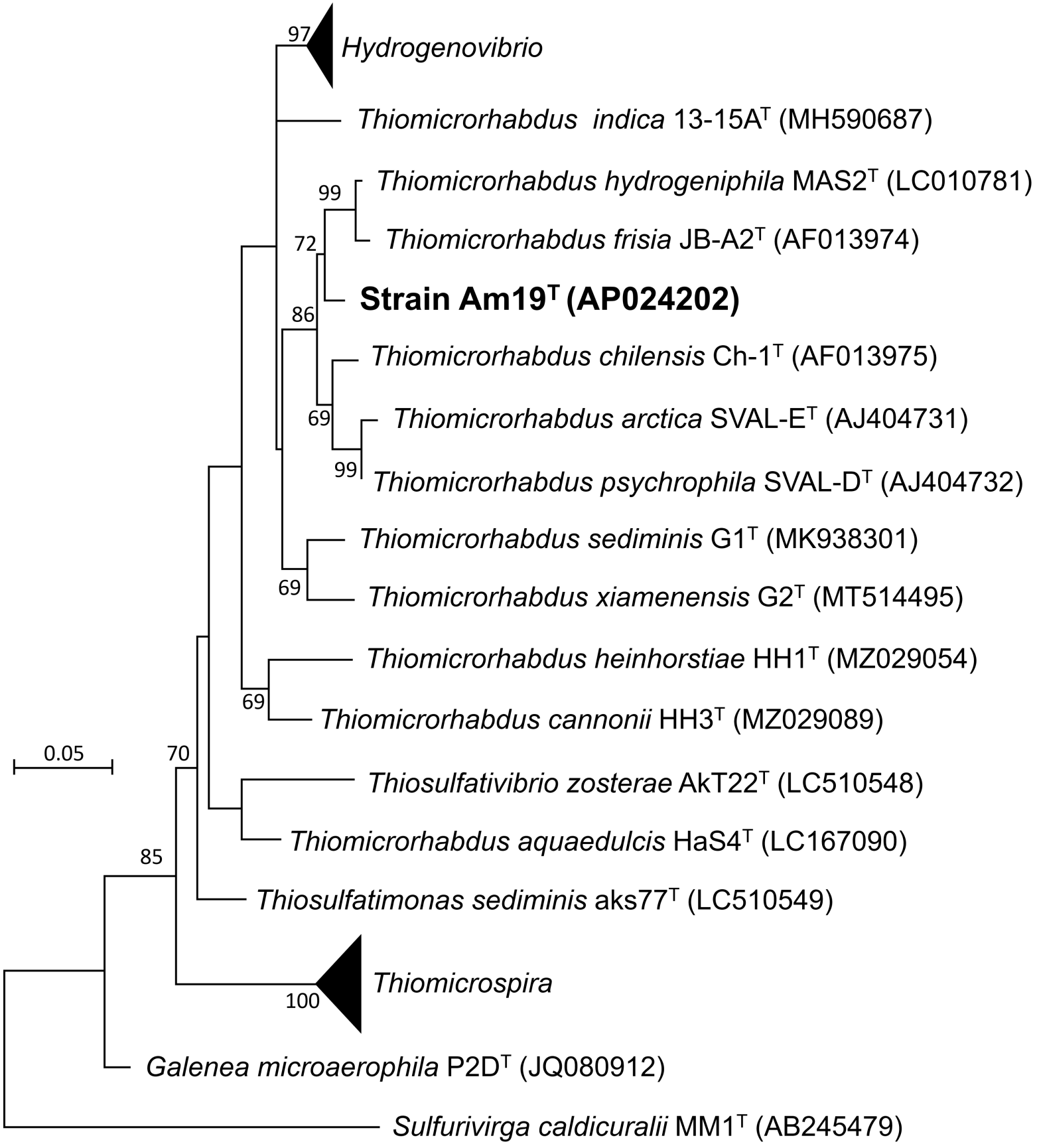
Fig. 1 Phylogenetic tree of strain Am19^T and related organisms, based on the 16S rRNA gene sequences. This tree was constructed with the maximum likelihood method based on Kimura 2-parameter model, and the tree displayed has the lowest log-likelihood (-5947.74). A discrete gamma distribution was used to model differences in evolutionary rates among sites (5 categories, parameter = 0.2510). The rate variation model allowed for some sites to be invariable (49.03% sites). All positions containing gaps and missing data were eliminated, leaving a total of 1,334 positions in the final dataset. Numbers on nodes represent percentage values of 1,000 bootstrap resampling (values lower than 70 are not shown). Names of all 27 strains included in the analysis are shown in full version of the tree provided as Figure S2.

