Novel bacterial group potentially dominates sulfur cycling in the dark ocean

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Article

Keywords:

Posted Date: April 20th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1561045/v1

Version of Record: A version of this preprint was published at Nature Microbiology on April 24th, 2023. See the published version at https://doi.org/10.1038/s41564-023-01374-2.

Abstract

The dark ocean (>200 m depth) is the largest habitat on Earth. Dissolved inorganic carbon (DIC) fixation in the oxygenated waters of the dark ocean is in the same order of magnitude as heterotrophic microbial biomass production 1,2. Recent evidence suggests sulfur oxidation could be a major energy source for deep ocean microbes 3. However, the global relevance and the identity of the major players in sulfur oxidation in the oxygenated deep-water column remain elusive. Here we combined single-cell genomics, community metagenomics, metatranscriptomics and single-cell activity measurements to present a novel, ubiquitous mixotrophic bacterial group (UBA868) dominating the total expression of RuBisCO genes (up to 40%) and of key sulfur oxidation (soxB and rdsrA) genes (up to 100%) in the global dark ocean. Our study also underscores the unrecognized importance of mixotrophic microbes, such as UBA868, in the biogeochemical cycles of the deep ocean.

Introduction

The dark ocean (> 200 m depth) makes up around 90% of the global ocean's volume, constituting the largest habitat of the biosphere (~ 1.3 x 10^{18} m^3) and contains one of the largest reservoirs of microorganisms on the planet and the largest in aquatic systems [4]. Microorganisms are the major transformers of energy and matter in the dark ocean, remineralizing organics produced in sunlit surface waters and chemoautotrophically fixing dissolved inorganic carbon (DIC) [5]. This microbial dark DIC fixation is a globally relevant process comparable, based on rate and budget calculations, to the microbial heterotrophic carbon biomass production [1]. Also, dark DIC fixation is similar in magnitude to the sinking particulate organic carbon flux [2]. However, the energy sources required to support dark DIC fixation in the oxygenated global ocean remain largely enigmatic since its discovery almost two decades ago [5]. Originally, ammonia was thought to be the only energy source of dark DIC fixation, motivated by the discovery of the autotrophic ammonia oxidizing Crenarchaeota Group I [6,7]. However, the low ammonia supply to the lower mesopelagic ocean was found insufficient to account for the DIC fixation rates [5]. Similarly, nitrite oxidation by bacteria (*Nitrospina* and *Nitrospira*), another process fueling dark DIC fixation, cannot account for the measured DIC fixation rates outside the oxygen minimum zones of the global ocean [8,9].

Another potential energy source for DIC fixation is the oxidation of reduced sulfur compounds, widespread in hydrothermal vent plumes and oxygen minimum zones [10–13]. However, in the oxygenated oceanic water column the presence and efficient utilization of reduced sulfur compounds has been more difficult to explain, unless chemolithoautotrophic activity is linked to suboxic microniches (e.g. deep water marine snow) [14]. Current evidence also indicates that the degradation of dissolved organic sulfur compounds from sinking phytoplankton biomass [15] as well secretion by zooplankton [16,17] produces reduced and oxidized forms of inorganic sulfur species (e.g., sulfide, thiosulfate and sulfite) in the oxic water column [15]. Also a recent study suggests the potential for sulfite generation from organic compounds, such as taurine, to be further oxidized to sulfate by members of the SAR324 and the

gammaproteobacterial ARCTIC96BD-19 clades [18]. Recently, a positive correlation between the abundance of sulfur oxidation genes and RuBisCO with depth was reported across the Atlantic Ocean, suggesting an increasing potential for sulfur oxidation in the dark ocean [3]. Yet, the potential for sulfur oxidation and the key taxa responsible remain largely elusive, particularly in the global ocean outside oxygen minimum zones, i.e., the oxygenated deep-water column.

We combined single-amplified genomes (SAGs), metagenome assembled genomes (MAGs) and microautoradiography with catalyzed-reported-deposition fluorescence *in situ* hybridization (MICRO-CARD-FISH) to explore the potential of sulfur oxidation and activity in the global dark ocean. This approach was complemented by a global analysis of the expression of sulfur oxidation genes compiled from the *Tara* Oceans expedition; see Material and Methods [19]. These analyses revealed the presence of a novel but ubiquitous mixotrophic gammaproteobacterial clade, temporarily classified in the family UBA868 as per the Genome Taxonomy Database (GTDB [20]).

