Supplementary Information for

Global evolutionary pattern and distinct metabolic strategies of sulfur-cycling microorganisms across ecosystems

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Supplementary Text

The results presented below and in the figures are based on analyses of GTDB r95 genomes. However, taxon names mentioned in the text follow the GTDB r226 nomenclature. Where names differ between the two releases, the corresponding GTDB r95 names are provided in brackets (Data S4).

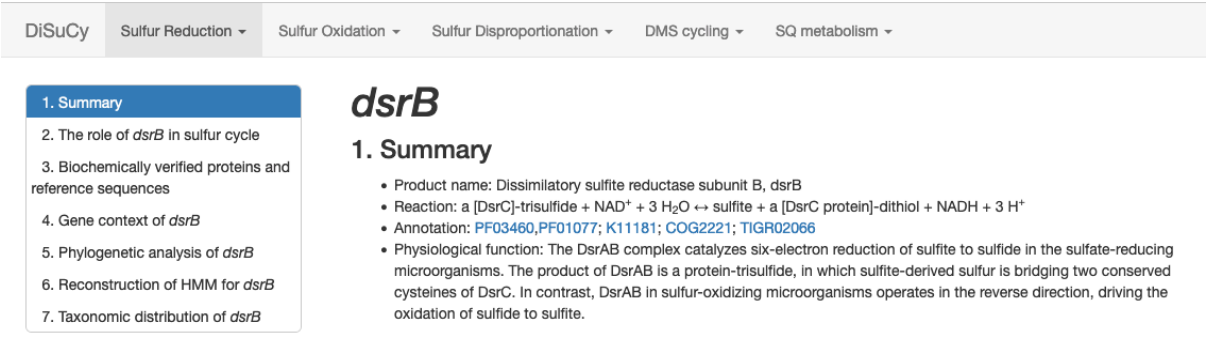
Reconstruction of DiSuCy knowledgebase to discern sulfur-cycling genes from (meta)genomic data

DiSuCy is a manually curated knowledgebase of sulfur-cycling genes (n = 116), comprehensively capturing the major pathways involved in dissimilatory metabolism of inorganic and selected organic sulfur compounds (e.g., sulfoquinovose and dimethylsulfide). For each gene, DiSuCy integrates (1) experimentally validated information on the gene function, (2) a manually curated phylogenetic framework, (3) publicly available HMM sets, and (4) homologs rigorously inferred from GTDB genome database. The multidimensional data is intuitively structured in seven sections on the Github website (https://songcanchen11.github.io/DissimilatoryScycle/; **password: songcanchen2026**). Below, we use *dsrB* (encoding the beta subunit of the dissimilatory sulfite reductase) to exemplify the information DiSuCy provides for each gene, and demonstrate how it can be utilized to analyze (meta)genomic data (https://songcanchen11.github.io/DissimilatoryScycle/IvuzE.html; **password: songcanchen2026**). Snapshots of each section from the webpage were embedded in the text.

Section 1: Summary

The summary section describes (1) the full name of the gene product, (2) the metabolic reaction catalyzed by the DsrAB complex, (3) annotation of the gene in the publicly available databases, such as PFAM, KEGG, COG, and TIGRFAM [1–4], and (4) literature-based summary on the physiological function of the target gene. For DsrB, the functional summary subsection highlights the complexity of its functional role. DsrB orthologs are known to catalyze directionally opposed transformations.

Specially, DsrB from sulfate-reducing microorganisms is involved in the dissimilatory reduction of sulfite to sulfide, yielding DsrC-trisulfide as the end-product, whereas orthologs in sulfur-oxidizing microorganisms drive the reversed reaction [5]. This enzymatic reversibility demonstrates the inherent difficulty to infer the precise functional roles of the uncharacterized DsrB homologs from (meta)genomic data [6]. Although placing the query sequence to the phylogenetic framework (see section 5) partially resolves the directional ambiguity, we advocate more conservative interpretation of the metabolic function and physiological roles of the predicted DsrB homologs and their host microbes.



Section 2: The role of the target gene in the sulfur cycle

DiSuCy provides two distinct visualizations to contextualize the functional role of the target sulfur-cycling gene. First, it places the gene within a broad, microbially mediated sulfur transformation network, encompassing the reduction and oxidation of various sulfur compounds. Second, DiSuCy offers a comprehensive plot detailing the major enzymatic pathways involving the target gene. Using *dsrB* as an example, the first plot enables researchers to interpret the overarching biological function of *dsrB* within a metabolic context of varied redox transformations involving inorganic sulfur species. The second plot shows the enzymatic pathway for dissimilatory sulfate reduction (Dsr) and the reversed Dsr (rDsr) for chemolithoautotrophic sulfur oxidation, where *dsrB* acts as a marker. Because both pathways are complex and require the coordinated action of over 10 Dsr-related proteins, this detailed visualization facilitates the functional interpretation of the DsrB-catalyzed reaction in the context of the full Dsr or rDsr pathway.

2. The role of *dsrB* in sulfur cycle

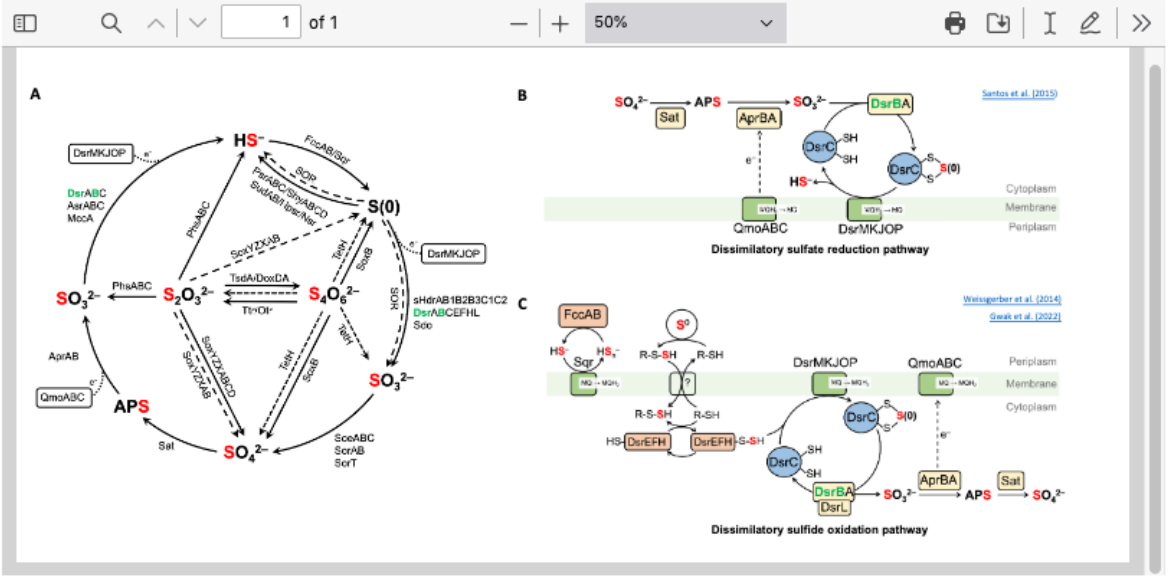


Figure 1. The role of *dsrB* (highlighted with green) in sulfur cycle

Section 3: Biochemically verified proteins and reference sequences

The section gathers experimentally verified proteins and selected reference sequences from model sulfur microorganisms belonging to a particular sulfur-cycling gene (e.g., *dsrB*). Each sequence entry is cross-referenced to the key literature providing genetic, biochemical and/or physiological evidence supporting its metabolic function. All sequences can be downloaded from DiSuCy in FASTA format, and are also directly linked to Uniprot or NCBI based on their accession.

3. Biochemically verified proteins and reference sequences

Organisms	Accession	Gene_name	Literature
Desulfovibrio vulgaris str. Hildenborough	P45575.3	*dsrB*	Santos, A.A.; Karkhoff-Schweizer,R.R.; Lee,J.P.; Wolfe,B.M.; Oliveira,T.F.;
Archaeoglobus fulgidus DSM 4304	Q59110.1	*dsrB*	Dahl,C.; Schiffer,A.; Parey,K.;
Allochromatium vinosum	O33999	*dsrB*	Dahl,C.; Hipp,W.M.;

Download FASTA

Section 4: Genomic context of reference sequences

The section visualizes the genomic context for each of the reference sequences. The functionally associated sulfur-cycling genes are often arranged as gene clusters within microbial genomes, and information on gene co-localization can thus be leveraged to assign sulfur-related function to uncharacterized homologs. By providing the genomic context of these reference sequences, DiSuCy helps to guide and refine assignment of gene function when the genomic context of the uncharacterized homologs is available. For example, the reference *dsrB* sequences typically co-localizes with other *dsr* genes, forming conserved gene clusters such as *dsrAB(DN)* in sulfite/sulfate-reducers and *dsrABEFHMKLJOP* in sulfur-oxidizers [7]. When an uncharacterized *dsrB* homolog shares similar genomic context with experimentally verified relatives, it strongly supports the functional assignment of that homolog as *dsrB*.

4. Gene context of *dsrB*

Section 5: Phylogenetic framework

The section provides a reference phylogeny to discern sulfur-cycling genes from their functionally divergent homologs. Within each gene phylogeny, the monophyletic clade(s) spanned by functionally verified reference sequences and their functional orthologs are pinpointed, based on their (1) gene neighboring patterns and (2) phylogenetic position relative to functionally divergent homologs [8]. The utility of the phylogenetic framework is exemplified for DsrB. In the DsrB phylogeny, clades are annotated to differentiate true DsrB homologs from functional divergent homologs (outgroups), including the alpha subunit of dissimilatory sulfite reductase (DsrA), sulfite reductase subunit C (AsrC) and coenzyme F_{420}-dependent sulfite reductase

(Fsr). Moreover, the DsrB clade is further divided into reductive type, oxidative type, and archaeal type, which primarily encompass sequences from sulfite/sulfate-reducing microorganisms, sulfur-oxidizing bacteria, and sulfur-metabolizing archaea, respectively [9]. Placing the uncharacterized homologs to this reference phylogeny distinguish DsrB from outgroups in a more accurate manner than e.g. the BLAST-based methods, and help to predict the functional directionality (oxidative vs. reductive). For instance, homologs falling within the oxidative-type clade likely catalyze the sulfur oxidation reaction. However, functional assignments for uncharacterized DsrB homologs must be approached with caution, because: (1) reductive-type DsrB clade contains some members that operate the enzyme in reverse direction (e.g., *Desulfurivibrio alkaliphilus*) [6], and (2) DsrB homologs from uncultured microorganisms frequently branch between the reductive-type and oxidative-type clades, complicating their function inference [6,10]. The DsrB example highlights the need for sequence analyses beyond simple homology searches when evaluating uncharacterized sulfur-cycling genes from (meta)genomes, alongside a conservative interpretation on their functional roles. By integrating phylogenetic framework and experimentally validated information, DiSuCy facilitates biological interpretation on the function of uncharacterized homologs. Sequences used for tree reconstruction, the corresponding Newick-formatted tree, and detailed sequence metadata are available for download from DiSuCy.

5. Phylogenetic analysis of *dsrB*

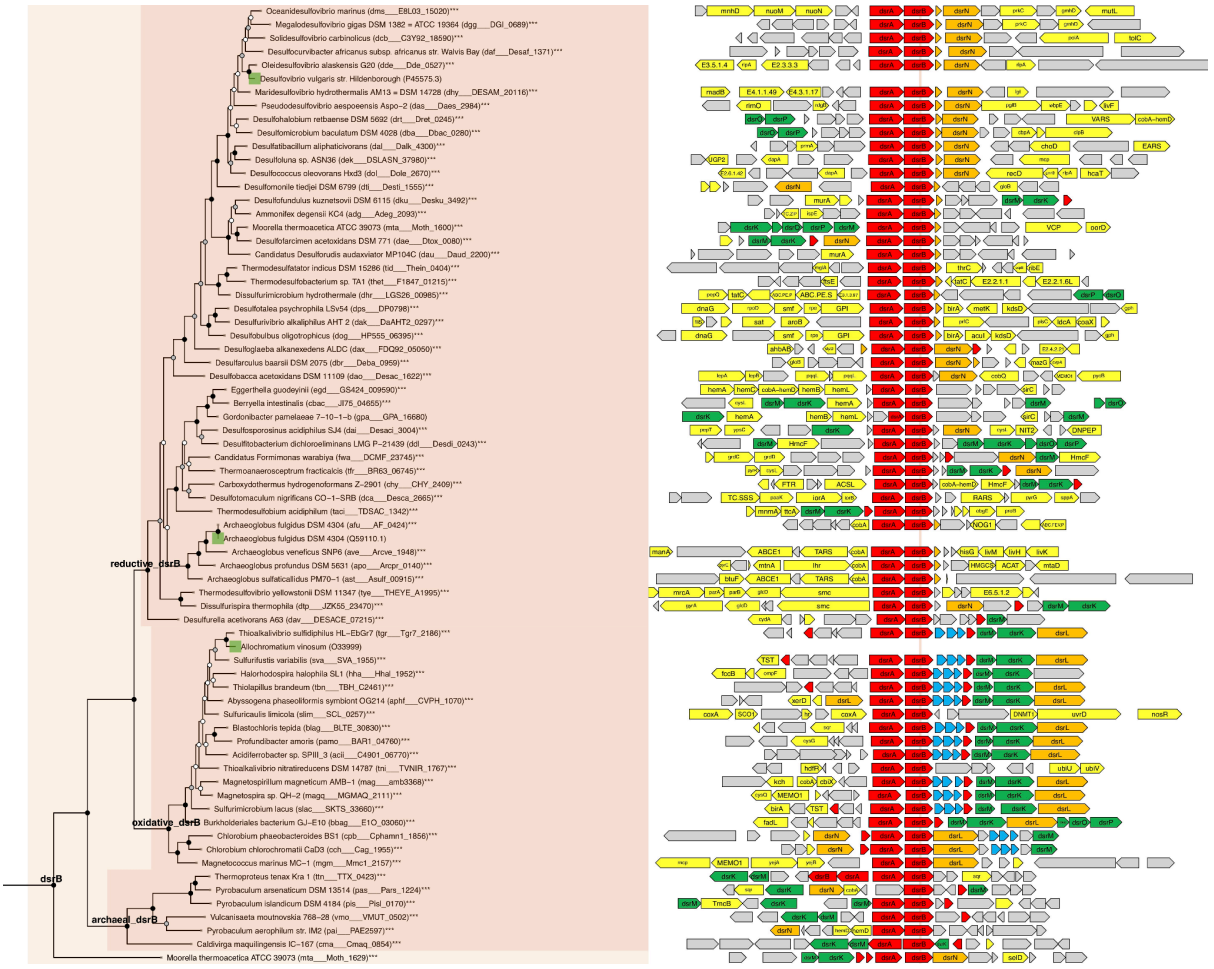


Figure 2. Phylogenetic tree of DsrB homologs. DsrB is homologous to the alpha subunit of dissimilatory sulfite reductase (DsrA), sulfite reductase subunit C (AsrC) and coenzyme F420-dependent sulfite reductase (Fsr). The experimentally characterized DsrB from model sulfate-reducing microorganisms (i.e., *Desulfovibrio vulgaris* and *Archaeoglobus fulgidus*) and sulfur-oxidizing bacteria (i.e., *Allochromatium vinosum*), as well as their homologs encoded in a *dsr* gene cluster from cultured microorganisms form a well-supported monophyletic clade, clearly separating from the outgroups (i.e., DsrA and AsrC). Within this DsrB-clade, there are four main branches that delineate three major protein families, namely the reductive bacterial type, the oxidative bacterial type and the reductive archaeal type (Loy, A.). The fourth branch is represented by the second DsrA copy of *Moorella thermoacetica* (Loy, A.). Notably, recent metagenome-based survey has greatly expanded the phylogenetic diversity of DsrB. Homologs from uncultured microbial groups (i.e., *Verrucomicrobia* and two candidate phyla, *Candidatus* Rokubacteria and *Candidatus* Hydrothermarchaeota) form completely novel lineages outside the four known DsrB clusters (Anantharaman, K). The DsrB phylogeny is rooted using the minimal ancestor deviation method for visualization. Branches with bootstrap value over 70%, between 50% and 70%, and below 50% are indicated by black, grey, and white circles, respectively. The NCBI or KEGG accession number of each sequence is shown in parentheses. Organisms encoding DsrA (a functional marker protein for dissimilatory sulfur metabolism) are marked with ***. The experimentally verified DsrB are highlighted by green squares in the tree; known outgroups with different, verified functions (i.e., AsrC; P0A1Y3) are denoted by red squares. The gene context for each homolog is placed next to the tree, with the coloring scheme shown in the figure legend.

Tree File | FASTA File | Metadata File

Section 6: Gene-specific hidden markov models (HMM)

This section provides the HMM allowing large-scale homology search for sulfur-cycling genes in (meta)genomic datasets. For each sulfur-cycling gene, HMM(s) were reconstructed to specifically target the members within monophyletic

clade(s) of functional orthologs [8]. Two types of cutoff are provided for homology search: the more stringent threshold aims at retrieving high-fidelity homologs of conserved functions, and a relaxed threshold intended primarily to filter out the functionally divergent homologs. Using *dsrB* as an example, multiple HMMs were reconstructed to target the entire DsrB clade, and the oxidative-, the reductive-, and the archaeal-type subclades. Both the stringent and relaxed thresholds have been directly incorporated into the downloadable HMM(s) files as gathering cutoff (GA) and noise cutoff (NC) parameters, respectively. An example *hmmsearch* command used to retrieve homologs of DsrB from a sequence collection using GA threshold is also provided.

6. Reconstruction of HMM for *dsrB*

6.1 HMM reconstructed for the **archaeal_dsrB** clade

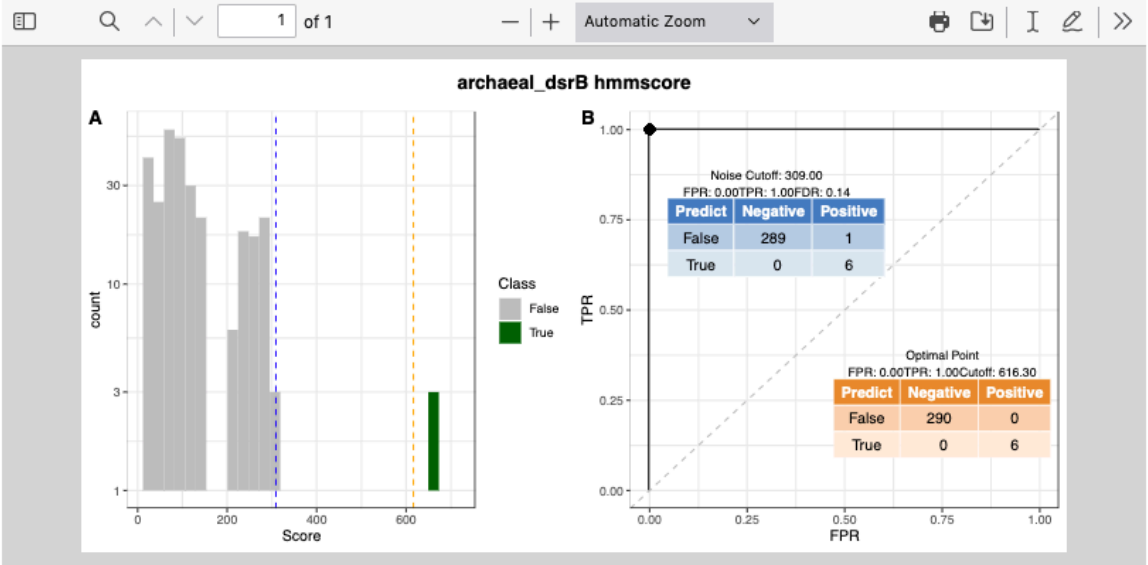


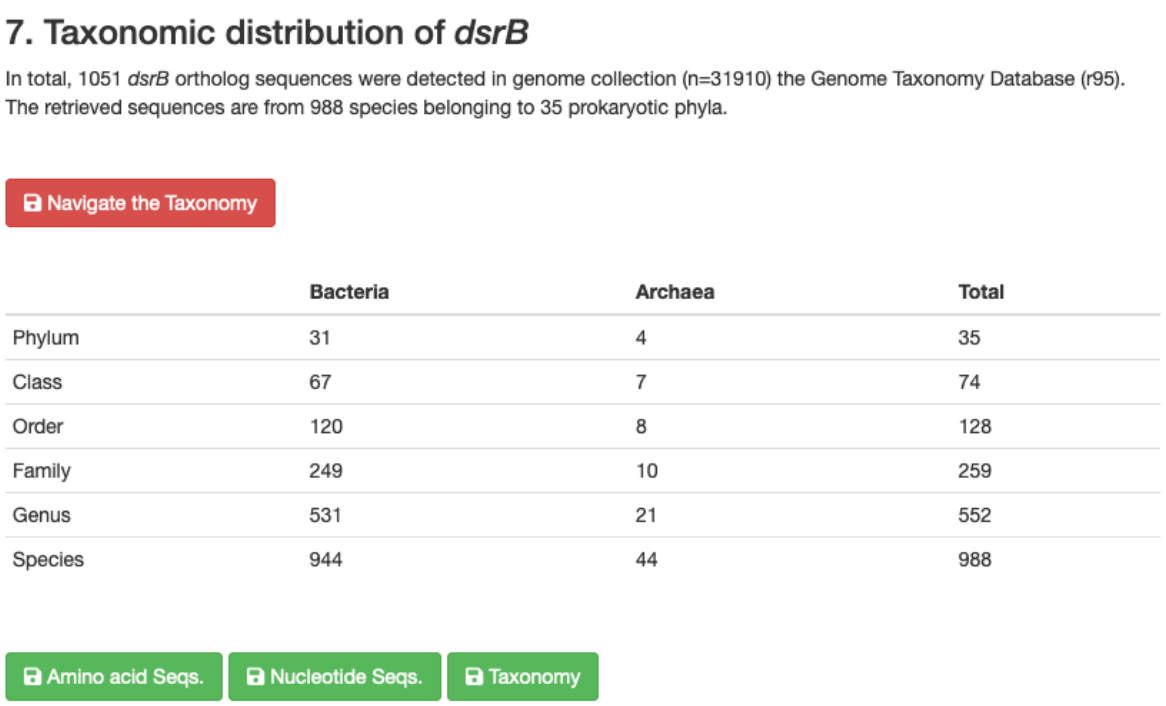
Figure 3-1. Distribution of hmmsearch score (A) and receiver operating characteristic (ROC) curve of the **archaeal_dsrB HMM model** (B). A. The HMM model was searched against a set of dsrB homologs curated by phylogenetic and gene context analyses (Figure 3). Hmmsearch score of each homolog was recorded for distribution analyses. In the histogram, true *dsrB* are indicated by green bars and paralogs (false) of *dsrB* are marked as grey. B. ROC method was used to determine the cut-off the HMM model. The optimal cut-off takes a trade-off between true positive rate (TPR) and false postive rate (FPR) of the model. The noise cut-off takes the highest hmmsearch score among paralogs below the optimal cut-off.

Download HMM model

Section 7: Taxonomic distribution

This section provides a compilation of the homologs for a specified sulfur-cycling gene retrieved from the GTDB database (n=31910 genomes; release 95). Nucleotide

and protein sequences for these homologs, and their GTDB taxonomic classification are available for download from DiSuCy. Additionally, the distribution of the retrieved homologs across different taxonomic ranks is visualized via an interactive Krona plot. Using *dsrB* as a representative example, a total of 1051 *dsrB* homologous sequences were detected in GTDB release 95 using the DsrB-specific HMMs. These sequences originate from 988 species of 35 prokaryotic phyla.



7. Taxonomic distribution of *dsrB*

In total, 1051 *dsrB* ortholog sequences were detected in genome collection (n=31910) the Genome Taxonomy Database (r95). The retrieved sequences are from 988 species belonging to 35 prokaryotic phyla.

