Sulfuricystis multivorans gen. nov., sp. nov. and *Sulfuricystis thermophila* sp. nov., facultatively autotropic sulfur-oxidizing bacteria isolated from a hot spring, and emended description of the genus *Rugosibacter*

Hisaya Kojima*[1,2], Miho Watanabe[2], Naoyuki Miyata[2] and Manabu Fukui[1]

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

2. Department of Biological Environment, Faculty of Bioresource Sciences, Akita Prefectural University. Shimo-Shinjyo Nakano, Akita, Akita, 010-0195 Japan

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*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

Strains J5B^T and M52^T are facultatively autotrophic sulfur-oxidizing bacteria isolated from a microbial mat from a hot spring. They were isolated and partially characterized in previous studies, as facultative anaerobes which use nitrate as electron acceptor. In this study, additional characterizations were made to determine their taxonomic status. In both strains, major cellular fatty acids were $C_{16:1}$ ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) and $C_{16:0}$. Their chemolithoautotrophic growth was supported by thiosulfate and elemental sulfur. They used some organic acids as growth substrates. Their 16S rRNA gene sequences indicated the highest sequence identities to species in the family *Sterolibacteriaceae*, but the identities were 95% or lower. Phylogenetic analysis indicated that these strains do not belong to any existing genera. Values of average nucleotide identity and digital DNA–DNA hybridization between strains J5B^T and M52^T were 87.93 % and 34.3 %, respectively. On the basis of phenotypic and genomic characteristics, *Sulfuricystis multivorans* gen. nov. sp. nov., and *Sulfuricystis thermophila* sp. nov. are proposed, with type strains of J5B^T and M52^T, respectively. An emended description of the genus *Rugosibacter* is also proposed, for its reclassification to the family *Sterolibacteriaceae*.

Introduction

The family *Sterolibacteriaceae* was proposed to encompass six genera, as a part of major reconstruction of the order *Nitrosomonadales* (Boden et al., 2017). According to the List of Prokaryotic Names with Standing in Nomenclature (as of 29 March 2022), the family still comprises six genera, *Sterolibacterium*, *Denitratisoma*, *Methyloversatilis*, *Georgfuchsia*, *Sulfuritalea* and *Sulfurisoma*. Before the reclassification, some of these genera had been regarded as members of the family *Rhodocyclaceae* in the order *Rhodocyclales*. Shortly before establishment of the family *Sterolibacteriaceae*, a genus was proposed in the family *Rhodocyclaceae*, with the name of *Rugosibacter* (Corteselli et al., 2017). In that study, phylogenetic analysis of the 16S rRNA gene indicated that *Rugosibacter* belongs to a clade consisting of the six genera mentioned above. The genus *Rugosibacter* was not taken into consideration in the proposal of *Sterolibacteriaceae*, and it is still placed in the family *Rhodocyclaceae* as noted in the original description. The close relatedness between *Rugosibacter* and genera of *Sterolibacteriaceae* has also been indicated by a phylogenetic analysis of concatenated ribosomal proteins (Okubo & Takami, 2021).

The original description of family *Sterolibacteriaceae* states that the family is circumscribed on the basis of 16S rRNA gene sequences, and includes physiologically

61 diverse organisms (Boden et al., 2017). It refers to methylotrophy, autotrophy and
62 anaerobic respiration (with nitrate, ferric iron or manganic manganese), as varied
63 metabolism observed in the family. As other notable functions, anaerobic degradation
64 of aromatic compounds (Weelink et al., 2009; Sperfeld et al., 2019) and arsenate
65 respiration (Watanabe et al., 2017) have also been demonstrated in species belonging
66 to this family.