Results And Discussion

First evidence of the major role of UBA868 in sulfur cycling and DIC fixation came from our recent analysis of the microbiome in the oceanic cavity underneath the ca. 400 m of ice of the Ross Sea Ice Shelf [21]. Figure 1 shows that UBA868 (formerly called UBA10353) was among the most active microbes transcribing RuBisCO and also dominated the transcription of genes indicative of sulfur oxidation (sulfur oxidation - *soxB*, reverse dissimilatory sulfur reductase - *rdsrA* and adenylylsulfate reductase - *aprA*). Transcripts mapped to a UBA868 SAG retrieved from the water column under the Ross Sea Ice Shelf (Fig. 1F) indicated sulfur oxidation and revealed colocalization of both *sox* and *rdsr* genes, together with genes indicative of taurine utilization (*tauD*; Fig. 1F). Interestingly, recent evidence suggests the potential for sulfite generation from taurine to be further oxidized to sulfate by mixotrophic marine bacteria in the oceans [18], indicating a potential means for UBA868 to gain energy from organic sulfur compounds in the oxygenated water column. Furthermore, UBA868 expressed both, *rbcL* type I and II genes, the latter dominating the overall gene expression for the RuBisCO large subunit (Fig. 1E). Our data thus shows that, in the dark ocean below the Ross Sea Ice Shelf, UBA868 plays a disproportional role in sulfur oxidation and DIC fixation.

Yet, to confirm the global relevance of UBA868 in the expression of sulfur oxidation and DIC fixation genes we performed a further more general and global ocean analysis of this enigmatic group.

First we performed a phylogenetic analysis based on a number of publicly available genomes (see supplementary Materials and Methods), as well as our SAGs and MAGs from the Ross Sea Ice Shelf study [21]. The results of this analysis places UBA868 within the order *Arenicellales*, closely related to other sulfur oxidizing bacteria, including *Thioglobus* (SUP05; Fig. 2). The genus *Thioglobus* contains cultured representatives and is abundant in oxygen minimum zones, where they grow chemoautotrophically by oxidizing inorganic sulfur [12]. The deep branching pattern in Fig. 2 suggests instead that UBA868 might form a separate taxonomic order of sulfur oxidizers.

Then we screened all available genomes of this group to derive information about their functional and metabolic capabilities. Suppl. Table 1 contains the number of *Arenicellales* in our genome database and key genes that describe the physiological adaptations to the environment where these bacteria thrive. Out of 683 *Arenicellales*, most, 616 genomes, belong to the family UBA868, which does not contain any isolate. The rest belong to three more families. *Arenicellaceae* includes 38 genomes, with the two described species in the order, *Arenicella chitinovorans* and *Arenicella xantha*. This genus and *Perspicuibacter* are the only formal genera in the order. The family classified as UBA5680 according to the GTDB is represented by 23 genomes, 4 in the family LS-SOB and one in the family BMS3Bbin11. RuBisCO genes were readily found in UBA868 genomes (*rcbL-I* in 21, and *rcbL-I* in 88 genomes) (Fig. S1) indicating the potential for DIC fixation via chemoautotrophy besides its capability to utilize organic carbon. Interestingly, while most UBA868 genomes contained *soxB* (399), *rdsrA* (433) or *aprA* (398) involved in sulfur oxidation, none of these sulfur oxidation, or *rbcL* genes for CO_2 fixation were detected in the family *Arenicellaceae*, nor were detected genes involved in the carboxysome formation. Lithoautotrophs are known to synthetize carboxysomes, a specialized bacterial micro-compartment with a high concentration of RuBisCO and carbonic anhydrase, the latter, an enzyme that converts CO_2 to HCO_3^- [22]. Fewer UBA868 genomes were predicted to harbor the *sqr* gene (sulfide:quinone oxidoreductase gene) (122 UBA868 genomes) involved in sulfide oxidation to elemental sulfur, and the *hdr* system (13 UBA868 genomes, not detected at all in the *Aranicella* family) also likely to be involved in oxidation of sulfur, including the atom bound to organic compounds [23]. These genes would allow the bacterium to derive energy from the oxidation at least from thiosulfate, elemental sulfur, sulfide and sulfite to sulfate. Another predicted physiological feature of the UBA868 group is its ability to grow on C1 compounds since the key enzymes of the serine pathway were identified, hydroxypyruvate reductase and serine–glyoxylate transaminase, as well as genes involved in the oxidation of trimethylamine, methanesulfonate or methanethiol. The last two are both C1 compounds and a source of inorganic sulfur for energy. Genetic content suggests an aerobic life style as most genomes are not expected to respire nitrate or nitrite. A total of 120 genomes harbor the proteorhodopsin gene making the bacterium a potential phototroph. Among the organic sulfur and nitrogen compounds of interest, most, 536 genomes, are predicted to contain the dimethylsulfoniopropionate (DMSP) demethylase gene (*dmdA*). Bacteria with the ability to demethylate DMSP can obtain reduced sulfur from this compound bypassing the need to spend energy to reduce sulfate [24]. *tauD* is involved in the release of sulfite from taurine and is predicted in 319 genomes. *tmm* encodes trimethylamine monooxygenase, an enzyme for the transformation of trimethylamine into trimethylamine *N*-oxide or dimethyl sulfide (DMS) to DMSO [25]. Both *tmm* or *tauD* was not, however predicted in the family *Arenicellaceae*. Collectively, this reconstruction of UBA868 physiological features based on its gene content draws a multifaceted organism, different to the *Arenicellaceae* family, capable of a lithoautotrophic, mixotrophic, aerobic, methylotrophic and phototrophic lifestyle (Fig. 3).