Navigate the Taxonomy

	Bacteria	Archaea	Total
Phylum	31	4	35
Class	67	7	74
Order	120	8	128
Family	249	10	259
Genus	531	21	552
Species	944	44	988

Amino acid Seqs. Nucleotide Seqs. Taxonomy

Sulfur metabolism potential of less-well-studied bacterial phyla

Phylum *Methylomirabilota*

Metagenome-assembled genomes (MAGs) of all representative species classified as *Methylomirabilota* were retrieved from GTDB r95. Out of 41 species-level representatives, nine belong to the order *Methylomirabilales*, including

Methylomirabilis lanthanidiphila (*M. oxyfera*_A), *M. oxygeniifera* (*M. oxyfera*_B), *M. tolerans* (*M.* sp002634395), and *Methylomirabilis limnetica* (Fig. S2, Data S4). These species are known for their capacity to couple anaerobic methane oxidation with nitrite reduction in anoxic environments (i.e., groundwater, freshwater sediments, and wastewater treatment sludge) [11–14]. The rest are affiliated with the order *Rokubacteriales*, which does not contain any cultured representatives. *Rokubacteriales* members are primarily found in terrestrial ecosystems (i.e., soil, freshwater sediment, and wetland) (Fig. S2). *Rokubacteriales* and *Methylomirabilales* appear to have distinct sulfur metabolisms. Sulfur-cycling genes are relatively rare in *Methylomirabilales* genomes. Exceptions are genes for the oxygen-dependent methanethiol oxidase (*mtoX*) and sulfur dioxygenase (*sdo*) that may be involved in detoxification of methanethiol and sulfide, or in energy metabolism. The oxygen for MtoX and Sdo may derive from a unique denitrification pathway that produces N_2 and O_2 intracellularly from nitric oxide (NO) [13]. In contrast, members of the order *Rokubacteriales* encode a diverse suite of genes involved in dissimilatory oxidation of sulfur compounds. Sulfide dehydrogenase flavocytochrome c (*fccBA*), *sdo*, the thiosulfate-oxidizing system (*soxXYZABCD*), and sulfite dehydrogenase (*sorAB*) genes are found in 12 out of 32 *Rokubacteriales* genomes. The encoded enzymes constitute a complete pathway for oxidizing sulfide to sulfate, which is similar to the characterized pathway in the heterotrophic bacterium *Cupriavidus pinatubonesis* [15]. In this pathway, sulfide is initially oxidized by FccBA into polysulfide, which is further oxidized to sulfite by Sdo. The spontaneous reaction between sulfite and polysulfide produces thiosulfate, which is further oxidized into sulfate by the Sox system. Similar to previous findings, two *Rokubacteriales* members from the candidate genera CSP1-6 and UBA12499 encode a Dsr-dependent pathway putatively involved in sulfur oxidation [10]. In addition, selected *Rokubacteriales* genomes also contain genes for thiosulfate dehydrogenase (*tsdA*) and dimethylsulfide (DMS) monooxygenase (*dmoA*), suggesting the ability to derive energy from the oxidation of thiosulfate and DMS. Complementary genomic analyses predicted *Rokubacteriales* as obligate heterotrophs growing by oxygen respiration. We detected genes for glycolysis, the tricarboxylic acid cycle (TCA) cycle, beta oxidation, and electron transport complexes required for aerobic respiration in nearly all *Rokubacteriales* genomes. Yet, no genome encoded the complete genetic setup for an autotrophic metabolism, i.e.,

Wood-Ljungdahl pathway, Calvin-Benson-Bassham cycle, reductive tricarboxylic acid cycle, 3-hydroxypropionate bicycle, 3-hydroxypropionate-4-hydroxybutyrate cycle, and dicarboxylate-4-hydroxybutyrate cycle. The presence of genes for diverse sulfur-oxidizing enzymes and the potential to utilize organic compounds suggest the capability for conserving energy by heterotrophic sulfur oxidation. Another predicted physiology in a few members of *Rokubacteriales* is their ability to oxidize both sulfur and alkanes. The genome of candidate species 20CM−2−70−11 sp003220675 contains genes for the Sox system and particulate methane monooxygenase (Pmo). Phylogenetic analysis of the catalytic subunit of Pmo revealed that the Pmo from 20CM−2−70−11 sp003220675 is closely related to hydrocarbon monooxygenases encoded in alkane-oxidizing *Mycolicibacterium chubuense* and *Nocardioides* sp. CF8, which forms a monophyletic clade distinct from Pmo in *Methylomirabilales* (Fig. S3). The presence of thiosulfate oxidizing system (Sox) and Pmo putatively involved in alkane degradation suggest a combination of thiotrophy and alkanotrophy in a single microbe, similar to the sulfur-oxidizing methanotroph *Methylovirgula thiovorans* [16].

Phylum *Vulcanimicrobiota* (*Eremiobacterota*)

The phylum *Eremiobacterota* was recently renamed to *Vulcanimicrobiota* (Data S4) [17] and is represented by 34 species-level MAGs in GTDB r95, with few cultured representatives . 28 of *Vulcanimicrobiota* species belong to the order *Vulcanimicrobiales* (UBP12), whereas the remaining species are classified as *Xenobiales* (UBA4705) and *Eremiobacterales* (Fig. S4; Data S4). *Vulcanimicrobiales* and *Eremiobacterales* are primarily found in soil ecosystems (i.e., soil, wetland, bog, permafrost, and moss), whereas members of *Xenobiales* are associated with industrial (i.e., bioreactor and activated sludge) or gut environments (i.e., human and pig gut). Genomes of *Vulcanimicrobiota*, in particular *Vulcanimicrobiales*, have a range of genetic systems indicative of sulfur oxidation, including genes for thiosulfate dehydrogenase (*tsdA*), sulfur dioxygenase (*sdo*), and sulfite dehydrogenase (*sorA*). Phylogenetic analysis revealed the inferred protein sequences clustered with experimentally validated members of each protein family (Fig. S5a-c), supporting their functional roles in sulfur oxidation. Three *Vulcanimicrobiota* genomes have the gene cluster for the thiosulfate-oxidizing Sox system (*soxXYZABCD*) for oxidation of

thiosulfate to sulfate. Some genomes carry genes for a sulfide dehydrogenase flavocytochrome c (*fccBA*), indicating their potential to oxidize sulfide to elemental sulfur. Another predicted physiological feature of *Vulcanimicrobiota* is the ability to utilize C_1 organosulfur compounds. They have key genes involved in DMS metabolism, including methanesulfonate monooxygenase (*msm*) and $FMNH_2$-dependent dimethylsulfone monooxygenase (*sfn*). Genes encoding subunits of these enzymes and their auxiliary metabolic module were found within a putative *msm* and *sfn* operon (Fig. S5d-e), supporting a functional role in oxidizing methanesulfonate and dimethylsulfone. Consistent with a previous genomic study, most *Vulcanimicrobiota* within *Vulcanimicrobiales* are predicted to be aerobic heterotrophs capable of utilizing sugars and/or fatty acids [18–20]. Genes for aerobic respiration (*coxABCD* and *cydAB*), beta oxidation, glycolysis, and/or the TCA cycle were present in 28 of 34 *Vulcanimicrobiota* genomes. The potential to use organic substances and the presence of various sulfur-oxidizing genes suggest these microorganisms use sulfur compounds as supplementary energy sources during heterotrophic growth. This sulfur-dependent chemolithoheterotrophic lifestyle has been described in *Arcobacter peruensis* and *Thermus scotoductus*, whose growth on organic carbon can be promoted by the presence of sulfur compounds [21,22]. In addition, a few *Vulcanimicrobiota* also encode ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) and Calvin cycle enzymes, indicative of the capability to fix carbon. In this scenario, the reduced sulfur compounds could serve as the sole energy source during autotrophic growth.

Phylum *Zhuqueibacterota* (KSB1)

The candidate phylum KSB1 was recently renamed to *Zhuqueibacterota* [23] and was represented by thirteen genomes in GTDB r95, belonging to four orders AABM5-25-91, CR04bin15, JdFR-76, and UBA2214 (Fig. S6). The candidate orders AABM5-25-91 and UBA2214 have been reclassified as *Thermofontimicrobiales* and *Residuimicrobiales*, respectively (Data S4). The former candidate order CR04bin15 is now split into the orders *Zhuqueibacterales* (e.g., QEVD01 sp003576975; Data S4) and *Oceanimicrobiales* (e.g., N075bin58 sp004356825 and CR04bin15 sp004357015; Data S4). The order JdFR-76 remains unchanged, representing a single order within the candidate class JdFR-76. The species-level genomes

included in our analysis were recovered from diverse environments. *Residuimicrobiales* (UBA2214) appears to be associated with terrestrial and freshwater ecosystems (i.e., soil, wetland, freshwater sediments), while the remaining orders are from activated sludge (bioreactors), marine environments, hot springs or hydrothermal vents (Fig. S6). *Thermofontimicrobiales* (AABM5-25-91) and *Residuimicrobiales* (UBA2214) genomes contain the gene for an octaheme tetrathionate reductase (*otr*). The inferred Otr-proteins harbour eight heme-binding motifs, and are closely related to the Otr from *Shewanella* species that are capable of respiring tetrathionate (Fig. S7a) [24]. Genes encoding the catalytic subunit of the polysulfide/thiosulfate/selenite reductase family (*psrA/phsA/srrA*) were detected in three genomes, within a putative *psr* operon containing *psrC* (Fig. S7b), which encodes a functional component supposed to be associated specifically with polysulfide reductase Psr [25]. Such gene arrangement pattern suggests these *psrA/phsA/srrA* homologs encode a polysulfide reductase. The presence of *otr* and/or *psrA* would enable the use of tetrathionate and elemental sulfur as electron acceptor, respectively. Additionally, four genomes have a gene for sulfide:quinone oxidoreductase (*sqr*). The inferred Sqr sequences form a monophyletic group with type III Sqr from the green sulfur bacterium *Chloroherpeton thalassium* (Fig. S7c). Due to the lack of a photosynthetic system, the Sqr may be involved in sulfide detoxification rather than bioenergetics. Gene content analyses suggested a heterotrophic lifestyle for the *Zhuqueibacterota* species included in our analysis. Many genomes encode diverse extracellular glycoside hydrolases (GHs), suggesting the ability to degrade complex carbohydrates such as cellulose (via GH5, GH8, GH9, and GH74), xylan (via GH10, GH30 and GH131), and chitin (via GH18, GH19, and GH23). The monosaccharide hydrolysis products of GHs, could be further metabolized via the Embden-Meyerhof-Parnas pathway and the TCA cycle, yielding reducing equivalents (i.e., NADH) for dissimilatory reduction of sulfur. Alternatively, H_2 may also serve as electron donors for sulfur reduction. In support of this, genes for H_2-uptake hydrogenase, including [FeFe] hydrogenase group A, and/or [NiFe] hydrogenase group 1, 3b and 3c, were detected in nine of the thirteen genomes. The hydrogen-evolving group 4 hydrogenase found in some genomes (e.g. RPQV01 sp003818595) may be involved in the fermentation of organic compounds. Another predicted feature of *Zhuqueibacterota* members is the ability to use O_2, nitrate or iron(III) oxides as electron acceptor, in addition to sulfur compounds. Seven

genomes have genes for aa_3 cytochrome c oxidase (*coxABCD*) and/or cytochrome bd oxidase (*cydAB*), which may support growth by oxygen respiration under high-oxygen and low-oxygen environments, respectively. Genes involved in dissimilatory reduction of nitrate to ammonia and in flavin-based extracellular electron transfer pathway were detected in two and six genomes, respectively, suggesting the ability to respire nitrate and iron(III) oxides.

Phylum *Chloroflexota*

Chloroflexota is among a few phyla beyond the well-characterized *Pseudomonadota* (*Proteobacteria*) and *Euryarcheota* [26,27] that have genes for dimethylsulfide/dimethylsulfoxide (DMS(O)) metabolism. GTDB r95 contained 520 species-level genomes/MAGs of *Chloroflexota*. Here, we focused our analyses on twelve *Chloroflexota* MAGs showing potential to produce DMS(O) and/or to utilize various C_1 intermediates derived from DMS. These MAGs derive from six different orders, including UBA3495, *Lucifugimonadales* (UBA1151), *Aggregatilineales* (SBR1031), CAJWPE01 (SAR202), *Promineofiales*, and P2-11E (Fig. S8). Most members are associated with marine ecosystems (e.g., UBA3495), whereas those from *Promineofiales* and P2-11E are primarily found in terrestrial and freshwater ecosystems (e.g., soil, freshwater sediments, and/or permafrost). Among all analyzed *Chloroflexota* species, UBA9611 sp002721995, *Lucifugimonas* sp009392785 (GCA-002725925 sp002714085), and *Leptofilum* sp002699125 (GCA-2699125 sp002699125) encode a homolog of the dimethylsulfoniopropionate/dimethylsulfoxonium propionate (DMS(O)P) lyase DddP. Phylogenetic analysis revealed that these homologs formed a well-supported clade with the functionally ratified DddPs from *Roseobacter* species and their close relatives (Fig. 2d), and separated clearly from function divergent homologs, such as Xaa-Pro aminopeptidases that cleavage any amino acid N-terminally adjacent to a proline residue [28]. Consistently, sequence alignment combined with structure modelling indicated that DddP homologs from UBA9611 sp002721995 and *Lucifugimonas* sp009392785 (GCA-002725925 sp002714085) harbour a catalytic cavity similar to DddP from *Ruegeria lacuscaerulensis* [29]. The key residue (Asp377) of DddP, which initiates nucleophilic attack on the hydrogen of the DMSP carboxyl C_α atom, was found in the predicted DddP structure from both *Chloroflexota* species.

This suggests the *Chloroflexota* DddPs have the catalytic capability to cleave the C-S bond in DMS(O)P and generate DMS(O) as an end product. In addition, three other *Chloroflexota* were predicted to degrade methanesulfonate or respire DMSO, based on presence of genes for methanesulfonate monooxygenase (Msm) or DMSO reductase (Dms), respectively. Phylogeny of the catalytic subunit (MsmA and DmsA) showed that the retrieved Msm and Dms clustered with the functionally validated homologs, and shared the same gene synteny (Fig. S9a-b). This prediction is in line with the detection of *msmA* and *dmsA* in *Chloroflexota* from the deep ocean [30]. Moreover, multiple homologs of the catalytic subunit of DMS monooxygenase (DmoA) were detected among *Chloroflexota* members. However, phylogenetic analysis placed them within the clade of alkane monooxygenase (LadA) or monooxygenases involved in oxidation of other substrates, instead of DmoA (Fig. S9c). This clustering pattern suggests they oxidize other substrates (e.g., alkanes). Many of the *Chloroflexota* species with predicted DMS(O) metabolism also encode genes for aerobic respiration (*coxABCD*), suggesting an aerobic lifestyle similar to the cultivated bacteria involved in DMS(O) metabolism/production (i.e., *Ruegeria lacuscaerulensis*) [26].

Expanded diversity of putative sulfur-oxidizing microorganisms

Nitrate-dependent sulfur oxidizers

Nitrate-dependent, chemolithoautotrophic sulfur-oxidizers simultaneously mediate dark carbon fixation, nitrate reduction, and sulfur oxidation, and thereby play critical roles in global element cycles [31,32]. To describe the distribution of these ecologically relevant microorganisms across bacteria and archaea, we screened GTDB r95 genomes for co-occurrence of pathways involved in autotrophic carbon assimilation (i.e., six known carbon fixation pathways), nitration respiration (NapAB/NarGH), and dissimilatory sulfur oxidation (Sqr, Fcc, Sox, and oxidative Dsr). Consistent with previous literature, we detected genomic potential for nitrate-dependent sulfur oxidation in members of the phyla *Campylobacterota*, *Desulfobacterota*, and *Pseudomonadatoda* (*Proteobacteriota*), for which this physiological capability has already been confirmed. Representatives include *Sulfurimonas* [33,34] and *Sulfurovum*

[35,36] from *Campylobacterota*; *Desulfurivibrio alkaliphilus* [6] and *Electronema* (cable bacteria) [37,38] from *Desulfobacterota*; *Paracoccus pantotrophus* [39] from *Alphaproteobacteria*; and diverse clades of *Gammaproteobacteria* (e.g., *Thioglobus* formerly known as SUP-05 clade [32,40–42], *Thiobacillus* [43], *Thiomargarita* [44–46], *Thioalkalivibrio* [47], *Sedimenticola* [48], *Thioalbus* [49], *Thiohalomonas* [50], *Sulfuricella* [51], and *Thiohalorhabdus* [52].

Although extensively studied, we found genomic evidence of the nitrate-dependent sulfur oxidation pathway also in other taxa (Fig. S10). These include a member from the phylum *Myxococcota* (SURF-8) and a member from the *Alphaproteobacteria* family UBA2156. Both MAGs have a *dsrAB* falling within a clade of oxidative-type DsrAB from green-sulfur bacteria and sulfur-oxidizing *Magnetospiraceae*, suggesting a role in dissimilatory sulfide oxidation. Additionally, the same MAGs also contain *narGH*/*napAB* for nitrate respiration and genes for the Wood-Ljungdahl pathway potentially involved in carbon fixation. Such genetic makeup would allow for nitrate-dependent sulfide oxidation via a similar pathway that has been proposed for *D. alkaliphilus* and *Electronema* [6,53].

Other examples are (i) gammaproteobacterial members of the orders SZUA-229 and *Arenicellales* (UBA10353), and the families *Usitatibacteraceae* (UKL13-2) and Ga0077523 from the order *Burkholderiales*, and (ii) alphaproteobacterial members of the families UBA2165 and WMHbin7 from the order *Rhodospirillales*, and the families *Methyloligellaceae* and *Ancalomicrobiaceae* from the order *Rhizobiales*. Unlike the members from the phyla *Myxococcota* (SURF-8) and *Desulfobacterota*, these MAGs appear to use the oxidative type DsrAB and/or Sox system for dissimilatory sulfur oxidation (Fig. S11a-c), and the Calvin cycle for carbon fixation. A similar gene repertoire was detected in their functionally characterized relatives such as *Thioalkalivibrio nitratireducens* [47], *Thiomargarita nelsonii* [44–46], and *Thioglobus autotrohicus* [54], suggesting a shared mechanism underlying nitrate-dependent sulfur oxidation.

A full set of genes for nitrate-dependent sulfur oxidation was also detected in a MAG of the family *Sulfurospirillaceae* in the phylum *Campylobacterota*. The sulfur oxidation is probably achieved by the Sox system and the carbon fixation could be

performed by the reversed TCA cycle. Cultivated members of *Sulfurospirillaceae* are known for their capacity of sulfur reduction [55]. Sulfur oxidation coupled with nitrate reduction thus seems to be an uncommon feature of the family, and will require experimental validation.

Light-dependent sulfur oxidizers

Light-dependent sulfur oxidation is an energy-yielding process of specialized microorganisms, which capture photon energy to boost the reducing power of sulfur compounds up to the levels required for carbon fixation. Such photolithotrophic sulfur oxidation metabolism occurs under anoxic conditions, and has been characterized in diverse groups of anoxygenic phototrophic bacteria. These include green sulfur bacteria (e.g., *Chlorobi* [56]), green non-sulfur bacteria (e.g., *Chloroflexaceae* [57–59]), purple sulfur bacteria (e.g., *Chromatiaceae* and *Ectothiorhodospiraceae* [60]), and purple non-sulfur bacteria (e.g., *Rhodovulum sulfidophilum* [61]). Other anoxygenic phototrophic bacteria, including aerobic anoxygenic phototrophs [62] and *Heliobacteria* [63], are primarily photoheterotrophs that often lack the ability to grow photoautotrophically with sulfur, although photoheterotrophic oxidation of sulfur has been observed [64]. Here, we restricted our search to microorganisms with metabolic potential of sulfur-based photoautotrophy. Our screening criteria required the co-occurrence of genes for (1) type I *(pscABCD)* or type II *(pufML)* photosynthetic reaction center; (2) at least one of the six autotrophic metabolisms for carbon fixation; and (3) marker genes for dissimilatory sulfur oxidation (*sqr*, *fccB*, *dsrAB* or *soxB*). As expected, the procedure identified known photoautotrophic sulfur oxidizers, while also revealing this metabolic potential in taxa that were previously not known for this function (Fig. S12).

Multiple genera belonging to the orders *Burkholderiales* (genera Ga0077523, Ga0077526 and UBA4615), *Rhodobacterales* (the genus GCA-002705045), and *Rhizobiales* (the genus *Zhengella*) encode key enzymes required for sulfur-based photoautotrophy. These include large and small subunits of type II anoxygenic photosynthetic reaction center (PurLM), Calvin cycle for carbon fixation, and SoxB/FccB/Sqr indicative of dissimilatory sulfur oxidation (Fig. S13a-d). Moreover, most of these taxa also contain genes for aa_3-type cytochrome oxidase (*coxABCD*).

A combination of these functional systems would permit different modes of metabolism for growth, including sulfur-based photoautotrophy and chemoautotrophy. This predicted flexibility is similar to many metabolically versatile purple non-sulfur bacteria from the same orders, including the *Rhizobiales* bacteriums *Rhodopseudomonas palustris* [65]. Alternatively, the interaction of all detected functional systems may allow aerobic photoautotrophy on sulfur, resembling an unusual aerobic anoxygenic phototrophic bacterium, strain B3 from the class *Alphaproteobacteria*, which are known as photoheterotrophs, yet were recently demonstrated to be capable of fixing CO_2 via the Calvin cycle [66].

Chromatiaceae is one of two families of purple sulfur bacteria that are obligate or facultative light-dependent sulfur oxidizers. *Lamprobacter* sp003562815 (PWTO01 sp003562815) within family *Chromatiaceae* was found to harbour genetic potential for sulfur-based photoautotrophy, and probably represents a new species of purple sulfur bacteria. The presence of key genes for the sulfur oxidation system (Dsr, Sox, Sqr, and Fcc) implies its capacity of using sulfide or thiosulfate as electron donors during photoautotrophic growth (Figure S13a-d). Similar to closely related purple sulfur bacteria (i.e., *Allochromatium vinosum*), the genome also has genes for low-oxygen adapted cytochrome oxidase (*ccoPQNO*). This may allow the bacterium to switch from photolithotrophic to chemotrophic oxidation of sulfur under reduced oxygen tension, as demonstrated for *A. vinosum* [67].

Arsenate-dependent sulfur oxidizers

Sulfur-derived inorganic electron donors could fuel dissimilatory arsenate reduction, which may demobilize arsenic sorbed on iron minerals in organic-poor subsurface aquifers, affecting the health of millions of people worldwide [68]. Strain MLMS-1, affiliated to the genus *Desulfurivibrio*, is the only isolate that was proven of coupling chemoautotrophic sulfur oxidation with arsenate respiration [69,70]. Here, we screened for this metabolic potential among GTDB genomes based on the co-occurrence of genes for dissimilatory sulfur oxidation and arsenate reduction. In addition to *Desulfurivibrio* strain MLSM-1 and *Thiovibrio* members (UBA2262) [71], also members of the yet uncultured genera UBA5628 within the family *Desulfurivibrionaceae* and BM506 within the family SURF-16), harbour the genomic potential for

arsenate-dependent sulfur oxidation (Fig. S14). The genes detected in these genomes encode a dissimilatory sulfite reductase (DsrAB) and the catalytic subunit of arsenate respiratory reductase (ArrA), a marker for arsenate respiration [72]. Phylogenetic analysis revealed that the DsrAB sequences are closely related to sulfur-oxidizing bacteria *Desulfurivibrio alkaliphilus* and *Desulfurivibrio* MLMS-1 (Fig. S15a-b), suggesting the utilization of DsrAB in a reverse direction for dissimilatory sulfur oxidation [6,53]. Inferred ArrA sequences from the same genomes formed a well-supported monophyletic clade with function validated ArrAs from known arsenate respiring bacteria (Fig. S15c). This suggests that the uncultured taxa have a functional arsenate reduction pathway, which might also be used for antimony reduction, as proposed for a few uncultured *Desulfurivibrio* species [73].

In fact, arsenate- and antimonate-dependent thiotrophy seems more taxonomically widespread than currently appreciated. For example, members of the gammaproteobacterial families *Sedimenticolaceae*, SG8-39, *Rhodocyclaceae*, and *Burkholderiaceae* encode a diverse array of pathways for sulfur oxidation (*soxB*, oxidative *dsrAB*, and *fccBA*), and may conserve energy by respiring arsenate/antimonate using *arrA* (Fig. S14). Among these, the cultivated member known to use arsenate as electron acceptor or sulfur as electron donor, e.g., *Sulfuritalea hydrogenivorans* [74], represent candidate strains for physiological testing arsenate-dependent thiotrophy beyond *Desulfurivibrionaceae*.

Microbial sulfur-cycling across ocean waters

By examining the taxa responsible for sulfur-transforming reactions in marine ecosystems - primarily from seawater metagenome samples, we uncovered several previously unrecognized key players involved in DMS(O) cycling and sulfur oxidation (Fig. S18). More detailed analyses were performed focusing on these microbial processes, including MsmABCD-dependent methanesulfonate oxidation, DsyB-dependent dimethylsulfoniopropionate (DMSP) production, DsrAB-dependent sulfide oxidation, and TsdA-dependent thiosulfate oxidation.

The catalytic subunit of methanesulfonate monooxygenase is encoded by *msmA*, representing a key gene indicative of aerobic methanesulfonate degradation [75]. The ten most prevalent *msmA* unigenes are geographically widespread across different oceanic regions (Fig. S19a). Phylogenetic placement revealed close association of the *msmA* unigene sequences with three bacterial families; UBA868 [76], *Parvicellaceae* (UBA10066), and Casp-alpha2 (UBA3470) (Fig. S19b). Among these, *Parvicellaceae* (UBA10066) and Casp-alpha2 (UBA3470) appear to be previously unrecognized bacterial lineages participating in marine methanesulfonate metabolism. Genomic analysis indicated their *msmA* genes are present in a gene cluster containing *msmEFGH* for a methanesulfonate transporter, and *msmABCD* for a methanesulfonate monooxygenase (Fig. S19c), which together allow cellular uptake and oxidation of methanesulfonate into formaldehyde and sulfite [77]. Formaldehyde could be either assimilated via the serine pathway or oxidized into CO_2 for energy generation by coordinated actions of aldehyde and formate dehydrogenases (Fig. S19b). Sulfite may be further oxidized to sulfate via adenylylsulfate reductase (AprABM). Consistent with this oxygen-dependent degradation pathway, the *msmA*-carrying *Parvicellaceae* (UBA10066), and Casp-alpha2 (UBA3470) genomes encode aa_3-type cytochrome c oxidase (CoxABCD) for aerobic respiration. Casp-alpha2 (UBA3470) genomes further harbor a taurine monooxygenase (TauD) and a sulfolactate sulfolyase (SuyAB), which cleave the C-S bond from taurine and sulfolactate and release sulfite. Overall, our findings support the involvement of bacteria from the families *Parvicellaceae* (UBA10066), and Casp-alpha2 (UBA3470) in oxidation of various sulfonates in the ocean.