67 Besides the organisms mentioned above, some strains have been isolated and
68 characterized as members of this family. *Sterolibacteriaceae* bacterium strain J5B^T is
69 a facultatively autotrophic sulfur-oxidizing bacterium, isolated from a microbial mat
70 collected in a hot spring in Japan (Watanabe et al, 2019). As for strain J5B^T, some
71 physiological characteristics have been reported, along with genomic characteristics
72 related to sulfur oxidation (Watanabe et al, 2019). The closest relative of strain J5B^T
73 is also sulfur oxidizer, isolated from the same microbial mat. The sulfur-oxidizing
74 bacterium, strain M52^T, can oxidize arsenite anaerobically (Ospino et al., 2019). The
75 previous studies reported complete genome sequences of these two strains. The
76 genome sequences have been publicized and incorporated in the genome taxonomy
77 database (GTDB) (Parks et al., 2018). In the GTDB release 06-RS202, they are both
78 classified in a genus-level taxon (UBA2250) which has no species with validly

79 published name and regarded as representatives of independent species.

80 In this study, additional characterizations of strains J5B^T and M52^T were made
81 to determine their taxonomic status in the family *Sterolibacteriaceae*.

82

83 **Materials and methods**

84

85 Isolation and maintenance of strains

86 The strains J5B^T and M52^T were isolated from a microbial mat obtained from a hot
87 spring in Japan (42° 57′ 53″ N 141° 09′ 47″ E). They were isolated into pure cultures
88 under nitrate-reducing conditions, by using bicarbonate and thiosulfate as sole carbon
89 source and electron donor (Watanabe et al, 2019; Ospino et al., 2019). The strains were
90 maintained in the laboratory with a bicarbonate-buffered medium referred to as "S5
91 medium" (Kojima et al., 2017), supplemented with 10 mM nitrate. Purity and identity
92 of the cultures were periodically checked by microscopic observation and direct
93 sequencing of the 16S rRNA gene.

94

95 Cellular fatty acid analysis

96 For fatty acid analysis, cells of strains J5B^T and M52^T were grown at 45°C,
97 in a medium consisting of the following constituents (l^{-1}): 1 g $NaNO_3$, 0.5 g yeast

98 extract, 0.5 g Casamino acids, 0.5 g disodium fumarate, 0.5 g disodium succinate, and
99 50 μg cyanocobalamin. Headspace of the culturing bottles was filled with N_2 gas, and
100 pH of the medium was adjusted to 7.0. Their cellular fatty acid profiles were obtained
101 with the Sherlock Microbial Identification System (MIDI) version 6.0 (database;
102 TSBA6). As for J5B^T, cells grown in the S5 medium under oxic conditions were also
103 subjected to the same analysis.
104
105 Physiological characterizations
106 Effects of temperature on growth of strain M52^T were examined by
107 culturing at various temperatures (15, 18, 22, 25, 28, 30, 32, 35, 37, 40, 42, 45, 48, 50,
108 53, 55, 57 and 60°C), in the medium used for maintenance. The other tests for
109 phenotypic characterization were all conducted at 45°C.
110 Chemolithoautotrophic growth under nitrate-reducing conditions was tested
111 in the medium used for the maintenance, by replacing thiosulfate with one of electron
112 donors listed below; elemental sulfur (0.5 g l^{-1}), sulfide (2 mM), tetrathionate (10 mM)
113 and hydrogen gas ($H_2/N_2/CO_2$ 50:40:10 v/v/v; 200 kPa in total pressure). Utilization
114 of organic substrates for heterotrophic growth was tested in 10 mM MOPS-NaOH
115 buffer (pH 7.0), supplemented with $NaNO_3$ (1 g l^{-1}), yeast extract (0.1 g l^{-1}), Casamino
116 acids (0.1 g l^{-1}) and cyanocobalamin (50 μg l^{-1}). The medium was dispensed in closed

117 culture bottles and the headspace was filled with N_2 gas. One of the following organic
118 substrates were added to the medium (mM); pyruvate (5), lactate (5), acetate (5),
119 propionate (2.5), succinate (2.5), fumarate (2.5), malate (2.5), butyrate (2.5), benzoate
120 (2.5), isobutyrate (2.5), methanol (5), ethanol (2.5), formate (5), citrate (5), glucose
121 (2.5), xylose (2.5), phenol (2), *o*-cresol (1), *m*-cresol (1).

