



Title	Sulfuricystis multivorans gen. nov., sp. nov. and Sulfuricystis thermophila sp. nov., facultatively autotrophic sulfur-oxidizing bacteria isolated from a hot spring, and emended description of the genus Rugosibacter
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2 ***Sulfuricystis multivorans* gen. nov., sp. nov. and *Sulfuricystis***  
3 ***thermophila* sp. nov., facultatively autotrophic sulfur-oxidizing**  
4 **bacteria isolated from a hot spring, and emended description**  
5 **of the genus *Rugosibacter***

6

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23

24 **Abstract**

25 Strains J5B<sup>T</sup> and M52<sup>T</sup> are facultatively autotrophic sulfur-oxidizing bacteria isolated  
26 from a microbial mat from a hot spring. They were isolated and partially  
27 characterized in previous studies, as facultative anaerobes which use nitrate as  
28 electron acceptor. In this study, additional characterizations were made to determine  
29 their taxonomic status. In both strains, major cellular fatty acids were C<sub>16:1</sub> (C<sub>16:1</sub>ω7c  
30 and/or C<sub>16:1</sub>ω6c) and C<sub>16:0</sub>. Their chemolithoautotrophic growth was supported by  
31 thiosulfate and elemental sulfur. They used some organic acids as growth substrates.  
32 Their 16S rRNA gene sequences indicated the highest sequence identities to species  
33 in the family *Sterolibacteriaceae*, but the identities were 95% or lower. Phylogenetic  
34 analysis indicated that these strains do not belong to any existing genera. Values of  
35 average nucleotide identity and digital DNA–DNA hybridization between strains  
36 J5B<sup>T</sup> and M52<sup>T</sup> were 87.93 % and 34.3 %, respectively. On the basis of phenotypic  
37 and genomic characteristics, *Sulfuricystis multivorans* gen. nov. sp. nov., and  
38 *Sulfuricystis thermophila* sp. nov. are proposed, with type strains of J5B<sup>T</sup> and M52<sup>T</sup>,  
39 respectively. An emended description of the genus *Rugosibacter* is also proposed, for  
40 its reclassification to the family *Sterolibacteriaceae*.

41

42

## 43 **Introduction**

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

59 The original description of family *Sterolibacteriaceae* states that the family is  
60 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<sup>T</sup> 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<sup>T</sup>, 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<sup>T</sup>  
73 is also sulfur oxidizer, isolated from the same microbial mat. The sulfur-oxidizing  
74 bacterium, strain M52<sup>T</sup>, 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<sup>T</sup> and M52<sup>T</sup> 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<sup>T</sup> and M52<sup>T</sup> 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<sup>T</sup> and M52<sup>T</sup> were grown at 45°C,  
97 in a medium consisting of the following constituents (l<sup>-1</sup>): 1 g NaNO<sub>3</sub>, 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<sub>2</sub> 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<sup>T</sup>, 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<sup>T</sup> 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<sup>-1</sup>), sulfide (2 mM), tetrathionate (10 mM)  
113 and hydrogen gas (H<sub>2</sub>/N<sub>2</sub>/CO<sub>2</sub> 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<sub>3</sub> (1 g l<sup>-1</sup>), yeast extract (0.1 g l<sup>-1</sup>), Casamino  
116 acids (0.1 g l<sup>-1</sup>) and cyanocobalamin (50 µg l<sup>-1</sup>). The medium was dispensed in closed

117 culture bottles and the headspace was filled with N<sub>2</sub> 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<sup>T</sup> 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<sup>T</sup> and M52<sup>T</sup> 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<sup>T</sup>  
153 and M52<sup>T</sup> were input into the pipeline, along with those of the type strains in the family

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

159

## 160 **Results and Discussion**

161

### 162 Physiological and chemotaxonomic characteristics

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

170           The strain M52<sup>T</sup> was isolated and maintained under thiosulfate-oxidizing  
171 conditions, without organic carbon source. Chemolithoautotrophic growth of the  
172 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<sup>T</sup>. As  
174 reported previous, strain M52<sup>T</sup> 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<sup>T</sup>.