Reversely-operating dissimilatory sulfite reductase, encoded by phylogenetically distinct *dsrAB* genes, is commonly used as a functional marker for a dissimilatory sulfur oxidation [9,10,78]. The top ten most prevalent *dsrB* unigenes detected across global ocean regions (Fig. S20a) belong to the oxidative DsrB clade including known sulfur oxidizers such as *Allochromatium vinosum* and *Thioalkalivibrio denitrificans* (Fig. S20b). Close relatives of these *dsrB* sequences were found in species from four *Pseudomonadota* families; UBA868, GCA-002705445, GCA-002716945, and *Rhodobacteraceae* (genus MED-G52). While UBA868 was shown to be a ubiquitous mixotrophic bacterial group involved in sulfur oxidation and carbon fixation in the mesopelagic ocean [76], the other three taxa (GCA-002705445, GCA-002716945, and MEG-G52) represent previously uncharacterized lineages in the oceans that also have sulfur oxidation potential and are of global relevance. Metabolic reconstruction revealed *dsrB*-carrying GCA-002705445, GCA-002716945, and MEG-G52 possess alternative genetic systems for sulfur oxidation (*soxB*, *fccB*, *sdo*, *sqr*, or *soeA*; Fig. S20b), as well as multiple genes for organosulfur catabolism (*mtoX*, tmm, *tauD*, *xsc*, *hpsN*, *dddP*, or *dmdA*; Fig. S20b). These genes would allow the bacteria to (1) carry out oxidation of thiosulfate (*soxB*), element sulfur (*sdo*) or sulfite (*soeA*); (2) metabolize methanethiol (*mtoX*), taurine (*tauD*) or sulfoacetaldehyde (*xsc*); (3) generate DMS by demethylating DMSP (*dmdA*); and (4) transform DMS to dimethyl sulfoxide (*tmm*). The predicted sulfur oxidation and organosulfur metabolism pathways are likely coupled with oxygen respiration for energy conservation, based on co-occurrence with genes for the high-oxygen adopted terminal oxidase (CoxABCD). Moreover, genomes of GCA-002705445, GCA-002716945, and MEG-G52 lack genes for autotrophy, but contain genetic systems for organic carbon utilization (EMP pathway, TCA cycle, alcohol dehydrogenase, or serine cycle; Fig. S20b), suggesting a heterotrophic lifestyle. A few genomes also encode genes for a type II anoxygenic photosynthetic reaction center (pufML) and proteorhodopsin, suggesting a capability for light-dependent growth. Collectively, these findings uncovered three enigmatic, yet ubiquitous *dsrB*-carrying lineages potentially capable of a thiotrophic, heterotrophic or phototrophic lifestyle.

The gene (*dsyB*) for a key methyltransferase in the methionine transamination pathway is a functional marker for bacterial DMSP production [79]. The top ten most

prevalent *dsyB* unigenes in seawater metagenomes were affiliated to alphaproteobacterial lineages, including the genera *Salipiger* (*Rhodobacterales*), *Pseudooceanicola* (*Rhodobacterales*), *Albimonas (Rhodobacterales)*, *Stappia* (*Rhizobiales*), *Roseibium (Rhizobiales)*, and the family Casp-alpha2 (UBA1479) (*Rhodospirillales*) (Fig. S21). The family Casp-alpha2 (UBA1479) contains a genome (GCA_002433335.1) that is ubiquitous across ocean regions and has previously unrecognized DMSP production potential (Fig. S21a). To derive information about the functional capacities of the *dsyB*-encoding UBA1479 species, its genome was screened for key genes indicative of carbon, sulfur and energy metabolism (Fig. S21b). The results predicted UBA1479 as a heterotroph capable of utilizing organic carbon for DMSP biosynthesis. This is supported by the presence of genes involved in EMP pathway, TCA cycle, formation utilization, and formate-assimilating serine pathway in the UBA1479 genome. These genes would allow the bacterium to utilize sugars, intermediates of TCA cycle or formate as carbon source. Another predicted physiological feature of UBA1479 is its ability to gain energy from reduced sulfur compounds. The UBA1479 genome harbors *fccB* and *soxB* indicative of dissimilatory oxidation of sulfide and thiosulfate, *tauD* and *xsc* for releasing sulfite from taurine and sulfoacetaldehyde, and *soeA* for oxidizing sulfonate-derived sulfite to sulfate. The predicted DMSP biosynthesis and sulfur oxidation processes may occur under various oxygen levels, given the presence of genes encoding both high-oxygen- (CoxABCD) and low-oxygen-adapted (CcoNOQP) cytochrome c oxidase. Overall, *dsyB*-carrying UBA1479 may be a ubiquitous heterotrophic bacterial group in marine environments, capable of deriving energy from organic and inorganic sulfur compounds, alongside with its ability to produce DMSP.

The gene (*tsdA*) for a bidirectional thiosulfate dehydrogenase is a functional marker of either thiosulfate oxidation or tetrathionate reduction [80]. Among the top ten most prevalent *tsdA* unigenes among seawater metagenomes (Fig. S22a), one unigene (GMGC10.286_216_443.UNKNOWN) is affiliated to the genus *Diaphorobacter*_A (UBA2121) from *Burkholderiaceae*, and another unigene (GMGC10.078_611_114.CYTA) to the family UBA6960 from *Gemmatimonadota* (Fig. S22b). The genus *Diaphorobacter*_A (UBA2121) and the family UBA6960 harbor previously unrecognized sulfur microorganisms of global relevance in seawater. The *tsdA*-containing *Diaphorobacter*_A (UBA2121) genome encodes aa_3- and cbb_3-type

cytochrome c oxidase for aerobic respiration, a wide suite of genes for sulfur oxidation (*sorA*, *soxB*, *sqr* and *fccB*), as well as metabolic modules for organic carbon utilization (EMP pathway, TCA cycle, and beta oxidation) (Fig. S22b), suggesting an aerobic, thiotrophic, heterotrophic lifestyle. In contrast, all four *tsdA*-carrying UBA6960 genomes consistently lack genes for terminal oxidase, despite their high genome completeness. This suggests a general anaerobic lifestyle of UBA6960 bacteria, with TsdA probably acting as a tetrathionate reductase, similar to *Campylobacter jejuni* [80].

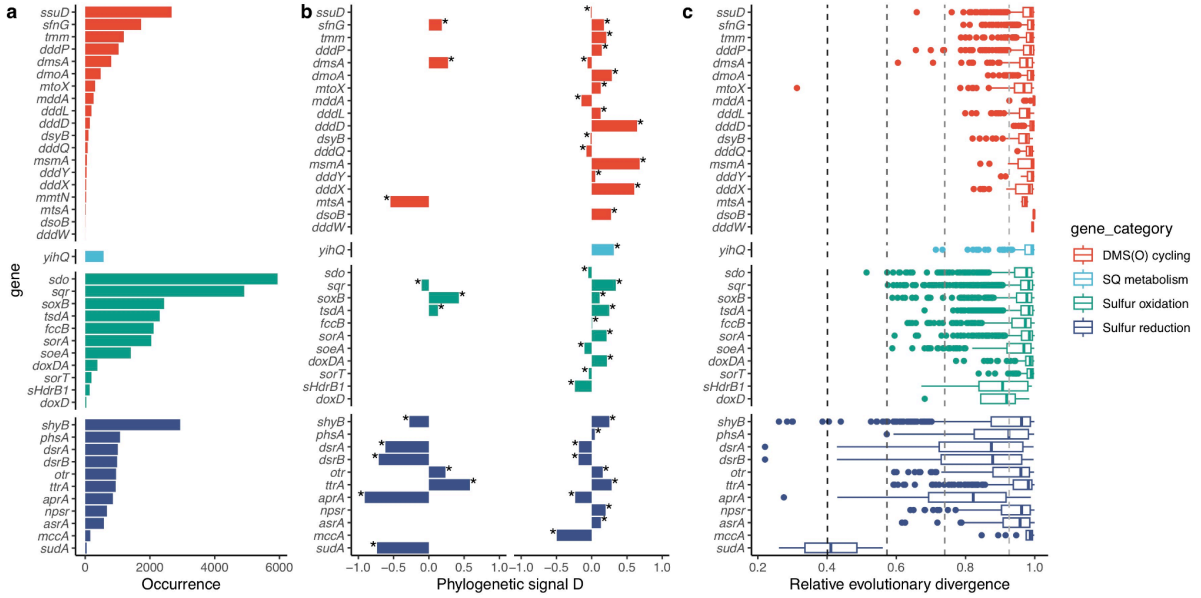


Fig. S1 | Occurrence, phylogenetic signal D, and conservatism of sulfur-cycling genes in archaeal and bacterial genomes. a, The occurrence plot shows the number of GTDB genomes encoding a particular sulfur-cycling gene; **b**, phylogenetic signal D measures the deviation of the observed distribution pattern from pattern expected under random (D = 1) or Brownian (D = 0) mode of evolution. Genes deviating significantly from random distribution ($P < 0.05$) along the archaeal (left) and bacterial (right) tree are marked with a star. The absence of bars indicates genes that are not detected in archaea (e.g., *soeA* and *doxD*) or bacteria (i.e., *sudA* and *mtsA*); **c**, Relative evolution divergence (RED) of the clades where a particular sulfur-cycling gene is conserved in >90% of constituent members. The median relative evolutionary divergence of taxa at taxonomic ranks from class (RED = 0.401) to genus (RED = 0.926) is shown as dash lines.

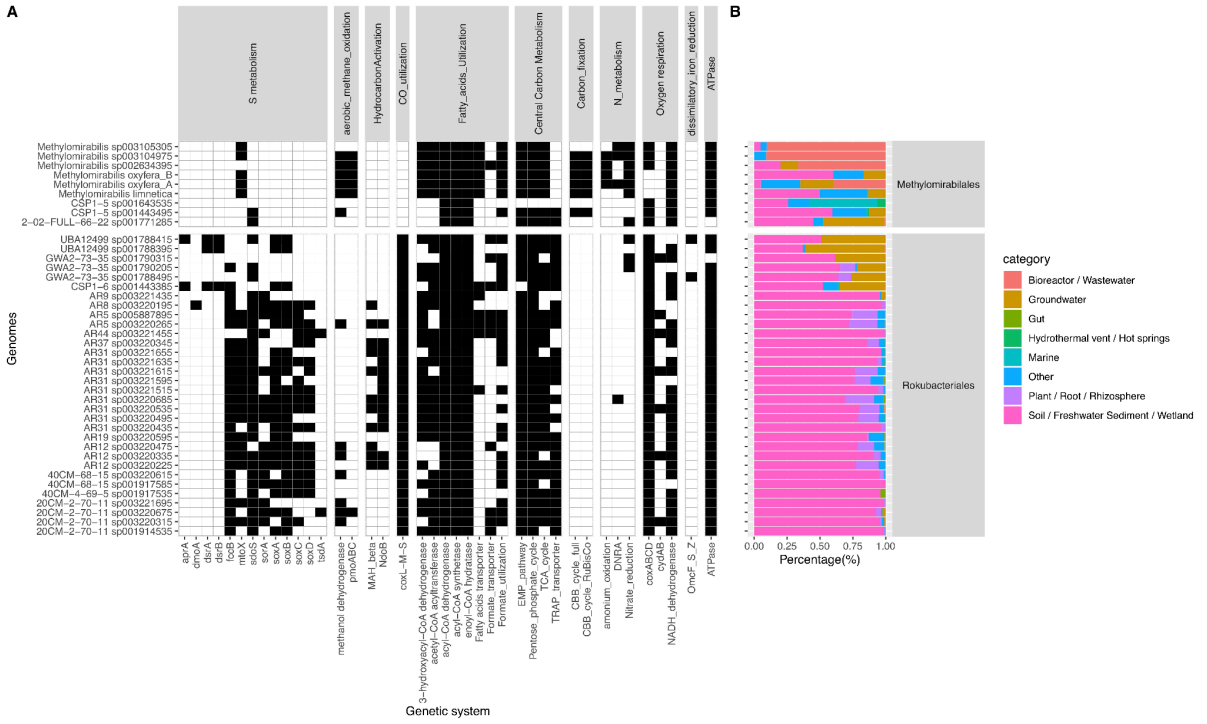


Fig. S2 | Metabolic traits and environmental distribution of *Methylomirabilota*. **a**, The occurrence of metabolic pathways involved in sulfur, carbon, and energy metabolism across *Methylomicrobiota* species. The metabolic reconstruction was performed using the representative genomes of *Methylomirabilota* species from GTDB r95. The species names for following genomes have been revised in GTDB r226 (the GTDB r95 taxa are shown in brackets): AR9 sp003221455 (AR44 sp003221455), *Methylomirabilis oxygeniifera* (*Methylomirabilis oxyfera*_B), *Methylomirabilis tolerans* (*Methylomirabilis* sp002634395), *Methylomirabilis lanthanidiphila* (*Methylomirabilis oxyfera*_A). **b**, The environmental distribution of each GTDB species was retrieved from Sandpiper (https://sandpiper.qut.edu.au/) and is shown as a bar chart. aprA, adenylylsulfate reductase subunit alpha; dmoA, dimethylsulfide monooxygenase; dsrAB, dissimilatory sulfite reductase; fccB, sulfide dehydrogenase [flavocytochrome c] flavoprotein chain; mtoX, methanethiol oxidase; sdoS, sulfur dioxygenase; sorAB, sulfite dehydrogenase; soxB, S-sulfosulfanyl-L-cysteine sulfohydrolase; soxC, sulfane dehydrogenase subunit SoxC; soxD, S-disulfanyl-L-cysteine oxidoreductase SoxD; tsdA, thiosulfate dehydrogenase; pmoABC, particulate methane monooxygenase; MAH_beta, benzene/toluene/naphtalene dioxygenase subunit beta; NdoB, benzene/toluene/naphtalene dioxygenase subunit alpha; coxL-M-S, aerobic carbon-monoxide dehydrogenase large/medium/small subunit; EMP_pathway, Embden-Meyerhof-Parnas pathway; TCA_cycle, tricarboxylic acid cycle; TRAP_transporter, tripartite ATP-independent periplasmic transporter; CBB_cycle_full, Calvin-Benson-Bassham cycle; CBB_cycle_RuBisCo, ribulose-bisphosphate carboxylase; DNRA, dissimilatory nitrate reduction to ammonium; coxABCD, aa_3-type cytochrome oxidase; cydAB, cytochrome d

ubiquinone oxidase; OmcF_S_Z, outer membrane cytochrome OmcF, OmcS, or OmcZ; ATPase, adenosine 5'-triphosphatase.

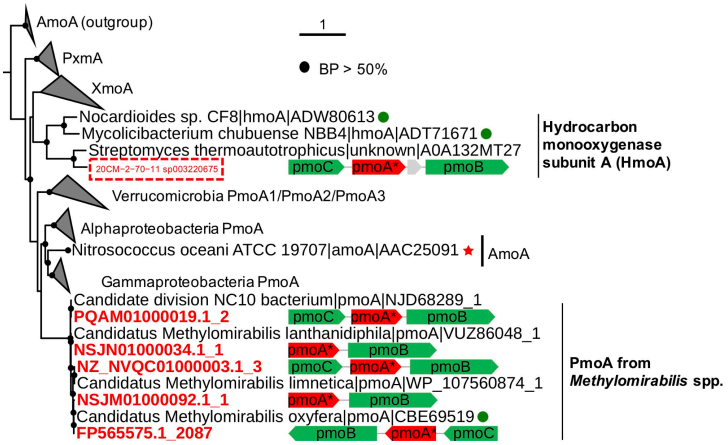


Fig. S3 | Phylogeny of particulate methane monooxygenase catalytic subunit (PmoA) homologs encoded in members of *Methylomirabilota*. The accession of PmoA homologs from GTDB *Methylomicrabilis* MAGs (e.g., PAQM01000019.1_2) are highlighted in red, whereas the *Methylomirabilis* PmoAs with NCBI accession numbers (e.g., NJD68289_1) are used as reference. The PmoA homolog from the candidate species 20CM−2−70−11 sp003220675 clusters with the hydrocarbon monooxygenase catalytic subunit (HmoA) from alkane-oxidizing *Mycolicibacterium chubuense* and *Nocardioides* species CF8. The neighbouring genes of *pmoA* from *Methylomirabilota* are shown as arrows. The phylogenetic tree was rooted using sequences of the ammonia monooxygenase catalytic subunit (AmoA) from *Nitrosomonadaceae* as an outgroup. PmoA/HmoA from characterized hydrocarbon oxidizers (e.g., *Norcardioides* species CF8 and *Methylomirabilis oxyfera*) are highlighted with green circles. Amo from *Nitrosococcus oceani* ATCC19707 are indicated using filled red stars. Branches with bootstrap (BP) values over 50% are indicated by black circles. The scale bar indicates the number of amino acids substitution per site.

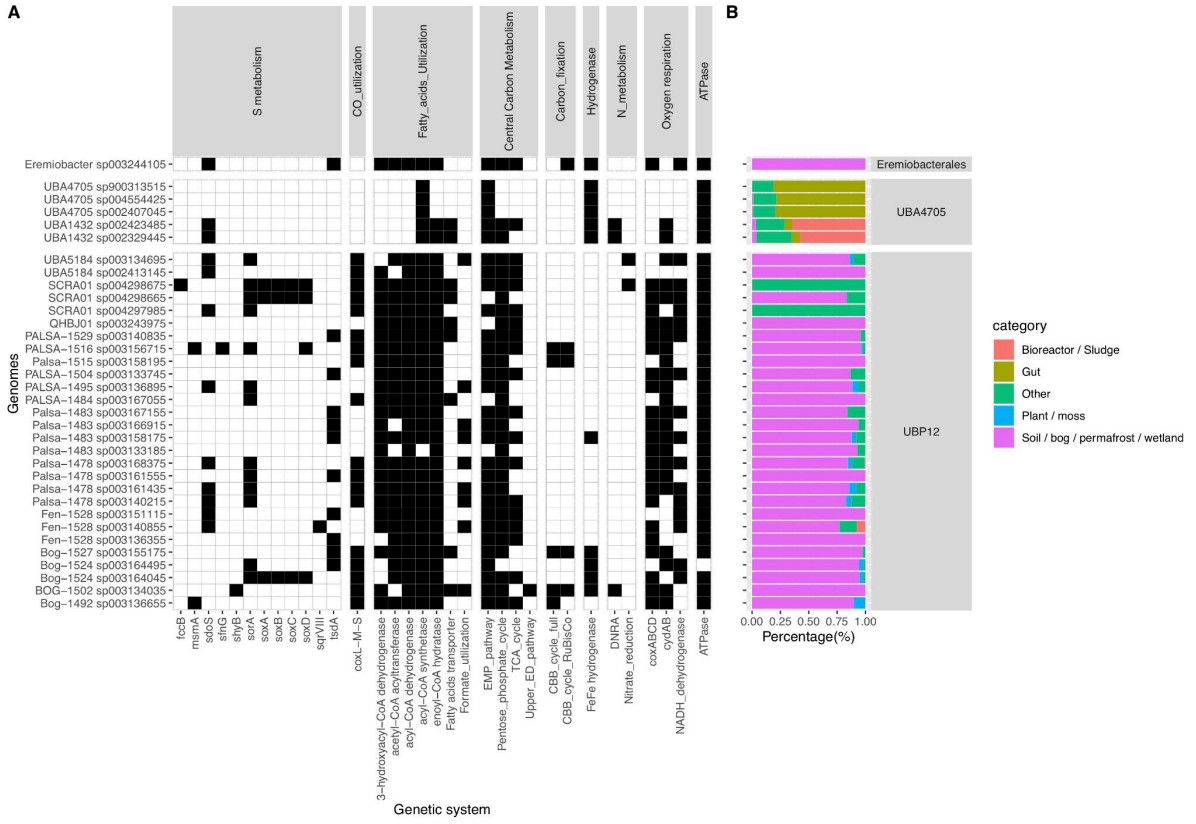
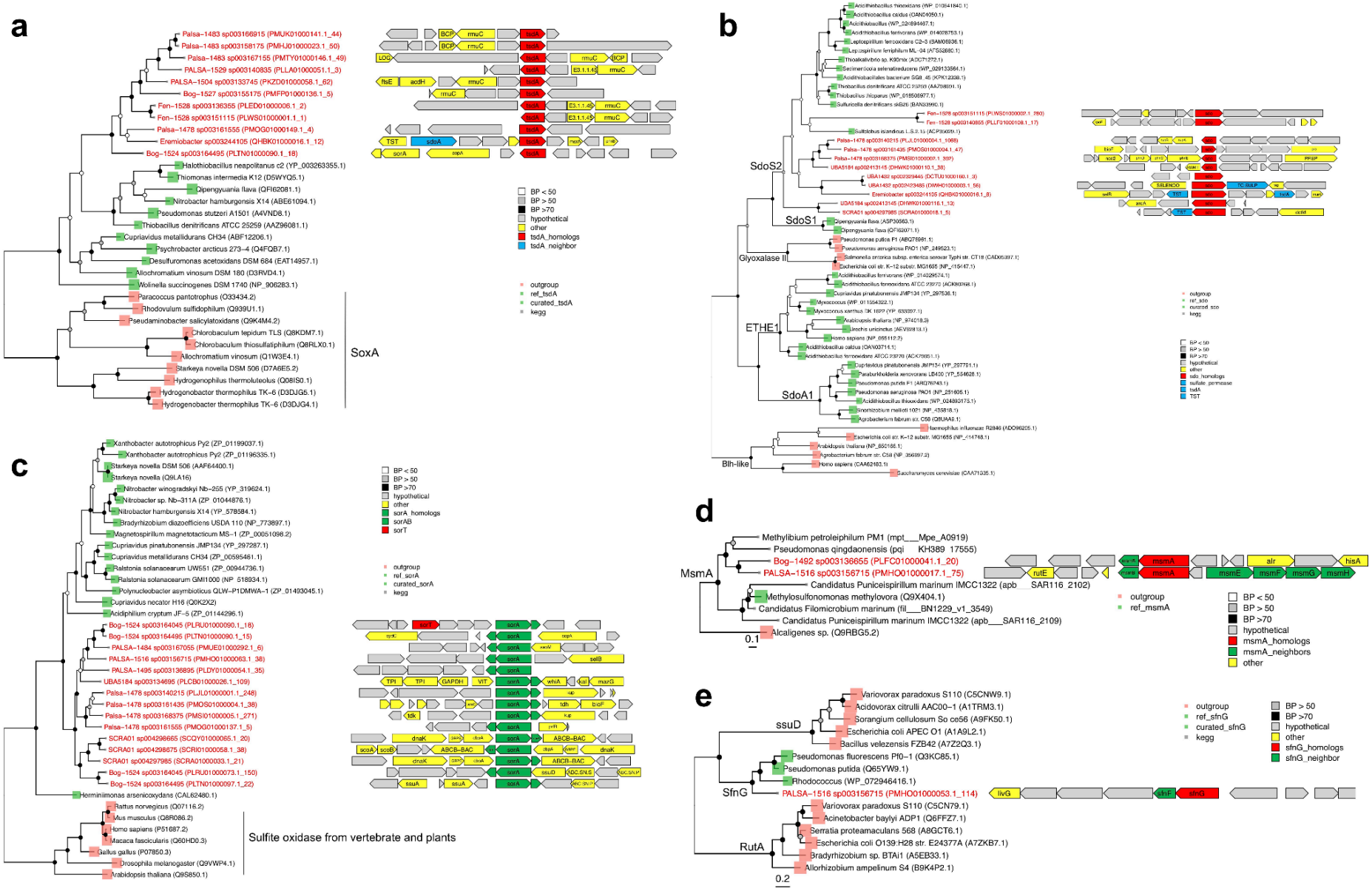


Fig. S4 | Metabolic traits and environmental distribution of *Vulcanimicrobiota* (*Eremiobacterota* in GTDB r95). **a**, The occurrence of metabolic pathways involved in sulfur, carbon and energy metabolism across *Vulcanimicrobiota* species. The metabolic reconstruction was performed using the representative genomes of *Vulcanimicrobiota* species from GTDB r95. The species names for following genomes have been revised in GTDB r226 (the GTDB r95 taxa are shown in brackets): *Lustribacter telmatis* (Bog-1524 sp003164045); *Bruticola papionis* (UBA4705 sp002407045); *Hesperobacter lustricola* (Bog-1492 sp003136655); *Hemerobacter limicola* (Bog-1527 sp003155175); *Rubrimentiphilum* sp003136355 (Fen-1528 sp003136355); *Bruticola* sp004554425 (UBA4705 sp004554425); *Tumulicola scandinaviensis* (PALSA-1529 sp003140835); *Cybelea palsarum* (Palsa-1483 sp003166915); *Lustribacter caenicola* (Bog-1524 sp003164495); *Vulcanimicrobium vanleeuwenhoeki* (Palsa-1478 sp003161555); *Zemelea* sp002413145 (UBA5184 sp002413145); *Eremiobacter antarcticus* (*Eremiobacter* sp003244105); *Rubrimentiphilum* sp003140855 (Fen-1528 sp003140855); *Erabacter solicola* (PALSA-1495 sp003136895); *Vulcanimicrobium pasteuri* (Palsa-1478 sp003161435); *Zemelea palustris* (UBA5184 sp003134695); *Cybelea tyrphae* (Palsa-1483 sp003133185); *Cybelea septentrionalis* (Palsa-1483 sp003158175); *Cybelea tumulisoli* (Palsa-1483 sp003167155); *Palsibacter borealis* (Palsa-1515 sp003158195); *Aquilonibacter stordalenmirensis* (PALSA-1484 sp003167055); *Tyrphobacter aquilonaris* (PALSA-1504 sp003133745); *Tityobacter terrigena* (PALSA-1516 sp003156715); *Vulcanimicrobium winogradskyi* (Palsa-1478 sp003168375); *Xenobium purgamenti* (UBA1432 sp002329445); *Vulcanimicrobium beijerinckii* (Palsa-1478 sp003140215); *Meridianibacter frigidus* (QHBJ01