122 Effect of pH on growth of strain M52^T was tested with pH-buffered media
123 modified from the medium used for the fatty acid analysis. The media were buffered
124 with 10 mM of MES, MOPS or Tricine, and adjusted to varying pH (with 0.1- or 0.2-
125 unit intervals) by adding NaOH. The tested pH were as follows: 5.4–7.3 with MES;
126 6.4–8.0 with MOPS; 7.0–9.0 with Tricine.

127

128 Genomic characterization

129 The complete genome sequences of strains J5B^T and M52^T were obtained in
130 the previous studies (Watanabe et al, 2019; Ospino et al., 2019). As overall genome
131 relatedness indices between them, values of average nucleotide identity (ANI) and
132 digital DNA–DNA hybridization (dDDH) were calculated by using online tools as
133 follows. The ANI was computed by ANI calculator available in EzBioCloud, based on
134 the OrthoANIu algorithm (Yoon et al., 2017). The dDDH value was calculated using
135 Genome-to-Genome Distance Calculator (GGDC) provided by DSMZ (Meier-

136 Kolthoff et al., 2013), applying the formula 2. To evaluate genomic similarity with
137 related bacteria, values of average amino acid identity (AAI) were calculated with
138 EzAAI version 1.0 (Kim et al. 2021).

139 The genome sequences were annotated with DFAST (Tanizawa et al., 2018),
140 to identify protein coding sequences and RNA genes. Genes for sulfur oxidation were
141 identified as described previously (Watanabe et al, 2019).

142 The full sequences of the 16S rRNA gene identified in the genomes were
143 subjected to the blastn search at NCBI against the nucleotide collection (nr/nt) database.
144 Phylogenetic analyses were conducted using the program MEGA version 11 (Tamura
145 et al., 2021). The 16S rRNA gene sequences were aligned with reference sequences,
146 using the MUSCLE algorithm. The references sequences were those of type strains of
147 species with validly published names in the order *Nitrosomonadales* and the genus
148 *Rugosibacter*. The best model for calculation of genetic distances was selected by
149 using the model selection tool in MEGA, as the model with the lowest score of the
150 Bayesian Information Criterion (BIC).

151 Genome-based phylogenetic analysis of family *Sterolibacteriaceae* was
152 conducted by using ezTree pipeline (Wu, 2018). The genome sequences of strains J5B^T
153 and M52^T were input into the pipeline, along with those of the type strains in the family

and *Rugosibacter aromaticivorans* Ca6^T. *Thiobacillus thioparus* DSM 505^T was also included in the analysis as an outgroup. In the pipeline with default settings, single-copy marker genes were automatically identified, and their coding sequences were separately aligned. The resulting alignments were concatenated to generate a maximum-likelihood tree.

Results and Discussion

Physiological and chemotaxonomic characteristics

The fundamental characteristics of strains J5B^T and M52^T are presented in the species descriptions, and some of them are summarized in Table 1. Their cells grown in the maintenance medium were both motile rods, with width of 0.4–0.5 μm. The cell lengths of strain J5B^T and M52^T were 0.8–2.0 μm and 1.8–3.2 μm, respectively. The strain M52^T grew at 18–55°C, with optimum growth at 50°C. Its pH range for growth was 5.5–8.6, and optimum pH was 6.7–6.9. These characteristics of strain J5B^T have been reported previously.

The strain M52^T was isolated and maintained under thiosulfate-oxidizing conditions, without organic carbon source. Chemolithoautotrophic growth of the strain was also supported by electron donors of tetrathionate and elemental sulfur. On

173 the other hand, hydrogen gas and sulfide did not support growth strain M52^T. As
174 reported previous, strain M52^T can grow on acetate and lactate. The culturing
175 experiments in this study revealed that the following organic acids are also used as
176 growth substrates; pyruvate, propionate, succinate, fumarate, malate, and butyrate
177 and isobutyrate. None of tested sugars, alcohols or cresols supported growth of strain
178 M52^T.

