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## **A pan-genomic approach reveals novel *Sulfurimonas* clade in the ferruginous meromictic Lake Pavin**

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### ► To cite this version:

Corinne Biderre-Petit, Damien Courtine, Claire Hennequin, Pierre Galand, Stefan Bertilsson, et al.. A pan-genomic approach reveals novel *Sulfurimonas* clade in the ferruginous meromictic Lake Pavin. *Molecular Ecology Resources*, In press, 10.1111/1755-0998.13923 . hal-04381944v2

**HAL Id: hal-04381944**

**<https://hal.science/hal-04381944v2>**

Submitted on 10 Jan 2024

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1 **Title**

2 A pan-genomic approach reveals novel *Sulfurimonas* clade in the ferruginous meromictic Lake

3 Pavin

4

5 **Running title:** *Sulfurimonas* pan-genomic analysis

6

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## 21 **Abstract**

22 The permanently anoxic waters in meromictic lakes create suitable niches for the growth of  
23 bacteria using sulfur metabolisms like sulfur oxidation. In Lake Pavin, the anoxic water mass  
24 hosts an active cryptic sulfur cycle that interacts narrowly with iron cycling, however the  
25 metabolisms of the microorganisms involved are poorly known. Here we combined  
26 metagenomics, single-cell genomics, and pan-genomics to further expand our understanding  
27 of the bacteria and the corresponding metabolisms involved in sulfur oxidation in this  
28 ferruginous sulfide- and sulfate-poor meromictic lake. We highlighted two new species within  
29 the genus *Sulfurimonas* that belong to a novel clade of chemotrophic sulfur oxidizers exclusive  
30 to freshwaters. We moreover conclude that this genus holds a key-role not only in limiting  
31 sulfide accumulation in the upper part of the anoxic layer but also constraining carbon,  
32 phosphate, and iron cycling.

33

34 **Keywords:** Meromictic lake, freshwater, sulfur oxidation, *Sulfurimonas*, pan-genome.

35

## 36 **Introduction**

37 Sulfur cycling is an important driver of microbial growth and influences element conversions  
38 in most environments (Berben et al., 2019). In the absence of oxygen, the sulfide produced as  
39 part of this cycle (Booker et al., 2017; Grein et al., 2013; Ravot et al., 2006) is further oxidized  
40 mostly in environments with low oxygen levels and/or limited free transition metals. In some  
41 aquatic environments, biological rates of sulfide oxidation can be orders of magnitude higher  
42 than rates of abiotic sulfide oxidation. Microbial oxidation can thereby be of vital importance,  
43 as sulfide is potentially toxic to aerobic organisms and consumption in anoxic waters can

44 effectively prevent its efflux to the aerobic waters. Bacteria involved in sulfur oxidation (SOBs)  
45 are phylogenetically and metabolically diverse and spread across various environments.  
46 Moreover, although sulfur-based metabolism appeared almost 3.5 billion years ago, many  
47 enzymes and enzymatic pathways involved in this process remain to be discovered (Meier et  
48 al., 2017; Nosalova et al., 2023; Wacey et al., 2011).

49 In most anaerobic sub-surface systems, the dominant chemolithotrophic SOBs belong to the  
50 co-occurring *Sulfurovum*, *Sulfuricurvum* and *Sulfurimonas*-related species (*Campylobacteria*  
51 formerly *Epsilonproteobacteria*). *Sulfurimonas* representatives thrive in a broad spectrum of  
52 environments (Han & Perner, 2015) including pelagic redox zones (Grote et al., 2012; Hamilton  
53 et al., 2014; Henkel et al., 2021; Salmaso, 2019), hydrothermal vents (Hu et al., 2021; Sikorski  
54 et al., 2010; Takai et al., 2006; S. Wang, Jiang, et al., 2021; S. Wang, Shao, et al., 2021),  
55 petroleum reservoirs (Lahme et al., 2019; Tian et al., 2017), groundwaters (Anantharaman et  
56 al., 2016; Probst et al., 2018) and mud volcanoes (Ratnikova et al., 2020). They are particularly  
57 abundant in oxygen minimum zones (15 to 30% of all microorganisms (Callbeck et al., 2021;  
58 van Vliet et al., 2021)) and deep-sea hydrothermal vents (up to 70% of all microorganisms  
59 (Campbell et al., 2006; Han & Perner, 2015; L. Wang et al., 2017)). Their widespread  
60 occurrence is largely explained by their versatile metabolisms linked to the use of a wide  
61 variety of electron donors (e.g. sulfide, thiosulfate,  $S^0$ ,  $H_2$ ) and acceptors (e.g.  $NO_3^-$ ,  $O_2$ ,  $MnO_2$ )  
62 and by their adaptative abilities (e.g. aerobic to anoxic growth conditions, osmotic stress, etc)  
63 (Götz et al., 2018; Molari et al., 2023; Rogge et al., 2017; Taylor et al., 2018; Zeng et al., 2021).  
64 In addition to chemolithoautotrophic oxidation of sulfur compounds, they also perform  
65 chemolithoautotrophic disproportionation of thiosulfate and elemental sulfur that may  
66 represent a previously unrecognized primary production process (S. Wang et al., 2023).

67 *Sulfurimonas* spp. have mostly been isolated from hydrothermal vents and marine sediments.  
68 However, high-scale DNA sequencing showed that they are widespread in fresh waters such  
69 as aquifers (Anantharaman et al., 2016; Probst et al., 2018; Tian et al., 2017), springs (Hahn et  
70 al., 2022), and lakes (Diao et al., 2018; Fang et al., 2022), especially in the anoxic zone of  
71 meromictic (permanently stratified) systems (Berg et al., 2019; Block et al., 2021; Hamilton et  
72 al., 2014; Salmaso, 2019). In the meromictic Lake Pavin, the co-occurring *Sulfuricurvum* and  
73 *Sulfurimonas*-related species comprise up to 12% of the total bacterial community at the oxic-  
74 anoxic boundary (Biderre-Petit, Boucher, et al., 2011). However, compared to most other  
75 euxinic meromictic lakes (both anoxic and sulfidic), Lake Pavin contains very low free sulfide  
76 concentrations (<15 $\mu$ M) in the upper part of its monimolimnion, thus representing a good  
77 model for studying the ecology and metabolic traits of *Sulfurimonas*.

78 The small surface (44 ha) and great depth (92m) of Lake Pavin impede light penetration below  
79 the oxycline, resulting in the fixation of carbon predominantly by chemolithoautotrophic  
80 microorganisms in the anoxic bottom layer (*i.e.* monimolimnion). In this sulfide and sulfate-  
81 poor (<20  $\mu$ m) water mass, about 80% of total sulfide detected is in the electroactive FeS  
82 colloidal form (Bura-Nakić et al., 2012). Berg et al. (2019) showed that the cryptic sulfur cycle  
83 in these waters is tightly interacting with the biogeochemical iron cycle, suggesting that the  
84 microorganisms involved in these two cycles contribute to mineral formation through  
85 biomineralization processes (Miot et al., 2016). The bacteria and metabolisms involved remain  
86 however poorly known.

87 The main goal of our study was to test the hypothesis that *Sulfurimonas* strains that thrive in  
88 Lake Pavin are specific to freshwater systems, and act as key-players in tightly linked sulfur,  
89 carbon, phosphate, and iron cycles. We therefore investigated the distribution of

90 *Sulfurimonas* spp. and their adaptation processes along the anoxic water column. Ribotypes  
91 and metabolisms of this genus were studied *via* metabarcoding, genome-resolved  
92 metagenomics, and single cell genomics, and compared with publicly available data from all  
93 environments shown to host *Sulfurimonas* spp by using a pan-genomic approach.

94

## 95 **Materials and methods**

96

### 97 **Sample collection**

98 Water samples for Single Amplified Genomes (SAGs) were collected from Lake Pavin on  
99 December 2015 at 65 and 80m depth, while samples for metagenomes were collected on  
100 October 2015 at 50, 65, 70 and 80m. All samples were recovered from a platform positioned  
101 above the deepest point of the lake (*i.e.* 92m deep) using a Van Dorn bottle. Samples for SAGs  
102 were cryopreserved in 1X TE, 5% glycerol (final concentration) for later cell-sorting, and  
103 biomass for metagenome sequencings (called MetaG2, N=4) were concentrated by filtration  
104 onto 0.22µm pore size polycarbonate (47-mm diameter, TSTP, Millipore, Billerica,  
105 Massachusetts, USA) and stored at -80°C for later DNA extraction.

106

### 107 **Single amplified genomes (SAGs)**

108 Single-cell sorting, whole genome amplification and SAG screening were mainly performed as  
109 previously described ((Alneberg et al., 2018), for details see Supplemental material). Thirty-  
110 one SAGs that carried 16S rRNA sequences, among which one affiliated to the genus  
111 *Sulfurimonas*, were selected for genome sequencing. Their DNA content was quantified with  
112 Qubit dsDNA HS assay kit (ThermoFisher Scientific, Waltham MA) and diluted to 0.2ng/µl in

113 nuclease-free water. MiSeq sequencing libraries were prepared with Nextera XT DNA Library  
114 Preparation Kit and Nextera XT Index Kit (Illumina, San Diego CA), and run according to the  
115 manufacturer's instructions. After Qubit quantification, the libraries were pooled in equimolar  
116 concentration and sequenced on a MiSeq with the V3 2x300bp strategy. Subsequently, we  
117 used the *bbduck2* and *bbnorm* programs from the BBTools package (release 12.10.2015)  
118 (<http://jgi.doe.gov/data-and-tools/bbtools/>) to remove sequencing adapters and discard the  
119 low quality reads (option: trimq = 28, maq = 20, maxns = 0, and minlength = 25) as well as to  
120 normalize the quality-controlled reads (parameters target = 40, and mindepth = 0). The  
121 remaining reads were then assembled with SPAdes v3.5.0 with `--careful` and `--sc` options  
122 (Bankevich et al., 2012). Completeness and contamination were estimated by CheckM v1.0.5  
123 (Parks et al., 2015). Gene prediction for the SAGs was performed using Prodigal V2.6.3 (Hyatt  
124 et al., 2010).

125

### 126 **Metagenome sequencing, assembly, and binning**

127 For MetaG2 metagenomes (N=4), genomic DNA was extracted using a standard phenol-  
128 chloroform method as previously described (Biderre-Petit, Jézéquel, et al., 2011). DNA  
129 libraries were prepared and sequenced by GenoScreen (Lille, France), with an Illumina  
130 HiSeq2500 sequencer to generate 2x150bp paired-end reads. After sequencing, reads were  
131 preprocessed to remove Nextera adapters using the *bbduck* program as described above. Prior  
132 to assembly, raw paired-end reads were filtered using Trimmomatic v.0.33 (option:  
133 LEADING=28, TRAILING=28, SLIDINGWINDOW=4:15 and MINLEN=30) (Bolger et al., 2014).  
134 Then, IDBA-UD v1.1.1 was used to assemble each sample (Peng et al., 2012) with the default  
135 parameters and both pair-end reads (`-r` entry) plus single-end reads (`--long` entry).

136 To further assemble additional (low coverage or less abundant) contigs, we performed an  
137 additional co-assembly of all Lake Pavin metagenomes samples, *i.e.* MetaG2 obtained in the  
138 present study and MetaG1 obtained from a previous study (Biderre-Petit et al., 2019). The  
139 MetaG1 (N=4) and MetaG2 (N=4) datasets were co-assembled using MEGAHIT v.1.1.1 with  
140 the ‘meta-sensitive’ preset option (D. Li et al., 2015) followed by the filtering of the resulting  
141 contigs by length (>2.5kb, *i.e.* the minimum contig length generally used for the initial binning  
142 (Kang et al., 2015)).

143 To bin the contigs, the process was as follows: (i) metagenomic reads were individually  
144 mapped against contigs using the Burrows-Wheeler-Algorithm (BWA) v.0.7.17 (Li and Durbin  
145 2009) and stored as BAM files using Samtools, (ii) each BAM file was processed by *anvi-profile*  
146 as implemented in *anvi'o* to get the coverage and abundance information for each contig, (iii)  
147 *anvi-merge* was used to combine single profiles into a merged profile database, (iv) contigs  
148 were clustered *via* the automatic binning algorithm CONCOCT v.1.0.0 (Alneberg et al., 2014)  
149 with a maximal number of clusters set to 100 to minimize ‘fragmentation errors’, finally (v)  
150 the resulting bins were manually curated through the *Anvi'o* interactive interface using *anvi-*  
151 *refine* v6.1 (Eren et al., 2015) and as detailed by the *Anvi'o* tutorial  
152 (<https://merenlab.org/2015/05/11/anvi-refine/>). The manually curated bins will hereafter be  
153 referred to as Metagenome-Assembled Genomes (MAGs). MAG abundance across MetaG1  
154 and MetaG2 datasets was assessed with the *anvi-summarize* program from *Anvi'o*.

155

### 156 **Quality evaluation and MAG taxonomic analysis**

157 The SAGs and MAGs genome quality was evaluated using both *Anvi'o* and the ‘lineage  
158 workflow’ of CheckM v1.0.12 (Parks et al., 2015). Completeness/completion and  
159 contamination/redundancy metrics were then used to compute the mean completeness



160 (mcpl) and the mean contamination (mctn) values to classify genome quality according to  
161 established community standards (Bowers et al., 2017). Finally, quality scores were calculated  
162 as follows:  $Q\text{-score} = mcpl - 5 \times mctn$ ; and  $Q'\text{-score} = mcpl - 5 \times mctn + mctn \times (\text{Checkm strain}$   
163  $\text{heterogeneity})/100 + 0.5 \times \log[N50]$ .

164 The Genome Taxonomy Database (GTDB, release r202) (Parks et al., 2022) and associated  
165 taxonomic classification toolkit (GTDB-Tk, v1.7.0, default parameters) (Chaumeil et al., 2020)  
166 were adopted to perform MAGs taxonomic classification on the basis of a set of 120 bacterial  
167 and 122 archaeal gene markers.

168 To produce *Sulfurimonas* MAG/SAG hybrid assemblies of superior contiguity and accuracy, we  
169 tested two different pipelines allowing genome assembly, reconciliation, and merging, namely  
170 CISA v1.3 (Lin & Liao, 2013) and GARM v0.7.5 (Soto-Jimenez et al., 2014). Hybrid genome  
171 quality was evaluated as described before for SAGs and MAGs.

172

### 173 ***Sulfurimonas* MAGs and SAG abundance**

174 Prediction of 16S rDNA genes was performed on *Sulfurimonas* SAG and MetaG2 contigs, using  
175 HMM search (Huang et al., 2009) with the SILVA database (Yarza et al., 2014) in rna\_hmm  
176 tools ([https://github.com/meb-team/Public\\_Tools](https://github.com/meb-team/Public_Tools)). Predicted sequences were checked with  
177 BLASTN+ v.2.2.31+ (Altschul et al., 1990) searches against the SILVA database (option :  
178 perc\_identity 97% and evaluate  $1e^{-10}$ ) (Yarza et al., 2014). 16S rDNA sequences were clustered  
179 using cd-hit-est (v4.6) (-c 0,95) (W. Li & Godzik, 2006, p.). Quality-controlled metagenomic  
180 reads from all samples were individually mapped against the clustered sequences. Mapping  
181 was performed using BWA (v.0.7.17) with default parameters and relative abundances were  
182 calculated with BAM-Tk (<https://github.com/meb-team/BAM-Tk>).

183

## 184 **Comparative genomic analysis of *Sulfurimonas* MAGs, SAGs and reference genomes**

185 For performing a comparative genomic analysis between the *Sulfurimonas* MAGs and SAG  
186 (identified here) and the reference genomes, we first downloaded the 17 *Sulfurimonas*  
187 genomes available from NCBI Genbank (December 2022). These genomes include 15 species  
188 among which one is represented by two strains (*S. xiamenensis* 1-1N and *S. xiamenensis* CVO),  
189 as well as one strain (*Sulfurimonas* sp. SWIR-19) (Table S1). We onwards refer to these 17  
190 datasets as *Sulfurimonas* reference genomes for convenience. *Sulfurimonas* sp. HSL-3221  
191 (CP087998.1) was excluded from the study because of its high GC% (>57%) and low 16S rRNA  
192 gene sequence similarity with *Sulfurimonas* representatives (GC% ranging from 33.3 to 38.8%;  
193 up to 93% 16S rRNA identity with *S. lithotrophica*), suggesting that it belongs to another genus.  
194 We also downloaded 55 MAGs and one additional SAG affiliated with *Sulfurimonas*  
195 (completeness >70%, redundancy <4%, and GC% <40%) from NCBI genome database and  
196 IMG/M (<https://img.jgi.doe.gov>) web portals (Table S2).

197 The average nucleotide identity (ANI) and amino acid identity (AAI) enabled delineation of  
198 operational units at the genome level. ANIb was calculated using pyANI v0.2.12 (Pritchard et  
199 al., 2015). It was estimated by aligning fragments of 1020nt (Goris et al., 2007) with BLASTN+  
200 (Altschul et al., 1990; Camacho et al., 2009) and averaging the sequence identity between  
201 pairs of genomes. AAI was calculated using the program CompareM v0.1.2  
202 (<https://github.com/dparks1134/comparem>) and the parameters aai\_wf --proteins --  
203 sensitive. The matrices were then plotted as a heatmap and clustered using clustermap from  
204 the Seaborn statistical data visualization package, v0.11.2, using default parameters (Waskom,  
205 2021).

206 Analyses of acquired resistance genes were performed by ResFinder v4.1 (Zankari et al., 2012).

207 Insertion sequence (IS) elements were manually annotated according to the ISfinder database

208 (<http://www-is.biotoul.fr/>). Icefinder v2 (M. Liu et al., 2019) was used to identify putative  
209 integrative and conjugative elements (ICE) and integrative mobilizable elements (IME). Finally,  
210 VFDB (B. Liu et al., 2019) was used, with the genus *Helicobacter* as reference, to highlight  
211 putative virulence factors and TAFinder v2 (Xie et al., 2018) with the option “4.2 unannotated  
212 complete genome” to identify the presence of toxin-antitoxin (TA) loci. Contigs were also  
213 manually analyzed to complete automatic annotations performed by the different tools, using  
214 both BLAST searches and predictions performed by Prodigal (Hyatt et al., 2010).