711 sp003243975); *Velthaea versatilis* (BOG-1502 sp003134035); *Xenobium occultum*
712 (UBA1432 sp002423485); *Rubrimentiphilum* sp003151115 (Fen-1528 sp003151115);
713 *Bruticola* sp900313515 (UBA4705 sp900313515). The changed order-level
714 classifications in GTDB r226 included *Vulcanimicrobiales* (formerly known as UBP12
715 in GTDB r95) and *Xenobiales* (UBA4705). **b**, The environmental distribution of each
716 GTDB species was retrieved from Sandpiper (https://sandpiper.qut.edu.au/) and is
717 shown as a bar chart. fccB, sulfide dehydrogenase [flavocytochrome c] flavoprotein
718 chain; msmA, methanesulfonate monooxygenase subunit A; sdoS, sulfur
719 dioxygenase; sfnG, $FMNH_2$-dependent dimethylsulfone monooxygenase subunit
720 alpha; shyB, sulfhydrogenase subunit beta (sulfur reductase); soxA, L-cysteine
721 S-thiosulfotransferase, soxB, S-sulfosulfanyl-L-cysteine sulfohydrolase; soxC,
722 sulfane dehydrogenase subunit SoxC; soxD, S-disulfanyl-L-cysteine oxidoreductase
723 SoxD; sqrVIII, sulfide quinone reductase clade VIII; tsdA, thiosulfate dehydrogenase;
724 coxL-M-S, aerobic carbon-monoxide dehydrogenase large/medium/small subunit;
725 EMP_pathway, Embden-Meyerhof-Parnas pathway; TCA_cycle, tricarboxylic acid
726 cycle; ED_pathway, Entner-Doudoroff Pathway; CBB_cycle_full,
727 Calvin-Benson-Bassham cycle; CBB_cycle_RuBisCo, ribulose-bisphosphate
728 carboxylase; coxABCD, aa_3-type cytochrome oxidase; cydAB, cytochrome d
729 ubiquinone oxidase; ATPase, adenosine 5'-triphosphatase.


a
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Eremiobacter sp003244105 (QHBK01000016.1_12)
Bog−1524 sp003164495 (PLTN01000090.1_18)
Halothiobacillus neapolitanus c2 (YP_003263355.1)
Thiomonas intermedia K12 (D5WYQ5.1)
Dipengyuania flava (QFI62081.1)
Nitrobacter hamburgensis X14 (ABE61094.1)
Pseudomonas stutzeri A1501 (A4VND8.1)
Thiobacillus denitrificans ATCC 25259 (AAZ96081.1)
Cupriavidus metallidurans CH34 (ABF12206.1)
Psychrobacter arcticus 273−4 (Q4FQB7.1)
Desulfuromonas acetoxidans DSM 684 (EAT14957.1)
Allochromatium vinosum DSM 180 (D3RVD4.1)
Wolinella succinogenes DSM 1740 (NP_906283.1)
Paracoccus pantotrophus (O33434.2)
Rhodovulum sulfidophilum (Q939U1.1)
Pseudaminobacter salicylatoxidans (Q9K4M4.2)
Chlorobaculum tepidum TLS (Q8KDM7.1)
Chlorobaculum thiosulfatiphilum (Q8RLX0.1)
Allochromatium vinosum (Q1W3E4.1)
Starkeya novella DSM 506 (D7A6E5.2)
Hydrogenophilus thermoluteolus (Q08IS0.1)
Hydrogenobacter thermophilus TK−6 (D3DJG5.1)
Hydrogenobacter thermophilus TK−6 (D3DJG4.1)
SoxA
BP < 50
BP > 50
BP >70
hypothetical
other
tsdA_homologs
tsdA_neighbor
outgroup
ref_tsdA
curated_tsdA
kegg
b
SdoS2
SdoS1
Glyoxalase II
ETHE1
SdoA1
Blh-like
outgroup
ref_sdo
curated_sdo
kegg
BP < 50
BP > 50
BP >70
hypothetical
other
sdo_homologs
sulfate_permease
tsdA
TST
c
Xanthobacter autotrophicus Py2 (ZP_01199037.1)
Xanthobacter autotrophicus Py2 (ZP_01196335.1)
Starkeya novella DSM 506 (AAF64400.1)
Starkeya novella (Q9LA16)
Nitrobacter winogradskyi Nb−255 (YP_319624.1)
Nitrobacter sp. Nb−311A (ZP_01044876.1)
Nitrobacter hamburgensis X14 (YP_578584.1)
Bradyrhizobium diazoefficiens USDA 110 (NP_773897.1)
Magnetospirillum magnetotacticum MS−1 (ZP_00051098.2)
Cupriavidus pinatubonensis JMP134 (YP_297287.1)
Cupriavidus metallidurans CH34 (ZP_00595461.1)
Ralstonia solanacearum GMI1000 (NP_518034.1)
Ralstonia solanacearum UW551 (ZP_00944736.1)
Polynucleobacter asymbioticus QLW−P1DMWA−1 (ZP_01493045.1)
Cupriavidus necator H16 (Q0K2X2)
Acidiphilium cryptum JF−5 (ZP_01144296.1)
Bog−1524 sp003164045 (PLRU01000090.1_18)
Bog−1524 sp003164495 (PLTN01000090.1_15)
PALSA−1484 sp003167055 (PMUE01000292.1_6)
PALSA−1516 sp003156715 (PMHO01000063.1_38)
PALSA−1495 sp003136895 (PLDY01000054.1_35)
UBA5184 sp003134695 (PLCB01000026.1_109)
Palsa−1478 sp003140215 (PLJL01000001.1_248)
Palsa−1478 sp003161435 (PMOS01000004.1_38)
Palsa−1478 sp003168375 (PMSI01000005.1_271)
Palsa−1478 sp003161555 (PMOG01000137.1_5)
SCRA01 sp004298665 (SCQY01000065.1_20)
SCRA01 sp004298675 (SCRI01000058.1_38)
SCRA01 sp004297985 (SCRA01000033.1_21)
Bog−1524 sp003164045 (PLRU01000073.1_150)
Bog−1524 sp003164495 (PLTN01000097.1_22)
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Rattus norvegicus (Q07116.2)
Mus musculus (Q8R086.2)
Homo sapiens (P51687.2)
Macaca fascicularis (Q60HD0.3)
Gallus gallus (P07850.3)
Drosophila melanogaster (Q9VWP4.1)
Arabidopsis thaliana (Q9S850.1)
Sulfite oxidase from vertebrate and plants
BP < 50
BP > 50
BP >70
hypothetical
other
sorA_homologs
sorAB
sorT
outgroup
ref_sorA
curated_sorA
kegg
d
Methylibium petroleiphilum PM1 (mpt___Mpe_A0919)
Pseudomonas qingdaonensis (pqi___KH389_17555)
Bog−1492 sp003136655 (PLFC01000041.1_20)
PALSA−1516 sp003156715 (PMHO01000017.1_75)
Candidatus Puniceispirillum marinum IMC1322 (apb___SAR116_2102)
Methylosulfonomonas methylovora (Q9X404.1)
Candidatus Filomicrobium marinum (fil___BN1229_v1_3549)
Candidatus Puniceispirillum marinum IMC1322 (apb___SAR116_2109)
Alcaligenes sp. (Q9RBG5.2)
MsmA
0.1
outgroup
ref_msmA
BP < 50
BP > 50
BP >70
hypothetical
msmA_homologs
msmA_neighbors
other
e
Variovorax paradoxus S110 (C5CNW9.1)
Acidovorax citrulli AAC00−1 (A1TRM3.1)
Sorangium cellulosum So ce56 (A9FK50.1)
Escherichia coli APEC O1 (A1A9L2.1)
Bacillus velezensis FZB42 (A7Z2Q3.1)
Pseudomonas fluorescens Pf0−1 (Q3KC85.1)
Pseudomonas putida (Q65YW9.1)
Rhodococcus (WP_072946416.1)
PALSA−1516 sp003156715 (PMHO01000053.1_114)
Variovorax paradoxus S110 (C5CN79.1)
Acinetobacter baylyi ADP1 (Q6FFZ7.1)
Serratia proteamaculans 568 (A8GCT6.1)
Escherichia coli O139:H28 str. E24377A (A7ZKB7.1)
Bradyrhizobium sp. BTAi1 (A5EB33.1)
Allorhizobium ampelinum S4 (B9K4P2.1)
ssuD
SfnG
RutA
0.2
outgroup
ref_sfnG
curated_sfnG
kegg
BP > 50
BP >70
hypothetical
other
sfnG_homologs
sfnG_neighbor

730

Fig. S5 | Phylogenetic analysis of selected sulfur-cycling genes predicted in members of *Vulcanimicrobiota* (*Eremiobacterota* in GTDB r95). a, Phylogeny of TsdA. Homologs of TsdA from *Vulcanimicrobiota* (shown with red text) cluster with the experimentally verified TsdAs from thiosulfate-oxidizing bacteria (highlighted with green squares; e.g., *Thiomonas intermedia* K12 and *Pseudomonas stutzeri* A1501). The gene context for each *Vulcanimicrobiota* TsdA is placed next to the tree; the colouring scheme is denoted in the figure legend. The name of neighbouring genes is based on the KEGG annotation. The phylogenetic tree was rooted using sequences of the L-cysteine S-thiosulfotransferase subunit SoxA (indicated with red squares). **b,** Phylogeny of Sdo. Homologs of Sdo from *Vulcanimicrobiota* (shown with red text) are placed within the SdoS2 clade comprising the experimentally verified member from *Acidithiobacillus caldus* [81]. The gene context for each *Vulcanimicrobiota* Sdo is placed next to the tree; the colouring scheme is denoted in the figure legend. The name of neighbouring genes is based on the KEGG annotation. ETHE1 and SdoA1 represent two additional clades of Sdo, each containing members with verified enzymatic activity of Sdo [81,82]. The phylogenetic tree was rooted using sequences of beta-lactamase domain proteins (Blh; red squares). **c,** Phylogeny of SorA. Homologs of SorA from *Vulcanimicrobiota* (shown with red text) branch sister to the experimentally verified SorA, i.e., those from *Starkeya novella* [83,84]and *Cupriavidus necator* H16 [85]. The gene context for each *Vulcanimicrobiota* SorA is placed next to the tree; the colouring scheme is denoted in the figure legend. The name of neighbouring genes is based on the KEGG annotation. The phylogenetic tree was rooted using sequences of sulfite oxidase from vertebrates and plants (indicated with red squares). **d,** Phylogenetic analysis of MsmA. MsmA from two *Vulcanimicrobiota* members (shown with red text) clusters with experimentally verified MsmA from *Methylosulfonomonas methylovora* [75]. Gene context analysis revealed a *msmB* locating downstream of *msmA* in both *Vulcanimicrobiota* species, and a putative *msmEFGH* operon for methanesulfonate (MSA) transporter system locating upstream of *msmA* from PALSA-1516 sp003156715 [77] The close association with structural genes for a MSA transporter resembles the arrangement of *msm* genes in the MSA-degrading bacterium *M. methylovora*, and supports *msmAB* from *Vulcanimicrobiota* as a MSA monooxygenase. The phylogenetic tree was rooted using sequences of 2-aminobenzenesulfonate 2,3-dioxygenase subunit alpha from *Alcaligenes* sp. (indicated with red squares). **e,** Phylogenetic analysis of SfnG. SfnG from PALSA-1516 sp003156715 (shown with red text) clusters with experimentally verified SfnG (green squares) in *Pseudomonas* spp. [86,87], and is clearly separating from functionally divergent homologs (red squares), such as pyrimidine monooxygenase RutA and alkanesulfonate monooxygenase SsuD. Gene context analysis shows *sfnG* from *Tityobacter terrigena* (PALSA-1516 sp003156715) located adjacent to *sfnF*, forming a putative *sfnFG* operon responsible for dimethyl sulfone oxygenation as characterized in *Pseudomonas putida* DS1 [88]. Branches with bootstrap value over 70% and between 50% and 70% are indicated by black and grey circles, respectively. The species names for following genomes have been revised in GTDB

r226 (the GTDB r95 taxa are shown in brackets): *Lustribacter telmatis* (Bog-1524 sp003164045); *Bruticola papionis* (UBA4705 sp002407045); *Hesperobacter lustricola* (Bog-1492 sp003136655); *Hemerobacter limicola* (Bog-1527 sp003155175); *Rubrimentiphilum* sp003136355 (Fen-1528 sp003136355); *Bruticola* sp004554425 (UBA4705 sp004554425); *Tumulicola scandinaviensis* (PALSA-1529 sp003140835); *Cybelea palsarum* (Palsa-1483 sp003166915); *Lustribacter caenicola* (Bog-1524 sp003164495); *Vulcanimicrobium vanleeuwenhoeki* (Palsa-1478 sp003161555); *Zemelea* sp002413145 (UBA5184 sp002413145); *Eremiobacter antarcticus* (Eremiobacter sp003244105); *Rubrimentiphilum* sp003140855 (Fen-1528 sp003140855); *Erabacter solicola* (PALSA-1495 sp003136895); *Vulcanimicrobium pasteuri* (Palsa-1478 sp003161435); *Zemelea palustris* (UBA5184 sp003134695); *Cybelea tyrphae* (Palsa-1483 sp003133185); *Cybelea septentrionalis* (Palsa-1483 sp003158175); *Cybelea tumulisoli* (Palsa-1483 sp003167155); *Palsibacter borealis* (Palsa-1515 sp003158195); *Aquilonibacter stordalenmirensis* (PALSA-1484 sp003167055); *Tyrphobacter aquilonaris* (PALSA-1504 sp003133745); *Tityobacter terrigena* (PALSA-1516 sp003156715); *Vulcanimicrobium winogradskyi* (Palsa-1478 sp003168375); *Xenobium purgamenti* (UBA1432 sp002329445); *Vulcanimicrobium beijerinckii* (Palsa-1478 sp003140215); *Meridianibacter frigidus* (QHBJ01 sp003243975); *Velthaea versatilis* (BOG-1502 sp003134035); *Xenobium occultum* (UBA1432 sp002423485); *Rubrimentiphilum* sp003151115 (Fen-1528 sp003151115); *Bruticola* sp900313515 (UBA4705 sp900313515). The changed order-level classifications in GTDB r226 included *Vulcanimicrobiales* (formerly known as UBP12 in GTDB r95) and *Xenobiales* (UBA4705).

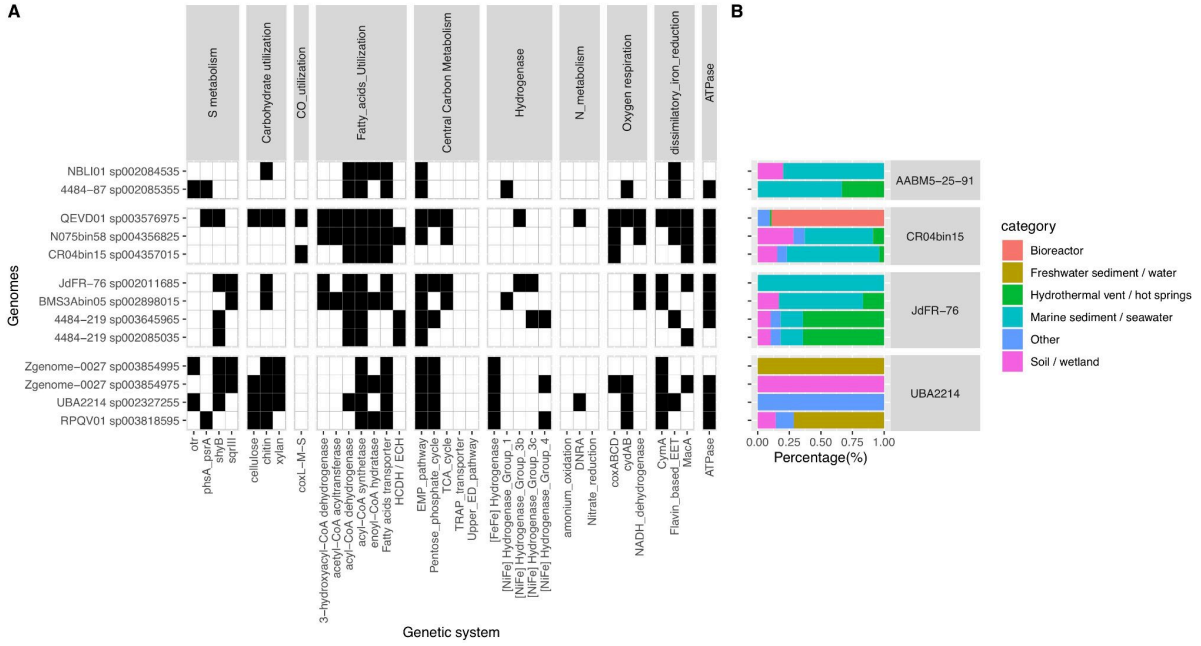


Fig. S6 | Metabolic traits and environmental distribution of *Zhuqueibacterota* (KSB1 in GTDB r95). a, The occurrence of metabolic pathways involved in sulfur, carbon and energy metabolism across *Zhuqueibacterota* species. The metabolic reconstruction was performed using 13 representative genomes of *Zhuqueibacterota* species from GTDB r95. The species names for following genomes have been revised in GTDB r226 (the GTDB r95 taxa are shown in brackets): *Oceanimicrobium pacificum* (N075bin58 sp004356825), *Kriniovivens* sp003854995 (Zgenome-0027 sp003854995), 4484-219 sp002085035 (4484-219 sp003645965), *Fontimicrobium primus* (RPQV01 sp003818595), *Coneutiohabitans* sp003576975 (QEVD01 sp003576975), *Residuimicrobium suncorense* (UBA2214 sp002327255). The changed order-level classifications in GTDB r226 included: (1) the split of GTDB r95 order CR04bin15 into orders *Zhuqueibacterales* and *Oceanimicrobiales*, and (2) the reclassification of the order AABM5-25-91 and UBA2214 as *Thermofontimicrobiales* and *Residuimicrobiales*, respectively. **b**, The environmental distribution of each GTDB species grouped at order level was retrieved from Sandpiper (https://sandpiper.qut.edu.au/) and is shown as a bar chart. The phylum KSB1 has recently been renamed to *Zhuqueibacterota*. The orders AABM5-25-91 and UBA2214 have been renamed to *Thermofontimicrobiales* and *Residuimicrobiales*, respectively. Members of the orders CR04bin15 have been reclassified to the orders *Zhuqueibacterales* (*Ca*. QEVD01 sp003576975) and *Oceanimicrobiales* (*Ca*. N075bin58 sp004356825 and *Ca*. CR04bin15 sp004357015). otr, octaheme tetrathionate reductase; phsA_psrA, thiosulfate reductase / polysulfide reductase chain A; shyB, sulfhydrogenase subunit beta (sulfur reductase); sqrIII, sulfide quinone reductase clade III; coxL-M-S, aerobic carbon-monoxide dehydrogenase large/medium/small subunit; HCDH/ECH, 3-hydroxyacyl-CoA dehydrogenase/enoyl-CoA hydratase; EMP_pathway, Embden-Meyerhof-Parnas pathway; TCA_cycle, tricarboxylic acid cycle; TRAP_transporter, tripartite

ATP-independent periplasmic transporter; ED_pathway, Entner-Doudoroff Pathway; DNRA, DNRA, dissimilatory nitrate reduction to ammonium; coxABCD, aa_3-type cytochrome oxidase; cydAB, cytochrome d ubiquinone oxidase; CymA, tetraheme quinol dehydrogenase; EET, extracellular electron transfer; MacA, diheme cytochrome c involved in iron(III) reduction; ATPase, adenosine 5'-triphosphatase.

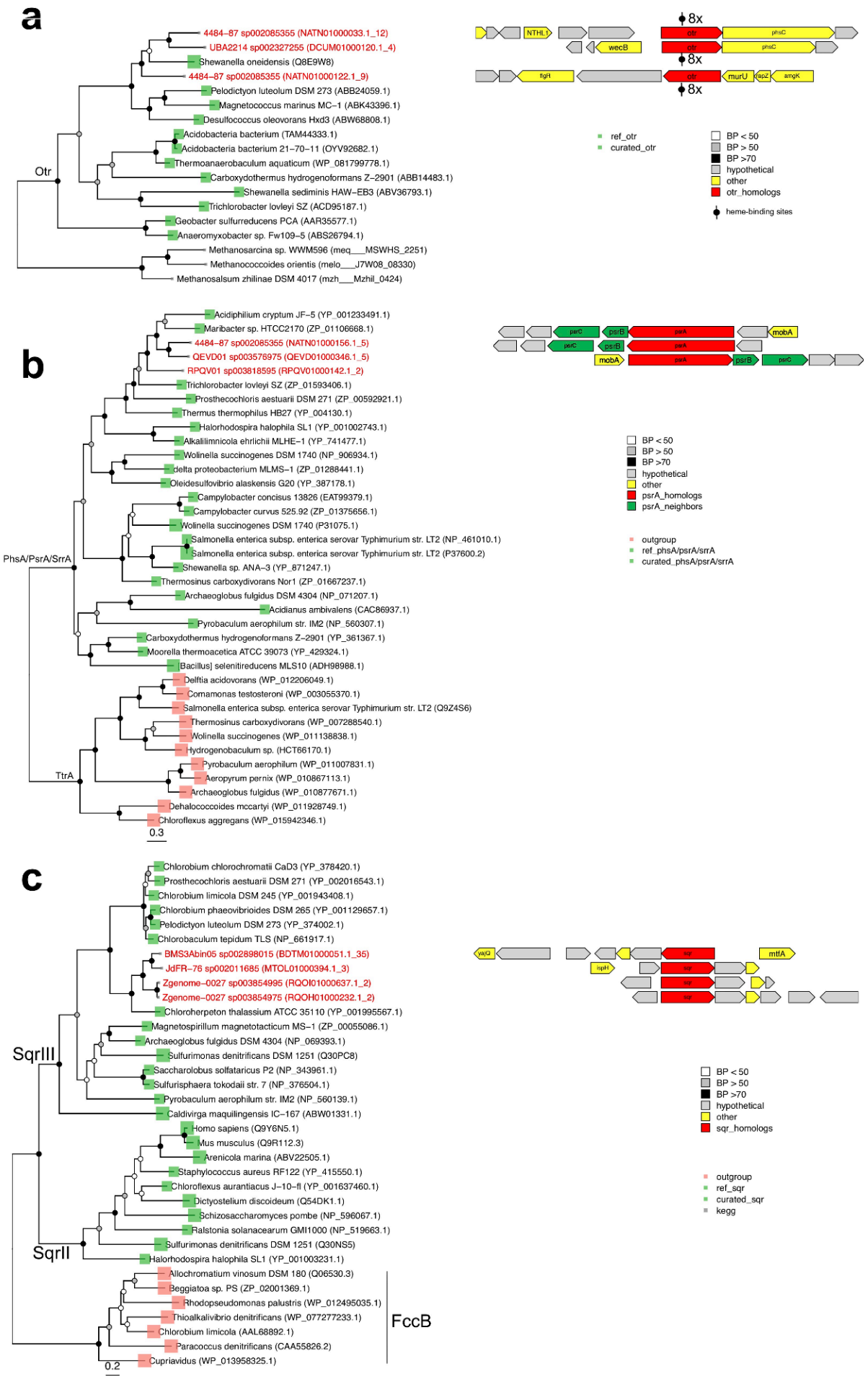

a
4484–87 sp002085355 (NATN01000033.1_12)
UBA2214 sp002327255 (DCUM01000120.1_4)
Shewanella oneidensis (Q8E9W8)
4484–87 sp002085355 (NATN01000122.1_9)
Pelodictyon luteolum DSM 273 (ABB24059.1)
Magnetococcus marinus MC–1 (ABK43396.1)
Desulfococcus oleovorans Hxd3 (ABW68808.1)
Acidobacteria bacterium (TAM44333.1)
Acidobacteria bacterium 21–70–11 (OYV92682.1)
Thermoanaerobaculum aquaticum (WP_081799778.1)
Carboxydothermus hydrogenoformans Z–2901 (ABB14483.1)
Shewanella sediminis HAW–EB3 (ABV36793.1)
Trichlorobacter lovleyi SZ (ACD95187.1)
Geobacter sulfurreducens PCA (AAR35577.1)
Anaeromyxobacter sp. Fw109–5 (ABS26794.1)
Methanosarcina sp. WWM596 (meq___MSWHS_2251)
Methanococcoides orientis (melo___J7W08_08330)
Methanosalsum zhilinae DSM 4017 (mzh___Mzhil_0424)
Otr
NTHL1
wecB
otr
phsC
figH
murU
kepZ
amgK
8x
ref_otr
curated_otr
BP < 50
BP > 50
BP >70
hypothetical
other
otr_homologs
heme-binding sites
b
Acidiphilium cryptum JF–5 (YP_001233491.1)
Maribacter sp. HTCC2170 (ZP_01106668.1)
4484–87 sp002085355 (NATN01000156.1_5)
QEVD01 sp003576975 (QEVD01000346.1_5)
RPQV01 sp003818595 (RPQV01000142.1_2)
Trichlorobacter lovleyi SZ (ZP_01593406.1)
Prosthecochloris aestuarii DSM 271 (ZP_00592921.1)
Thermus thermophilus HB27 (YP_004130.1)
Halorhodospira halophila SL1 (YP_001002743.1)
Alkalilimnicola ehrlichii MLHE–1 (YP_741477.1)
Wolinella succinogenes DSM 1740 (NP_906934.1)
delta proteobacterium MLMS–1 (ZP_01288441.1)
Oleidesulfovibrio alaskensis G20 (YP_387178.1)
Campylobacter concisus 13826 (EAT99379.1)
Campylobacter curvus 525.92 (ZP_01375656.1)
Wolinella succinogenes DSM 1740 (P31075.1)
Salmonella enterica subsp. enterica serovar Typhimurium str. LT2 (NP_461010.1)
Salmonella enterica subsp. enterica serovar Typhimurium str. LT2 (P37600.2)
Shewanella sp. ANA–3 (YP_871247.1)
Thermosinus carboxydivorans Nor1 (ZP_01667237.1)
Archaeoglobus fulgidus DSM 4304 (NP_071207.1)
Acidianus ambivalens (CAC86937.1)
Pyrobaculum aerophilum str. IM2 (NP_560307.1)
Carboxydothermus hydrogenoformans Z–2901 (YP_361367.1)
Moorella thermoacetica ATCC 39073 (YP_429324.1)
Bacillus] selenitireducens MLS10 (ADH98988.1)
Delftia acidovorans (WP_012206649.1)
Comamonas testosteroni (WP_003055370.1)
Salmonella enterica subsp. enterica serovar Typhimurium str. LT2 (Q9Z4S6)
Thermosinus carboxydivorans (WP_007288540.1)
Wolinella succinogenes (WP_011138838.1)
Hydrogenobaculum sp. (HCT66170.1)
Pyrobaculum aerophilum (WP_011007831.1)
Aeropyrum pernix (WP_010867113.1)
Archaeoglobus fulgidus (WP_010877671.1)
Dehalococcoides mccartyi (WP_011928749.1)
Chloroflexus aggregans (WP_015942346.1)
PhsA/PsrA/SrrA
TtrA
0.3
psrC
psrB
psrA
mobA
BP < 50
BP > 50
BP >70
hypothetical
other
psrA_homologs
psrA_neighbors
outgroup
ref_phsA/psrA/srrA
curated_phsA/psrA/srrA
c
Chlorobium chlorochromatii CaD3 (YP_378420.1)
Prosthecochloris aestuarii DSM 271 (YP_002016543.1)
Chlorobium limicola DSM 245 (YP_001943408.1)
Chlorobium phaeovibrioides DSM 265 (YP_001129657.1)
Pelodictyon luteolum DSM 273 (YP_374002.1)
Chlorobaculum tepidum TLS (NP_661917.1)
BMS3Abin05 sp002898015 (BDTM01000051.1_35)
JdFR–76 sp002011685 (MTOL01000394.1_3)
Zgenome–0027 sp003854995 (RQOI01000637.1_2)
Zgenome–0027 sp003854975 (RQOH01000232.1_2)
Chloroherpeton thalassium ATCC 35110 (YP_001995567.1)
Magnetospirillum magnetotacticum MS–1 (ZP_00055086.1)
Archaeoglobus fulgidus DSM 4304 (NP_069393.1)
Sulfurimonas denitrificans DSM 1251 (Q30PC8)
Saccharolobus solfataricus P2 (NP_343961.1)
Sulfurisphaera tokodaii str. 7 (NP_376504.1)
Pyrobaculum aerophilum str. IM2 (NP_560139.1)
Caldivirga maquilingensis IC–167 (ABW01331.1)
Homo sapiens (Q9Y6N5.1)
Mus musculus (Q9R112.3)
Arenicola marina (ABV22505.1)
Staphylococcus aureus RF122 (YP_415550.1)
Chloroflexus aurantiacus J–10–fl (YP_001637460.1)
Dictyostelium discoideum (Q54DK1.1)
Schizosaccharomyces pombe (NP_596067.1)
Ralstonia solanacearum GMI1000 (NP_519663.1)
Sulfurimonas denitrificans DSM 1251 (Q30NS5)
Halorhodospira halophila SL1 (YP_001003231.1)
Allochromatium vinosum DSM 180 (Q06530.3)
Beggiatoa sp. PS (ZP_02001369.1)
Rhodopseudomonas palustris (WP_012495035.1)
Thioalkalivibrio denitrificans (WP_077277233.1)
Chlorobium limicola (AAL68892.1)
Paracoccus denitrificans (CAA55826.2)
Cupriavidus (WP_013958325.1)
SqrIII
SqrII
FccB
0.2
vajQ
sqr
mtfA
ispH
BP < 50
BP > 50
BP >70
hypothetical
other
sqr_homologs
outgroup
ref_sqr
curated_sqr
kegg