Table 1. Differential properties of strain Am19^T and type strains of *Thiomicrorhabdus* species. Strains: 1, Am19^T; 2, *T. frisia* JB-A2^T (Brinkhoff et al., 1999a); 3, *T. chilensis* Ch-1^T (Brinkhoff et al., 1999b); 4, *T. arctica* SVAL-E^T (Knittel et al., 2005); 5, *T. psychrophila* SVAL-D^T (Knittel et al., 2005); 6, *T. hydrogeniphila* MAS2^T (Watsuji et al., 2016); 7, *T. aquadulcis* HaS4^T (Kojima & Fukui, 2019); 8, *T. indica* 13-15A^T (Liu et al., 2020); 9, *T. sediminis* G1^T (Liu et al., 2021); 10, *T. xiamenensis* G2^T (Liu et al., 2021); 11, *T. heinhorstiae* HH1^T (Updegraff et al., 2022); 12, *T. cannonii* HH3^T (Updegraff et al., 2022). All strains are obligate chemolithoautotrophs which oxidize thiosulfate, tetrathionate and sulfur. NR, not reported.

	1	2	3	4	5	6	7	8	9	10	11	12
Optimum pH for growth	6.6-7.0	6.5	7	7.3-8.0	7.5-8.5	6	6.6-7.4	7	7.5	6.5	7.4	7.5
pH range for growth	5.6-8.5	4.2-8.5	5.3-8.5	6.5-9.0	6.5-9.0	5.0-8.0	6.2-8.8	5.0-9.0	6.0-9.0	5.5-8.0	6.5-7.5	6.0-8.0
Optimum temperature for growth (°C)	30	32-35	32-37	11.5-13.2	14.6-15.4	30	22	28	30	28	32.8	32.0
Temperature range for growth (°C)	5-37	3.5-39	3.5-42	-2.0-20.8	-2.0-20.8	2-40	0-25	10-45	10-40	4-45	15-35	15-35
Growth on H_2	-	-	-	-	-	+	-	-	-	-	+	-
Anaerobic growth	-	-	-	-	-	-	-	-	+	-	-	-
Fourth most abundant fatty acid	$C_{17:1}$	NR	$C_{18:0}$	$C_{14:1}$	$C_{12:1}$	$C_{12:0}$	$C_{18:0}$	$C_{10:0}$ 3-OH	$C_{18:0}$	$C_{10:0}$ 3-OH	$C_{10:0}$ 3-OH	$C_{12:0}$
Percentage 16S rRNA gene identity to that of strain Am19^T (%)	100	97.7	96.5	97.3	96.7	97.1	95.3	94.9	96.2	94.7	93.4	94.6

Table 2. Values of dDDH and ANI between strain Am19^T and genome-sequenced strains in the genus *Thiomicrorhabdus*

Strain (accession number)	dDDH (%)	ANI (%)
Thiomicrorhabdus sp. Kp2 (GCA_000478585.1)	21.1	80
Thiomicrorhabdus sp. Milos-T2 (GCA_000702325.1)	20.9	80
Thiomicrorhabdus sp. HH3 (GCA_013391695.1)	15.8	79
Thiomicrorhabdus sp. HH1 (GCA_013391765.1)	16.2	78
T. aquaedulcis HaS4^T (GCA_004001325.1)	16.2	78
T. indica 13-15A^T (GCA_004293625.1)	16.5	78
T. sediminis G1^T (GCA_005885815.1)	16.8	79
T. xiamenensis G2^T (GCA_013282625)	16.4	79
T. arctica DSM 13458^T (GCA_000381085.1)	16.4	78
T. chilensis DSM 12352^T (GCA_000483485.1)	16.3	79
T. heinhorstiae HH1^T (GCA_013391765.2)	20.6	78
T. cannonii HH3^T (GCA_013391695.1)	19.8	78


97
Hydrogenovibrio
Thiomicrorhabdus indica 13-15A^{T} (MH590687)
99
Thiomicrorhabdus hydrogeniphila MAS2^{T} (LC010781)
72
Thiomicrorhabdus frisia JB-A2^{T} (AF013974)
Strain Am19^{T} (AP024202)
86
Thiomicrorhabdus chilensis Ch-1^{T} (AF013975)
69
Thiomicrorhabdus arctica SVAL-E^{T} (AJ404731)
99
Thiomicrorhabdus psychrophila SVAL-D^{T} (AJ404732)
Thiomicrorhabdus sediminis G1^{T} (MK938301)
69
Thiomicrorhabdus xiamenensis G2^{T} (MT514495)
Thiomicrorhabdus heinhorstiae HH1^{T} (MZ029054)
69
Thiomicrorhabdus cannonii HH3^{T} (MZ029089)
0.05
70
Thiosulfativibrio zosterae AkT22^{T} (LC510548)
Thiomicrorhabdus aquaedulcis HaS4^{T} (LC167090)
85
Thiosulfatimonas sediminis aks77^{T} (LC510549)
Thiomicrospira
100
Galenea microaerophila P2D^{T} (JQ080912)
Sulfurivirga caldicuralii MM1^{T} (AB245479)