215

### 216 **Pan-genomics analysis**

217 The pan-genome was computed to identify the clusters of shared genes (GC) according to  
218 amino acid sequence homology. Firstly, anvio v7.1 (Eren et al., 2015) was used to determine  
219 the pan-genome for the 17 complete reference genomes using Diamond (*--sensitive*) for  
220 sequence alignment (Buchfink et al., 2021) and the Markov cluster algorithm (MCL) for  
221 clustering (Van Dongen, 2008). The other parameters were kept as default except the  
222 inflation, set to 8. Gene collections were classified as follows: core genome included GCs  
223 present in all genomes; accessory genome included two collections – dispensable (present in  
224 at least two genomes, *hereafter referred to as* shell GCs) and unique (singletons, present in  
225 just one genome, *hereafter referred to as* cloud GCs). Next, these results were used to assign  
226 a category to the proteins predicted from the Lake Pavin SAG and MAGs assemblies using a  
227 BLASTP v2.9.0+ sequence alignment. Proteins without BLASTP hit were compared in an all-  
228 against-all BLASTP, e-value  $<10^{-5}$  and query coverage  $>80\%$ , to extract GCs present only in a  
229 unique Lake Pavin strain. GC functional annotation was performed using eggNOG-mapper  
230 (version emapper-2.1.9) based on eggNOG orthology data (Huerta-Cepas et al., 2019).

231

## 232 **Phylogeny**

233 A phylogenomic tree was generated with the program GToTree v1.8.2 (Lee, 2019) using a set  
234 of 74 markers spanning all bacterial genomes. The representative *Sulfurimonas* genomes were  
235 downloaded from NCBI RefSeq (Tables S1 and S2). GToTree identified target genes from  
236 genomes and MAGs using HMMER3, aligned each gene set using MUSCLE program v5.1  
237 (Edgar, 2022) and performed automated trimming with trimAl v1.4.1 ("-automated1")  
238 (Capella-Gutiérrez et al., 2009). Individual gene alignments were concatenated to construct a  
239 species tree using FastTree 2.1.11 the default parameters (Price et al., 2010).

240 Protein sequences to the NapA, PiT, SulP, YeeE, ferritin, sulfide: quinone reductases, and DsrE  
241 proteins of *Sulfurimonas* genomes were collected using accession numbers found in literature  
242 and for those not present in the literature, by searching the NCBI non-redundant database by  
243 protein BLAST search. A phylogenetic tree was generated for each protein, from aligned  
244 protein sequences (MUSCLE program (Edgar, 2004)) using MEGA software package version 7.0  
245 (Kumar et al., 2016). Maximum likelihood (ML) method was used based on the Whelan and  
246 Goldman model (JTT model, discrete Gamma distribution (5 categories)) (Whelan & Goldman,  
247 2001), with a bootstrap iteration of 1000. All positions with less than 95% site coverage were  
248 eliminated.

249

## 250 **Results**

251

### 252 **Reconstruction of *Sulfurimonas* genomes from Lake Pavin and species delineation**

253 The metagenome assembly from all depths represented  $\approx 1000$  bins (Table S3) among which  
254 two binned MAGs were affiliated to the genus *Sulfurimonas*, one (Bin58) being >94% complete

255 and high-quality and another (Bin76), >57% complete and medium quality (Table S4). One SAG  
256 (733-F2, 27.5% completeness, 0.2% single-marker-gene redundancy) recovered from the  
257 water column was also affiliated to this genus (Table S5). Across the genome regions of Bin58  
258 and SAG 733-F2 showing homology, the within-cluster sequence identity was close to or 100%  
259 (sequence inversions were observed at some contig extremities), suggesting that both  
260 represent the same genomic population (hereafter referred to as LP strain 1). However,  
261 merging SAG 733-F2 and Bin58 genomic data led to lower estimates of completeness, higher  
262 contamination, and lower Q-score (Table S6), these two genomes were therefore kept  
263 separate in further analyses. For Bin76, the within-cluster sequence identity dropped below  
264 85%, suggesting that this MAG corresponds to a second strain (referred to as LP strain 2).

265 Bin58 and SAG 733-F2 had ANI<sub>b</sub> and AAI of 99.8% and 98.7% respectively, and >99% with MAG  
266 *LacPavin\_0419\_WC90* (Jaffe et al., 2022). The corresponding numbers for Bin76 fall below  
267 84%, while it shares >97% with MAG *LacPavin\_0818\_WC45* (Jaffe et al., 2022) (Fig. 1). These  
268 results confirm that Lake Pavin hosts two distinct *Sulfurimonas* species (threshold used for  
269 species demarcation >95% (Kim et al., 2014; Thompson et al., 2013)). Additionally, these  
270 values did not exceed 83% when compared to publicly available data for the genus  
271 *Sulfurimonas* (Fig. S1 and S2), suggesting that LP strains 1 and 2 represent novel species. This  
272 conclusion was supported by analyses of the 16S rRNA sequence from SAG 733-F2 with the  
273 closest database hit being *S. gotlandica* at 96.3% sequence identity. Moreover, the  
274 phylogenomic analysis assigned the LP strains to a clade which includes mostly strains from  
275 natural freshwater systems (Fig. 2A).

276 Unlike SAG 733-F2, no 16S rRNA gene was found in the recovered MAGs. By contrast, we  
277 detected three 16S rRNA sequences in the metagenome assemblies that exhibited 100%  
278 homology with the one from SAG 733-F2 (Fig. 2B). Regarding 5S rRNA genes, a copy was

279 present in Bin58. We also isolated contigs with 5S rRNA genes from metagenome assemblies  
280 that were 100% identical between each other and with Bin58. These rRNA genes were flanked  
281 by various genomic regions (Fig. 2B).

282 Abundance profiles based on mapping reads from the Lake Pavin metagenomes revealed a  
283 similar vertical pattern for both SAG 733-F2 and Bin58, with a maximum relative abundance  
284 between ~65m and 70m (Fig. 3A and B). A similar pattern was observed for a 16S rRNA OTU  
285 with 100% sequence similarity to the SAG 733-F2 (*i.e.* *Sulfu.* 1, Fig. 3C) based on amplicons  
286 from both DNA and RNA samples previously obtained for the Lake Pavin water column  
287 (Biderre-Petit et al., 2016). This OTU also showed a significant abundance in the upper part of  
288 the anoxic zone (~60-63m), suggesting that LP strain 1 (*i.e.* SAG 733-F2/Bin58) expand across  
289 a wider zone along the depth profile. In comparison, Bin76 had a marked peak in relative  
290 abundance at 65m (Fig. 3A). Its distribution pattern was similar to the one of the second most  
291 abundant *Sulfurimonas* OTU (*Sulfu.* 2, Fig. 3C), suggesting that they represent the same  
292 phylotype.

293 Among the 17 *Sulfurimonas* reference genomes, *S. sediminis* S2-6 and *S. sp.* strain SWIR-19  
294 have ANI<sub>b</sub> and AAI  $\geq$  96% (Fig. 1), and 16S rRNA gene sequences that are 99.3-99.6% identical  
295 (considering only the most similar copies of SWIR-19, Table S7). This suggests that they belong  
296 to the same species. The question of heterotypic synonymy for *S. sediminis* S2-6 and *S.*  
297 *hydrogeniphila* might also arise. Indeed, they share similar genome wide identity (94% AAI,  
298 92% ANI) and 16S rRNA genes that are 99.3% identical (Table S7) along with many shared  
299 biochemical and physiological features (S. Wang, Jiang, et al., 2021; S. Wang, Shao, et al.,  
300 2021).

301

## 302 **Metabolic potential of the LP strains**

303 Lake Pavin SAG and MAGs were assessed for the presence of specific genome-encoded  
304 functional traits involved in numerous processes, including mediation of carbon, nitrogen,  
305 sulfur, and dihydrogen (H<sub>2</sub>) cycles, oxidative stress, motility, vitamin biosynthesis and  
306 transport (Table S8, Fig. 4).

### 307 ***Sulfur metabolism.***

308 Bin58 had the potential to use bisulfide (HS<sup>-</sup>) as electron donor *via* either sulfide:quinone  
309 reductase (Sqr) or flavocytochrome c sulfide dehydrogenase (Fcc) pathways. Like in most  
310 *Sulfurimonas* representatives, Type II (SqrB), IV (SqrD) and VI (SqrF) (Fig. S3, Table S9) were  
311 identified, with two copies of Type II as in *S. marina*, *S. hongkongensis* and *S. gotlandica*.  
312 Moreover, the presence of a FccB homolog suggests that this pathway could also be used to  
313 catalyze initial sulfide oxidation (Fig. 5A). The less complete Bin76 genome had only one Type  
314 II and one Type IV Sqr. Only the *soxCDY<sub>2</sub>Z<sub>2</sub>H* gene cluster for oxidation of reduced sulfur  
315 compounds was found in both LP strains. In *S. xiamenensis* CVO and *S. denitrificans*, that  
316 display this phenotype, S<sup>0</sup> oxidation correlates to high expression of an OprD-like porin  
317 (CVO\_00715 and Suden\_1917, respectively, both sharing 57% amino acid sequence identity),  
318 for which a homolog (55% amino acid similarity) was found in Bin58. The elemental sulfur  
319 reduction in LP strains could also be mediated by the polysulfide reductase-like  
320 molybdoenzymes (psrBCA) (Fig. 5A, Table S8).

321 Among the three major protein families identified for the transport of oxidized sulfur  
322 compounds, two (SulP and PiT) clustered with clades involved in the bicarbonate:Na<sup>+</sup> symport  
323 and inorganic phosphate transport, respectively (Fig. S4). The only similar sequence to our PiT  
324 protein was identified in a MAG (90% protein identity, accession number OHE15791.1). By

325 **contrast**, the third family of transporters, encoding the YeeE/YedE proteins, clustered with  
326 thiosulfate transporters (Tanaka et al., 2020) and is predicted to be involved in thiosulfate ion  
327 transfer into the cytoplasm. In Bin76, the *yeeE* genes were tandemly organized in an operon  
328 with other genes involved in sulfur metabolism, *i.e.* rhodanese (*rhd*)-like and thioredoxin  
329 genes (Fig. S5). Only *Ca. S. marisnigri* contained *yeeE* homologs with a similar tandem  
330 organization (HUE87\_05335 and HUE87\_05340, > 72% amino acid sequence identity). Finally,  
331 TauE-like exporters might transport sulfite from cytoplasm to the periplasm (Gwak et al.,  
332 2022).

333 Both LP strains possessed a single homolog of the DsrE-like protein (Fig. S6A), involved in  
334 cytoplasmic sulfur trafficking during the sulfur oxidation. By comparison, zero to five DsrE-like  
335 proteins were detected in the full range of *Sulfurimonas* genomes and MAGs, with the lowest  
336 number typically found in freshwater strains (Table S10). Among all proteins identified in  
337 *Sulfurimonas* genomes, only one grouped in a cluster previously determined by L.-J. Liu et al.  
338 (2014), *i.e.* DsrE2A in a MAG (SAMN18877703), while the others formed eight additional  
339 clusters, herein referred to as DsrEa to DsrEh (Fig. S6B). Their distribution may not be random,  
340 as some of them are absent in for instance the freshwater strains (Table S10). The LP strain  
341 DsrE proteins belonged to the cluster DsrEa and contained three strictly conserved cysteine  
342 residues (Fig. S6A).

343 Regarding other cytoplasmic processes, a sulfur metabolizing pathway for assimilatory sulfate  
344 reduction was also present in the LP strains with homologs to Cys proteins (CysD, E, K, I) and  
345 a sulfate adenylytransferase (Sat, Fig. 5A) (Han & Perner, 2015).

346 ***Nitrogen metabolism.***



347 All components required for the denitrification pathway were detected in LP strains (Fig. 5B,  
348 Table S11), with a periplasmic nitrate reductase catalytic subunit (NapA) organized in a well  
349 conserved operon. Only *S. pluma* is currently known to lack this operon (Molari et al., 2023).  
350 Moreover, as previously shown, NapA from hydrothermal vent strains represent a  
351 monophyletic group distinct from genomes obtained from other ecosystems (Fig. S7). The LP  
352 strains also contain genes encoding ammonium (*atmB1* and *atmB2* genes) and nitrate (*nark*  
353 homolog) uptake. These compounds can subsequently be funneled into amino acid  
354 metabolism *via* the ferredoxin-nitrite reductase (*nirA*) and the GS-GOGAT/GDH pathways (Fig.  
355 5B). Unlike Bin58 and most other *Sulfurimonas* genomes, Bin76 contains a high-affinity nitrate  
356 ABC transporter (NrtABC) for nitrate/nitrite uptake for which homologs have only been found  
357 in *Ca. S. marisnigri*, *S. litotrophica*, *S. marina*, *S. pluma* and *S. gotlandica*. Bin76 genes also  
358 encode key proteins for N<sub>2</sub> fixation (*nifHD*, *nifA*, *nifT*) with homologs only detected in *Ca. S.*  
359 *marisnigri*, *Ca. S. baltica*, *S. litotrophica* and *S. marina*. However, while both *Candidatus* strains  
360 can fix N<sub>2</sub> (Henkel et al., 2021), *S. marina* was seemingly unable to grow with N<sub>2</sub> as the sole  
361 nitrogen source (Z. Wang et al., 2022).

### 362 ***H<sub>2</sub>*, *O<sub>2</sub>*, oxidative stress and central carbon metabolisms.**

363 Most *Sulfurimonas* can grow with H<sub>2</sub> as an energy source. The LP strains contained [NiFe]-  
364 hydrogenases of groups 1a and 1b that mediate respiratory H<sub>2</sub> uptake, and group 4e,  
365 representing a multisubunit membrane-bound Energy-Conserving Hydrogenase (Ech) that  
366 couples ferredoxin oxidation to H<sub>2</sub> evolution (Table S8). Unlike group 1 [NiFe]-hydrogenases,  
367 which is ubiquitous in *Sulfurimonas spp.*, group 4e is present only in marine pelagic species (*S.*  
368 *gotlandica*, *Candidatus S. marisnigri* and *Ca. S. baltica*) and in both LP strains.

369 Like other *Sulfurimonas* genomes (Molari et al., 2023), LP strains carry various systems for  
370 uptake and intracellular availability control of iron and manganese. Among them, a gene

371 encodes a DNA-binding protein belonging to the ferritin-like diiron-carboxylate protein  
372 superfamily (known as Dps). This gene is found in almost half of the *Sulfurimonas* genomes  
373 (Fig. S8). In contrast, a second member within this gene superfamily (*i.e.* a gene encoding for  
374 a 24-subunit non-heme ferritin) was only found in four *Sulfurimonas* reference genomes (Fig.  
375 S8). In addition to the ferritin, LP genomes have genes encoding a superoxide dismutase (SOD),  
376 producing H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub> as byproducts, and a superoxide reductase (SOR), producing only H<sub>2</sub>O<sub>2</sub>  
377 (Table S8, Fig. 4A), both designed to specifically minimize stress and cell damage from reactive  
378 oxygen species (ROS) (Johnson & Hug, 2019; Lushchak, 2011).

379 In assessing the potential for aerobic respiration, both LP strains have a gene coding for the  
380 cbb3-like high-O<sub>2</sub> affinity cytochrome, as all other *Sulfurimonas* spp. This cytochrome is active  
381 in anaerobic processes such as dinitrogen (N<sub>2</sub>) fixation and denitrification (Bell et al., 2020). In  
382 addition, two hemerythrins, non-heme intracellular O<sub>2</sub>-binding proteins, were present in LP  
383 strains, one with a single-domain and one with a multi-domain that belongs to the  
384 hemerythrin-GGDEF family (Table S8). We also detected an Aer-PAS domain primary energy  
385 sensor for motility as well genes coding for a flagellum and chemotaxis (Fig. 4A, Table S8).

386 Finally, both LP strains contain all essential genes for carbon fixation *via* the reverse  
387 tricarboxylic acid (rTCA) cycle, a common autotrophic carbon fixation pathway of all  
388 *Sulfurimonas* spp. (Fig. 4A).

389

## 390 **Mobilome and defense mechanism analysis**

### 391 ***MGE in LP strains***

392 MGE is a generic term for a multitude of genomic sequences (plasmid, prophage, ICE, IME,  
393 insertion sequences, etc.) transmitted vertically with cell division or through horizontal

394 transfer (HGT). Most of such genomic features were identified in the LP strain genomes *via*  
395 either automatic or manual annotations (Table S12). An IME was found in both LP strains while  
396 a prophage was present only in Bin58. The IME encodes a tyrosine integrase and harbors a  
397 compact mobilization module with two genes encoding relaxases (one of which is a canonical  
398 MobC) and one gene encoding a non-canonical coupling protein (Type IV secretory system  
399 conjugative DNA transfer family protein) (Fig. 6). Only one MAG affiliated to *Arcobacter* sp.,  
400 reconstructed from cold oxic seafloor (Tully et al., 2018), have been shown to host a similar  
401 element with a conserved gene order (Fig. 6). With regards to the prophage, genomic  
402 elements with a similar organization and content have only been reported for *Sulfurimonas*  
403 MAGs (Fig. S9). Both the IME and prophage carried many *flexible* genes, mostly encoding  
404 proteins of unknown function. Therefore, their role in the acquisition of novel adaptive  
405 phenotypes remains unknown.