Fig. S7 | Phylogenetic analysis of selected sulfur-cycling genes predicted in members of *Zhuqueibacterota* (KSB1 in GTDB r95). a, Phylogeny of Otr. Homologs of Otr from *Zhuqueibacterota* (shown with red text) are closely related to experimentally verified Otr from *Shewanella oneidensis* [24]. All three Otr from *Zhuqueibacterota* harbour eight heme-binding motifs (CXXCH), similar to the Otr from *S. oneidensis*. Experimentally verified Otr and reference Otr sequences collected from a previous study [89] are highlighted with green squares. The gene context for each *Zhuqueibacterota* Otr is placed next to the tree; the colouring scheme is denoted in the figure legend. The name of neighbouring genes is based on the KEGG annotation. The phylogenetic tree was rooted using Otr homologs from methanogens (indicated with red squares), which are not known to be capable of respring tetrathionate. **b,** Phylogeny of PsrA. Homologs of PsrA from *Zhuqueibacterota* (shown with red text) branches within a clade comprising experimentally verified thiosulfate reductase PhsA, polysulfide reductase (PsrA), respiratory selenite reductase (SrrA). Gene context analysis revealed PsrA-encoding genes from KSB1 are located within a putative *psr* operon containing *psrC* (shown as arrows beside the tree), which encodes a functional component supposed to be associated specifically with the polysulfide reductase (Psr). This genomic arrangement supports a functional role of KSB1 PsrA in respiring polysulfide/elemental sulfur. The phylogenetic tree was rooted using tetrathionate reductase TtrA (indicated with red squares). **c,** Phylogeny of Sqr. Homologs of Sqr from *Zhuqueibacterota* (shown with red text) cluster with the type III Sqr (Sqr III) from green sulfur bacteria, such as *Chloroherpeton thalassium* [90]. Type II Sqr (Sqr II), a sister clade of Sqr III, is also shown in the tree for comparison [90,91]. The gene context for each *Zhuqueibacterota* Sqr is placed next to the tree; the colouring scheme is denoted in the figure legend. The name of neighbouring genes is based on the KEGG annotation. The phylogenetic tree was rooted using sequences of flavocytochrome c sulfide dehydrogenase FccB (indicated with red squares). Branches with bootstrap value over 70%, between 50% and 70%, and below 50% are indicated by black, grey, and white circles, respectively. The species names for following genomes have been revised in GTDB r226 (the GTDB r95 taxa are shown in brackets): *Oceanimicrobium pacificum* (N075bin58 sp004356825), *Kriniovivens* sp003854995 (Zgenome-0027 sp003854995), 4484-219 sp002085035 (4484-219 sp003645965), *Fontimicrobium primus* (RPQV01 sp003818595), *Coneutiohabitans* sp003576975 (QEVD01 sp003576975), *Residuimicrobium suncorense* (UBA2214 sp002327255).

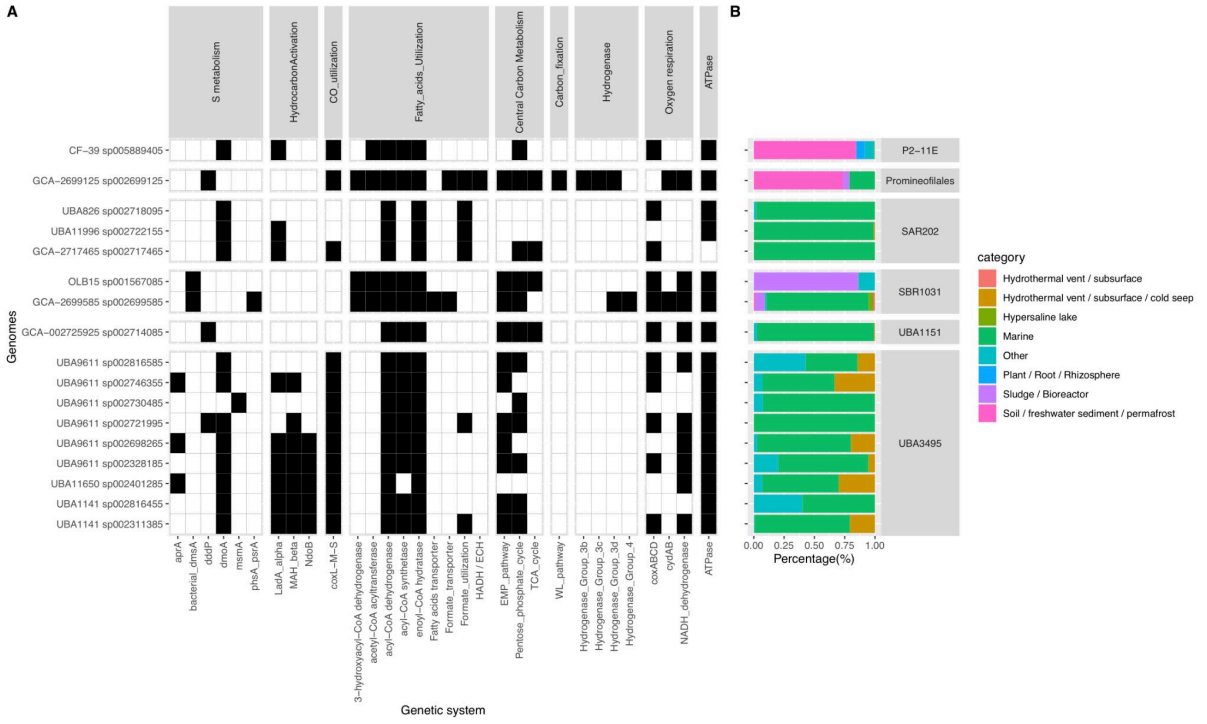


Fig. S8 | Metabolic traits and environmental distribution of *Chloroflexota* exhibiting potential to produce DMS(O) or to metabolize C_1 intermediates derived from DMS metabolism. a, The occurrence of metabolic pathways involved in sulfur, carbon and energy metabolism across selected *Chloroflexota* species. The metabolic reconstruction was performed using the representative genomes of *Chloroflexota* species from GTDB r95. The species names for following genomes have been revised in GTDB r226 (the GTDB r95 taxa are shown in brackets): *Flexifilum* sp001567085 (OLB15 sp001567085), *Phototrophicus* sp002699585 (GCA-2699585 sp002699585), *Leptofilum* sp002699125 (GCA-2699125 sp002699125), and *Lucifugimonas* sp009392785 (GCA-002725925 sp002714085). The changed order-level classifications in GTDB r226 included: *Lucifugimonadales* (known as UBA1151 in GTDB r95), *Aggregatilineales* (SBR1031), CAJWPE01 (SAR202). **b**, The environmental distribution of each GTDB species was retrieved from Sandpiper (https://sandpiper.qut.edu.au/) and is shown as a bar chart. aprA, adenylylsulfate reductase, subunit A; dmsA, anaerobic dimethyl sulfoxide reductase subunit A; dddP, dimethylsulfoniopropionate (DMSP) / dimethylsulfoxonium propionate (DMSOP) lyase; dmoA, dimethyl-sulfide monooxygenase; msmA, methanesulfonate monooxygenase subunit alpha; phsA_psrA, thiosulfate reductase / polysulfide reductase chain A; LadA, long-chain alkane monooxygenase; MAH_beta, benzene/toluene/naphtalene dioxygenase subunit beta; NdoB, benzene/toluene/naphtalene dioxygenase subunit alpha; coxL-M-S, aerobic carbon-monoxide dehydrogenase large/medium/small subunit; HCDH/ECH, 3-hydroxyacyl-CoA dehydrogenase/enoyl-CoA hydratase; EMP_pathway, Embden-Meyerhof-Parnas pathway; TCA_cycle, tricarboxylic acid cycle;

WL_pathway, Wood-Ljungdahl pathway; coxABCD, aa_3-type cytochrome oxidase; cydAB, cytochrome d ubiquinone oxidase; ATPase, adenosine 5'-triphosphatase.

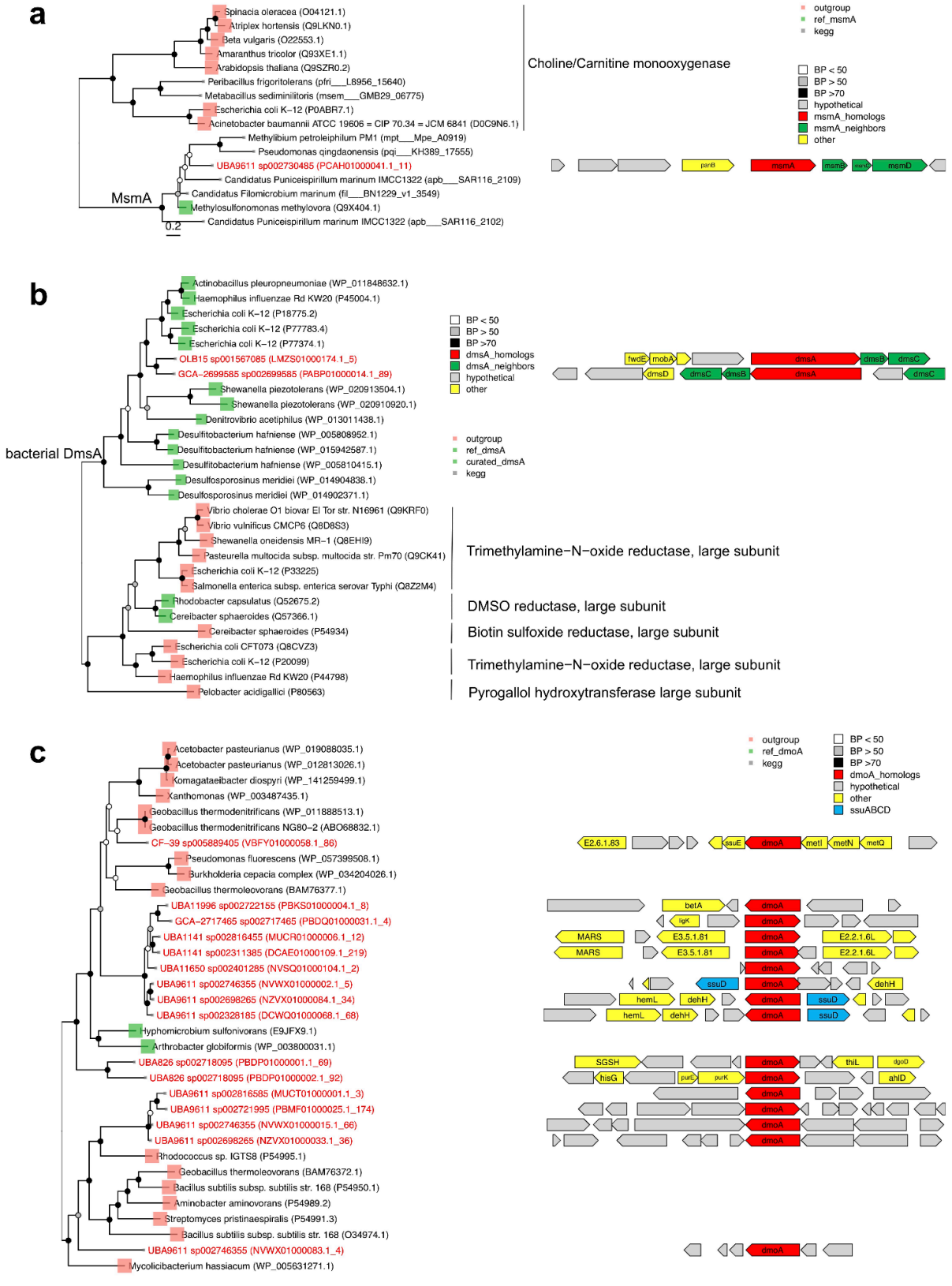


Fig. S9 | Phylogenetic analysis of selected sulfur-cycling genes predicted in members of *Chloroflexota*. a, Phylogenetic analysis of MsmA. MsmA from the uncultured species UBA9611 sp002730485 (shown with red text) clusters with

experimentally verified MsmA from *Methylosulfonomonas methylovora* [75]. Gene context analysis shows the arrangement of *msm* genes in the UBA9611 sp002730485 follows the gene synteny pattern (i.e., *msmABCD*) of *M. methylovora* [75] The phylogenetic tree was rooted using sequences of choline/carnitine monooxygenase (indicated with red squares). **b,** Phylogenetic analysis of DmsA. DmsA homologs from two uncultured *Chloroflexota* species (shown with red text) cluster with experimentally verified DmsAs, e.g., those from *Escherichia coli* [92] and *Haemophilus influenzae* [93]. Gene context analysis shows localization of *dmsA* within a *dms* operon for the dimethylsulfoxide reductase complex. The phylogenetic tree was rooted using functionally divergent homologs of DmsA (indicated with red squares), such as the large subunit of trimethylamine-N-oxide reductase. **c,** Phylogenetic analysis of DmoA. DmoA homologs from uncultured *Chloroflexota* species (shown with red text) share closer phylogenetic relatedness with monooxygenases involved in oxidation of alkane or other substrates (e.g., dibenzothiophen-sulfone [P54995.1; indicated by a red square] and nitrilotriacetate [P54989.2; indicated by a red square]), rather than the experimentally verified DmoA. The gene context for each *dmoA* homolog is placed next to the tree; the colouring scheme is denoted in the figure legend. The name of neighbouring genes is based on the KEGG annotation. The phylogenetic tree was rooted using functionally divergent monooxygenases (indicated with red squares), including putative monooxygenase MoxC (O34974.1), N-acetyl-S-(2-succino)cysteine monooxygenase (P54950.1), nitrilotriacetate monooxygenase component A (P54989.2), pristinamycin IIA synthase subunit A (P54991.3), dibenzothiophene-sulfone monooxygenase (P54995.1), Alkane monoxygenase (ABO68832.1, BAM76372.1, BAM76377.1, and ABO68832.1), LLM class flavin-dependent oxidoreductase (WP_005631271.1, WP_057399508.1, WP_011888513.1, WP_003487435.1, WP_141259499.1, and WP_019088035.1), and NtaA/DmoA family FMN-dependent monooxygenase (WP_034204026.1 and WP_012813026.1). Branches with bootstrap value over 70%, between 50% and 70%, and below 50% are indicated by black, grey, and white circles, respectively. The species names for following genomes have been revised in GTDB r226 (the GTDB r95 taxa are shown in brackets): *Flexifilum* sp001567085 (OLB15 sp001567085), *Phototrophicus* sp002699585 (GCA-2699585 sp002699585), *Leptofilum* sp002699125 (GCA-2699125 sp002699125), and *Lucifugimonas* sp009392785 (GCA-002725925 sp002714085). The changed order-level classifications in GTDB r226 included: *Lucifugimonadales* (known as UBA1151 in GTDB r95), *Aggregatilineales* (SBR1031), CAJWPE01 (SAR202).

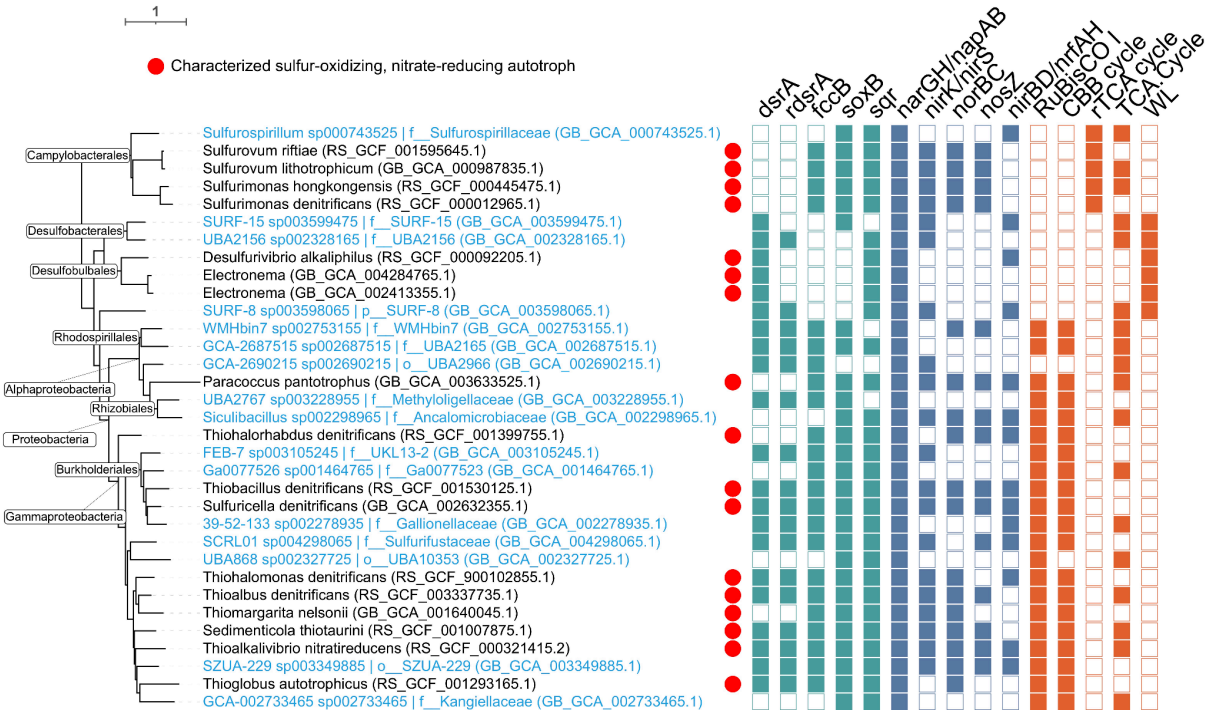
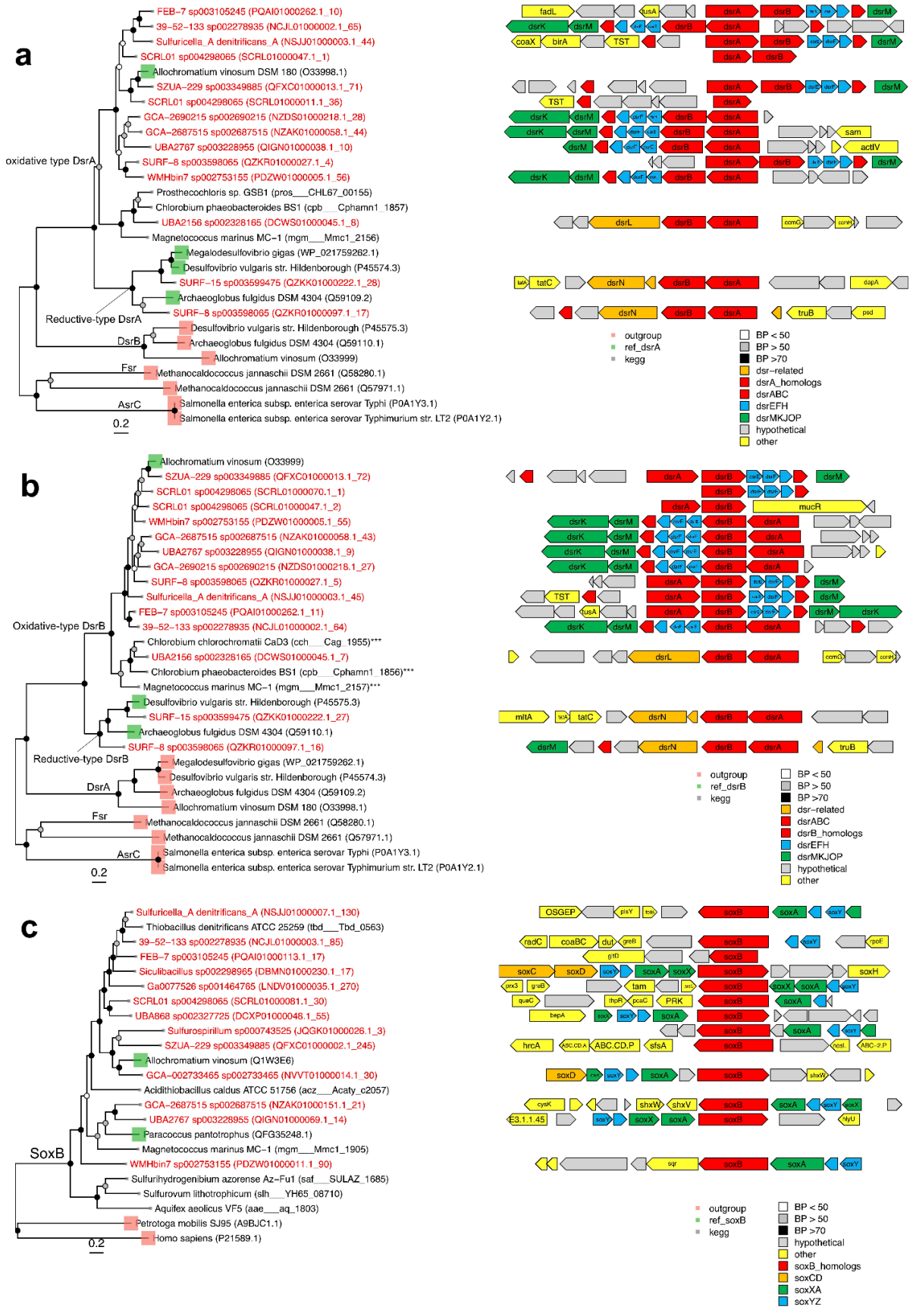


Fig. S10 | Phylogenomic tree and distribution of metabolic traits among microorganisms showing metabolic potential for nitrate-dependent sulfur oxidation. The phylogenomic tree showing the evolutionary relationship of the species is pruned from the GTDB r95 bacterial tree of life. Candidate sulfur-oxidizers are highlighted in cyan. The family (f__), order (o__), or phylum (p__) level classification from GTDB r95 of each species is shown in the taxa label. The species names for following genomes have been revised in GTDB r226 (the GTDB r95 taxa are shown in brackets): *Sulfurimicrobium denitrificans*_A (*Sulfuricella*_A *denitrificans*_A), *Pinisolibacter* sp002298965 (*Siculibacillus* sp002298965), *Thioalkalivibrio nitratireducens* (*Thioalkalivibrio*_B *nitratireducens*), Ga0077523 sp001464765 (Ga0077526 sp001464765). The changed nomenclature for high-rank taxa in GTDB r226 include the phylum *Myxococcota* (SURF-8 in GTDB r95), the order *Arenicellales* (UBA10353), and the family Usitatibacteraceae (UKL13-2). The functionally characterized nitrate-dependent, chemolithoautotrophic sulfur-oxidizers are indicated by red circles. The presence/absence of genetic systems for sulfur oxidation (cyan-green), nitrate reduction (dark blue), and carbon fixation (dark orange) is shown with filled/empty squares. dsrA, dissimilatory sulfite reductase, subunit A; rdsrA, reverse-acting (oxidative) dissimilatory sulfite reductase, subunit A; fccB, sulfide dehydrogenase flavocytochrome c; soxB, L-cysteine S-thiosulfotransferase subunit soxB; sqr, sulfide:qunione oxidoreductase; narGH/napAB, respiratory nitrate reductase/periplasmic nitrate reductase; nirK/nirS, copper-containing nitrite reductase / nitrite reductase; norBC, nitric oxide reductase, subunit B and C; nosZ, nitrous-oxide reductase; nirBD/nrfAH, nitrite reductase (NADH) large and small subunit /nitrite reductase (cytochrome c-552); RuBisCO, ribulose-bisphosphate carboxylase; CBB cycle, Calvin-Benson-Bassham cycle; rTCA cycle, reverse tricarboxylic acid cycle; WL, Wood-Ljungdahl pathway.