179 The cellular fatty acid profiles of strains J5B^T and M52^T are shown in Table
180 S1. For strain J5B^T, effects of growth conditions on fatty acid composition was
181 investigated by analyzing cells grown autotrophically and heterotrophically. Under
182 both conditions, $C_{16:0}$ was the most abundant fatty acid, followed by summed feature
183 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$). These two major components accounted for 78–80% of
184 total, irrespective of the culture conditions. The third most abundant fatty acid
185 differed depending on the growth conditions. That was cyclo-$C_{17:0}$ under the
186 heterotrophic condition, whereas that under the autotrophic condition was summed
187 feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$). The fatty acid profile of strain M52^T was
188 similar to that of J5B^T grown under the same conditions, sharing the major fatty
189 acids (Table S1).

190

191 Genomic characteristics

192 Complete genome sequences of strains J5B^T and M52^T were independently
193 obtained in the previous studies. The values of ANI and dDDH between them were
194 calculated to be 87.93 % and 34.3 %, respectively. These values are lower than
195 thresholds for species delineation (Richter & Rosselló-Móra, 2009; Meier-Kolthoff et
196 al., 2013), suggesting that strains J5B^T and M52^T are representatives of different
197 species.

198 As reported previously, strains J5B^T and M52^T are chemolithoautotrophs
199 growing on thiosulfate. According to these physiological properties, genes for sulfur
200 oxidation and carbon fixation were identified in their genomes. As those for sulfur
201 oxidation, genes encoding proteins involved in the Sox-Dsr-Soe pathway were
202 identified in the genome of strain M52^T. The Sox-Dsr-Soe pathway is one of the
203 three core pathways of sulfur oxidation, and shared by sulfur oxidizers in the family
204 *Sterolibacteriaceae*, i.e., *Sulfuritalea hydrogenivorans* sk43H^T, *Sulfurisoma*
205 *sediminicola* BSN1^T and strain J5B^T (Watanabe et al. 2019). These sulfur oxidizers
206 seem to fix inorganic carbon via the Calvin–Benson–Bassham cycle. They have
207 genes encoding key enzyme of the cycle, ribulose-1,5-bisphosphate
208 carboxylase/oxygenase (RuBisCO). They all have the *cbbM* gene which encodes

209 form II RuBisCO, whereas the *cbbLS* genes encoding form I RuBisCO were
210 identified only in genomes of strain M52^T and *Sulfurisoma sediminicola* BSN1^T.
211 *Sulfuritalea hydrogenivorans* sk43H^T is known to have key genes for anaerobic
212 aromatics degradation and arsenate respiration (Sperfeld et al., 2019; Watanabe et al.
213 2017). These genes were not identified in the genomes of strains J5B^T and M52^T.
214
215 Taxonomic assignment
216 The strains J5B^T and M52^T have two copies of the 16S rRNA gene in
217 their genomes, respectively. The copies of strain J5B^T are both 1544 nt in length, and
218 there are five mismatches between their sequences. Those of strain M52^T are
219 different in length, 1544 nt and 1534 nt respectively. These sequences indicated the
220 highest sequence identities to species belonging to family *Sterolibacteriaceae*, but
221 the identities were 95% or lower. The sequence identities between strains J5B^T and
222 M52^T ranged 98.3–98.8%. The sequence variations between the copies of each strain
223 did not affect the phylogenetic analysis described below, because they were located
224 at positions with gaps and thus excluded from the calculation.
225 Phylogenetic positions of strains J5B^T and M52^T within the order
226 *Nitrosomonadales* are shown in Fig. 1, as the maximum likelihood tree of the 16S

rRNA gene (full version is shown in Figure S1). The tree indicated that the strains belong to the family *Sterolibacteriaceae* but not to any existing genera. This means that a novel genus should be created to accommodate strains J5B^T and M52^T. The tree also indicate that the genus *Rugosibacter* should be reclassified to the family *Sterolibacteriaceae*, at least at present. These conclusions did not conflict with the phylogenetic analysis based on 92 marker genes identified in the genomes (Fig. S2).