179           The cellular fatty acid profiles of strains J5B<sup>T</sup> and M52<sup>T</sup> are shown in Table  
180 S1. For strain J5B<sup>T</sup>, effects of growth conditions on fatty acid composition was  
181 investigated by analyzing cells grown autotrophically and heterotrophically. Under  
182 both conditions, C<sub>16:0</sub> was the most abundant fatty acid, followed by summed feature  
183 3 (C<sub>16:1</sub>ω7*c* and/or C<sub>16:1</sub>ω6*c*). 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<sub>17:0</sub> under the  
186 heterotrophic condition, whereas that under the autotrophic condition was summed  
187 feature 8 (C<sub>18:1</sub>ω7*c* and/or C<sub>18:1</sub>ω6*c*). The fatty acid profile of strain M52<sup>T</sup> was  
188 similar to that of J5B<sup>T</sup> grown under the same conditions, sharing the major fatty  
189 acids (Table S1).

190

191 Genomic characteristics

192 Complete genome sequences of strains J5B<sup>T</sup> and M52<sup>T</sup> 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<sup>T</sup> and M52<sup>T</sup> are representatives of different  
197 species.

198 As reported previously, strains J5B<sup>T</sup> and M52<sup>T</sup> 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<sup>T</sup>. 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<sup>T</sup>, *Sulfurisoma*  
205 *sediminicola* BSN1<sup>T</sup> and strain J5B<sup>T</sup> (Watanabe et al. 2019). These sulfur oxidizers  
206 seem to fix inorganic carbon via the Calvin–Benson–Bascham 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<sup>T</sup> and *Sulfurisoma sediminicola* BSN1<sup>T</sup>.  
211 *Sulfuritalea hydrogenivorans* sk43H<sup>T</sup> 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<sup>T</sup> and M52<sup>T</sup>.

214

215 Taxonomic assignment

216           The strains J5B<sup>T</sup> and M52<sup>T</sup> have two copies of the 16S rRNA gene in  
217 their genomes, respectively. The copies of strain J5B<sup>T</sup> are both 1544 nt in length, and  
218 there are five mismatches between their sequences. Those of strain M52<sup>T</sup> 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<sup>T</sup> and  
222 M52<sup>T</sup> 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<sup>T</sup> and M52<sup>T</sup> within the order  
226 *Nitrosomonadales* are shown in Fig. 1, as the maximum likelihood tree of the 16S

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

233           As an attempt to evaluate reasonability of genus-level classification, AAI  
234 values were calculated for strains J5B<sup>T</sup> and M52<sup>T</sup>, against all type strains within the  
235 family *Sterolibacteriaceae* (Table 1). The calculated values ranged from 63.2 to  
236 71.5%. These results do not provide solid support for creation of a new genus,  
237 because AAI values between different species in a same genus are 60–80% in many  
238 cases (Luo et al., 2014). In the family *Sterolibacteriaceae*, AAI may not work as a  
239 key criterion for genus-level classification. The AAI values were also calculated in  
240 all combinations of the type strains in this family (Table S2). The values between  
241 *Sterolibacteriaceae* strains from different genera were within the range of 60–80%,  
242 in all combinations.

243

244 **Conclusion**

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

251

252   **Description of *Sulfuricystis* gen. nov.**

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

255 Grows by oxidation of sulfur compounds. Facultatively anaerobic and neutrophilic.  
256 Gram-stain-negative. Major cellular fatty acids are C<sub>16:0</sub> and C<sub>16:1</sub> (C<sub>16:1</sub>ω7c and/or  
257 C<sub>16:1</sub>ω6c). Phylogenetically, belongs the family *Sterolibacteriaceae*. The type species  
258 is *Sulfuricystis multivorans*.