970

971 **Fig. S11 | Phylogenetic analysis of selected sulfur-cycling genes predicted in**
972 **microorganisms with metabolic potential for nitrate-dependent sulfur**

oxidation. a, Phylogenetic analysis of DsrA**.** 12 of 14 DsrA homologs from candidate sulfur oxidizers fall within the clade spanned by oxidative-type DsrA from green-sulfur bacteria (e.g., *Prosthecochloris* and *Chlorobium*) and purple-sulfur bacteria (e.g., *Allochromatium vinosum*). The gene context for each DsrA is placed next to the tree; the colouring scheme is denoted in the figure legend. The name of neighbouring genes is based on the KEGG annotation. The phylogenetic tree was rooted using functionally divergent homologs (indicated with red squares), including anaerobic sulfite reductase subunit C (AsrC) and coenzyme F_{420}-dependent sulfite reductase (Fsr). **b,** Phylogenetic analysis of DsrB. 12 of 14 DsrB homologs from candidate sulfur oxidizers fall within the clade spanned by oxidative-type DsrB from green-sulfur bacteria (e.g., *Prosthecochloris* and *Chlorobium*) and purple-sulfur bacteria (e.g., *Allochromatium vinosum*). The gene context for each DsrB is placed next to the tree; the colouring scheme is denoted in the figure legend. The name of neighbouring genes is based on the KEGG annotation. The phylogenetic tree was rooted using functionally divergent homologs (indicated with red squares), including anaerobic sulfite reductase subunit C (AsrC) and coenzyme F_{420}-dependent sulfite reductase (Fsr). **c,** Phylogenetic analysis of SoxB**.** SoxB homologs from candidate sulfur oxidizers fall within the clade spanned by experimentally validated SoxB (e.g., *Allochromatium vinosum* and *Paracoccus pantotrophus*) and SoxB from known thiosulfate-oxidizing bacteria (e.g., *Sulfurovum lithotrophicum* and *Thiobacillus denitrificans*). The gene context for each SoxB is placed next to the tree; the colouring scheme is denoted in the figure legend. The name of neighbouring genes of *soxB* is based on the KEGG annotation. The phylogenetic tree was rooted using functionally divergent homologs (indicated with red squares), including 5-nucleotidase from *Homo sapiens* and mannosylglucosyl-3-phosphoglycerate phosphatase from *Petrotoga mobilis*. Branches with bootstrap value over 70%, between 50% and 70%, and below 50% are indicated by black, grey and white circles, respectively. The species names for following genomes have been revised in GTDB r226 (the GTDB r95 taxa are shown in brackets): *Sulfurimicrobium denitrificans*_A (*Sulfuricella*_A *denitrificans*_A), *Pinisolibacter* sp002298965 (*Siculibacillus* sp002298965), *Thioalkalivibrio nitratireducens* (*Thioalkalivibrio*_B *nitratireducens*), Ga0077523 sp001464765 (Ga0077526 sp001464765). The changed nomenclature for high-rank taxa in GTDB r226 include the phylum *Myxococcota* (SURF-8), the order *Arenicellales* (UBA10353), and the family Usitatibacteraceae (UKL13-2).

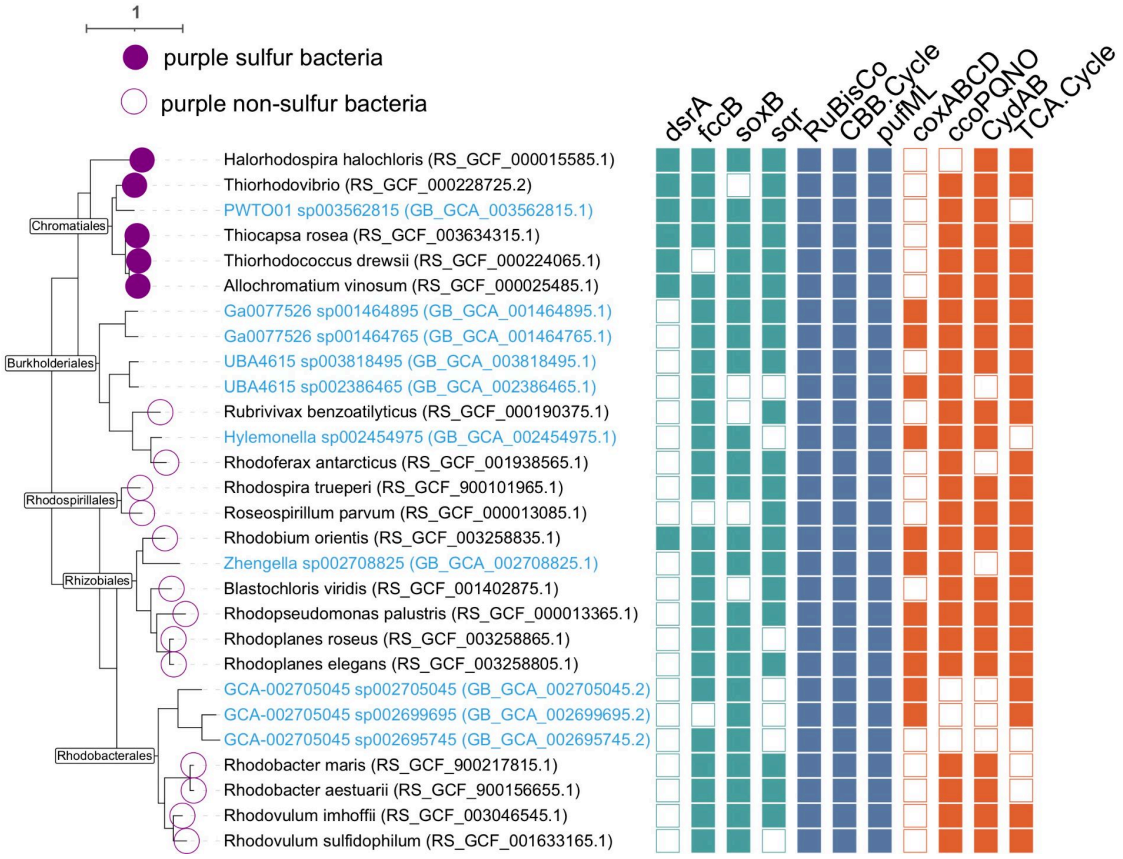
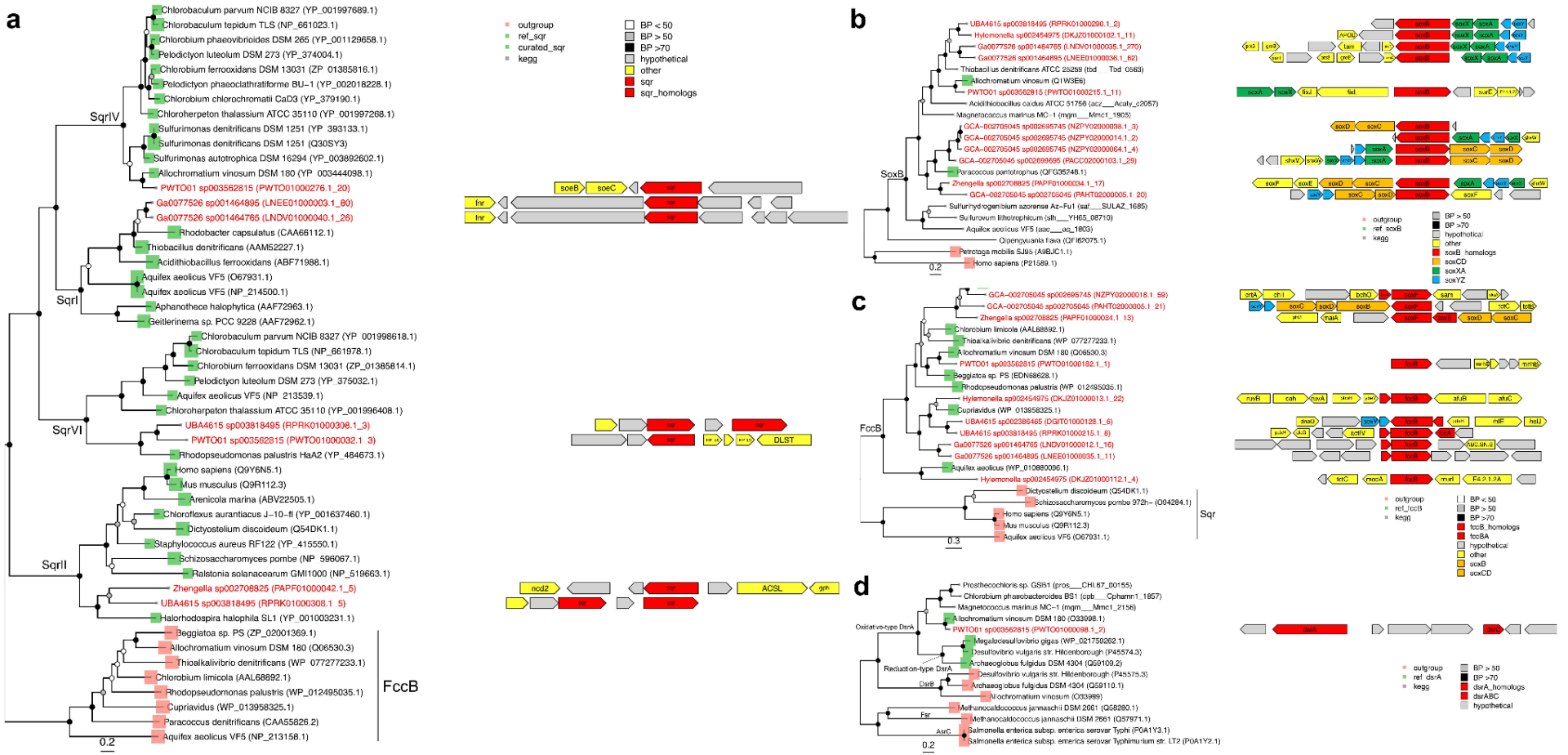


Fig. S12 | Phylogenomic tree and distribution of metabolic traits among microorganisms showing metabolic potential for photoautotrophic sulfur oxidation. The phylogenomic tree showing the evolutionary relationship of the species is pruned from the GTDB r95 bacterial tree of life. Candidate sulfur-oxidizing phototrophs are highlighted in cyan. The cultivated purple sulfur bacteria and purple non-sulfur bacteria are indicated by filled and empty purple circles. The presence/absence of genetic systems for sulfur oxidation (cyan-green), carbon fixation and photosynthetic systems (dark blue), and oxygen respiration (dark orange) is shown with filled/empty squares.dsrA, dissimilatory sulfite reductase, subunit A; fccB, sulfide dehydrogenase flavocytochrome c; soxB, L-cysteine S-thiosulfotransferase subunit soxB; sqr, sulfide:qunione oxidoreductase; RuBisCO, ribulose-bisphosphate carboxylase; CBB.cycle, Calvin-–Benson-–Bassham cycle; PufML, M and L subunit of the type II photosynthetic reaction center; CoxABCD, aa_3-type cytochrome oxidase; CcoPQNO, cbb_3-type cytochrome oxidase; CydAB, cytochrome bd ubiquinol oxidase; TCA.Cycle, tricarboxylic acid cycle. The species names for following genomes have been revised in GTDB r226 (the GTDB r95 taxa are shown in brackets): *Lamprobacter* sp003562815 (PWTO01 sp003562815), *Thermochromatium vinosum* (*Allochromatium vinosum*), CACIIZ01 sp002699695 (GCA-002705045 sp002699695), *Thiorhodovibrio frisius* (Thiorhodovibrio sp000228725), Ga0077523 sp001464895 (Ga0077526 sp001464895), *Imhoffiella*

drewsii (Thiorhodococcus drewsii), and Ga0077523 sp001464765 (Ga0077526 sp001464765).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SqrI
SqrVI
SqrII
FccB
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Pelodictyon luteolum DSM 273 (YP_374004.1)
Chlorobium ferrooxidans DSM 13031 (ZP_01385816.1)
Pelodictyon phaeoclathratiforme BU-1 (YP_002018228.1)
Chlorobium chlorochromatii CaD3 (YP_379190.1)
Chloroherpeton thalassium ATCC 35110 (YP_001997268.1)
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Cupriavidus (WP_013958325.1)
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soeB
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BP >70
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hypothetical

1033

1034

Fig. S13 | Phylogenetic analysis of selected sulfur-cycling genes predicted in candidate sulfur-oxidizing phototrophs. a, Phylogenetic analysis of Sqr**.** Sqr homologs from candidate anoxygenic phototrophs (highlighted in red) are affiliated with four distinct Sqr clades, namely SqrI, SqrII, SqrIV and SqrVI [90,91]. Functionally characterized Sqr and Sqr from well-studied sulfide-oxidizing genera (e.g., *Chlorobaculum*, *Sulfurimonas*, *Chloroflexus*, and *Allochromatium*) are indicated by green squares. The gene context for each Sqr is placed next to the tree; the colouring scheme is denoted in the figure legend. The name of neighbouring genes of *sqr* is based on the KEGG annotation. The phylogenetic tree was rooted using the flavocytochrome subunit of sulfide dehydrogenase (FccB; indicated with red squares). **b,** Phylogenetic analysis of SoxB**.** SoxB homologs from candidate phototrophs fall within the clade spanned by experimentally validated SoxB (e.g., those from *Allochromatium vinosum* and *Paracoccus pantotrophus*) and SoxB from known thiosulfate-oxidizing bacteria (e.g., *Sulfurovum lithotrophicum* and *Thiobacillus denitrificans*). The gene context for each SoxB is placed next to the tree; the colouring scheme is denoted in the figure legend. The name of neighbouring genes of *soxB* is based on the KEGG annotation. The phylogenetic tree was rooted using functionally divergent homologs (indicated with red squares), including 5-nucleotidase from *Homo sapiens* and mannosylglucosyl-3-phosphoglycerate phosphatase from *Petrotoga mobilis*. **c,** Phylogenetic analysis of FccB**.** FccB homologs from candidate phototrophs fall within the clade spanned by experimentally validated FccB (e.g., those from *Allochromatium vinosum* and *Paracoccus denitrificans*; green squares). The gene context for each FccB is placed next to the tree; the colouring scheme is denoted in the figure legend. The name of neighbouring genes of *fccB* is based on the KEGG annotation. The phylogenetic tree was rooted using sulfide:quinone reductase (indicated with red squares). **d,** Phylogenetic analysis of DsrA homologs detected in a candidate sulfur-oxidizing phototroph, PWTO01 sp003562815. The DsrA homolog is placed within the clade spanned by oxidative-type DsrA from green-sulfur bacteria (e.g., *Prosthecochloris* and *Chlorobium*) and purple-sulfur bacteria (e.g., *Allochromatium vinosum*; green squares). The gene context for each DsrA is placed next to the tree; the colouring scheme is denoted in the figure legend. The name of neighbouring genes is based on the KEGG annotation. The phylogenetic tree was rooted using functionally divergent homologs (indicated with red squares), including anaerobic sulfite reductase subunit C (AsrC) and coenzyme F_{420}-dependent sulfite reductase (Fsr). Branches with bootstrap value over 70%, between 50% and 70%, and below 50% are indicated by black, grey and white circles, respectively. Tree scale indicates the substitution of amino acids per site. The species names for following genomes have been revised in GTDB r226 (the GTDB r95 taxa are shown in brackets): *Lamprobacter* sp003562815 (PWTO01 sp003562815), *Thermochromatium vinosum* (*Allochromatium vinosum*), CACIIZ01 sp002699695 (GCA-002705045 sp002699695), *Thiorhodovibrio frisius* (*Thiorhodovibrio* sp000228725), Ga0077523 sp001464895 (Ga0077526 sp001464895), *Imhoffiella drewsii* (*Thiorhodococcus drewsii*), and Ga0077523 sp001464765 (Ga0077526 sp001464765).

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1079
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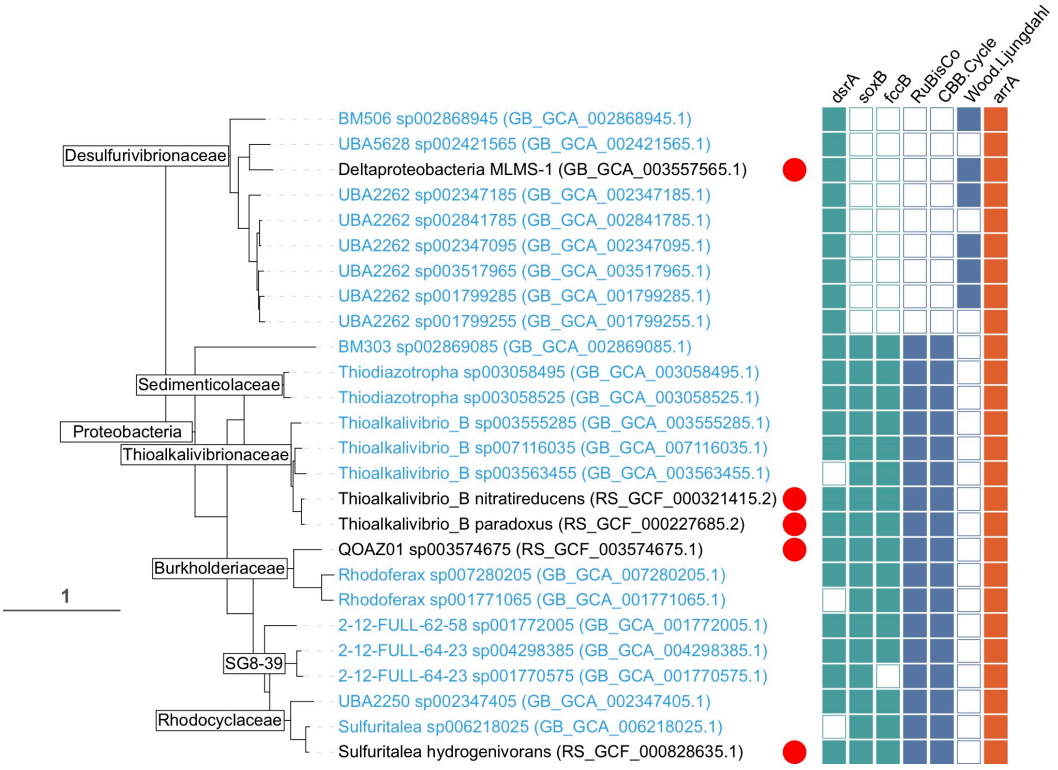
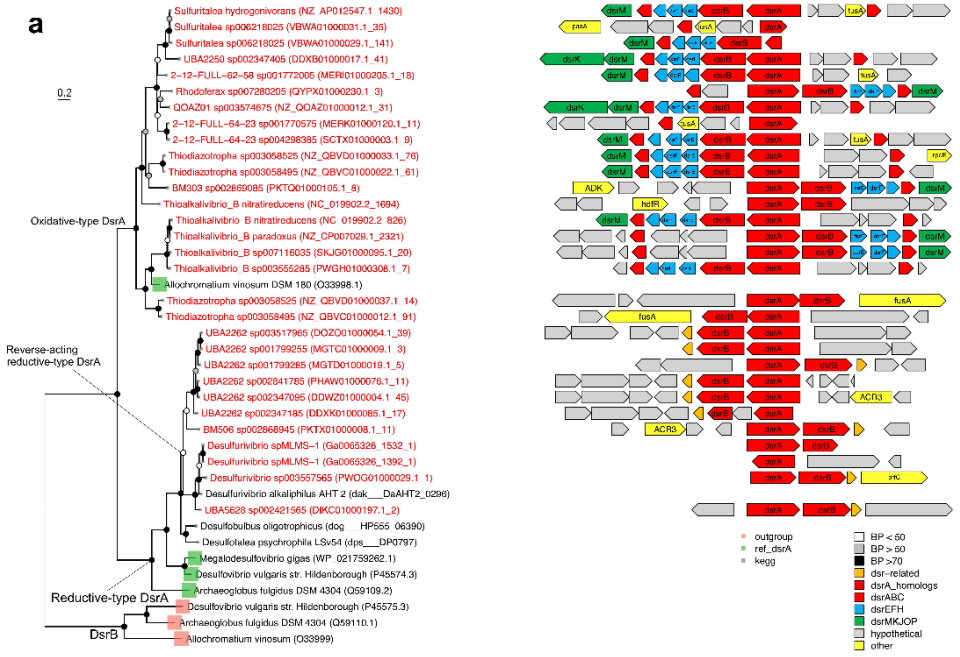