As an attempt to evaluate reasonability of genus-level classification, AAI values were calculated for strains J5B^T and M52^T, against all type strains within the family *Sterolibacteriaceae* (Table 1). The calculated values ranged from 63.2 to 71.5%. These results do not provide solid support for creation of a new genus, because AAI values between different species in a same genus are 60–80% in many cases (Luo et al., 2014). In the family *Sterolibacteriaceae*, AAI may not work as a key criterion for genus-level classification. The AAI values were also calculated in all combinations of the type strains in this family (Table S2). The values between *Sterolibacteriaceae* strains from different genera were within the range of 60–80%, in all combinations.

Conclusion

On the basis of the results of the previous and present studies, strain J5B^T is proposed as the type strain of a novel species of a new genus, with the name *Sulfuricystis multivorans* gen. nov., sp. nov. In addition, strain M52^T is proposed as the type strain of another species of the novel genus, with the name *Sulfuricystis thermophila* sp. nov. Further, emended description of the genus *Rugosibacter* is also proposed to indicate its affiliation to the family *Sterolibacteriaceae*.

Description of *Sulfuricystis* gen. nov.

Sulfuricystis (Sul.fu.ri.cys'tis. L. neut. n. *sulfur*, sulfur; Gr. fem. n. *kystis*, a bag; N.L. fem. n. *Sulfuricystis*, sulfur-oxidizing bag).

Grows by oxidation of sulfur compounds. Facultatively anaerobic and neutrophilic. Gram-stain-negative. Major cellular fatty acids are $C_{16:0}$ and $C_{16:1}$ ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$). Phylogenetically, belongs the family *Sterolibacteriaceae*. The type species is *Sulfuricystis multivorans*.

Description of *Sulfuricystis multivorans* sp. nov.

Sulfuricystis multivorans (mul.ti.vo'rans. L. masc. adj. *multus*, many; L. pres. part.

vorans, devouring, eating; N.L. part. adj. *multivorans*, devouring various substrates).

In addition to properties listed in the genus description, cells are rod-shaped, 0.8–2.0 μm long and 0.4–0.5 μm wide. Uses oxygen and nitrate as electron acceptor. Under nitrate-reducing conditions, grows chemolithoautotrophically on thiosulfate and elemental sulfur, but not on sulfide, tetrathionate, or hydrogen gas. Grows heterotrophically on pyruvate, lactate, acetate, propionate, succinate, fumarate, malate, and butyrate. Does not grow on benzoate, isobutyrate, methanol, ethanol, formate, citrate, glucose, xylose, phenol, *o*-cresol, and *m*-cresol. Temperature range for growth is 28–55°C, with an optimum of 45–50°C. Growth occurs at pH 5.8–8.7, with an optimum of pH 6.7–7.4. G + C content of genomic DNA of the type strain is 62.4 mol%.

The type strain J5B^T (= BCRC 81316^T = DSM 104688^T = NBRC 112605^T) was isolated from a microbial mat of a hot spring in Japan.

The GenBank/EMBL/DDBJ accession numbers for the chromosome and two plasmids of type strain are AP018718 and AP018719-AP018720, respectively.

Description of *Sulfuricystis thermophila* sp. nov.

Sulfuricystis thermophila (ther.mo'phi.la. Gr. masc. adj. *thermos*, hot; Gr. masc. adj. *philos*, loving; N.L. fem. adj. *thermophila*, heat-loving).