259

260   **Description of *Sulfuricystis multivorans* sp. nov.**

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

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

263 In addition to properties listed in the genus description, cells are rod-shaped, 0.8–2.0

264 µm long and 0.4–0.5 µm wide. Uses oxygen and nitrate as electron acceptor. Under

265 nitrate-reducing conditions, grows chemolithoautotrophically on thiosulfate and

266 elemental sulfur, but not on sulfide, tetrathionate, or hydrogen gas. Grows

267 heterotrophically on pyruvate, lactate, acetate, propionate, succinate, fumarate, malate,

268 and butyrate. Does not grow on benzoate, isobutyrate, methanol, ethanol, formate,

269 citrate, glucose, xylose, phenol, *o*-cresol, and *m*-cresol. Temperature range for growth

270 is 28–55°C, with an optimum of 45–50°C. Growth occurs at pH 5.8–8.7, with an

271 optimum of pH 6.7–7.4. G + C content of genomic DNA of the type strain is 62.4

272 mol%.

273 The type strain J5B<sup>T</sup> (= BCRC 81316<sup>T</sup> = DSM 104688<sup>T</sup> = NBRC 112605<sup>T</sup>) was

274 isolated from a microbial mat of a hot spring in Japan.

275 The GenBank/EMBL/DDBJ accession numbers for the chromosome and two

276 plasmids of type strain are AP018718 and AP018719-AP018720, respectively.

277

278 **Description of *Sulfuricystis thermophila* sp. nov.**



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

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

290 Type strain M52<sup>T</sup> (= BCRC 81317<sup>T</sup> = NBRC 114016<sup>T</sup>) was isolated from a  
291 microbial mat of a hot spring in Japan.

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

294

295 **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-stain-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<sub>16:1</sub>ω7c and/or C<sub>16:1</sub>ω7c) and C<sub>16:0</sub>. 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

### 306 **Acknowledgments**

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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<sup>T</sup> and M52<sup>T</sup> 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<sup>T</sup>, M52<sup>T</sup> and type strains of species in the family *Sterolibacteriaceae*. Strains: 1, J5B<sup>T</sup> (Watanabe et al., 2019); 2, M52<sup>T</sup> (Ospino et al., 2019; this study); 3, *Sterolibacterium denitrificans* Chol-1S<sup>T</sup> (Tarlera & Denner, 2003); 4, *Denitratisona oestradiolicum* AcBE2-1<sup>T</sup> (Fahrbach et al., 2006); 5, *Methyloversatilis universalis* FAM5<sup>T</sup> (Kalyuzhnaya et al., 2006); 6, *M. thermotolerans* 3t<sup>T</sup> (Doronina et al., 2014); 7, *M. discipulorum* FAM1<sup>T</sup> (Smalley et al., 2015); 8, *Georgfuchsia toluolica* G5G6<sup>T</sup> (Weelink et al., 2009); 9, *Sulfuritalea hydrogenivorans* sk43H<sup>T</sup> (Kojima & Fukui, 2011); 10, *Sulfurisoma sedimnicola* BSN1<sup>T</sup> (Kojima & Fukui, 2014); 11, *Rugosibacter aromaticivorans* Ca6<sup>T</sup> (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
pyruvate	+	+	-	+	-	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
glucose	-	-	-	-	+	+	+	-	-	-	ND
xylose	-	-	-	-	ND	ND	ND	-	-	-	ND
citrate	-	-	-	-	+	-	ND	-	-	-	-
formate	-	-	-	-	+	+	+	ND	-	-	+
AAI to J5B <sup>T</sup> (%)	100	89.9	66.4	67.4	63.5	63.2	63.4	67.0	69.1	71.1	67.0
AAI to M52 <sup>T</sup> (%)	89.9	100	66.2	68.1	63.6	63.6	63.5	67.3	69.6	71.5	67.8