#### 406 ***Defense systems***

407 Among all defense mechanisms identified, Toxin-Antitoxin (TA) systems were prominent,  
408 representing small genetic modules with implications in a multitude of bacterial lifestyle  
409 adaptation (Fraikin et al., 2020). Among the six TA types (types I-VI) currently classified, type  
410 II, that function *via* protein-protein interactions, is the most well characterized and the most  
411 prevalent across bacterial genomes (Kamruzzaman et al., 2021; Yeo, 2018). Here, we identified  
412 a repertoire of 51 different type II TA systems in the *Sulfurimonas* genomes involving 16 toxin  
413 families that are mostly involved in translational arrest (Table 1). Each of the 17 reference  
414 genomes had 1 to 28 TA pairs through its chromosome. For those genomes featuring plasmids,  
415 one (*S. parvalvinellae*) and five additional loci (*S. aquatica*) were identified. For LP strains, we  
416 identified 12 TA pairs in Bin76, nine in Bin58 and eight in SAG 733-F2, as well as the Clp  
417 proteases involved in their degradation (Table S12). Overall, the TA pattern was similar for all

418 species of a specific environment even if the TA pair number per species may varied (Table 1).  
419 In contrast, both TA number and pattern are kept across strains of a same species, regardless  
420 if they are from the same (*S. sediminis* and SWIR-19) or different habitats (*S. xiamenensis*  
421 strains). Interestingly, two species (*S. paralvinellae* and *S. marina*) have only one TA pair.  
422 **However**, compared to the species that live in the same environment, these species seem to  
423 be restricted to more specific niches. Indeed, *S. paralvinellae*, in hydrothermal vents, lives  
424 associated with a host, unlike any of the other species and *S. marina*, in marine sediments,  
425 thrives at 2000m depth while the other species were retrieved from less than 250m deep. In  
426 addition to type II, a putative type IV TA system (AbiE), **widespread in bacteria in which it**  
427 **ensures phage resistance (Dy et al., 2014)**, was present in seven genomes with an occurrence  
428 per genome ranging from one to five (Table 1).

#### 429 **Resistome**

430 The resistome analysis identified resistance to several antibiotic classes within the LP strain 1  
431 genomes including beta-lactams, quinolones, macrolides and fusaric acid (FA). We found a  
432 gene encoding an AmpC beta-lactamase (Table S12), with the only *Sulfurimonas* homolog  
433 being a gene found in a MAG from groundwater (Finland, SAMN12221366) **that exhibits** the  
434 same genomic context, *i.e.* associated with two amino acid ABC transporter substrate-binding  
435 proteins and a HAMP domain-containing histidine kinase. Interestingly, LP strain 1 is the only  
436 known *Sulfurimonas* representative harboring genes involved in FA resistance. The *fuaABC*  
437 operon consists of a transcription factor of the family MarR, an inner membrane efflux pump,  
438 a periplasmic efflux transporter and an outer membrane protein. Our strain also has other  
439 efflux pump systems belonging to MATE, ABC (MAcAB-TolC) and RND (AcrAB-TolC) families  
440 that could contribute to the generation of resistant phenotypes (Table S8).

#### 441 **Virulome**

442 Virulome prediction identified genes involved in adherence, lipid A synthesis and biofilm  
443 formation (Table S12). LP strain 1 features all traits required for biofilm formation capacity  
444 (chemotaxis, flagellum-assisted motility, EPS production and export). Moreover, we identified  
445 a *pgaBCD* locus that is required for a biofilm adhesin synthesis (poly- $\beta$ -1,6-GlcNAc or PGA)  
446 (Wang et al., 2004), but where the *pgaA* subunit encoding the outer membrane protein is  
447 lacking. Homologous genes were only detected in *S. gotlandica* and *S. denitrificans*, also  
448 devoid of the *pgaA* subunit.

449

#### 450 **Pan-genome analysis of the genus *Sulfurimonas***

451 Comparative analysis of the 17 *Sulfurimonas* reference genomes revealed a pan-genome  
452 composed of 9021 gene clusters (GCs) among which 915 core GCs, 3508 shell GCs and 4598  
453 cloud GCs (Fig. 7A), which is consistent with previous studies (Molari et al., 2023; S. Wang,  
454 Jiang, et al., 2021). With regards to the LP genomes (SAG 733-F2, Bin58 and Bin76), they  
455 contained 272 to 842 core GCs, 460 to 1152 shell GCs, and 57 to 192 cloud GCs (Fig. 7B).

456 Among the shell GCs identified in LP strains, 340 to 946 belonged to the shell GCs when  
457 analyzing the 17 *Sulfurimonas* reference genomes while 96 to 206 belonged to the cloud GCs  
458 (Fig. 7B). This is what is generally observed with the addition of new genomes in pan-genomic  
459 analyses: certain GCs firstly identified as cloud are subsequently shared by more than one  
460 genome after adding additional genomes resulting in their updated classification as shell GCs.

461 In this study, 316 distinct GCs classified as cloud GCs when analyzing the 17 *Sulfurimonas*  
462 reference genomes were reclassified into shell GCs when adding LP genomes (Table S13). The  
463 prediction of their function *via* assignment to the Clusters of Orthologous Groups (COGs)  
464 classification shows that 39% (123 GCs) could not be categorized due to the absence of  
465 common features with other proteins. The 61% remaining clusters fell into 18 COGs with the

466 categories « function unknown » (S, 18%), « signal transduction mechanisms » (T, 27.3%) and  
467 « cell wall, membrane, envelope biogenesis » (M, 10.8%) being the biggest three clusters  
468 (Table S13).

469 Comparative analysis based on the cloud GCs identified in LP strains revealed 104 GCs found  
470 only in SAG 733-F2, with 127 and 38 exclusive to Bin58 and Bin76, respectively (Fig. 7C). The  
471 vast majority of these were not categorized (76%, 66.2% and 71.1%, respectively). For the  
472 classified ones, nearly a quarter fall into the categories « S » and « R (only general predicted  
473 function) » (Table S14). The other most common COGs were related to « mobilome (X) » and  
474 « defense mechanisms (V) » (Fig. 8). For LP strain 1, proteins related to « amino-acid transport  
475 and metabolism (E) », « replication, recombination, and repair (L) » and « T » were also  
476 identified in lower proportions. In addition, a few cloud GCs (22 in total, Fig. 7C) were shared  
477 by both strains, most of which (95.4%) had a COG classification. The categories « inorganic ion  
478 transport and metabolism » (P), « cell cycle control, cell division, chromosome partitioning  
479 (D) », « intracellular trafficking, secretion, and vesicular transport (U) » and M were the main  
480 ones (Fig. 8, Table S9). Among these proteins, some could be relevant for the LP strain fitness,  
481 as for instance, a chromate-resistance protein, involved in the reduction of chromate  
482 accumulation (element with toxic and mutagenic effects), porins of the OprB family, central  
483 in carbohydrate transport, and the Min system proteins (MinE, MinC, MinD) (Fig. 4). LP strains  
484 differ from all *Sulfurimonas* genomes by the presence of the MinE protein (Fig. S5) that jointly  
485 with MinD enables oscillatory behavior of the Min system. In its absence, the system remains  
486 static (Taviti & Beuria, 2019). In addition to its involvement in cell division, this system is also  
487 crucial for cellular processes such as bacterial motility, RNA decay, colonization, and virulence.

488

489 **Discussion**

490 In this comparative study of Lake Pavin microbiota, we identified two uncharacterized  
491 *Sulfurimonas* species inhabiting the bottom anoxic zone using both MAGs and SAGs data. The  
492 clear difference we observed in level of completeness between SAG and MAGs affiliated to  
493 this genus is congruent with that was previously observed by Alneberg et al. (2018) and  
494 attributed this to constraints related to the WGA during single-genome amplification. The  
495 MAG quality was ensured by low level of redundancy, rejecting the possibility of contaminant  
496 metagenome contigs included in the binning and assembly. Moreover, our abundance profiles  
497 suggest that the two LP strains may differ in their ecological preferences related to oxygen  
498 (O<sub>2</sub>) and electron acceptor/donor availability. Such coexistence of *Sulfurimonas* species within  
499 a single ecosystem with overlapping niches has never been described before.

500 The presence of various genes in the vicinity of the ribosomal genes for the LP Lake Pavin  
501 strain 1 implies multiple copies per genome, as observed for most *Sulfurimonas* members  
502 (Table S1). Furthermore, phylogenomic analysis as well as the diversity and distribution  
503 pattern of specific genes (*e.g. dsrE* genes) suggest that LP strains form, with other freshwater  
504 representatives, a distinct freshwater clade within this genus. Regarding all the other  
505 *Sulfurimonas* strains analysed in this study, *S. sediminis* S2-6 and *S. sp.* strain SWIR-19 exhibit  
506 a high degree of genome relatedness. This finding allows us to propose renaming the latter to  
507 *S. sediminis* strain SWIR-19, similarly to the recent renaming of *S. sp.* strain CVO (renamed *S.*  
508 *xiamenensis* strain CVO (Henkel et al., 2021)). The high genetic similarity, also observed with  
509 the *S. hydrogeniphila* strain, could be explained by the fact that they all originate from the  
510 Indian Ocean (Table S1) with the most closely related *S. sediminis* S2-6 and SWIR-19 isolated  
511 from the same area (GPS coordinates 37.78S 49.64E and 37.28S 49.65E, respectively (S. Wang,  
512 Shao, et al., 2021)), while the third, *S. hydrogeniphila*, was recovered from a more distant  
513 region (6.21N 60.31E (S. Wang, Jiang, et al., 2021)). This is consistent with the identification of

514 ecotypes specific to an environment (as defined by Molari et al., (2023); e.g. brackish water  
515 samples, hydrothermal plume samples, etc...) defined on the basis of oligotypes (amplicon  
516 sequence variants distinguished on the basis of information entropy determined for each  
517 nucleotide position (Eren, Morrison, et al., 2015)).

518 From a functional point of view, like other *Sulfurimonas*-spp. from terrestrial subsurface  
519 environments, the LP strains display only the *soxCDY<sub>2</sub>Z<sub>2</sub>H* genes and not the *soxXY<sub>1</sub>Z<sub>1</sub>AB* genes  
520 (Lahme et al., 2020). The products of this gene cluster are involved in S<sup>0</sup> oxidation with  
521 thiosulfate as metabolic end product. Cell attachment to S<sup>0</sup> particles are required for their  
522 oxidation and different mechanisms were suggested, depending on the *Sulfurimonas* spp. (e.g.  
523 flagellar proteins, Type-IV pilin-like proteins). Recently, an outer membrane protein (OprD-like  
524 porin) was also implicated in the S<sup>0</sup> oxidation in strain CVO for which a homolog was found in  
525 LP strains. Here, the question whether this protein is directly involved in degradation of S<sup>0</sup> or  
526 facilitates its transport into the cell remains open. Data available for other proteins involved  
527 in the sulfur cycle are also poor. This is notably the case for the DsrE proteins for which  
528 investigations are needed to identify not only their function but also the proteins they may  
529 interact with. For instance, the DsrE2A protein, organized in a *rhd-tusA-dsrE2A* gene cluster,  
530 was shown to mediate sulfur transfer and sulfite oxidation in cytoplasm (Dahl, 2015; L.-J. Liu  
531 et al., 2014). Since the gene encoding DsrEa in LP strains is in a slightly different cluster (*tusA*  
532 gene replaced by the protein-disulfide reductase (*dsbD* gene)), its true function in the cell  
533 could be cryptic. We also detected in the LP strain genomes both a PiT transporter homolog,  
534 closer to inorganic phosphate transporters than to sulfur compounds (Marietou et al., 2018),  
535 and proteins involved in polyphosphate formation and degradation (Ppk). This suggests the LP  
536 strains have the capacity to accumulate polyphosphate under favorable conditions as a



537 cellular storage of energy, analogous to the *Sulfurimonas* subgroup GD17/GD1T isolated from  
538 Baltic Sea (Möller et al., 2019).

539 The presence of various systems for intracellular iron availability control in LP strain genome  
540 suggests its potential role as electron donors. Also, though essential, iron can be a harmful  
541 element for the cell by catalyzing the formation of ROS by reacting with H<sub>2</sub>O<sub>2</sub> in a Fenton  
542 reaction. Almost half of the *Sulfurimonas* genomes, including LP strains, encode a Dps-like  
543 protein known to minimize damage from such oxidative stress. Indeed, this protein quenches  
544 both Fe<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub> while physically protecting genomic DNA and hence protects the cell  
545 against Fenton-reaction (Bradley et al., 2020).

546 Numerous genes implicated in biofilm formation and motility are present in LP strain  
547 genomes. Some of these genes were found only in a very limited number of species (*e.g. pga*,  
548 hemerythrin genes). With regard to one of the two hemerythrins found, the GGDEF domain  
549 located at its C terminus is believed to be involved in the synthesis of a second messenger that  
550 contributes to the control of motility, virulence and biofilm formation (French et al., 2008;  
551 Römling et al., 2013). This suggests a link between O<sub>2</sub> levels and the cyclic-di-GMP signaling  
552 network in LP strains. However, how the interaction of the hemerythrin-like domain with O<sub>2</sub>  
553 alters the activity of the associated signaling domain is still not known and should be  
554 investigated. Furthermore, the importance of O<sub>2</sub> in *Sulfurimonas* strains motility is reinforced  
555 by the presence, in the few strains where this hemerythrin where detected (including LP  
556 strains), of an Aer-PAS domain primary energy sensor for motility, known to respond to  
557 changes in O<sub>2</sub> concentration. Hence, in cooperation with chemotaxis, such traits could confer  
558 an ability for the cell to position themselves in a microenvironment appropriate for their  
559 metabolic needs (Maschmann et al., 2022).

560 For the PGA adhesin, since it has been hypothesized that the PgaA subunit mediates its  
561 translocation and/or docking to the cell surface, it would be interesting to investigate how  
562 PGA is released at the surface in these strains in its absence. Moreover, both LP strains and *S.*  
563 *gotlandica* all thrive in the pelagic redox zone of the water column, whether in freshwater lake  
564 or the Baltic Sea. Therefore, although this has not yet been demonstrated experimentally,  
565 capacity to live in biofilm at such redox transition zones might confer ecological advantages,  
566 such as efficient nutrient cycling, stress tolerance and resistance to grazing. As *S. gotlandica* is  
567 a cultivated species, it would be possible to elucidate some of these issues and determine the  
568 importance of *pga* and hemerythrin genes in biofilm formation.

569 We suggest that MGEs might play important roles in the ability of *Sulfurimonas* spp. to  
570 disperse. Among them, IMEs have never been reported in *Sulfurimonas* before and,  
571 interestingly, the gene associations within IME in LP strains differs from the mandatory ones  
572 described by Coluzzi et al. (2017) in *Streptococci*. For TAs, [the wide variety of associated](#)  
573 [antitoxins suggests that each TA type II pair may function independently.](#) Moreover, since  
574 variations in their patterns are observed both between environments and between species,  
575 further work is needed to investigate how such genomic features contribute to *Sulfurimonas*  
576 strain acclimation to variable conditions and particular environment. [Indeed, these](#)  
577 [characteristics may, in part, explain the low number of TA pairs observed in certain strains.](#)

578 The LP strains also harbor a broad arsenal of virulence and resistance determinants that may  
579 demonstrate their capacity for genetic recombination and enable rapid and efficient  
580 adaptation to the local environment. Among the resistance determinants, the FA operon  
581 shows an organization that is consistent with the finding that Gram-negative bacteria  
582 resistance to high FA would be conferred by an efflux pump (Crutcher et al., 2017).

583 Nevertheless, the ability of our strain to grow when exposed to FA remains to be  
584 experimentally confirmed. Moreover, the similarity of the MarR sequences with those of  
585 *Sulfuricurvum kujiense* (Table S14) suggests an acquisition *via* HGT. Therefore, further studies  
586 are needed to shed light on the evolutionary and ecological forces underpinning the early  
587 evolution and differentiation of these freshwater strains, as well as their ability to disperse  
588 globally.

589 The upper boundary of the Lake Pavin monimolimnion, where *Sulfurimonas* live, is dynamic  
590 and uncertain as it responds to weather-induced mixing. Our results suggest that LP strain 1,  
591 like *S. gotlandica*, has a strategy based on polyphosphate storage capacity that can be used as  
592 an energy source to sustain cell integrity and reach favorable niches when substrate  
593 availability changes. This capacity to accumulate polyphosphate has also been demonstrated  
594 for magnetotactic bacteria in the monimolimnion of Lake Pavin (Rivas-Lamelo et al., 2017),  
595 suggesting that this process might be more widely used than expected in this ecosystem.  
596 Furthermore, the LP strain 1 was detected in a zone of the water column where conditions  
597 varied from anoxic, ferruginous with elemental sulfur ( $\approx 60\text{m}$ , S:  $3.3\mu\text{M}$ ) to slightly sulfidic  
598 ( $\approx 70\text{m}$ ,  $\text{H}_2\text{S}$ :  $15\mu\text{M}$ ). Together, this suggests *Sulfurimonas* could be key-players in the  
599 interconnected cycling of sulfur, carbon, phosphate, and iron cycles in Lake Pavin. Hence this  
600 lake may be a promising environment to gain new knowledge about freshwater *Sulfurimonas*  
601 strains and to elucidate their interactions with the environment.

602

### 603 **Funding**

604 The work was supported by the Agence Nationale de la Recherche (ANR) through the project  
605 EUREKA (ANR-14-CE02-0004-01).

606

## 607 Acknowledgements

608 The authors would like to acknowledge support from the Genomics infrastructure services at  
609 Science for Life Laboratory (<https://www.scilifelab.se>). Single Cell Sorting, whole genome  
610 amplification, screening and library preparation were performed at the Microbial Single Cell  
611 Genomics Facility (MSCG). Sequencing was performed by the SNP&SEQ Technology Platform.  
612 The facilities are part of the Uppsala node at the National Genomics Infrastructure (NGI)  
613 Sweden and Science for Life Laboratory. The SNP&SEQ Platform is also supported by the  
614 Swedish Research Council and the Knut and Alice Wallenberg Foundation. We are also grateful  
615 to the Mésocentre Clermont Auvergne University (<https://mesocentre.uca.fr/>) for providing  
616 help, computing, and storage resources. We thank Maria Hammond (MSCG, Upsala), H  l  ne  
617 Gardon (PhD, Universit   Clermont-Auvergne) and Vincent Dardot (M2 student, Universit    
618 Clermont-Auvergne) for efficient technical assistance and expertise.

619

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- 992

### 993 **Data accessibility**

994 The raw sequences of the metagenomic datasets MetaG1, previously described in Biderre-  
995 Petit et al (2019), are archived in MG-RAST under accession numbers mgm4580845.3 to  
996 mgm4580848.3 for the metagenomic reads and the accession numbers mgm4557752.3.100,  
997 mgm4557753.3.100, mgm4581104.3.100 and mgm4581106.3.100 for the assembled contigs.  
998 Metabarcoding data are available at the NCBI SRA database under the BioProject accession  
999 number PRJNA975855 and SRA accession numbers SRX20521566 to SRX20521576. Raw  
1000 sequences of the metagenomic datasets MetaG2 as well as the *Sulfurimonas* SAG and MAGs  
1001 produced as part of the present study are available at NCBI SRA database under the BioProject  
1002 accession PRJNA983208.