In addition to properties listed in the genus description, cells are rod-shaped, 1.8–3.2 μm long and 0.4–0.5 μm wide. Uses oxygen and nitrate as electron acceptor. Under nitrate-reducing conditions, grows chemolithoautotrophically on thiosulfate, tetrathionate and elemental sulfur, but not on sulfide or hydrogen gas. Grows heterotrophically on pyruvate, lactate, acetate, propionate, succinate, fumarate, malate, and butyrate and isobutyrate. Does not grow on benzoate, methanol, ethanol, formate, citrate, glucose, xylose, phenol, *o*-cresol, and *m*-cresol. Temperature range for growth is 18–55°C, with an optimum of 50°C. Growth occurs at pH 5.5–8.6, with an optimum of pH 6.6–6.9. G+C content of genomic DNA of the type strain is 63.6 mol%.

Type strain M52^T (= BCRC 81317^T = NBRC 114016^T) was isolated from a microbial mat of a hot spring in Japan.

The GenBank/EMBL/DDBJ accession number for the complete genome of the type strain is AP019373.

Emended description of *Rugosibacter* (Corteselli et al., 2017)

296 *Rugosibacter* (Ru.go.si.bac'ter. L. adj. *rugosus* wrinkled; N. L. masc. n. *bacter* a rod;
297 N. L. masc. n. *Rugosibacter* a wrinkled rod).

298 Cells are Gram-strain-negative and non-motile. Aerobic. Catalase-negative and
299 oxidase-positive. Heterotrophic growth occurs on organic acids. Predominant fatty
300 acids are summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega7c$) and $C_{16:0}$. The major polar
301 lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol
302 and phospholipid. The major respiratory quinone is ubiquinone-8. Phylogenetically,
303 belongs the family *Sterolibacteriaceae*. The type species is *Rugosibacter*
304 *aromaticivorans*.

305

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308

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402 **Statements and Declarations**

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405

406 **Figure legends**

407 Fig. 1. Maximum likelihood tree of the 16S rRNA gene sequences, showing

408 phylogenetic positions of strains J5B^T and M52^T within the order *Nitrosomonadales*.

409 The Kimura 2-parameter model with a discrete gamma distribution and invariable

410 sites was used as the best model with the lowest BIC. All positions containing gaps

411 and missing data were eliminated and there were 1,279 positions in the final dataset.