To determine the global relevance and distribution of this enigmatic and metabolically versatile bacterial group, we used public global gene libraries from both the '*Tara* Oceans' [26] and 'Malaspina' [27] expeditions (Fig. 4A). UBA868 was ubiquitous in all oceanic regions and depths, with a global average relative

abundance of 0.53% (Table S2), reaching a maximum relative abundance of up to 3.5% in the miTags and exhibiting a higher abundance in the mesopelagic than in epi- and bathypelagic waters (Fig. 4A). Interestingly, UBA868 belonged to the rare members of the microbial community (generally considered as taxa with relative abundances of < 1%) in > 80% of the *Tara* Ocean samples (Table S2, Fig. 4A). UBA868 is preferentially found at greater depths than the rest of the genomes across longitudes (Fig. 4B).

Based on our transcriptomic, phylogenetic and genomic analyses, and with the aim to empirically confirm and quantify the mixotrophic (chemoautotrophic and heterotrophic) capability of UBA868, we performed MICRO-CARD-FISH (microautoradiography combined with catalyzed reporter deposition-FISH) analyses of samples collected from different oceanic regions (North and Tropical Atlantic, Pacific and Southern Ocean) and depths (Fig. 5A). Our MICRO-CARD-FISH analyses, using a UBA868-specific oligonucleotide probe (see Material and Methods), confirmed the presence of UBA868 in 95% of the samples and depths examined ($n = 39$), reaching an average relative abundance of 1% with a maximum of up to 5% (Fig. S2). These relative abundances are similar to the ones detected by *Tara* Oceans using miTags (Fig. 4A). MICRO-CARD-FISH analyses using DI^{14}C- and ^{3}H-leucine-amended samples confirmed that UBA868 is fixing DIC as well as taking up leucine, hence indicating a mixotrophic lifestyle (Fig. 5B). This could indicate that there are either different trophic lifestyles of UBA868 strains (heterotrophic *versus* chemosynthetic) or individual strains that can shift between trophic states depending on conditions. Based on the MICRO-CARD-FISH analysis, we suggest that UBA868 is preferably metabolizing organic matter since 15–91% of the UBA868 cells exhibited heterotrophic activity, whereas < 27% of all UBA868 cells fixed DIC (Fig. 5B). Remarkably, despite their low relative abundance, UBA868 contributed a significant proportion (up to 10%) to both leucine- and DIC-positive microbial cells from the diverse oceanic regions we sampled (Fig. 5B). Using quantitative microautoradiography (see Supplemental Material and Methods), we determined the contribution of UBA868 to bulk community DIC fixation (S3) and leucine uptake (Table S4). Up to 12.8% and 5.6% of the total community's DIC fixation and leucine uptake, respectively, were mediated by UBA868 in the oceanic water column. This DIC fixation might not only be associated to chemoautotrophy but also to anaplerotic reactions [28]; although these heterotrophic fixation pathways are presumed to be of low relevance in the deep ocean due to the low availability of organic carbon [1], becoming important only in response to organic carbon inputs [29]. Yet, the MICRO-CARD-FISH tools used in this study provides a holistic representation of the total contribution of these microorganisms to the community DIC fixation (including the potential anaplerotic contribution from all community members).