1003

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1011

### 1012 **Conflict of interests**

1013 The authors declare that they have no known competing interests.

1014

### 1015 **Author contributions**

1016 **AM** conceived figures, involvement in writing-review and editing; **CBP** was responsible for  
1017 conceptualization, supervision, data curation, data analysis and interpretation and writing-  
1018 original draft; **Che** was involved in formal analysis and writing - review and editing; **CHo** was  
1019 responsible for metagenome assembly and analysis, data curation, methodology and writing  
1020 - review and editing; **CL** was involved in writing - original draft , **DC** participate to the pan-  
1021 genome analysis, data curation, methodology, writing - review and editing; **PEG** was  
1022 responsible for sampling and funding acquisition (ANR EUREKA), writing - review and editing;  
1023 **DD** was responsible for sampling and funding acquisition (ANR EUREKA); **SB, AMD** and **MH**  
1024 were responsible for SAG preparation and sequencing, writing - review and editing. All authors  
1025 contributed to the manuscript and approved the final version.

1026

### 1027 **Figure titles and legends**

1028 **Figure 1: Pairwise average similarity calculations for the 17 *Sulfurimonas* reference genomes**  
1029 **and the Lake Pavin strains.** The MAGs and SAG obtained in the present study are indicated in  
1030 orange (Bin58, Bin76 and SAG 733-F2) and MAGs from the Jaffe et al' study (2023), in blue  
1031 (MAG 0419\_WC90, and MAG 0818\_WC45). Upper matrix: pairwise average nucleotide  
1032 identity (ANIb); lower matrix: pairwise average amino acid identity (AAI). Lake Pavin strains  
1033 from the present study are indicated in orange and those from the Jaffe et al' study, in blue  
1034 (Jaffe et al., 2023).

1035 **Figure 2: Phylogenomic analysis of Lake Pavin strains and ribosomal genes detected in Lake**  
1036 **Pavin datasets. A)** Phylogenomic tree encompassing *Sulfurimonas* genomes (isolates and  
1037 MAGs) made using GToTree (Lee, 2019) and a set of 74 bacterial marker genes. MAGs  
1038 obtained in the present are indicated in orange and those from Jaffe et al. (2023), in blue.  
1039 Stars indicated *Sulfurimonas* strains found associated to a host. Bootstrap values based on  
1040 1000 replicates; only values above 75% are shown at branch nodes. **B)** Schematic  
1041 representation of the ribosomal RNA gene genomic context found in the MetaG2  
1042 metagenomes as well as in Bin58 and SAG 733-F2 assemblies. Boxed arrows indicate genes  
1043 and their direction of transcription. For each gene, the closest relative is indicated in the right  
1044 part of the figure, identified either by a letter or by a number.

1045 **Figure 3: Relative abundance of the Lake Pavin *Sulfurimonas* strains along the lake water**  
1046 **column. A)** Relative abundance of Bin58 and Bin76 MAGs in metagenomes produced in 2013  
1047 (MetaG1) and 2015 (MetaG2). **B).** Relative abundance of SAG 733-F2 in MetaG2 metagenomes  
1048 based on mapping the original 16S rRNA metagenomic reads onto the 16S rRNA cluster with  
1049 seed-sequence 100% identical to the SAG 733-F2 sequence. **C)** Relative abundance (% of total  
1050 community) in the 454 metabarcoding libraries constructed from gDNA (MetaB 16S gDNA)  
1051 and cDNA samples (MetaB 16S cDNA) collected along the Lake Pavin water column. Sulfu1:

1052 OTU with seed-sequence 100% identical to the SAG 733-F2 sequence; Sulfu2: the second most  
1053 abundant OTU affiliated to the genus *Sulfurimonas* and with less than 97% identity with the  
1054 SAG 733-F2 sequence.

1055 **Figure 4: Overview of the metabolism of the Lake Pavin *Sulfurimonas* strains inferred from**  
1056 **their genome. A)** General functions. Transporter families (ABC, MFS, P-type ATPase,  
1057 symporter, etc) are represented by different colors and forms. **B)** Functions only carried by  
1058 Lake Pavin (LP) strains (strain 1, strain 2 or both – black boxes) or shared by one or both LP  
1059 strains with only one other *Sulfurimonas* genome (blue box).

1060 **Figure 5: Overview of the putative pathways of oxidative and assimilation metabolisms for**  
1061 **sulfur (A) and of reductive and assimilation metabolisms for nitrogen (B) within the Lake**  
1062 **Pavin strains** (for details about genes involved in sulfur pathways see Table S9, and for  
1063 nitrogen pathways, Table S10).

1064 **Figure 6: Modular structure of integrative and mobilizable element (IME) found in**  
1065 ***Sulfurimonas* strains from Lake Pavin.** The gene blocks conserved between SAG 733-F2 and  
1066 Bin58, SAG 733-F2 and Bin76 and SAG 733-F2 and *Arcobacter* sp. are shown. Proteins not  
1067 found in other *Sulfurimonas* genomes appear in bold; those found only in a few genomes, in  
1068 bold italics.

1069 **Figure 7: Pan-genome analysis of the 17 *Sulfurimonas* reference genomes used in the**  
1070 **present study and the Lake Pavin strains. A)** Flower plot displaying the numbers of core gene  
1071 clusters (GCs) (in the center), flexible (shell) GCs (in the annulus) and unique (cloud) GCs (in  
1072 the petals) for the 17 *Sulfurimonas* genomes. **B)** Number of core, flexible (shell), and unique  
1073 (cloud) GCs for SAG 733-F2, Bin58 and Bin76 genomes. **C)** Venn diagram displaying the number  
1074 of cloud GCs shared by lake Pavin strains or specific to each. The number of genes  
1075 corresponding to the GC number is indicated in parentheses in order: SAG 733-F2, Bin58,  
1076 Bin76.

1077 **Figure 8: Clusters of Orthologous Groups (COGs) classification of the cloud GCs identified in**  
1078 **Lake Pavin strains.**

1079 **Table 1: Classification and distribution of Type II and IV toxin-antitoxin systems in**  
1080 ***Sulfurimonas* species.**

1081 **Figure S1: Pairwise average nucleotide identity (ANI<sub>b</sub>) heatmap of *Sulfurimonas* genomes,**  
1082 **MAGs and Bin58.**

1083 **Figure S2: Pairwise average amino acid identity (AAI) heatmap of *Sulfurimonas* genomes,**  
1084 **MAGs and Bin58.**

1085 **Figure S3: Maximum likelihood phylogenetic tree of deduced protein sequences of the**  
1086 **sulfide:quinone reductase (Spr).** Number at nodes represent bootstrap values (1 000  
1087 replicates of the original dataset), only number above 50% are shown. The scale bar  
1088 represents the average number of substitutions per site. The accession number for the  
1089 sequences are given at the right side of species name. The protein sequences from Lake Pavin  
1090 strains are indicated by a dot for Bin58 and a circle for Bin76, with colors depending on the  
1091 *sqr* type: type II in red (*SqrB*), type IV in green (*SqrD*) and type VI in blue (*SqrF*).

1092 **Figure S4: Maximum likelihood phylogenetic trees of deduced protein sequences of two**  
1093 **potential sulfate transporters (PiT and SulP). On the left:** PiT protein sequence tree. The two  
1094 protein clades are surrounded with different colors and given a letter label (A, B). Reference  
1095 sequences: PITA, inorganic phosphate transporter (UniProt P0AFJ7), PITB: inorganic  
1096 phosphate transporter (UniProt P0AFJ7), Cys Pit: sulfate transporter (BSUA\_01689). **On the**  
1097 **right:** SulP protein sequence tree. The three protein clades are surrounded with different  
1098 colors and given a letter label (A, B, C) while separate protein groupings are denoted by  
1099 subscript number (i, ii). Reference sequences: BICA (Aii), bicarbonate transporter (UniProt  
1100 Q14SY0), DAU\_Salm (B): dicarboxylic transporter (ACY88617), Riv1739c and SulP\_Pseudo  
1101 (PA1647) (C): sulfate transporter. Number at nodes represent bootstrap values (1 000  
1102 replications of the original dataset), only number above 50% are shown. The scale bar  
1103 represents the average number of substitutions per site. The accession number are given for  
1104 all sequences. Bin58 and SAG 733-F2 sequences are identified by a red dot.

1105 **Figure S5: Schematic representation of yee gene genomic context in the Bin76 genome.** All  
1106 genes potentially involved in sulfur cycling are in ochre-brown color. Each box represents  
1107 genes in the contig for which a homolog was present only in a restricted number of  
1108 *Sulfurimonas* genomes and MAGs. Their genomic context in the Lake Pavin strain 1 is also  
1109 shown (Node for SAG 733-F2 and locus tag, *i.e.* K119\_, for Bin58). Ochre arrows: genes  
1110 encoding YeeE proteins; green arrows: Min operon; black blue arrow: gene encoding a high  
1111 potential iron-sulfur protein.

1112 **Figure S6: Sequence alignment of DsrE homologs (A) and phylogenetic tree (B).** In **A**, the  
1113 sequences are identified by their locus tags. The active site cysteine conserved in all sequences  
1114 is labeled with an asterisk and highlighted in cyan. For clarity, only those parts of the alignment  
1115 are shown to contain conserved cysteine residues (N and C terminus, cysteines highlighted in  
1116 yellow). In blue: sequence identifiers of *Sulfurimonas* genomes. Bins are highlighted in red and  
1117 their motifs in bold italics. In **B**, the evolutionary history was inferred using the maximum  
1118 likelihood method. Number at nodes represent bootstrap values (1 000 replicates of the  
1119 original dataset), only number above 50% are shown. The scale bar represents the average  
1120 number of substitutions per site. The accession number are given for all sequences. Bin  
1121 sequences are identified by a red dot, *Sulfurimonas* genome sequences by a blue diamond.  
1122 Subclusters DsrE2A, DsrE3A, DsrE3B, DsrE4 and DsrE5 were those previously described by Liu  
1123 et al (2014). DsrE corresponds to the classical DsrE/TusD subunit from the DsrEFH operon that  
1124 acts as cytoplasmic acceptor for sulfur. C\* corresponds to the active site cysteine conserved  
1125 in all sequences.

1126 **Figure S7: Maximum likelihood phylogenetic trees of deduced protein sequences of**  
1127 **periplasmic nitrate reductase catalytic subunit (NapA).** Number at nodes represent bootstrap  
1128 values (1 000 replicates of the original dataset), only number above 50% are shown. The scale  
1129 bar represents the average number of substitutions per site. The accession number are given  
1130 for all sequences. Each dot color corresponds to an environmental category for *Sulfurimonas*  
1131 genomes: blue, freshwater (Bin58 sequence); yellow: marine sediment; orange: marine water;  
1132 light yellow: brackish water, green: oil field water, gray: mud volcano and light brown:  
1133 hydrothermal vent.

1134 **Figure S8: Maximum likelihood phylogenetic tree of deduced protein sequences of the**  
1135 **ferritin-like diiron-carboxylate protein superfamily.** Number at nodes represent bootstrap  
1136 values (1 000 replicates of the original dataset), only number above 50% are shown. The scale  
1137 bar represents the average number of substitutions per site. The accession numbers for the  
1138 sequences are given at the right side of species name. For the *Sulfurimonas* genomes, the  
1139 ferritin belonging to the DNA-binding protein from nutrient starved cells (Dps) family is  
1140 identified by a green dot while that belonging to the 24-subunit non-heme ferritin family, by  
1141 a red dot.

1142 **Figure S9: Modular structure of integrated prophage found in Lake Pavin strain 1.** The gene  
1143 blocks conserved in the two MAGs affiliated to the *Sulfurimonas* genus (UBA and RIFOXYD2)  
1144 are shown. Proteins found in no other *Sulfurimonas* genomes appear in red while those found  
1145 only in a few genomes, in blue.

1146 **Table S1:** Characteristics of the 17 *Sulfurimonas* reference genomes and strains from the water  
1147 column of Lake Pavin.

1148 **Table S2:** List of *Sulfurimonas* MAGs and SAGs downloaded from the public databases and  
1149 used in this study.

1150 **Table S3:** List of Bins produced by *de novo* assembly of pooled reads from all metagenomes  
1151 (MetaG1 and MetaG2) collected along the Lake Pavin water column.

1152 **Table S4:** Statistics on the assembly of SAG and MAGs of *Sulfurimonas* genus obtained from  
1153 Lake Pavin, completeness and contamination estimates.

1154 **Table S5:** Taxonomic affiliation of SAGs obtained at 65 and 80 m depth in Lake Pavin water  
1155 column based on their 16S rRNA gene sequence (Sanger sequencing).

1156 **Table S6:** Quality evaluation of hybrid assembly genomes and of Bin58 MAG and SAG\_733-F2  
1157 used to produce them.

1158 **Table S7:** Multiple sequence alignment of the different 16S rRNA gene copies from  
1159 *Sulfurimonas* sp. SWIR19, *S. sediminis* S2-6 and *S. hydrogeniphila* using Clustal Omega tool  
1160 (<https://www.ebi.ac.uk/Tools/msa/clustalo/>).

1161 **Table S8:** Specific functions involved in numerous processes, including: carbon, nitrogen,  
1162 sulfur, hydrogen (H<sub>2</sub>) cycling, oxidative stress, motility, vitamin biosynthesis, transport, etc,  
1163 identified in Lake Pavin strains.

1164 **Table S9:** List of genes involved in sulfur cycling identified in Lake Pavin strains.

1165 **Table S10:** DsrE-like protein diversity and distribution in *Sulfurimonas* strains.

1166 **Table S11:** List of genes involved in nitrogen cycling identified in Lake Pavin strains.

1167 **Table S12:** Predicted mobilome, resistome and virulome in MAGs and SAG sequences (\* genes  
1168 found only in Lake Pavin strains).

1169 **Table S13:** List of cloud gene clusters found both in *Sulfurimonas* reference genomes and Lake  
1170 Pavin strains to be reclassified into shell GC and their classification into COG categories.

1171 **Table S14:** Cloud gene clusters identified in Lake Pavin strains for which a COG classification  
1172 was found.  
1173

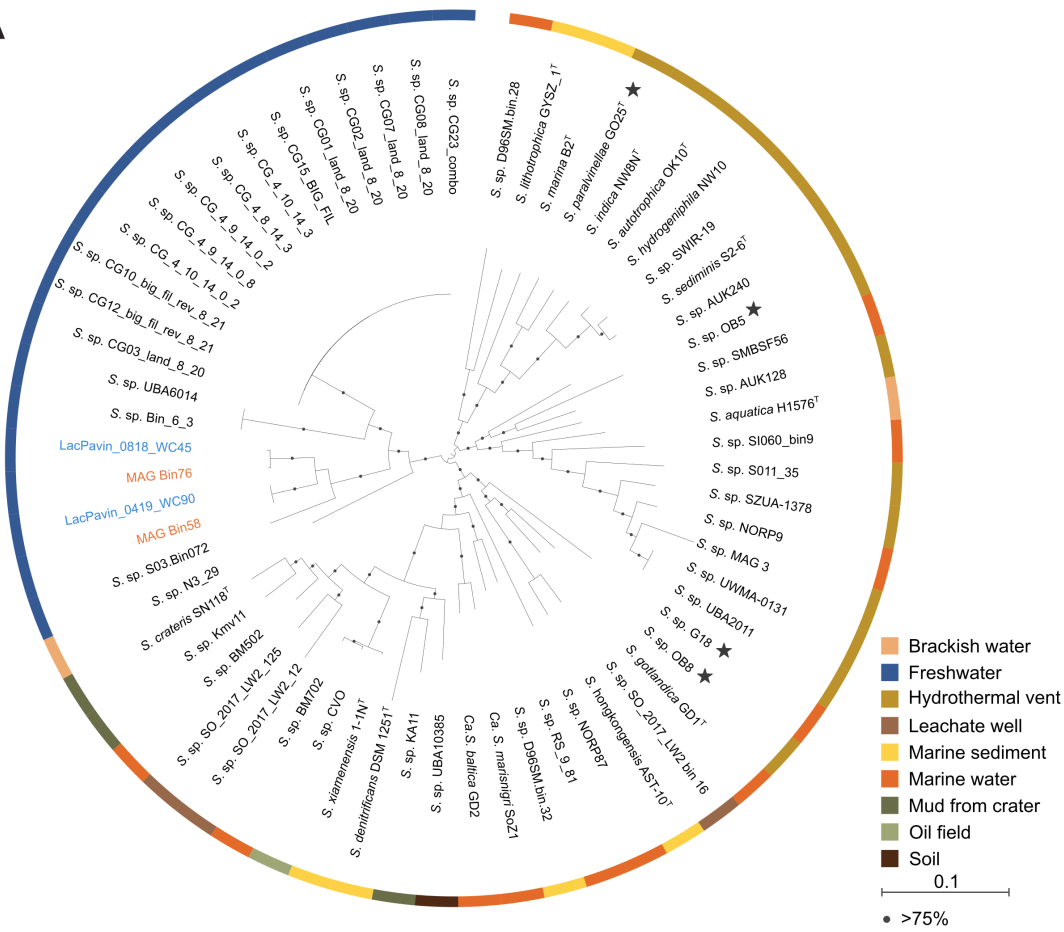


AAI (lower matrix) vs ANIb (upper matrix)