Fig. S14 | Phylogenomic tree and distribution of metabolic traits among microorganisms showing metabolic potential for arsenate-dependent sulfur oxidation. The phylogenomic tree showing the evolutionary relationship of the species is pruned from the GTDB r95 bacterial tree of life. The pure cultures are highlighted with red circles, with uncultured taxa colored in blue. The species names for following genomes have been revised in GTDB r226 (the GTDB r95 taxa are shown in brackets): *Thiovibrio* sp002347185 (UBA2262 sp002347185), *Thiovibrio* sp002347095 (UBA2262 sp002347095), *Thioalkalivibrio* sp007116035 (*Thioalkalivibrio*_B sp007116035), *Thiovibrio* sp003517965 (UBA2262 sp003517965), *Thiovibrio* sp002841785 (UBA2262 sp002841785), DSOT01 sp001772005 (2-12-FULL-62-58 sp001772005), *Calidifontimicrobium sediminis* (QOAZ01 sp003574675), *Thiovibrio* sp001799255 (UBA2262 sp001799255), *Thioalkalivibrio paradoxus* (*Thioalkalivibrio*_B *paradoxus*), *Thiovibrio* sp001799285 (UBA2262 sp001799285), *Thioalkalivibrio nitratireducens* (*Thioalkalivibrio*_B *nitratireducens*), *Sulfuricystis* sp002347405 (UBA2250 sp002347405), *Thioalkalivibrio* sp003563455 (*Thioalkalivibrio*_B sp003563455). The presence/absence of genetic systems for sulfur oxidation (cyan-green), carbon fixation (dark blue), and arsenate respiration (dark orange) is shown with filled/empty squares. dsrA, dissimilatory sulfite reductase, subunit A; fccB, sulfide dehydrogenase flavocytochrome c; soxB, L-cysteine S-thiosulfotransferase subunit soxB; RuBisCO, ribulose-bisphosphate carboxylase; CBB.cycle, Calvin-Benson-Bassham cycle; Wood Ljungdahl, Wood Ljungdahl pathway for carbon fixation; arrA, dissimilatory arsenate reductase.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Reverse-acting reductive-type DsrA
Reductive-type DsrA
DsrB
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Sulfuritalea sp006218025 (VBWA01000031.1_35)
Sulfuritalea sp006218025 (VBWA01000029.1_141)
UBA2250 sp002347405 (DDXB01000017.1_41)
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Rhodoferax sp007280205 (QYPX01000230.1_3)
QOAZ01 sp003574675 (NZ_QOAZ01000012.1_31)
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2-12-FULL-64-23 sp004298385 (SCTX01000003.1_9)
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BM303 sp002869085 (PKTQ01000105.1_8)
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Thioalkalivibrio_B paradoxus (NZ_CP007029.1_2321)
Thioalkalivibrio_B sp007116035 (SKJG01000095.1_20)
Thioalkalivibrio_B sp003555285 (PWGH01000308.1_7)
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Desulfurivibrio alkaliphilus AHT 2 (dak___DaAHT2_0296)
UBA5628 sp002421565 (DIKC01000197.1_2)
Desulfobulbus oligotrophicus (dog___HP555_06390)
Desulfotalea psychrophila LSv54 (dps___DP0797)
Megalodesulfovibrio gigas (WP_021759262.1)
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Archaeoglobus fulgidus DSM 4304 (Q59109.2)
Desulfovibrio vulgaris str. Hildenborough (P45575.3)
Archaeoglobus fulgidus DSM 4304 (Q59110.1)
Allochromatium vinosum (O33999)
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kegg
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BP > 50
BP >70
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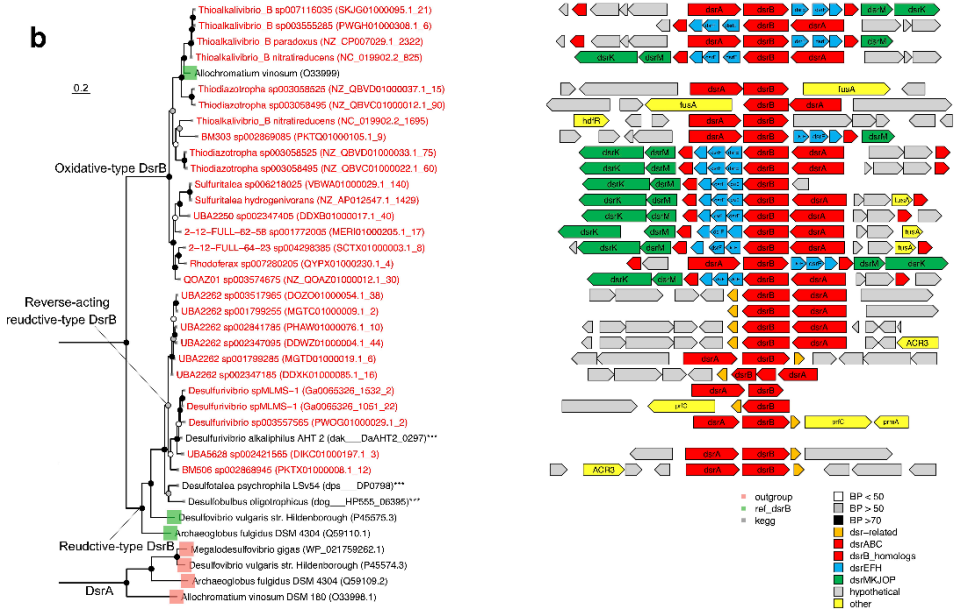
dsrABC
dsrEFH
dsrMKJOP
hypothetical
other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Oxidative-type DsrB
Reverse-acting reudctive-type DsrB
Reudctive-type DsrB
DsrA
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Megalodesulfovibrio gigas (WP_021759262.1)
Desulfovibrio vulgaris str. Hildenborough (P45574.3)
Archaeoglobus fulgidus DSM 4304 (Q59109.2)
Allochromatium vinosum DSM 180 (O33998.1)
0.2
outgroup
ref_dsrB
kegg
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BP > 50
BP >70
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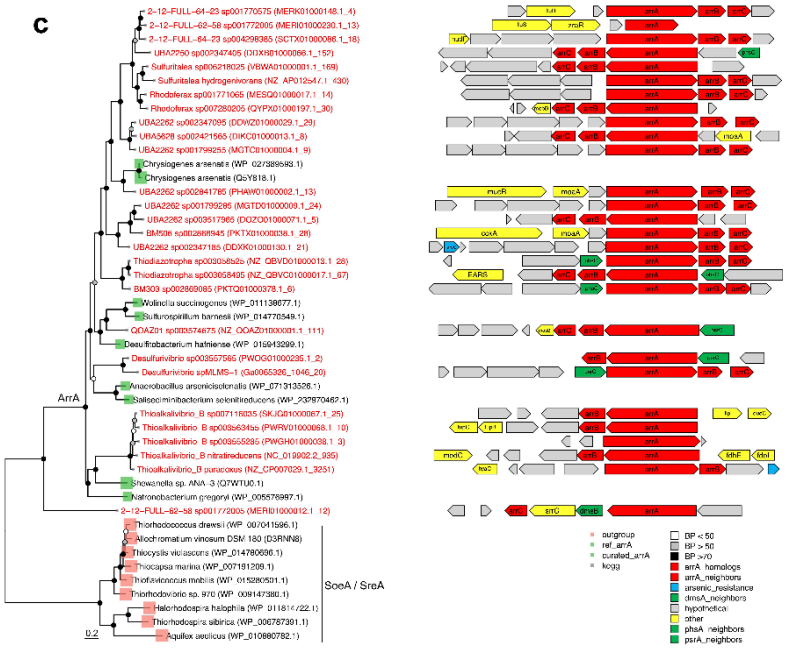
dsrB_homologs
dsrEFH
dsrMKJOP
hypothetical
other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Aquifex aeolicus (WP_010880782.1)
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curated_arrA
kegg
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BP > 50
BP >70
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arrA_neighbors
arsenic_resistance
dmsA_neighbors
hypothetical
other
phsA_neighbors
psrA_neighbors

Fig. S15 | Phylogenetic analysis of selected sulfur-cycling genes predicted in microorganisms with metabolic potential for arsenate-dependent sulfur oxidation. a, Phylogenetic analysis of DsrA. The DsrA homologs from proteobacterial lineages are placed within the clade spanned by oxidative-type DsrA from green-sulfur bacteria (e.g., *Prosthecochloris* and *Chlorobium*) and purple-sulfur bacteria (e.g., *Allochromatium vinosum*; green squares). The DsrA homologs from *Desulfurivibrionaceae* form a monophyletic clade branching within the reductive-type DsrA group. DsrA homologs from this clade may act in reverse for sulfur oxidation, as proposed in *Desulfurivibrio alkaliphilus*. The gene context for each DsrA is placed next to the tree; the colouring scheme is denoted in the figure legend. The name of neighbouring genes is based on the KEGG annotation. The phylogenetic tree was rooted using DsrB (indicated with red squares). **b,** Phylogenetic analysis of DsrB. The DsrB homologs from the proteobacterial lineages are placed within the clade spanned by oxidative-type DsrB from green-sulfur bacteria (e.g., *Prosthecochloris* and *Chlorobium*) and purple-sulfur bacteria (e.g., *Allochromatium vinosum*; green squares). The DsrB homologs from *Desulfurivibrionaceae* form a monophyletic clade within the reductive-type DsrB group. DsrB homologs from this clade may act in reverse for sulfur oxidation, as proposed in *Desulfurivibrio alkaliphilus*. The gene context for each DsrB is placed next to the tree; the colouring scheme is denoted in the figure legend. The name of neighbouring genes is based on the KEGG annotation. The phylogenetic tree was rooted using DsrA (indicated with red squares).**c,** Phylogenetic analysis of ArrA**.** The ArrA homologs are placed within the clade spanned by reference ArrA from bacterial species known to respire arsenate (e.g., *Shewanella* sp. ANA-3, *Chrysiogenes arsenatis*, and *Anaerobacillus arseniciselenatis* [72]. The gene context for each ArrA is placed next to the tree; the colouring scheme is denoted in the figure legend. The name of neighbouring genes is based on the KEGG annotation. The phylogenetic tree was rooted using functionally divergent homologs (indicated with red squares), including sulfite dehydrogenase subunit SoeA and sulfur reductase subunit SreA. Branches with bootstrap value over 70%, between 50% and 70%, and below 50% are indicated by black, grey and white circles, respectively. The scale bar indicates the number of amino acids substitution per site. The species names for following genomes have been revised in GTDB r226 (the GTDB r95 taxa are shown in brackets): *Thiovibrio* sp002347185 (UBA2262 sp002347185), *Thiovibrio* sp002347095 (UBA2262 sp002347095), *Thioalkalivibrio* sp007116035 (*Thioalkalivibrio*_B sp007116035), *Thiovibrio* sp003517965 (UBA2262 sp003517965), *Thiovibrio* sp002841785 (UBA2262 sp002841785), DSOT01 sp001772005 (2-12-FULL-62-58 sp001772005), *Calidifontimicrobium sediminis* (QOAZ01 sp003574675), *Thiovibrio* sp001799255 (UBA2262 sp001799255), *Thioalkalivibrio paradoxus* (*Thioalkalivibrio*_B *paradoxus*), *Thiovibrio* sp001799285 (UBA2262 sp001799285), *Thioalkalivibrio nitratireducens* (*Thioalkalivibrio*_B *nitratireducens*), *Sulfuricystis* sp002347405 (UBA2250 sp002347405), *Thioalkalivibrio* sp003563455 (*Thioalkalivibrio*_B sp003563455).

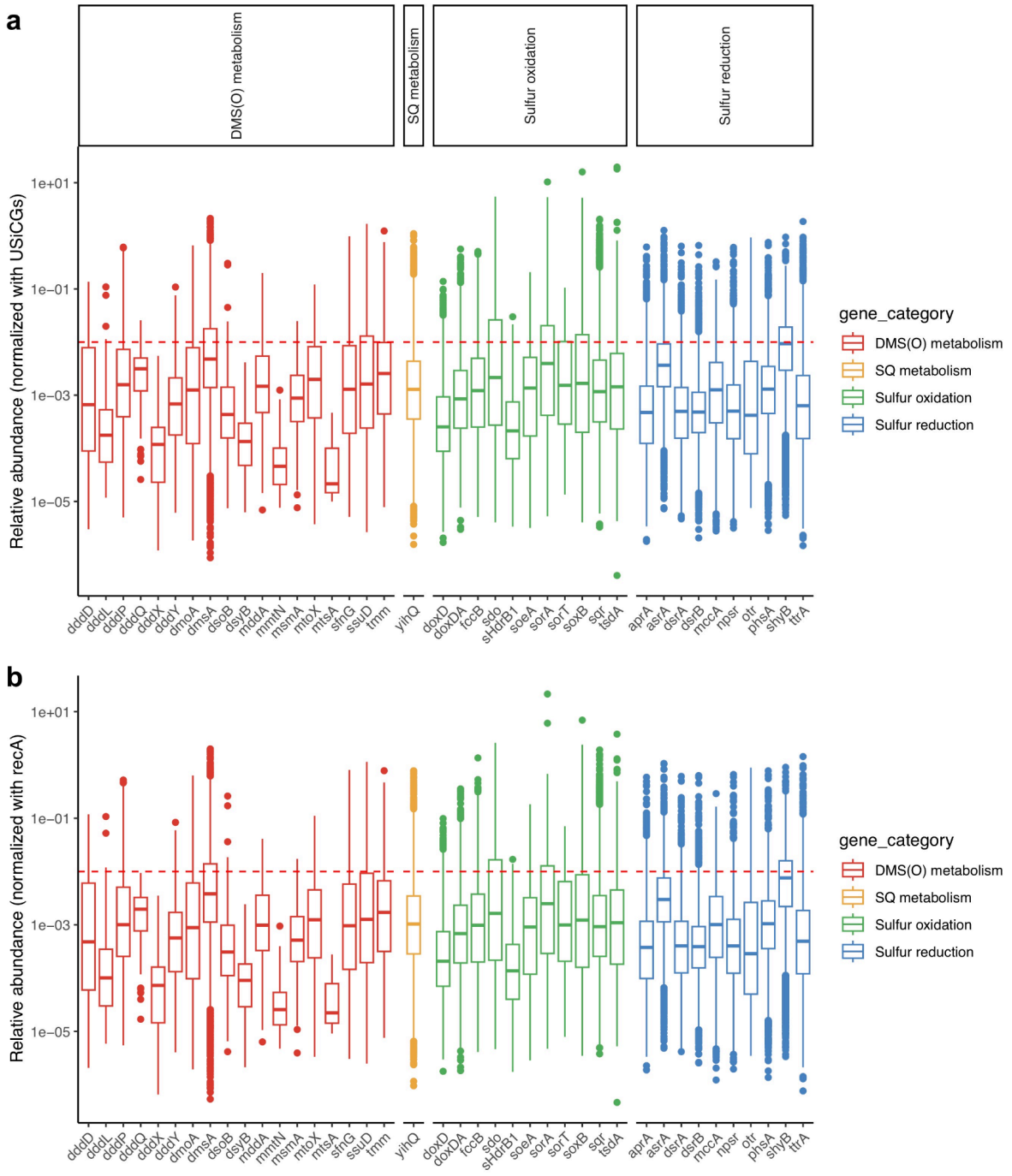


Fig. S16 | Relative abundance of each sulfur-cycling functional guild across >10000 metagenomes from the GMGC v1.0 database. The relative abundance in panel **a** is estimated by normalizing the abundance of sulfur-cycling genes against the median abundance of 76 universal single-copy genes (USiCGs). The relative abundance in panel **b** is estimated by normalizing the abundance of sulfur-cycling genes against the sum abundance of housekeeping gene *recA*. The dotted line indicates a 1% relative abundance. DMS, dimethylsulfide; DMSO, dimethyl sulfoxide SQ, sulfoquinovose. The full name of each sulfur-cycling gene is shown in Data S1.

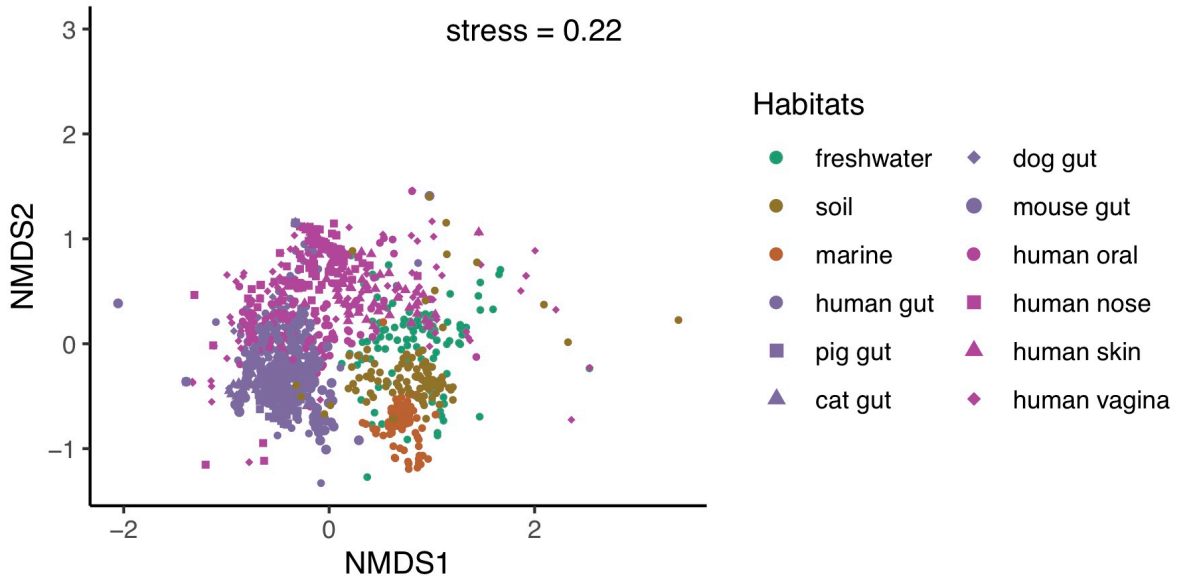


Fig. S17 | Beta diversity of 42 sulfur-cycling genes across metagenome samples from twelve major habitats. The Bray-Curtis dissimilatory was calculated from the abundance profile of sulfur-cycling genes across metagenome samples. The stress value, indicating the goodness-of-fit of the non-metric multidimensional scaling (NMDS) analysis, is shown in the top right corner. The differences in sulfur-cycling gene composition across habitats is statistically significant (PERMANOVA, $P < 0.01$; permutations = 100). For visualization purposes, maximally 100 randomly selected samples from each habitat are shown.

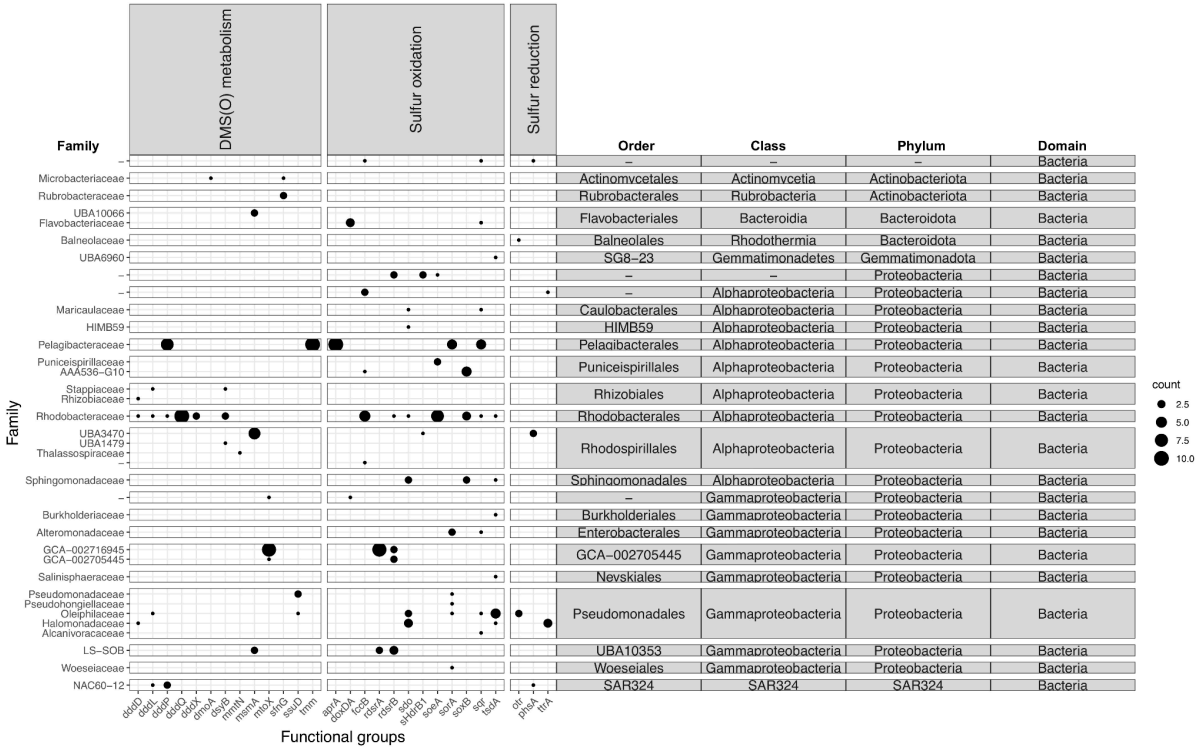


Fig. S18 | Taxonomic affiliation of the top ten most prevalent sulfur-cycling unigenes in the ocean waters. The prevalent unigenes were further filtered to include those detected in more than 30 out of 130 ocean metagenome samples. The taxonomy classifications down to family rank (i.e., domain, phylum, class, order, and family) from GTDB r95 is shown for unigenes. '-' indicates unigenes that were unclassified at this taxonomic rank. Only unigenes with family level taxonomic annotation are shown. The size of the bubble indicates the number of unigenes affiliated to a specific family. All retrieved *dsrA/B* unigenes are phylogenetically affiliated to oxidative-type, and thus are shown in the sulfur oxidation category. DMS, dimethylsulfide; DMSO, dimethyl sulfoxide. The changed taxonomy in GTDB r226 included: the phylum *Pseudomonadota* (*Proteobacteria* in GTDB r95), the order *Arenicellales* (UBA10353), the family *Parvicellaceae* (UBA10066), the family Casp-alpha2 (UBA3470), the family Casp-alpha2 (UBA1479), and the family UBA868 (LS-SOB).

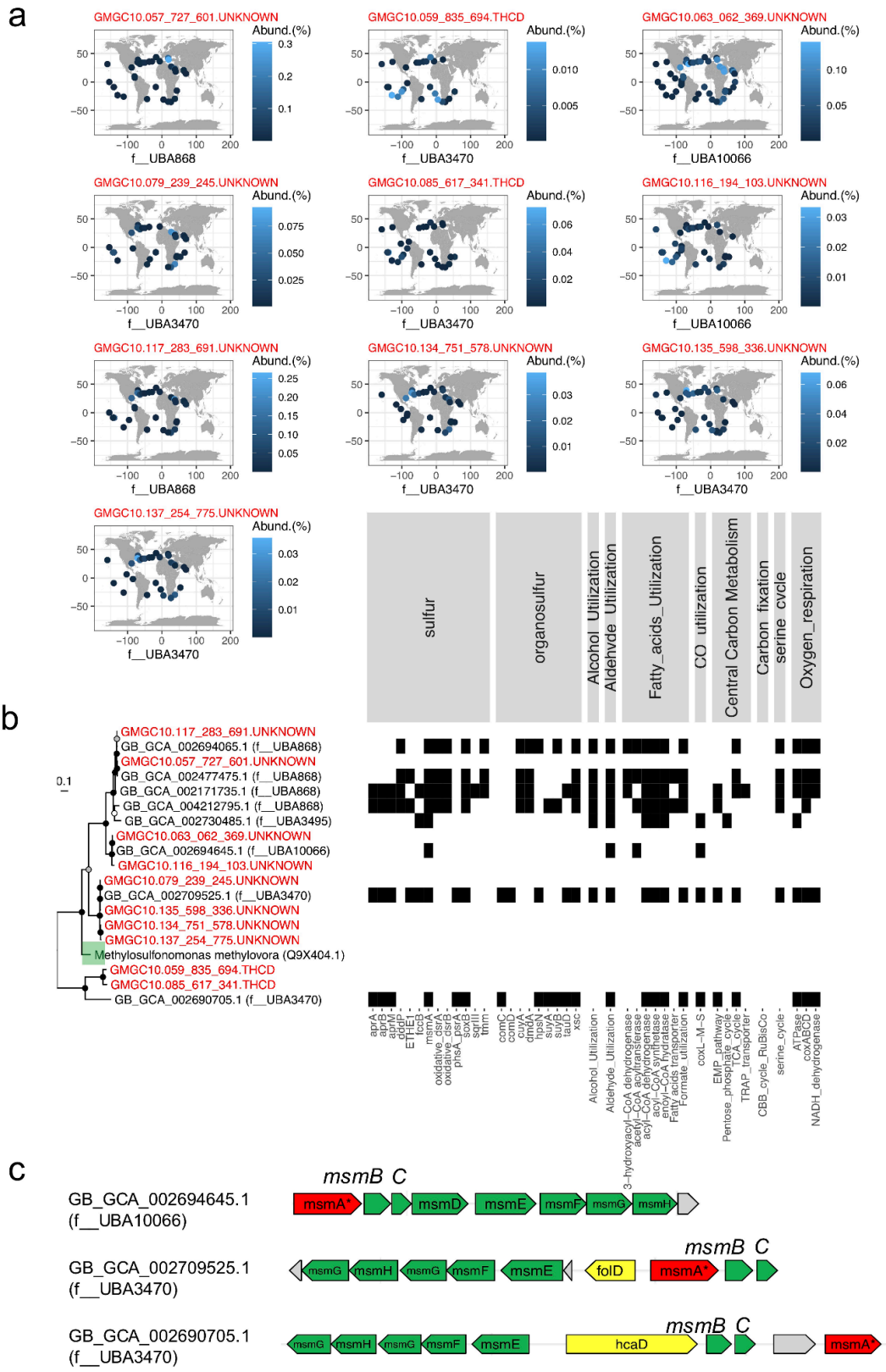
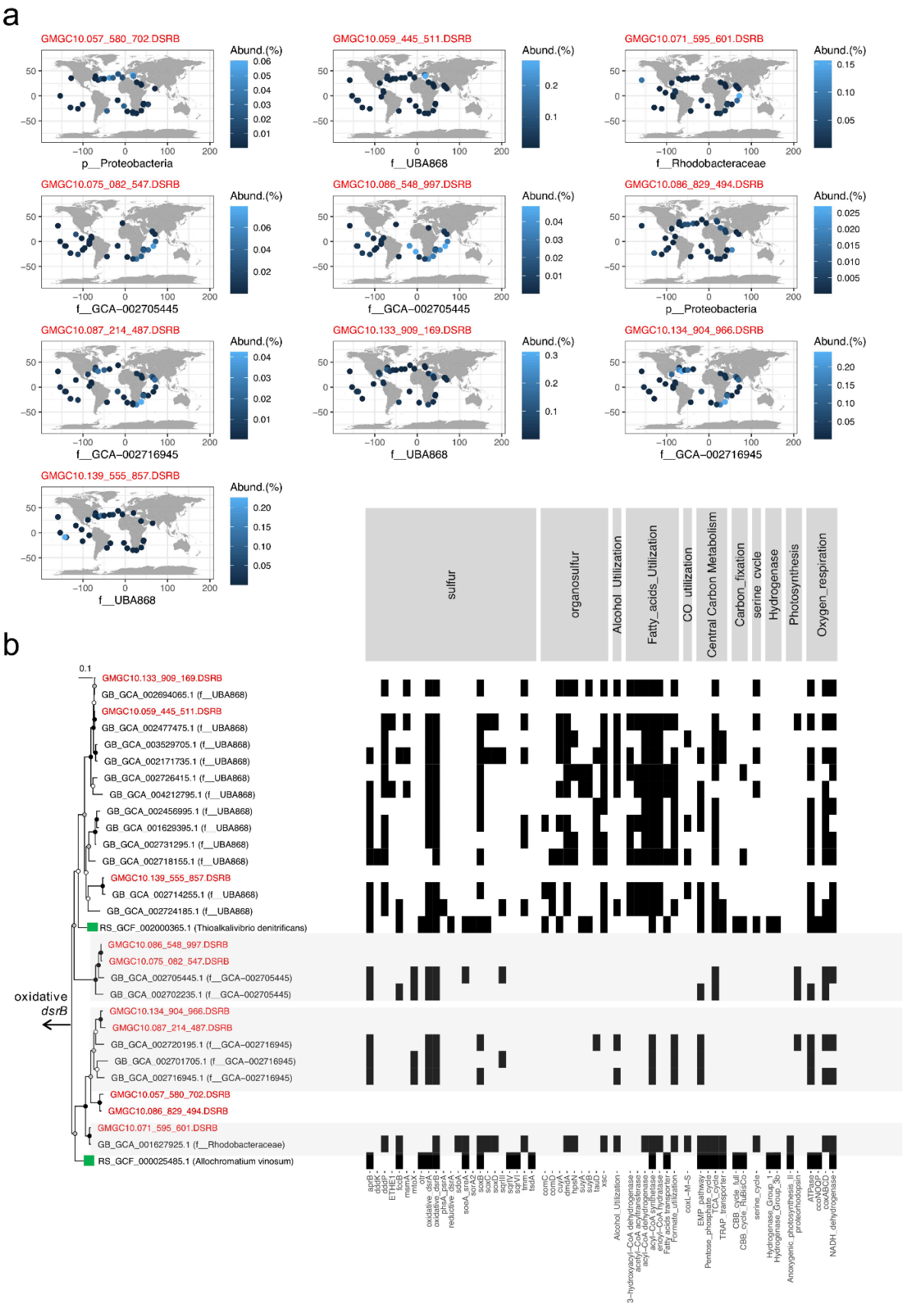