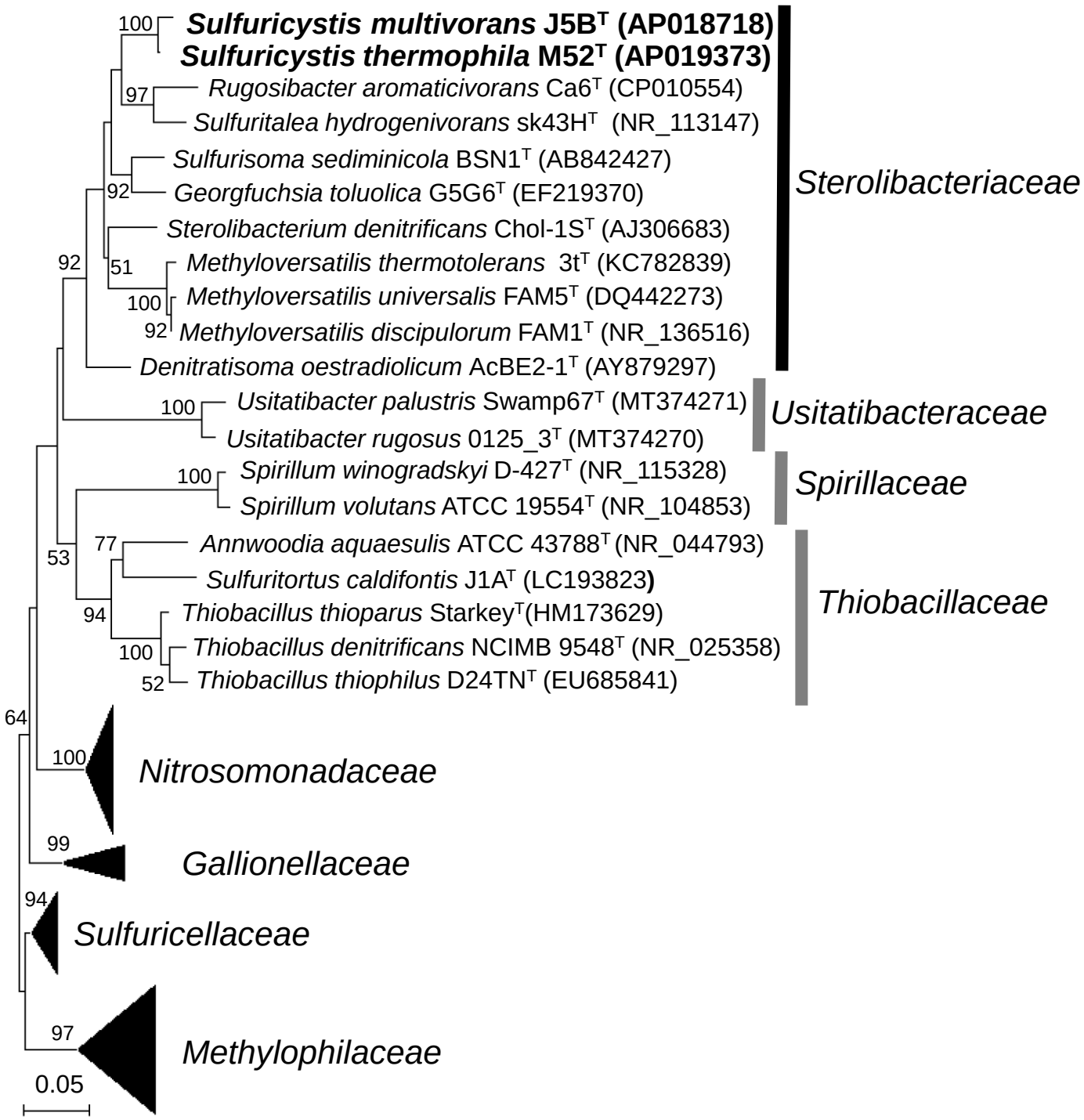
412 Numbers on nodes represent percentage values of 1,000 bootstrap resampling (values

413 greater than 50 are shown). Names of all strains included in the analysis are shown in

414 full version of the tree provided as Figure S1.

Table 1. Differential characteristics of strains J5B^T, M52^T and type strains of species in the family *Sterolibacteriaceae*. Strains: 1, J5B^T (Watanabe et al., 2019); 2, M52^T (Ospino et al., 2019; this study); 3, *Sterolibacterium denitrificans* Chol-1S^T (Tarlera & Denner, 2003); 4, *Denitratisoma oestradiolicum* AcBE2-1^T (Fahrbach et al., 2006); 5, *Methyloversatilis universalis* FAM5^T (Kalyuzhnaya et al., 2006); 6, *M. thermotolerans* 3t^T (Doronina et al., 2014); 7, *M. discipulorum* FAM1^T (Smalley et al., 2015); 8, *Georgfuchsia toluolica* G5G6^T (Weelink et al., 2009); 9, *Sulfuritalea hydrogenivorans* sk43H^T (Kojima & Fukui, 2011); 10, *Sulfurisoma sediminicola* BSN1^T (Kojima & Fukui, 2014); 11, *Rugosibacter aromaticivorans* Ca6^T (Corteselli et al., 2017). Data were retrieved from respective references, except for AAI values calculated in this study.