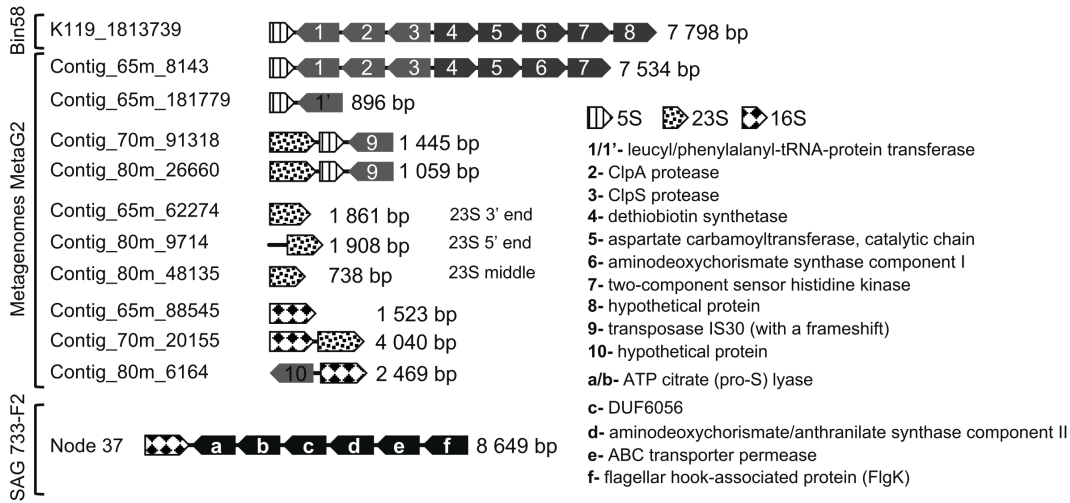
MAG 0419_WC90	100	100	100	82	81	73	72	73	73	75	75	72	73	73	72	74	74	74	73	74	73
SAG 733-F2	99	100	100	83	82	73	73	73	73	75	75	73	73	73	72	73	74	74	74	74	74
MAG BIN58	100	99	100	82	81	73	73	73	73	75	75	72	73	73	72	72	75	75	75	73	74
MAG BIN76	84	82	84	100	97	74	74	74	74	76	76	73	74	74	74	74	76	76	76	75	75
MAG 0818_WC45	82	82	83	97	100	74	73	73	73	75	75	72	73	73	73	73	75	75	75	74	74
<i>S. autotrophica</i>	69	68	69	71	70	100	82	82	82	74	74	76	76	74	74	73	74	74	74	73	73
<i>S. hydrogeniphila</i>	69	67	69	70	69	84	100	92	92	73	73	76	75	73	74	73	73	73	73	72	72
<i>S. sp SWIR-19</i>	69	68	69	70	69	84	94	100	96	73	73	76	75	73	74	73	73	73	73	72	73
<i>S. sediminis</i>	69	67	69	71	69	85	94	96	100	73	73	76	75	73	74	73	73	73	73	72	72
<i>S. sp CVO</i>	72	70	72	74	72	70	70	69	70	100	99	73	74	74	73	74	76	76	75	75	77
<i>S. xiamenensis</i>	72	70	72	74	73	70	70	70	70	99	100	73	74	73	73	74	76	76	75	75	77
<i>S. paralvinellae</i>	69	68	69	71	69	77	77	77	77	70	70	100	79	73	74	73	73	72	73	72	72
<i>S. indica</i>	69	67	70	71	70	77	76	76	76	70	70	82	100	74	75	73	73	73	73	73	72
<i>S. aquatica</i>	69	68	69	71	70	73	72	71	72	70	70	72	72	100	74	73	74	74	75	74	73
<i>S. marina</i>	68	66	68	70	68	72	72	71	71	69	69	73	73	70	100	74	73	73	74	73	73
<i>S. litotrophica</i>	68	67	69	71	68	69	68	68	68	70	71	68	69	69	69	100	73	73	74	73	73
Candidatus <i>S. baltica</i>	71	70	72	73	72	69	69	69	69	75	76	69	69	70	69	70	100	88	76	74	75
Candidatus <i>S. marisnigri</i>	72	69	72	73	72	70	69	69	69	76	76	69	70	70	69	70	90	100	77	75	74
<i>S. gotlandica</i>	72	71	72	74	72	70	70	70	70	73	74	70	70	71	69	70	75	75	100	77	75
<i>S. hongkongensis</i>	70	69	70	72	71	69	69	69	69	72	72	69	69	69	68	70	72	72	77	100	75
<i>S. denitrificans</i>	70	68	70	72	70	68	68	68	68	78	78	68	68	68	68	68	68	73	73	71	71
<i>S. crateris</i>	71	69	72	73	71	69	69	69	69	81	82	69	69	69	69	69	75	75	72	71	77
MAG 0419_WC90																					
SAG 733-F2																					
MAG BIN58																					
MAG BIN76																					
MAG 0818_WC45																					
<i>S. autotrophica</i>																					
<i>S. hydrogeniphila</i>																					
<i>S. sp SWIR-19</i>																					
<i>S. sediminis</i>																					
<i>S. sp CVO</i>																					
<i>S. xiamenensis</i>																					
<i>S. paralvinellae</i>																					
<i>S. indica</i>																					
<i>S. aquatica</i>																					
<i>S. marina</i>																					
<i>S. litotrophica</i>																					
Candidatus <i>S. baltica</i>																					
Candidatus <i>S. marisnigri</i>																					
<i>S. gotlandica</i>																					
<i>S. hongkongensis</i>																					
<i>S. denitrificans</i>																					
<i>S. crateris</i>																					

Figure 1

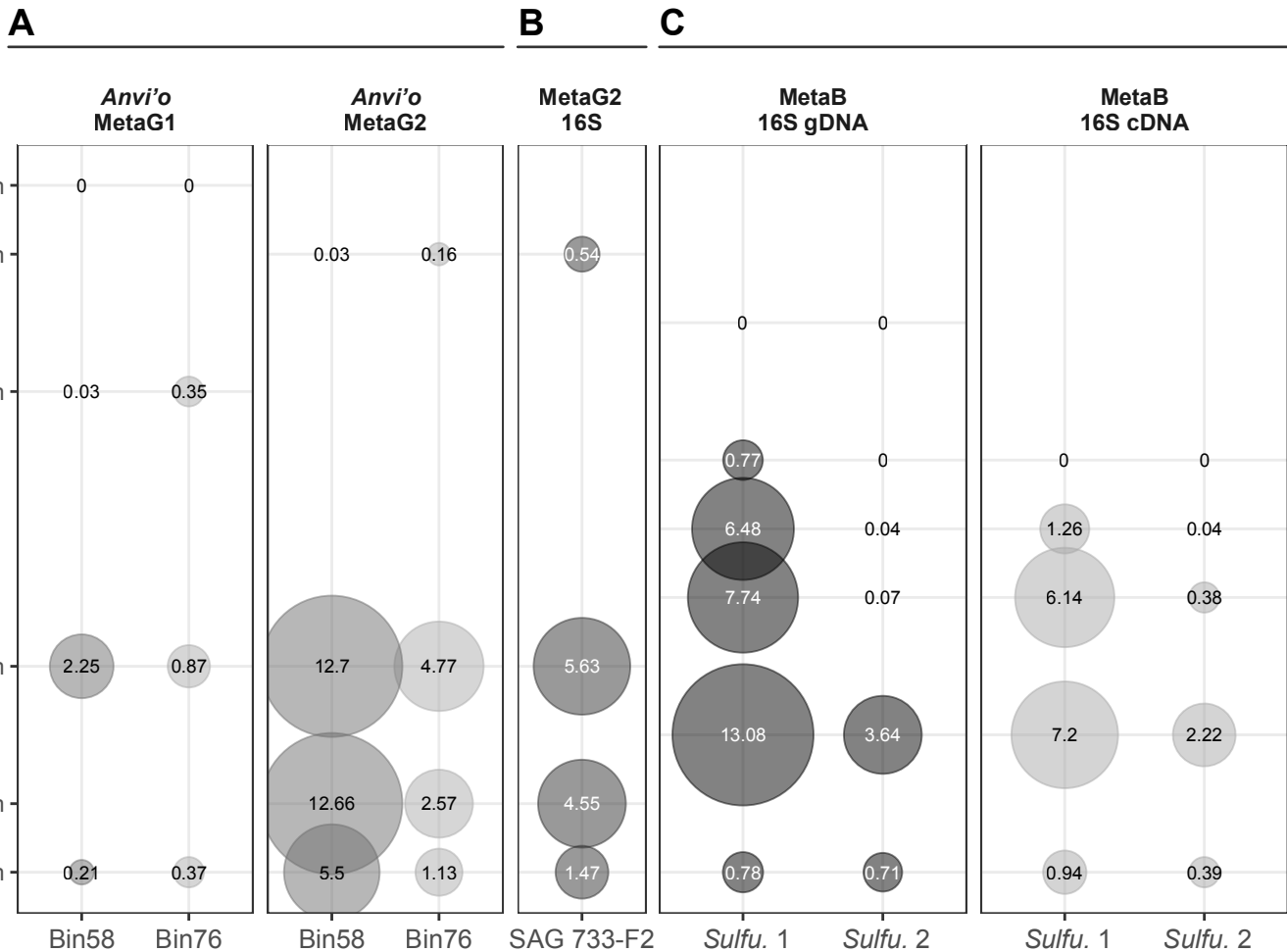
**A**



**B**



**Figure 2**



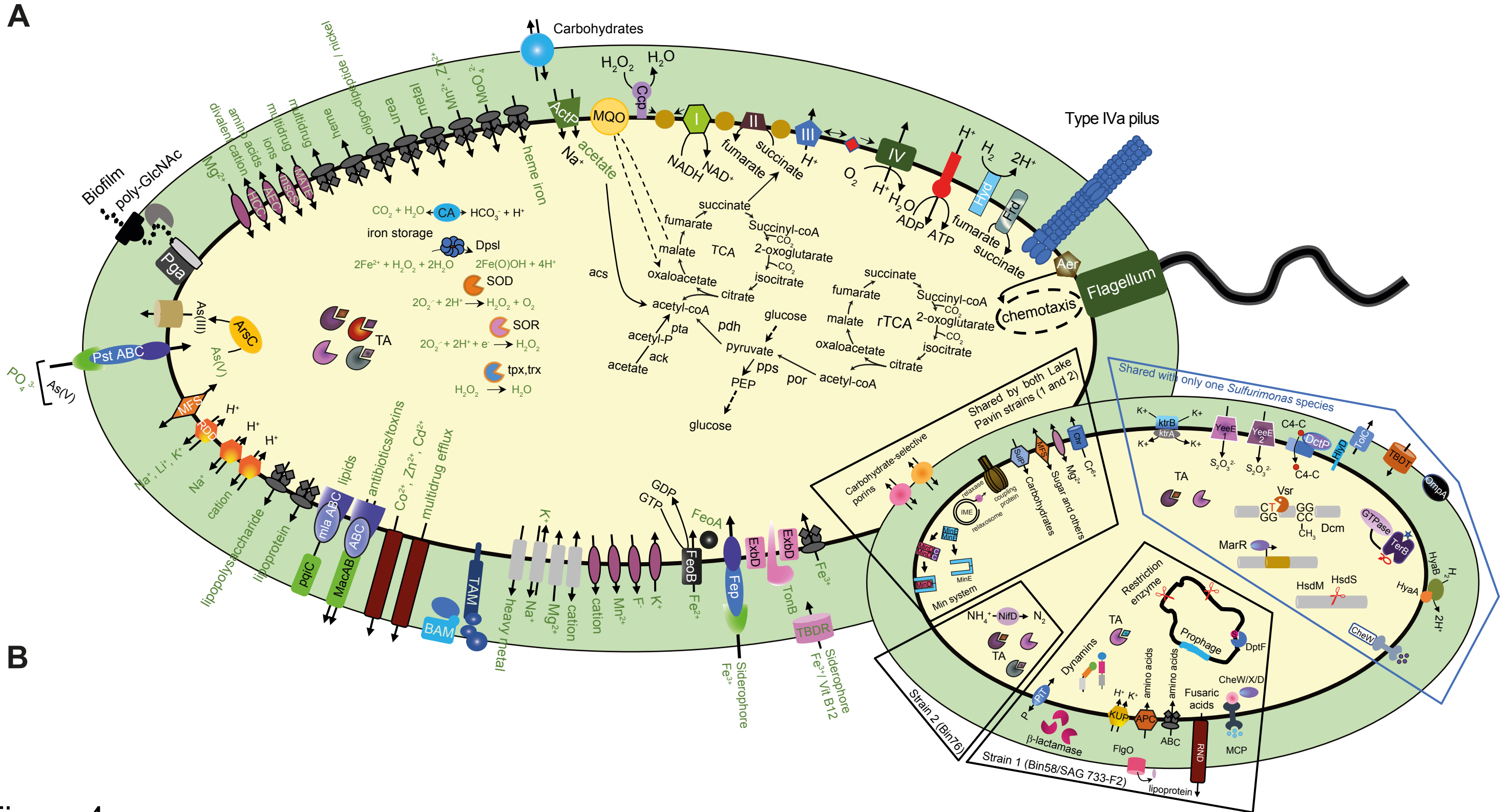


Figure 4

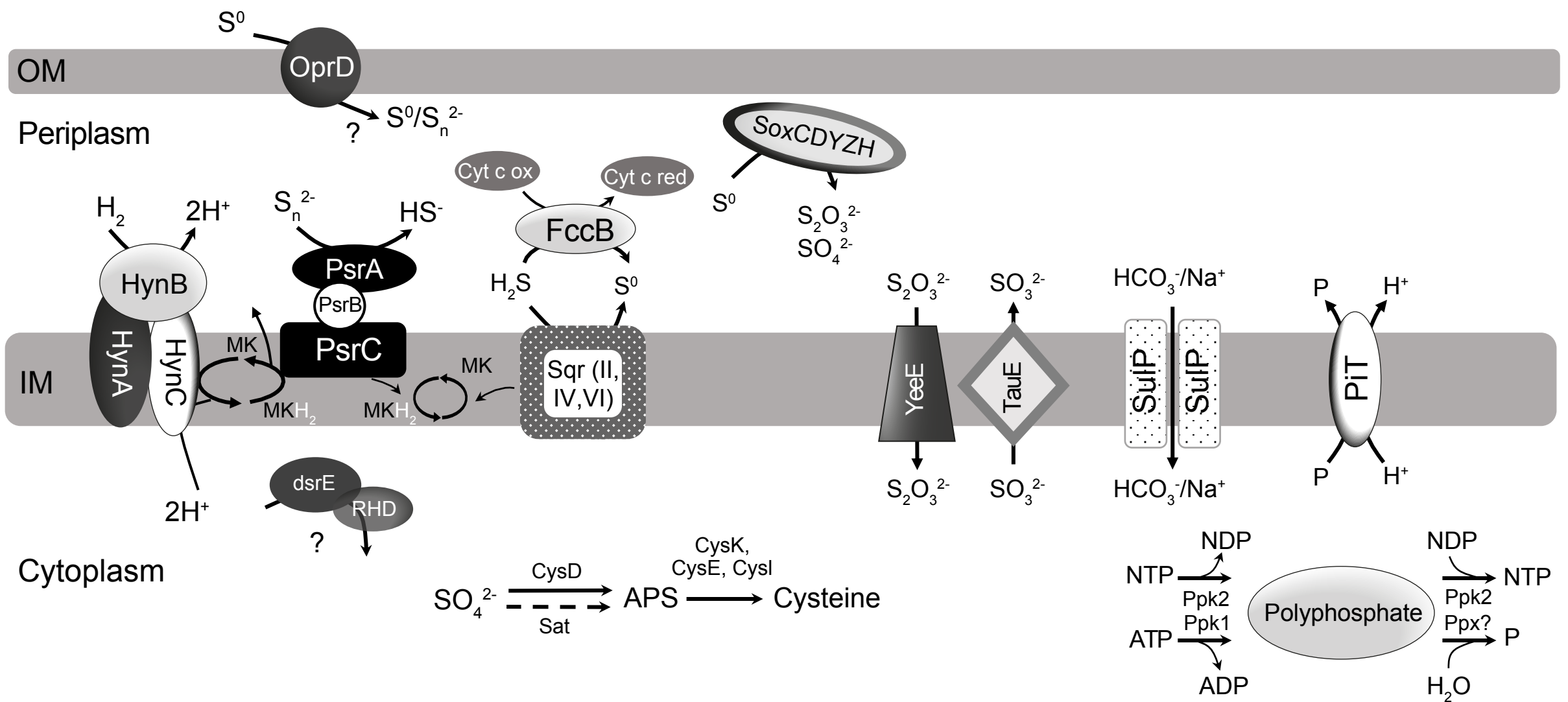
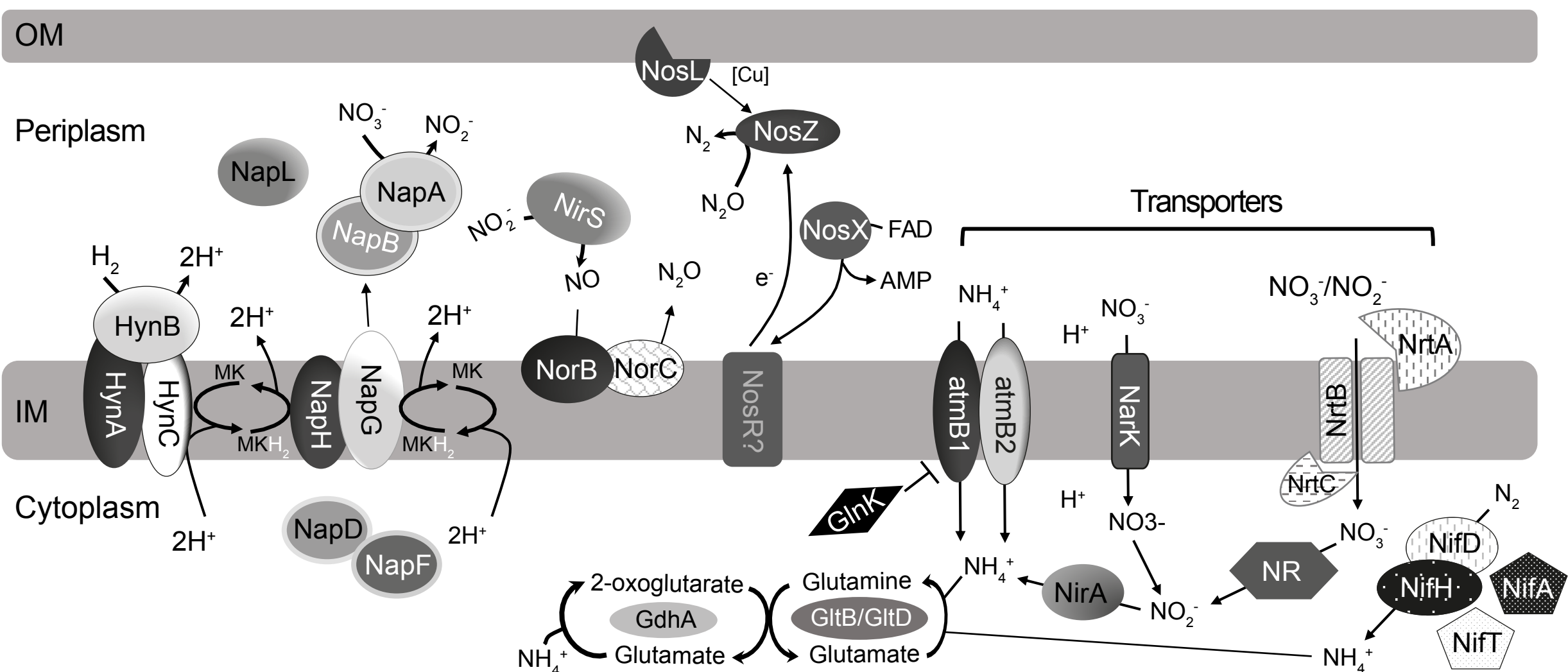
**A****B**