Fig. S19 | Biogeography, phylogeny, metabolic property, and genomic context of the ten most prevalent *msmA*-carrying microorganisms in seawater. a, *recA*-normalized *msmA* abundance across different ocean regions. The accession (red) and family-level classification from GTDB r95 of each *msmA* unigene are

shown on the top and bottom of the panel. **b**, The MsmA phylogeny was reconstructed using inferred amino acid sequences of the top ten most prevalent *msmA* unigenes (red) and their most closely related sequences in GTDB r95 species (black). Genome accessions of GTDB r95 species are denoted in parenthesis. Branches with bootstrap value over 70%, between 50% and 70%, and below 50% are indicated by black, grey, and white circles, respectively. Functionally characterized MsmA encoded in *Methylosulfonomonas methylovora* is indicated by green squares. The occurrence of metabolic pathways involved in sulfur, carbon, and energy metabolism in GTDB species is shown in the right panel. Detailed description of each metabolic module and its constituent genes can be found in Data S2. **c**, Genomic context of *msmA* from UBA10066 and UBA3470. The *msmABCD* operon encodes the large (α) and small (β) subunit hydroxylase components, the ferredoxin, and reductase of methanesulfonate monooxygenase, respectively; *msmEFGH* encodes a methanesulfonate transporter. *msmA* is highlighted in red, whereas other *msm* genes are colored in green. *folD*: methylenetetrahydrofolate dehydrogenase ($NADP^+$) / methenyltetrahydrofolate cyclohydrolase; *hcaD*: 3-phenylpropionate/trans-cinnamate dioxygenase ferredoxin reductase component. The changed family-level classifications in GTDB r226 included: *Parvicellaceae* (UBA10066) and Casp-alpha2 (UBA3470).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GMGC10.075_082_547.DSRB
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GMGC10.133_909_169.DSRB
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GMGC10.134_904_966.DSRB
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GMGC10.139_555_857.DSRB
f__UBA868
Abund.(%)
b
sulfur
organosulfur
Alcohol_Utilization
Fatty_acids_Utilization
CO_utilization
Central Carbon Metabolism
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serine_cycle
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Photosynthesis
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GB_GCA_002694065.1 (f__UBA868)
GMGC10.059_445_511.DSRB
GB_GCA_002477475.1 (f__UBA868)
GB_GCA_003529705.1 (f__UBA868)
GB_GCA_002171735.1 (f__UBA868)
GB_GCA_002726415.1 (f__UBA868)
GB_GCA_004212795.1 (f__UBA868)
GB_GCA_002456995.1 (f__UBA868)
GB_GCA_001629395.1 (f__UBA868)
GB_GCA_002731295.1 (f__UBA868)
GB_GCA_002718155.1 (f__UBA868)
GMGC10.139_555_857.DSRB
GB_GCA_002714255.1 (f__UBA868)
GB_GCA_002724185.1 (f__UBA868)
RS_GCF_002000365.1 (Thicalkalivibrio denitrificans)
GMGC10.086_548_997.DSRB
GMGC10.075_082_547.DSRB
GB_GCA_002705445.1 (f__GCA−002705445)
GB_GCA_002702235.1 (f__GCA−002705445)
oxidative dsrB
GMGC10.134_904_966.DSRB
GMGC10.087_214_487.DSRB
GB_GCA_002720195.1 (f__GCA−002716945)
GB_GCA_002701705.1 (f__GCA−002716945)
GB_GCA_002716945.1 (f__GCA−002716945)
GMGC10.057_580_702.DSRB
GMGC10.086_829_494.DSRB
GMGC10.071_595_601.DSRB
GB_GCA_001627925.1 (f__Rhodobacteraceae)
RS_GCF_000025485.1 (Allochromatium vinosum)
aprB
dsrK
dddP
ETHE1
soxA
moaA
mtoX
cutA
oxidative_dsrA
oxidative_dsrB
phsA_psrA
reductive_dsrK
sdoA
soeA_sreA
soxZ
soxB
soxC
soxD
sqrIII
sqrIV
sqrV
tmm
tsdA
comC
comD
cysA
dmdA
hpsN
suyB
tauA
tauD
xsc
Alcohol_Utilization
3−hydroxyacyl−CoA dehydrogenase
acetyl−CoA acyltransferase
acyl−CoA dehydrogenase
acyl−CoA synthetase
enoyl−CoA hydratase
Fatty_acids_Utilization
formate_utilization
coxL−M−S
EMP_pathway
Pentose_phosphate_cycle
TCA_cycle
TRAP_transporter
CBB_cycle_full
CBB_cycle_RuBisCo
serine_cycle
Hydrogenase_Group_1
Hydrogenase_Group_3b
Anoxygenic_photosynthesis_II
proteorhodopsin
ATPase
coxNOQP
coxMGB
NADH_dehydrogenase

Fig. S20 | Biogeography, phylogeny, and metabolic properties of the ten most prevalent *dsrB*-carrying microorganisms in seawater. a, *recA*-normalized *dsrB* abundance across different ocean regions. The accession (red) and family-level classification of GTDB r95 of each *dsrB* unigene are shown on the top and bottom of the panel (when available). The family-level taxonomy for GMGC10.057_580_702.DSRB and GMGC10.086_829_494.DSRB were not resolved based on dual-blast taxonomy classification algorithm, and their phylum-level are shown in the figure. **b**, The DsrB phylogeny was reconstructed using inferred amino acid sequences of the top ten most prevalent *dsrB* unigenes (red) and their closely related sequences in GTDB species (black). GTDB species are denoted using their genome accession and their family level classification (in parenthesis). Branches with bootstrap value over 70%, between 50% and 70%, and below 50% are indicated by black, grey, and white circles, respectively. Functionally characterized DsrB encoded in *Allochromatium vinosum* and *Thioalkalivibrio denitrificans* are indicated by green squares. The occurrence of metabolic pathways involved in sulfur, carbon, and energy metabolism in GTDB species is shown in the right panel. Detailed description of each metabolic module and its constituent genes can be found in Data S2. The changed taxonomy in GTDB r226 included: the phylum *Pseudomonadota* (*Proteobacteria*).

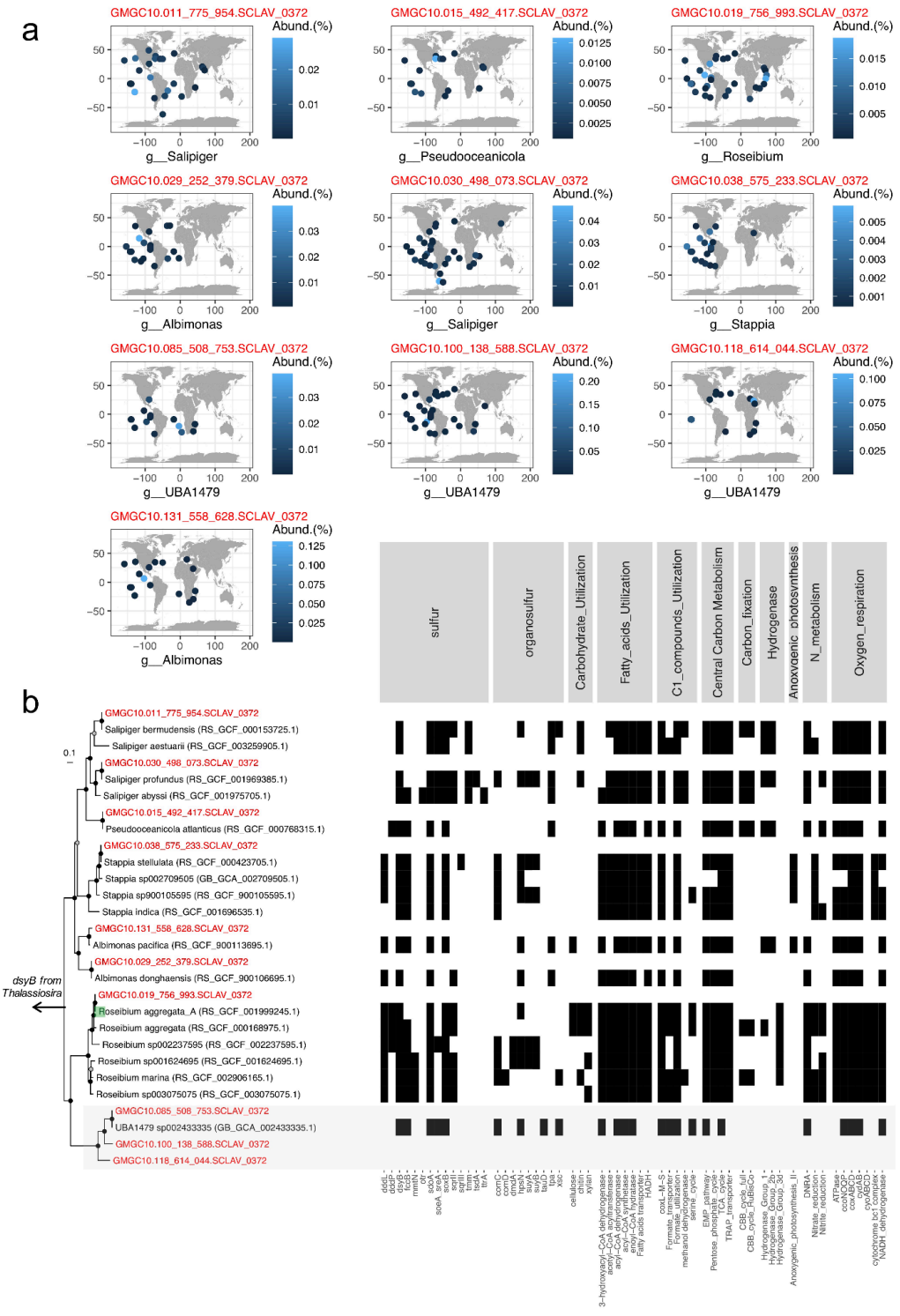

Fig. S21 | Biogeography, phylogeny, and metabolic properties of the ten most prevalent *dsyB*-carrying microorganisms in seawater. a, *recA*-normalized *dsyB* abundance across different ocean regions. The accession (red) and genus-level classification from GTDB r95 of each *dsyB* are shown on the top and bottom of the panel. **b**, The DsyB phylogeny was reconstructed using inferred amino acid sequences of the top ten most prevalent *dsyB* (red) and their most closely related sequences in GTDB species (black). Genome accessions of GTDB species are denoted in parenthesis. Branches with bootstrap value over 70%, between 50% and

70%, and below 50% are indicated by black, grey, and white circles, respectively. Functionally characterized DsyB encoded in *Roseibium aggregatum* (formerly *Labrenzia aggregata*) is indicated by green squares. The occurrence of metabolic pathways involved in sulfur, carbon, and energy metabolism in GTDB species is shown in the right panel. Detailed description of each metabolic module and its constituent genes can be found in Data S2.

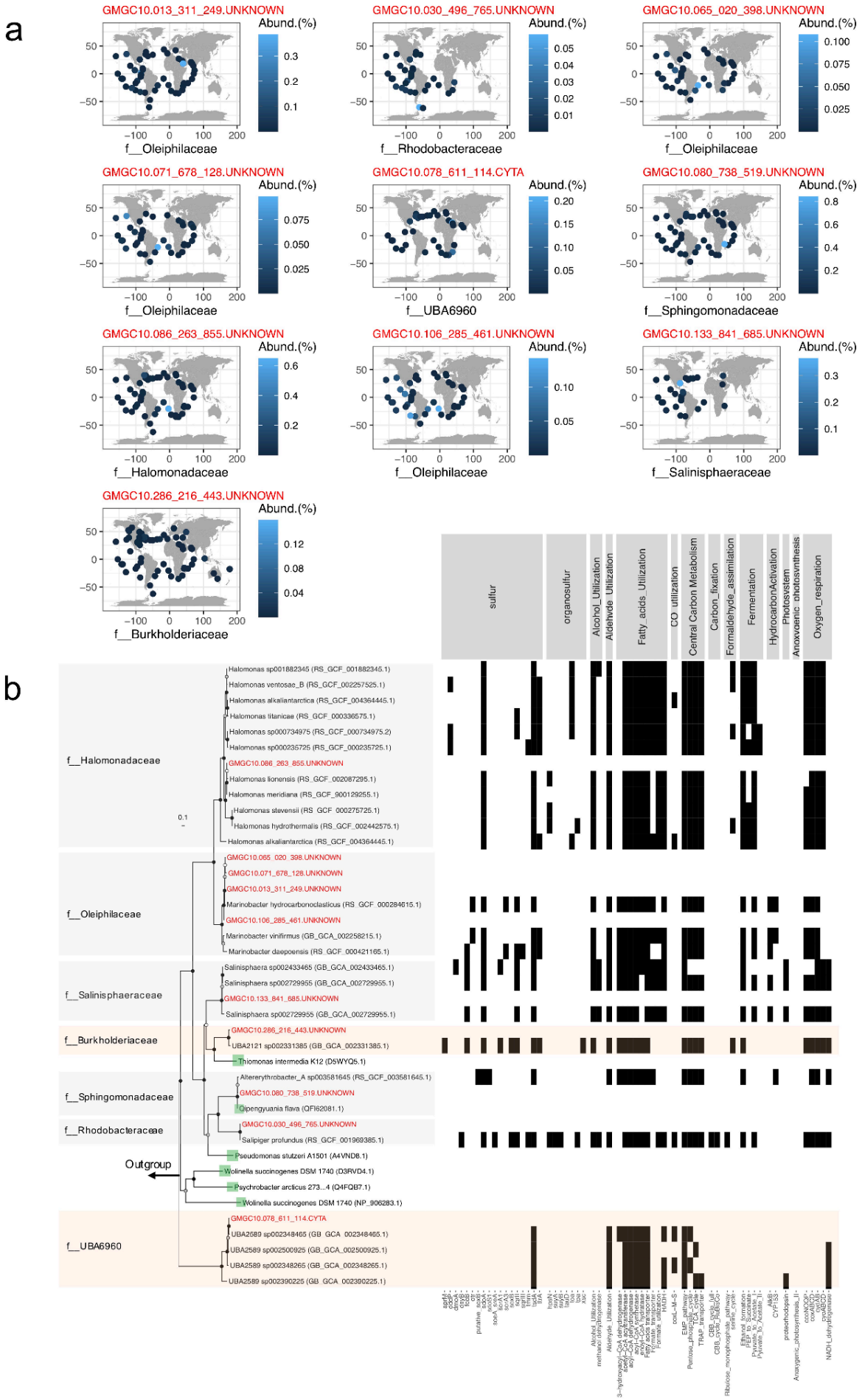
a
GMGC10.013_311_249.UNKNOWN
f__Oleiphilaceae
GMGC10.030_496_765.UNKNOWN
f__Rhodobacteraceae
GMGC10.065_020_398.UNKNOWN
f__Oleiphilaceae
GMGC10.071_678_128.UNKNOWN
f__Oleiphilaceae
GMGC10.078_611_114.CYTA
f__UBA6960
GMGC10.080_738_519.UNKNOWN
f__Sphingomonadaceae
GMGC10.086_263_855.UNKNOWN
f__Halomonadaceae
GMGC10.106_285_461.UNKNOWN
f__Oleiphilaceae
GMGC10.133_841_685.UNKNOWN
f__Salinisphaeraceae
GMGC10.286_216_443.UNKNOWN
f__Burkholderiaceae
Abund.(%)
b
sulfur
organosulfur
Alcohol_Utilization
Aldehyde_Utilization
Fatty_acids_Utilization
CO_utilization
Central Carbon Metabolism
Carbon_fixation
Formaldehyde_assimilation
Fermentation
HydrocarbonActivation
Photosystem
Anoxygenic_photosynthesis
Oxygen_respiration
f__Halomonadaceae
f__Oleiphilaceae
f__Salinisphaeraceae
f__Burkholderiaceae
f__Sphingomonadaceae
f__Rhodobacteraceae
Outgroup
f__UBA6960

Fig. S22 | Biogeography, phylogeny, and metabolic properties of the ten most prevalent *tsdA*-carrying microorganisms in seawater. a, *recA*-normalized *tsdA* abundance across different ocean regions. The accession (red) and family-level classification from GTDB r95 of each *tsdA* are shown on the top and bottom of the panel. **b**, The TsdA phylogeny was reconstructed using inferred amino acid sequences of the top ten most prevalent *tsdA* unigenes (red) and their most closely related sequences in GTDB species (black). Genome accessions of GTDB species are denoted in parenthesis. Branches with bootstrap value over 70%, between 50% and 70%, and below 50% are indicated by black, grey, and white circles, respectively. Functionally characterized TsdA are indicated by green squares. The occurrence of metabolic pathways involved in sulfur, carbon, and energy metabolism in GTDB species is shown in the right panel. Detailed description of each metabolic module and its constituent genes can be found in Data S2.

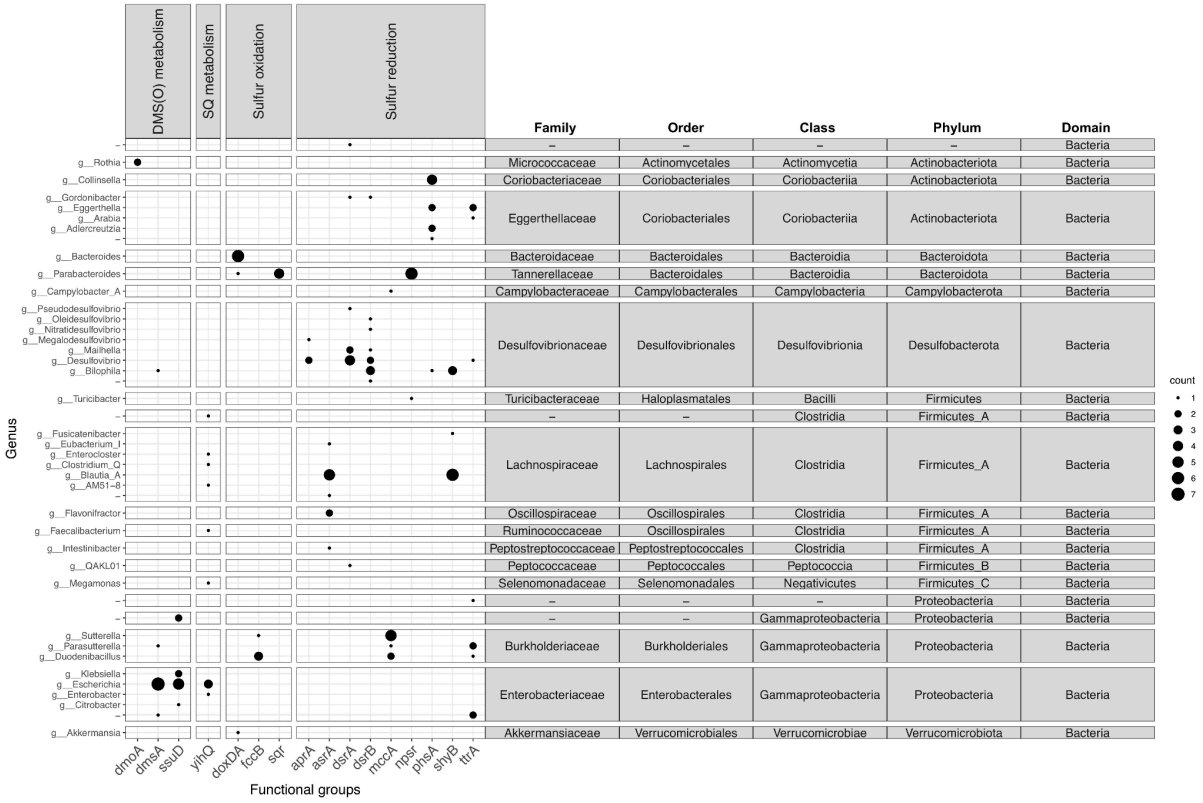

Fig. S23 | Taxonomic affiliation of the top ten most prevalent sulfur-cycling unigenes in the human gut. The prevalent sulfur-cycling unigenes were further filtered to include those detected in more than 350 out of 7052 human gut metagenome samples. The taxonomy classifications down to genus rank (i.e., domain, phylum, class, order, family, genus) is shown for unigenes. '-' indicates unigenes that were unclassified at this taxonomic rank. The extracted *dsrA/B* unigenes are classified as reductive-type, and thereby are shown in the sulfur reduction category. The size of the bubble indicates the number of unigenes affiliated to a specific family. DMS, dimethylsulfide; DMSO, dimethyl sulfoxide.

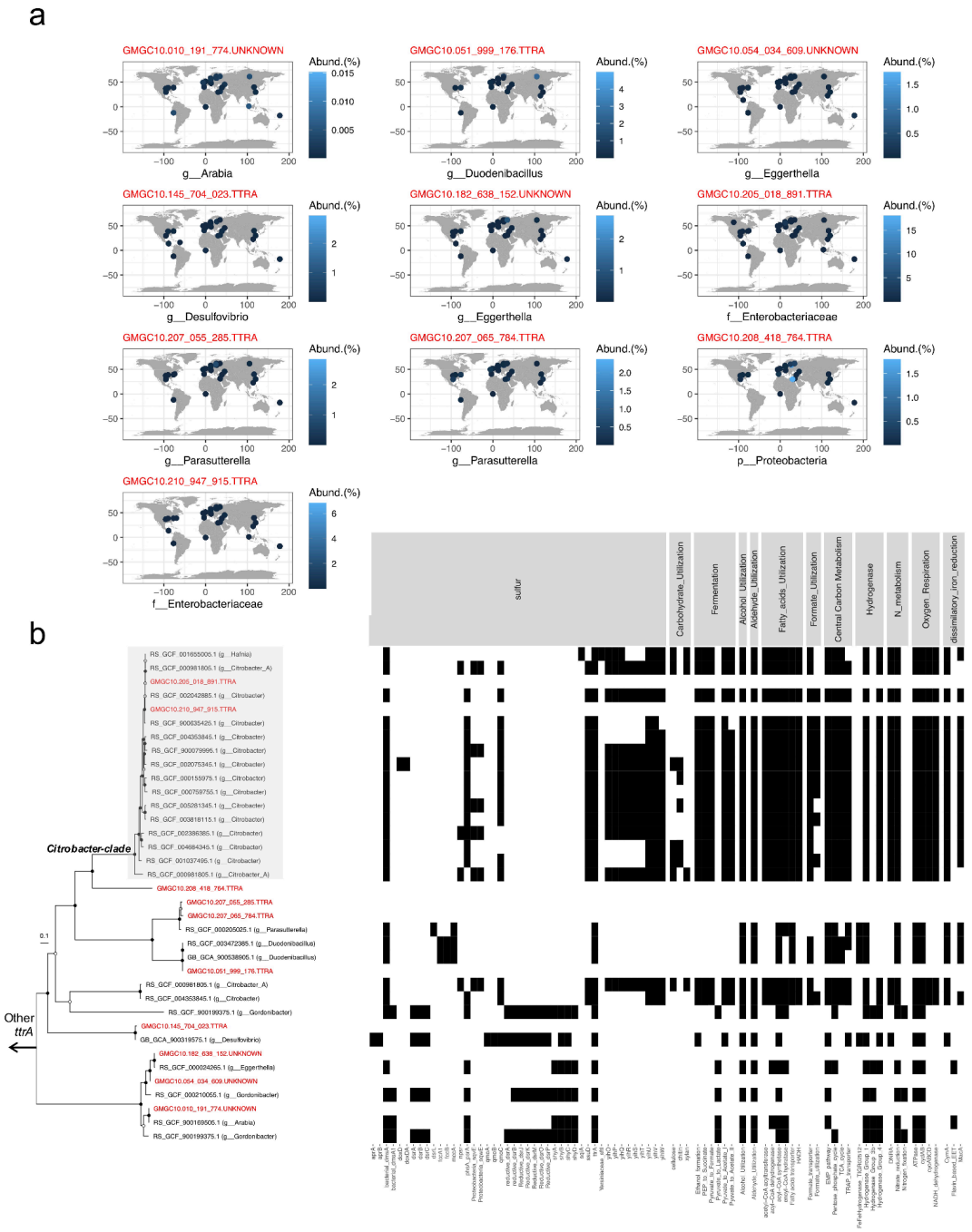

Fig. S24 | Biogeography, phylogeny, and metabolic properties of the ten most prevalent *ttrA*-carrying microorganisms in the human gut. a, *recA*-normalized *ttrA* abundance across different human gut metagenomes. The accession (red) and genus- (g__) or family-level (f__) classification from GTDB r95 of each *ttrA* are shown on the top and bottom of the panel. **b**, The TtrA phylogeny was reconstructed using inferred amino acid sequences of the top ten most prevalent *ttrA* unigenes (red) and their most closely related sequences in GTDB species (black). Genome accessions of GTDB species are denoted in parenthesis. Branches with bootstrap value over 70%, between 50% and 70%, and below 50% are indicated by black,

grey, and white circles, respectively. The occurrence of metabolic pathways involved in sulfur, carbon, and energy metabolism in GTDB species is shown in the right panel. Detailed description of each metabolic module and its constituent genes can be found in Data S2. We inferred that two *Enterobacteriaceae ttrA* unigenes (GMGC10.205_018_891.TTRA and GMGC10.210_947_915.TTRA) have *Citrobacter* origin, as they fall within a clade dominated by *Citrobacter* species (highlighted with gray background).

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