	1	2	3	4	5	6	7	8	9	10	11
Optimum temp.	45–50	50	30-32	28-30	37	30-37	25–37	25-30	25	30-32	30-34
Temp. range	28–55	18–55	15-35	4-38	10-42	10–45	7-37	20-37	8-32	8-34	20-35
Optimum pH	6.7–7.4	6.6–6.9	7.0	7.0-7.2	8.0	7.0-7.5	6.6-8.0	7.3	6.7-6.9	7.8-8.1	6.5
pH range	5.8–8.7	5.5–8.6	5.8-8.0	6.4-8.5	6.5-9.0	6.5-8.5	8.8-8.0	6.6-9.0	6.4-7.6	6.8–8.8	6.5-7.5
Electron acceptor											
oxygen	+	+	+	+	+	+	+	-	+	+	+
nitrate	+	+	+	+	-	+	+	+	+	+	-
Electron donor											
methanol	-	-	ND	-	+	+	+	-	-	-	ND
ethanol	-	-	-	-	+	+	+	-	-	-	ND
thiosulfate	+	+	ND	-	ND	ND	ND		+	+	ND
hydrogen	-	-	ND	ND	ND	ND	ND	-	+	+	ND
benzoate	-	-	-	-	ND	ND	ND	-	+	-	ND
phenol	-	-	-	-	ND	ND	+	+	-	-	ND
m-cresol	-	-	ND	-	ND	ND	ND	+	ND	-	ND
isobutyrate	-	+	-	+	ND	ND	ND	ND	+	-	ND
pyruvata	+	+	-	+	-	ND	+	-	+	+	ND
lactate	+	+	-	+	ND	ND	ND	-	+	+	-
acetate	+	+	+	+	-	+	+	-	+	+	-
propionate	-	+	+	+	ND	ND	ND	-	+	+	-
succinate	+	+	-	+	+	+	+	-	+	+	-
fumarate	+	+	-	+	ND	+	ND	-	+	+	ND
malate	+	+	-	ND	+	ND	+	-	+	+	ND
butyrate	+	+	+	+	ND	ND	ND	-	+	-	ND
glocose	-	-	-	-	+	+	+	-	-	-	ND
xylose	-	-	-	-	ND	ND	ND	-	-	-	ND
citrate	-	-	-	-	+	-	ND	-	-	-	-
formate	-	-	-	-	+	+	+	ND	-	-	+
AAI to J5B^T (%)	100	89.9	66.4	67.4	63.5	63.2	63.4	67.0	69.1	71.1	67.0
AAI to M52^T (%)	89.9	100	66.2	68.1	63.6	63.6	63.5	67.3	69.6	71.5	67.8


100
Sulfuricystis multivorans J5B^T (AP018718)
Sulfuricystis thermophila M52^T (AP019373)
97
Rugosibacter aromaticivorans Ca6^T (CP010554)
Sulfuritalea hydrogenivorans sk43H^T (NR_113147)
Sulfurisoma sediminicola BSN1^T (AB842427)
92
Georgfuchsia toluolica G5G6^T (EF219370)
Sterolibacterium denitrificans Chol-1S^T (AJ306683)
92
51
Methyloversatilis thermotolerans 3t^T (KC782839)
100
Methyloversatilis universalis FAM5^T (DQ442273)
92
Methyloversatilis discipulorum FAM1^T (NR_136516)
Denitratisoma oestradiolicum AcBE2-1^T (AY879297)
Sterolibacteriaceae
100
Usitatibacter palustris Swamp67^T (MT374271)
Usitatibacter rugosus 0125_3^T (MT374270)
Usitatibacteraceae
100
Spirillum winogradskyi D-427^T (NR_115328)
Spirillum volutans ATCC 19554^T (NR_104853)
Spirillaceae
77
53
Annwoodia aquaesulis ATCC 43788^T (NR_044793)
Sulfuritortus caldifontis J1A^T (LC193823)
94
Thiobacillus thioparus Starkey^T(HM173629)
100
Thiobacillus denitrificans NCIMB 9548^T (NR_025358)
52
Thiobacillus thiophilus D24TN^T (EU685841)
Thiobacillaceae
64
100
Nitrosomonadaceae
99
Gallionellaceae
94
Sulfuricellaceae
97
Methylophilaceae
0.05