Figure 5

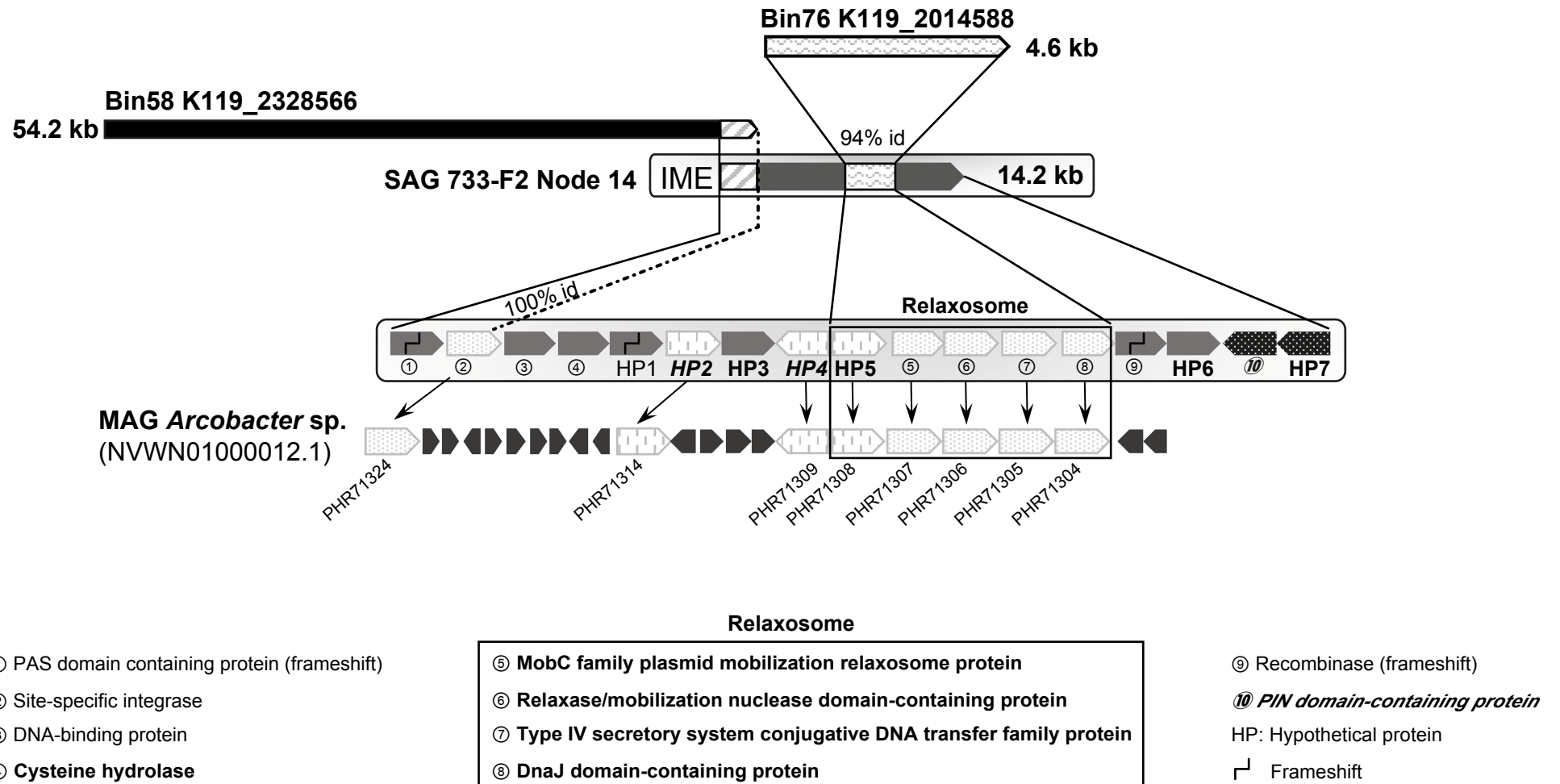


Figure 6

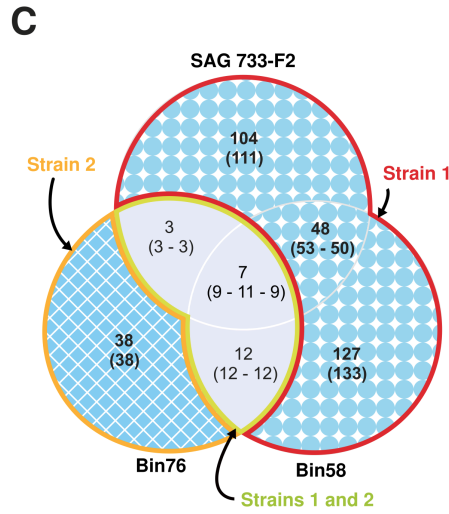
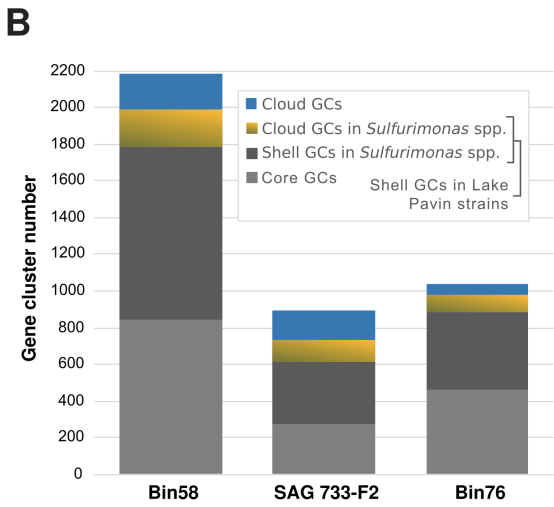
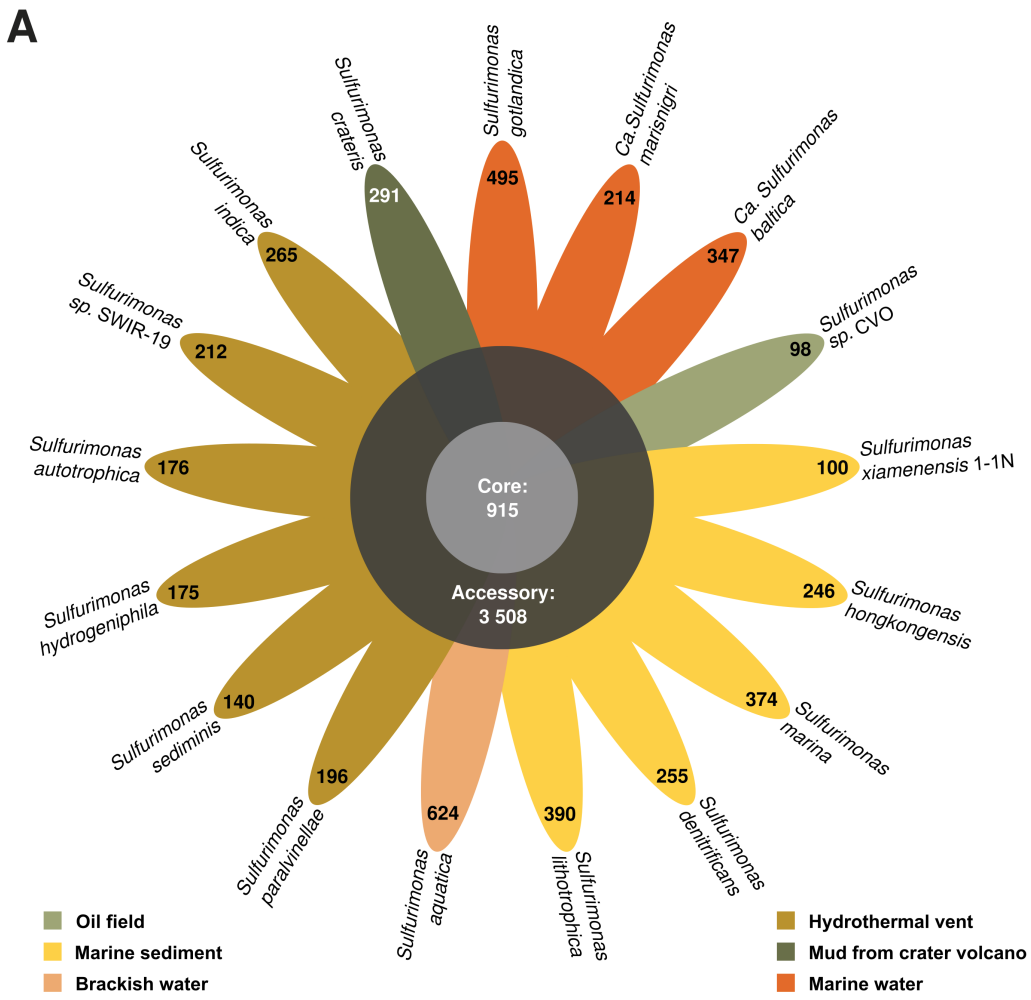


Figure 7

COG hierarchy	Function	COG category	Strain 1 Bin58/SAG 733-F2	Strain 2 Bin76	Strains 1 and 2	
<b>Information storage and processing</b>	Translation, ribosomal structure and biogenesis	J	1			
	Translation, ribosomal structure and biogenesis	JKL	1			
	Transcription	KT	1			
	Replication, recombination and repair	L	5			
<b>Cellular processes and signaling</b>	Cell cycle control, cell division, chromosome partitioning	D	1		3	
	Cell wall/membrane/envelope biogenesis	M			2	
	Cell wall/membrane/envelope biogenesis	MU	1			
	Cell motility	N	3			
	Posttranslational modification, protein turnover, chaperones	O	2			
	Signal transduction mechanisms	T	4			
	Intracellular trafficking, secretion, and vesicular transport	U	1		3	
	Defense mechanisms	V	7	5		
	Mobilome: prophages, transposons	X	17	1	2	
	<b>Metabolism</b>	Energy production and conversion	C		1	
		Energy production and conversion	CO	1		
		Amino acid transport and metabolism	E	3		2
		Nucleotide transport and metabolism	F	1		
Carbohydrate, transport metabolism		G	2		1	
Lipid transport and metabolism		I			1	
Inorganic ion transport and metabolism		P	1		4	
Secondary metabolites biosynthesis		Q	2			
<b>Poorly characterized</b>		Function unknown	S	14	1	2
		General function prediction only	IR	1		
<b>Figure 8</b>	General function prediction only	R	7	1	2	



# Table 1

Family	Toxin	Antitoxin	Cell process affected	Stage blocked	Loci number	Species																					
						<i>S. paravivellae</i>	<i>S. autotrophica</i>	<i>S. indica</i>	<i>S. hydrogeniphila</i>	<i>S. sediminis</i> ‡	<i>S. sp. SWIR19</i> ‡	<i>S. aquatica</i>	<i>S. gotlandica</i>	<i>S. Candidatus</i>	<i>S. Candidatus baltica</i>	<i>S. marina</i>	<i>S. denitrificans</i>	<i>S. hongkongensis</i>	<i>S. litotrophica</i>	<i>S. xiamenensis</i>	<i>S. xiamenensis 1-1N</i> *	<i>S. crateris</i>	SAG 733-F2	Bin58	Bin76		
Type II	RelE	HTH	Translation	RNase ribosome-dependent targeting mRNA	1+	10	9	18	22	19	5+5	3	25	11	1	13	13	9	7	7	28	8	9	12			
		RHH																									
		Phd/YefM																									
		CopG																									
		XRE																									
		HigA																									
		PHD																									
		CcdA																									
		AM																									
		DUF																									
	HP																										
	Orphan																										
	RelB/DinJ																										
	CopG																										
	PHD																										
	HP																										
	Orphan																										
	Txe/YoeB																										
	HigB	TR																									
		HigA																									
		DNAb																									
		HTH																									
		HP																									
	BrnT	BrnA																									
		CopG																									
		HTH																									
	PemK/MazF	HP																									
		Orphan																									
CopG																											
Phd/YefM																											
MazE																											
DNAb																											
HP																											
Orphan																											
VapC																											
HP																											
PIN	HTH																										
	HP																										
	HP																										
HipA	HicA																										
	HicB																										
GNAT	HTH																										
	XRE																										
	RR																										
NTase	HP																										
	HP																										
Zeta	HEPN																										
	DUF86																										
Yail/YqxD	HP																										
	Orphan																										
MMP	HTH																										
	HP																										
Type IV	AbiEii/AbiGii	AbiEi	Anti-phage defense	Viral replication																							
		HTH																									
		DUF																									
		HP																									
		Orphan																									

■: Hydrothermal vent, ■: Brackish water, ■: Marine water, ■: Marine sediment, ■: Oil field, ■: Mud from crater, ■: Freshwater

AM: Addition module, DNAb: DNA binding protein, DUF: Protein of unknown function, HP: Hypothetical protein, HTH: Helix turn helix, MMP: Mobile Mystery protein B, NTase: nucleotidyltransferase, PHD: Prevent-host-death family, RHH: Ribbon-helix-helix domain, RR: Response regulator transcription factor, TR: Transcription regulator  
nd: not determined

Chromosomal location:

●: 1 copy, ★: 2 copies, ◆: 3 copies, ■: 4 copies, ☆: 5 copies, ✦: 7 copies, ○: orphan toxin (1 copy).

Plasmid location:

●: 1 copy

‡ Strains of the species *S. sediminis*, delineated by double lines

\* Strains of the species *S. xiamenensis*, delineated by double lines

Y: black: chromosomal location, pink: plasmid location

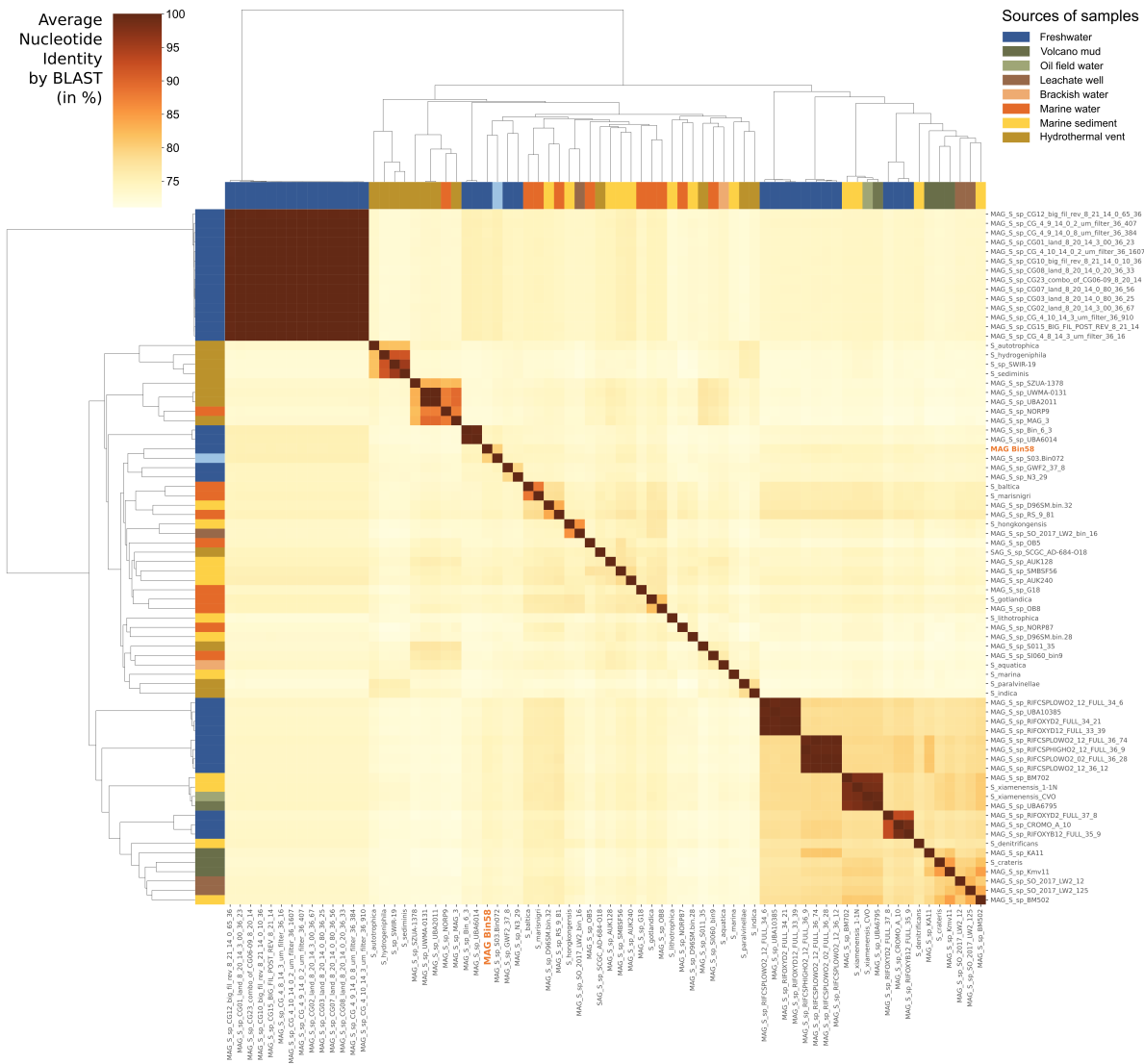


Figure S1

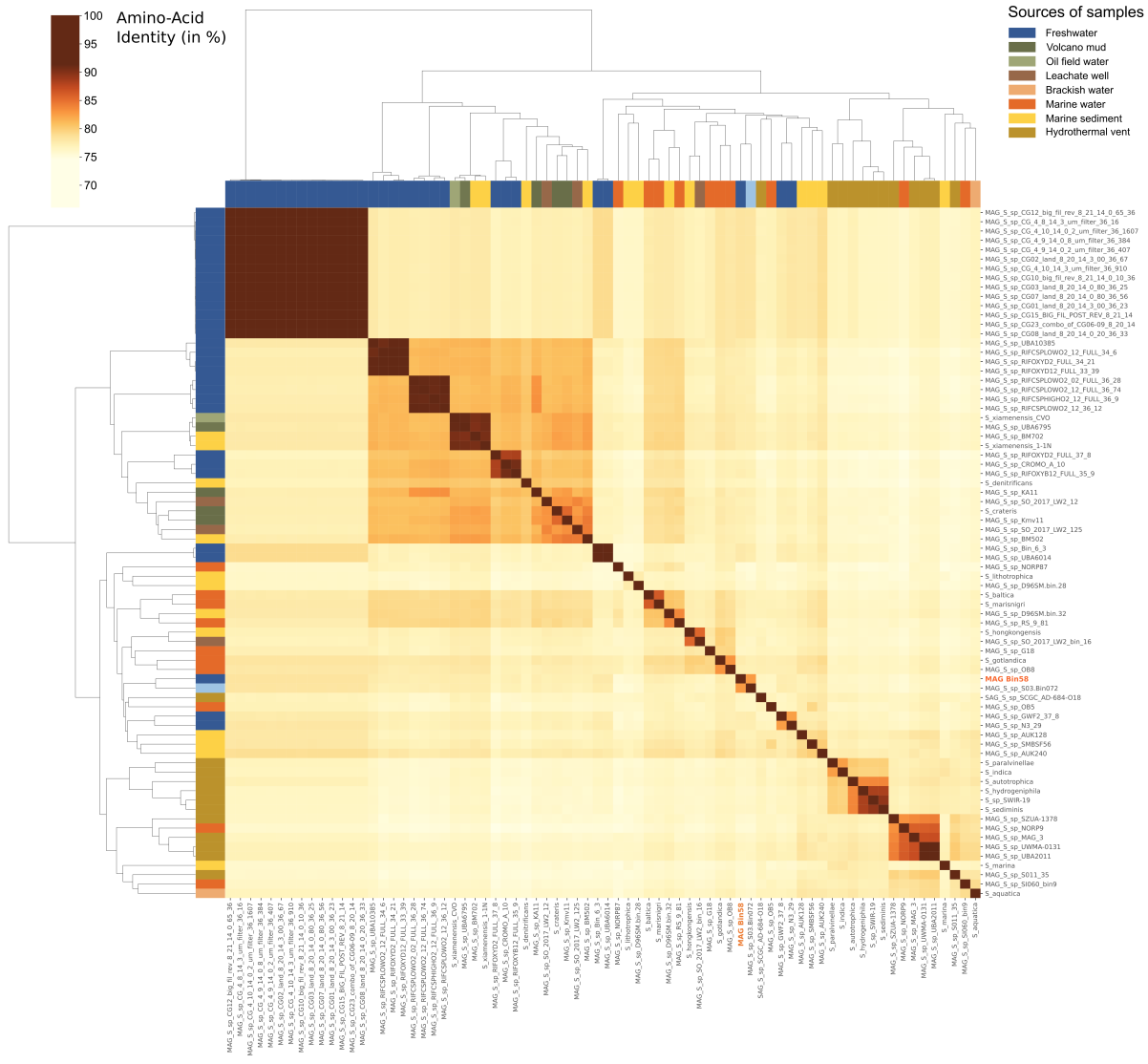


Figure S2

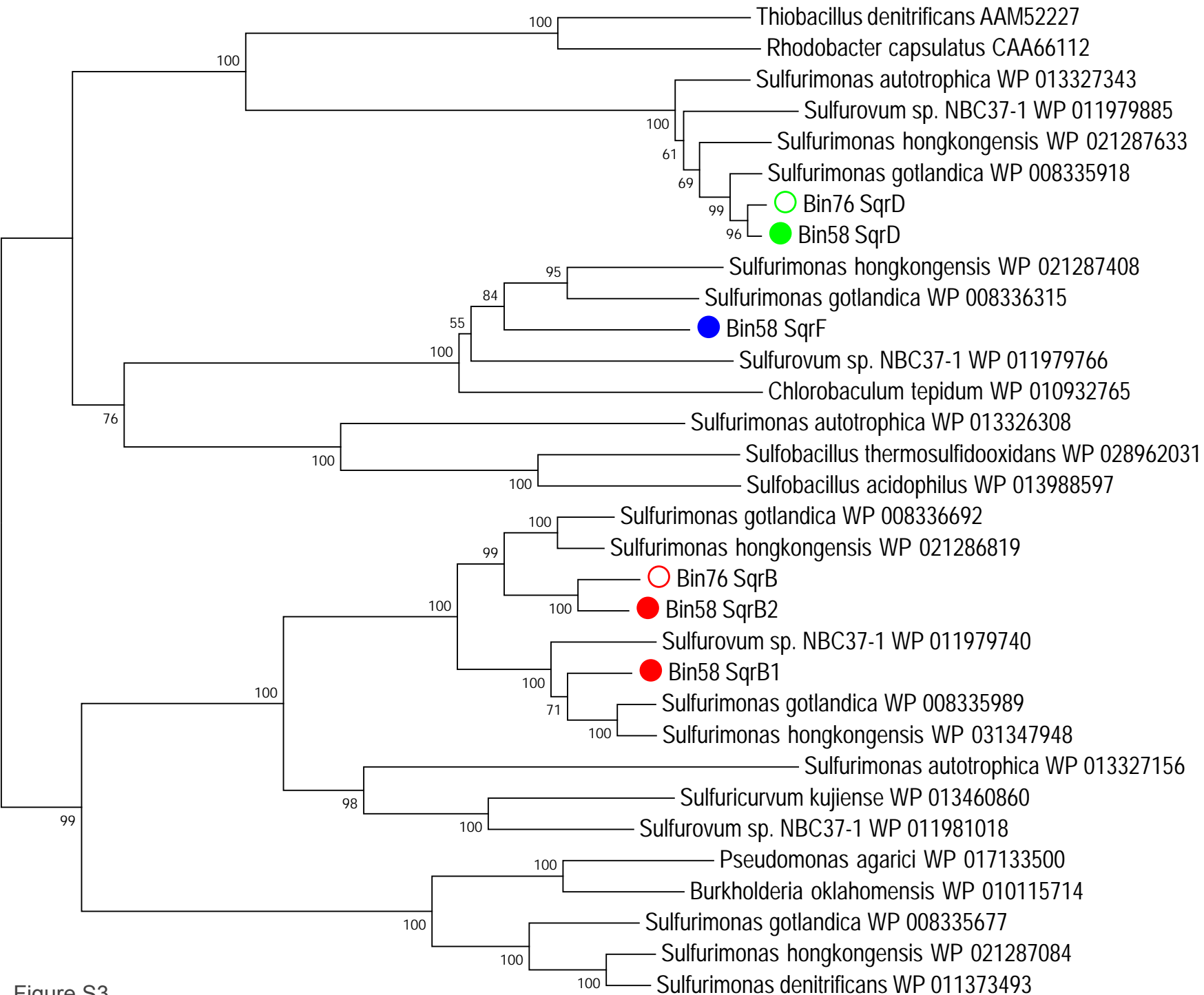
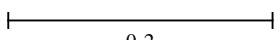


Figure S3



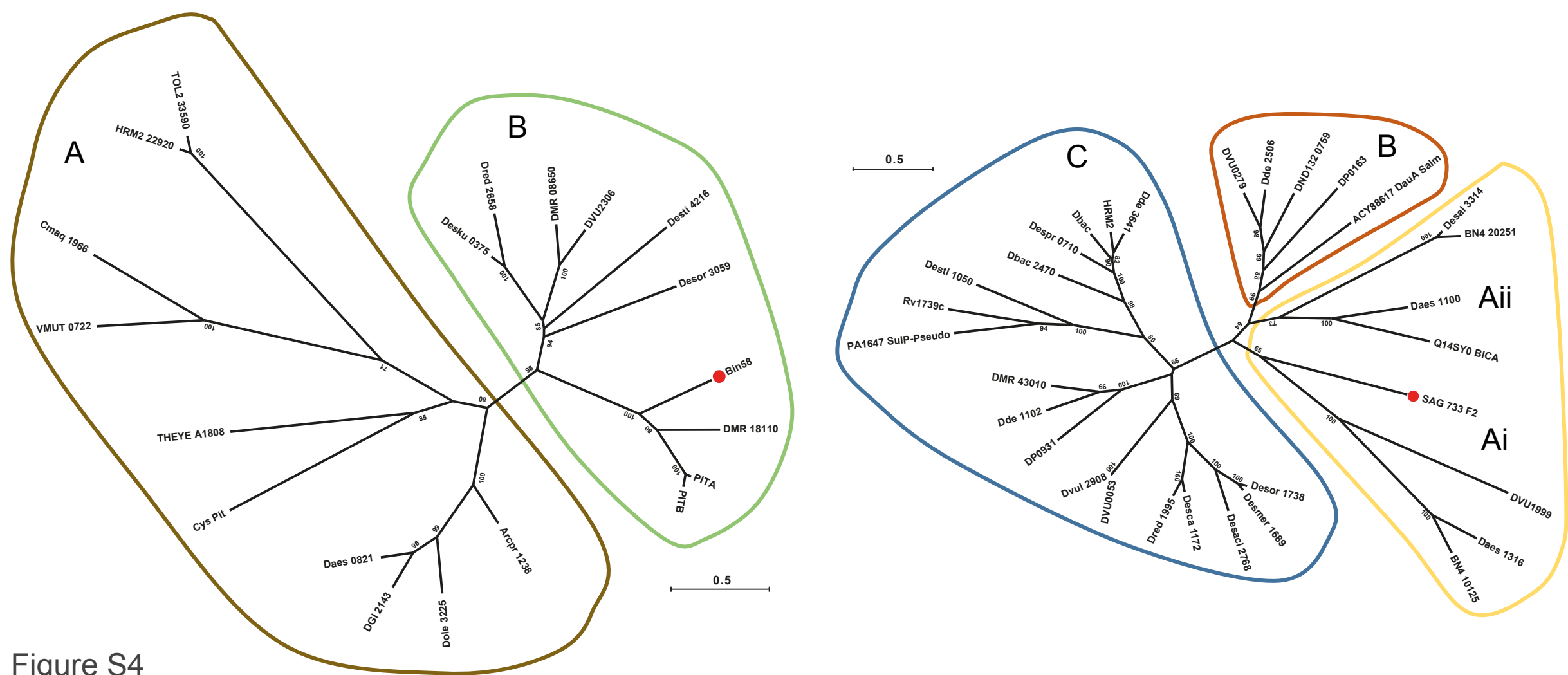
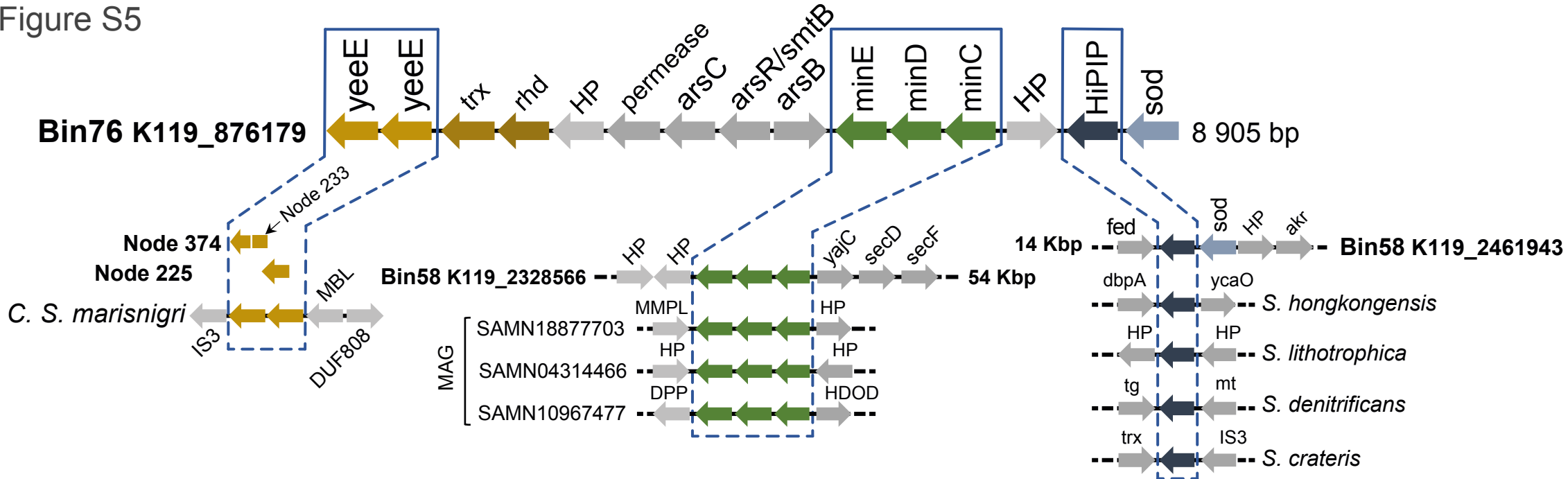


Figure S4

Figure S5



akr: aldo-keto reductase, fed: ferredoxin, ars: arsenate reductase genes, dbpA: decorin-binding protein A, DPD:divergent polysaccharide deacetylase, fed: federroxinHDOD:HDOD domain-containing protein, HiPIP: high potential iron-sulfur proteins, IS3: transposase, MAG: metagenome-assembled genome, MBL: MBL Fold metallo hydrolase, minCDE: Min system, MMPL:MMPL family transporter, sod: superoxide dismutase, rdh: rodhanese-like, tg: transglycosylase, mt:mutarotase, trx: thioredoxin, yajC/secCDF: secretion system, ycaO: ribosomal protein S12 methylthiotransferase accessory factor, HP: hypothetical protein.  
Node: contig from SAG 733-F2.

# A Figure S6

## N-terminus

TK90	27	TPHRCATPFYLGAVMASMDVDVY	DsrE3B
Atc_2345	24	TAHRCATPFYLGALLSSMDAEV	
<b>LCX93_RS03235</b>	36	IVYQCDFPDIQRIHMLNTINNA	DsrEd
ENK67_03485	36	VVYQCDFADPKRVHMLNTLNNA	
<b>LCX93_03195</b>	21	VVFDSSGNASYIKSRMLVIGKT	DsrEf
SUN_RS08545	20	AVFDCCSSDADYIKSRMLVIGKT	
AS592_RS10230	24	AVFDCCSSDNADYIKSRMLVIGKT	DsrEg
<b>FJR48_RS04845</b>	21	AVFDCAAGDMKFVLTTRMKLLDMT	
JXK04_04865	23	AVFDCAAKDMKFVASRMFLIEES	
<b>LCX_RS03235</b>	71	INVVALGPELQYMMKDFKGTG	DsrEd
ENK67_03485	71	IDIVALGPELQYVMKDFKDTG	
<b>LCX93_03195</b>	57	VALTLHGSQVAMVANDYDMIV	DsrEf
SUN_RS08545	56	FVITLHGGVSMVSKYKEFV	
AS592_RS10230	60	FAITLHGGVSMVSKYEMIV	DsrEg
<b>FJR48_RS04845</b>	56	FVLTIHSKCTPIVVKNSDNKD	
JXK04_04865	58	FVLTIHSGTPIAVEEGDDE	

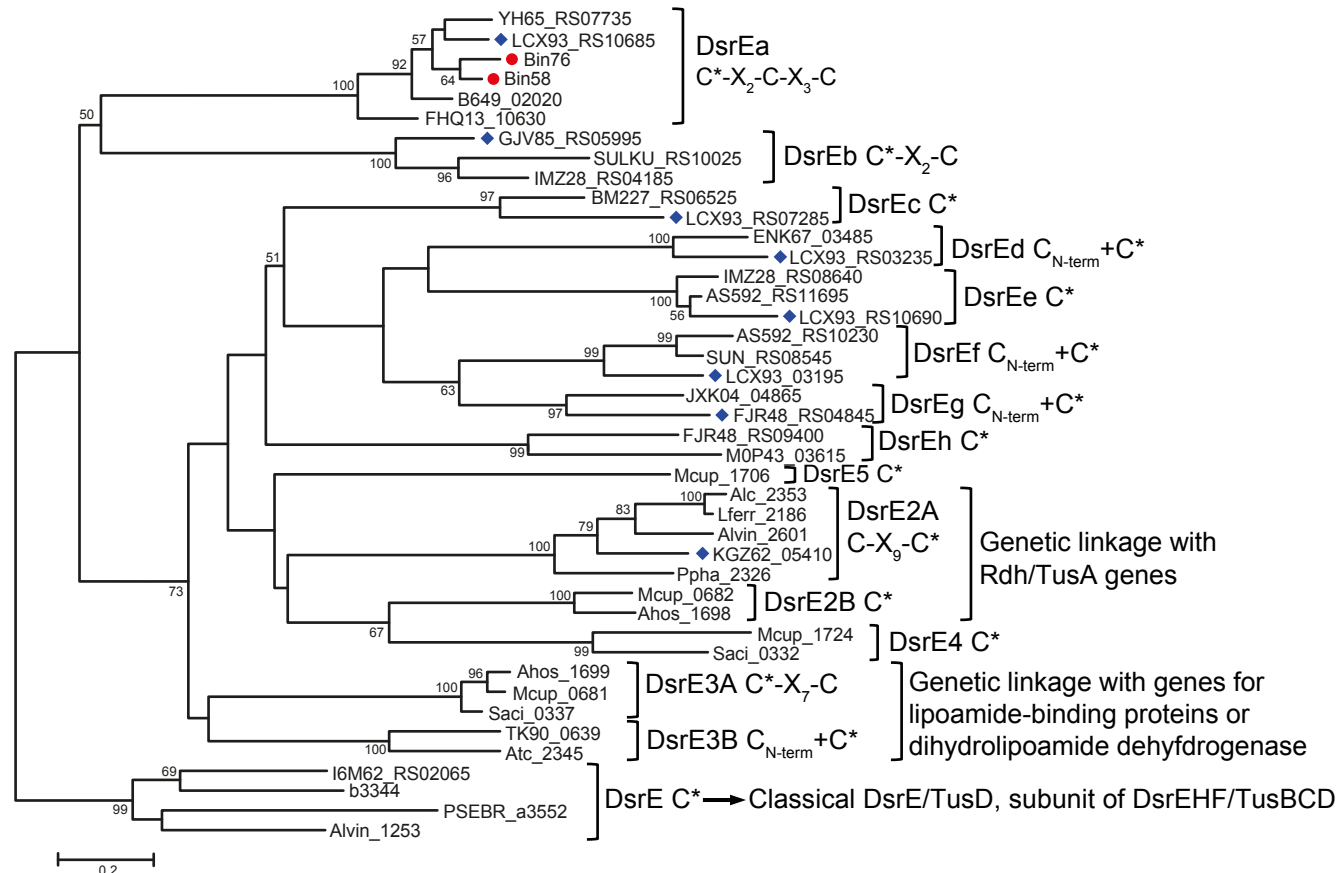
**Ahos** : *Acidianus hospitalis* W1, **Alvin** : *Allochromonium vinosum* DSM 180, **AS592** : *Sulfurovum rufiae* strain 1812E, **Atc** : *Acidithiobacillus caldus* SM-1, **b3344** : *Escherichia coli* str. K-12, **B649** : *Candidatus Sulfituricum* sp. RIFRC-1, **ENK67** : *Flavobacteriia* bacterium isolate HyVt-598, **FHQ18** : *Deferribacter autotrophicus*, **I6M62** : *Acinetobacter courvalinii*, **IMZ28** : *Sulfurovum indicum* strain ST-419, **JXK04** : *Campylobacteriales* bacterium isolate ZodW, **Lferr** : *Acidithiobacillus ferrooxidans* ATCC 53993, **Mcup** : *Metallosphaera cuprina*, **MOP43** : *Arcobacteraceae* bacterium isolate SO\_2017\_CLC, **Ppha** : *Pelodictyon phaeoclatratiforme* strain DSM 5477, **PSEBR** : *Pseudomonas brassicaearum*, **Saci** : *Sulfolobus acidocaldarius* DSM 639, **SUN** : *Sulfurovum* sp. NBC37-1, **TK90** : *Thioalkalivibrio* sp. K90mix, **YH65** : *Sulfurovum lithotrophicum* strain ATCC BAA-797, **KGZ62** : *Sulfurimonas* sp. CROMO\_A\_10, **LCX93** : *Sulfurimonas* sp. SWIR-19, **FJR48** : *Sulfurimonas lithotrophica*, **GJV85** : *Sulfurimonas aquatica*.

## C-terminus

<b>Bin58</b>	58	<b>NDLVEMLKKMYASGIKLQACGTCNARCGIFK</b>	DsrEa
<b>Bin76</b>	58	<b>YDLVEMLKKMYASGINLQACGTCNARCGIFK</b>	
FHQ18_10630	61	QDLVQMLKELYHSGVELKVGCTCQARCGVNK	DsrEb
<b>LCX93_RS10685</b>	58	<b>YDLAEMLQTMKDGISLQVCGTCNARCGIFK</b>	
YH65_RS07735	58	YDLLEMLKKMYTRGINLQVCGTCNARCGLFF	DsrEc
B649_02020	58	HDLVDMMLKKLYEEGISLQACGTCNARCGLFF	
<b>GJV85_RS05995</b>	56	SDLQERVKVQVATGVKFNACVVCDDYRVSK	DsrEd
IMZ28_RS04185	52	EALCEVTKVKQKSGVTFSCCIVCSDDYGVTE	
SULKU_RS10025	52	AELQTMVTKVLETVGKASVQVVCSDDYGVTE	DsrEe
<b>LCX93_RS07285</b>	84	KETAVRVDMALQYDVEFVACGNTMRTLHIKE	
BM227_RS06525	83	KKIAVVRVSLQTYDVEFVACGNTMRTLKIKP	DsrEf
<b>LCX93_RS03235</b>	109	VSREFKGLQLGGDNIHFFACGNTMCKKHVKD	
ENK67_03485	109	ASRIKGLQLGGDNIKMFACNNTMDKKNVKP	DsrEg
<b>LCX93_RS10690</b>	92	ELQKRIASADTYDVEFLMCKASMPKNHLEA	
IMZ28_RS08640	93	ILAKRIDSLIKTYDVEFLMCGAAMVKNKLAQA	DsrEh
AS592_RS11895	94	DLAKRIASMAKNYDVEFLMCGAAMPKNKLEA	
<b>LCX93_03195</b>	87	QSQQYLKLLAKRKNVTTITACMSLAHNAIEK	DsrE2A
SUN_RS08545	86	KAQEHLKTLATRKKVIVVCAMSLDANAIEK	
AS592_RS10230	90	KAQELRVLAEKRGVIVVCAMSLASNAIEQ	DsrE2B
<b>FJR48_RS04845</b>	79	AIHQKLTQLKREHNVKIEACKIATNKFYK	
JXK04_04865	82	GISQRLKLLHAEYGVKIEACEIALERFGEIK	DsrE3A
<b>FJR48_RS09400</b>	84	TSIKDMLSSLVKKGGVAVVCGMNAKQVEVKA	
MOP43_03615	85	KSIRDMLISLSKNGGTVMLCGMCASFEEIIT	DsrE3B
<b>KGZ62_05410</b>	112	ATIPELRELQAEVVFIAQMTVELFGFEH	
Alvin_2601	100	ASIEELRELQAEVVKMIACQMTVDLDFMPK	DsrE4
Lferr_2186	97	ASIEELRELQAEVVRMIACQMTVDLFEFDT	
Atc_2353	97	ASLEELRELQAEVVRMIACQMTVDLFEFDT	DsrE5
Ppha_2326	111	ATIEQLRGMQCFEGRFRIACQMTMEVFGFEK	
Ahos_1698	71	TWDQLIMQAKEVGEVVKVYACSTTMEFFGLKR	DsrE/TusD
Mcup_0682	80	MWHQLVKEAKDVGEVVKVYACSTTMEFFGIKK	
Saci_0337	74	NPFIRFFDMAKENGVMKYVCVQSLKDMCHMN	
Mcup_0681	74	NPFIHFDMARDNNGVMKYVCVQSLKDMCHMN	
Ahos_1699	68	NPFIHFDMARDNNGVMKYVCVQSLKDMCHMN	
TK90_0639	75	KLKIDFIDAKRAGVRLHVCQPALPGYRIDA	
Atc_2345	72	KKVIEFIRDANAGVEIHVCRPALPGYEIPA	
Saci_0332	67	DFTGLFKKAKKSGKVKIYACSYASKLFFNYSK	
Mcup_1724	66	NVFEHFKAKKSGKVKIYACSYASKIHGLDK	
Mcup_1706	62	KYYLDNLFELAGEDLEITACEFGMRVKDVHE	
I6M62_RS02065	53	DDQRNLKQEWQKLSIRLPVCSAALARGITD	
PSEBR_a3552	58	DLPQQWRTFVSDNQLDGVVCIAAALRRGVLN	
Alvin_1253	59	HIVNRWAELEAQYELDMVVCVAAALRRGIVD	
b3344	59	DLVRAWQQLNAHQHVALNICVAAALRRGVVD	

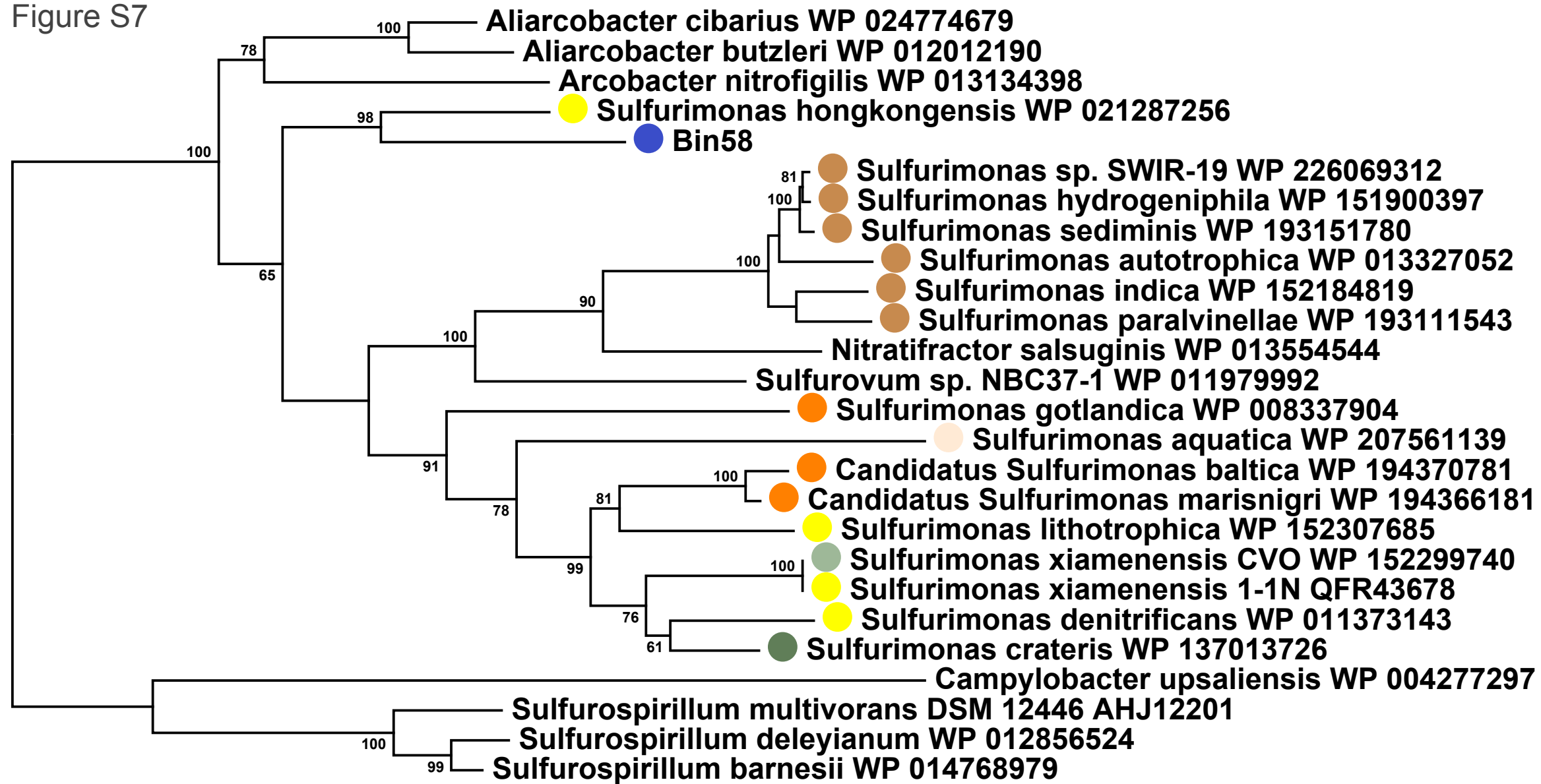
\* : active cysteine residue common to all DsrE-like proteins

# B



\* : active cysteine residue common to all DsrE-like proteins

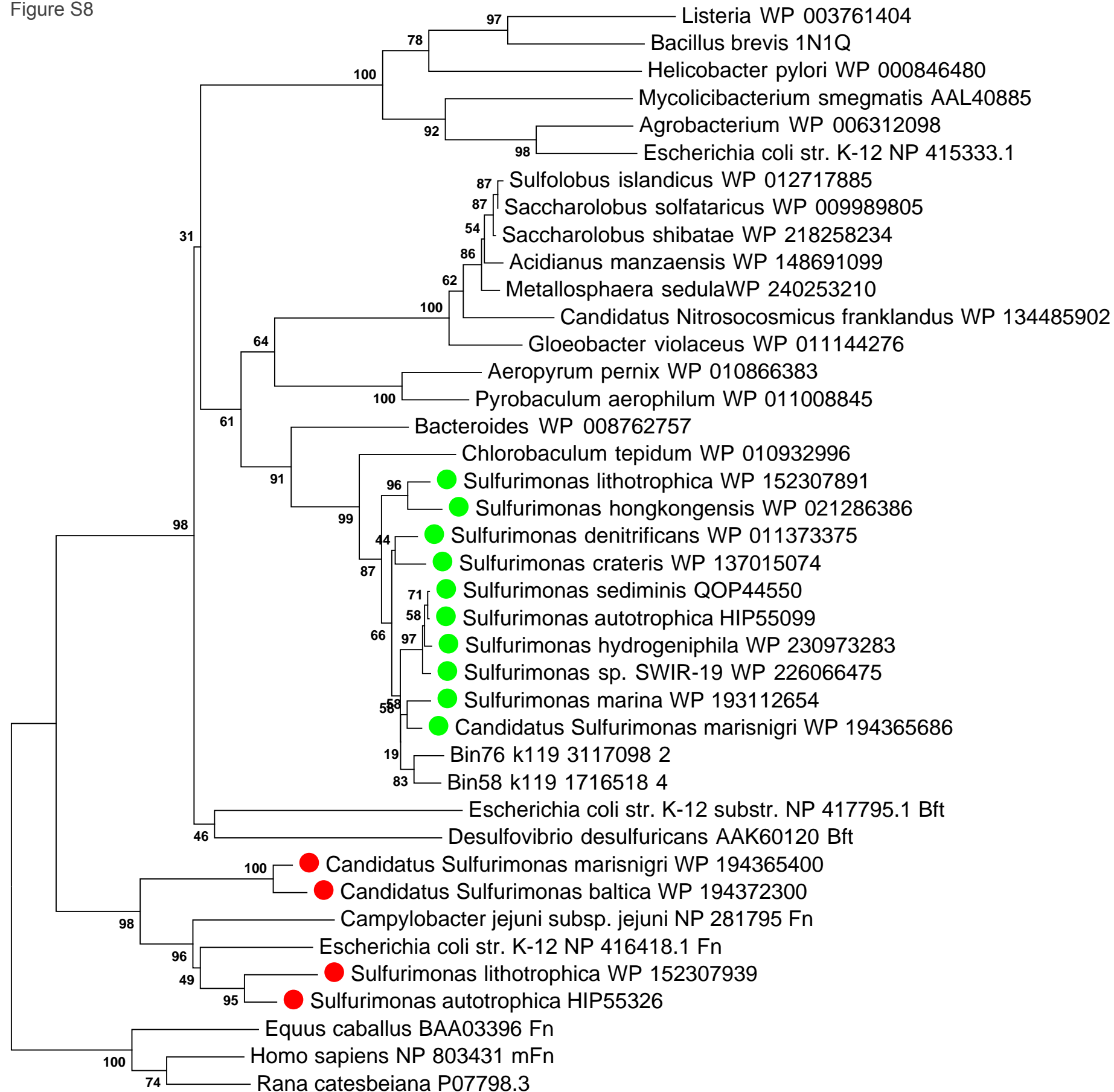
Figure S7



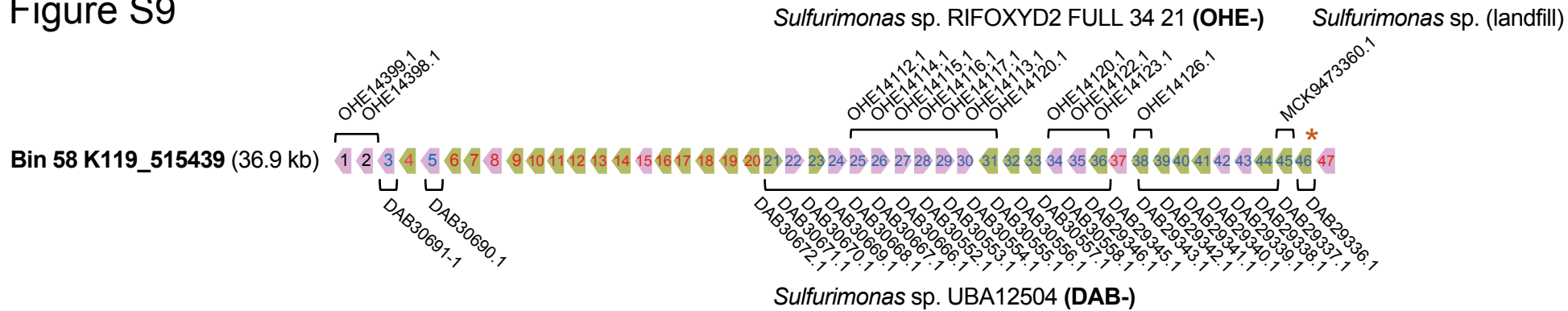
0.05



Figure S8



# Figure S9



▶ Hypothetical proteins    
 ★ Only *Sulfurimonas* MAG sequences in NR database (no *Sulfurimonas* species or other taxa sequences)

▶ Known proteins

Nb	Protein name
1	chaperonin GroL
2	co-chaperonin GroES
3	LexA family transcriptional repressor
5	DNA adenine methylase
8	lytic transglycosylase domain-containing protein
15	phage tail tape measure protein
22	phage protease

Nb	Protein name
24	major capsid protein
25	DUF1320 family protein
26	DUF1804 family protein
27	phage terminase large subunit
28	DUF935 family protein
29	phage minor head protein
30	phage virion morphogenesis protein

Nb	Protein name
34	regulatory protein GemA / DUF1018
35	helicase DNaB
37	DUF2528 family protein
42	DNA transposition protein
43	DDE-type integrase/transposase/recombinase
47	phage antirepressor N-terminal domain-containing protei