Finally, we confirmed the global relevance of UBA868 in the oxidation of sulfur compounds in the ocean and identified the potential energy sources sustaining the disproportionally high mixotrophic activity of UBA868 compared to its relative abundance. A global analysis on the expression of the main sulfur oxidation (*soxB*, *rdsrA* and *aprA*) and DIC fixation (*rbcL* types I and II) genes allowed quantifying how much of the expression of those genes is attributed to UBA868 in the global epi- and mesopelagic ocean (Fig. 6). UBA868 was one of the main groups transcribing *soxB*, *rdsrA* and *aprA* in both epi- and mesopelagic waters in all samples analyzed (Fig. 6). Although the number of metatranscriptomic

samples in the *Tara* Oceans libraries is limited (24 metatranscriptomic samples out of a total of 187 metagenomic samples), UBA868 consistently dominated the expression of most sulfur oxidation genes in the mesopelagic realm where they accounted for up to 100% of the total expression of *soxB* and *rdsrA*. As expected, *aprA* transcripts were dominated by HIMB59/SAR11. These analyses revealed an average contribution of UBA868 of 8% of the *soxB* (Fig. 6C), 14% of the *rdsrA* (Fig. 6F) and 0.7% of the *aprA* (Fig. 6I) transcripts to the total (epi- and mesopelagic) pool. Yet, this contribution of UBA868 increased in the mesopelagic to an average of 4%, 41% and 71% of *aprA*, *soxB* and *rdsrA*, respectively, exhibiting more than twice the number of transcripts than any other prokaryotic group. In addition, as observed in the Ross Sea Ice Shelf study, UBA868 expressed both *rbcL* types I and II genes and dominated the expression of the type II (40%) (Fig. 6O).

Collectively, we have uncovered a previously neglected but ubiquitous and metabolically versatile mixotrophic bacterial group (UBA868). Despite its relatively low abundance, UBA868 contributes substantially to the heterotrophic and autotrophic activity of the microbial community particularly in the mesopelagic ocean dominating the total expression of RuBisCO (*rbcL* types II) genes (up to 40%) and of key sulfur oxidation (*soxB* and *rdsrA*) genes (up to 100%) in the global dark ocean. We do not imply that all the energy UBA868 generates by the oxidation of sulfur compounds is used solely for the fixation of DIC, but we suggest that this completely unknown group remarkably dominates in the abundance of deep-ocean transcripts of the key genes involved in sulfur oxidation and in DIC fixation; now, whether these two processes are intimately linked or not deserved critical future attention. At any rate, the potential of UBA868 to use alternative energy sources (oxidation of sulfur compounds) makes it a major contributor to the bulk autotrophic and heterotrophic metabolic activity of the microbial community in the dark ocean, filling a critical gap in the oceanic elemental cycles. Our results also highlight the importance of mixotrophs (and not only obligate autotrophs and heterotrophs) in the biogeochemical cycles of the ocean.

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Declarations

Acknowledgments

General: We thank the Victoria University of Wellington Hot Water Drilling Team led by A. Pyne and D. Mendeno. We also thank L. Montiel and V. Balagué from the Institut de Ciències del Mar (ICM), CSIC, for extracting RNA, and the CNAG staff for RNAseq library preparation and sequencing. Bioinformatics analyses were performed at the LiSC Cluster (University of Vienna), and the MARBITS platform (ICM; http://marbits.icm.csic.es).

Funding: This research was facilitated by the New Zealand Antarctic Research Institute (NZARI) funded Aotearoa New Zealand Ross Ice Shelf Program. Samples for MICRO-CARD-FISH were collected on several research cruises led by Meinhard Simon (Sonne 248 cruise), Bernard Quéguiner and Ingrid Obernosterer (MobyDick) and Loes J.A. Gerringa (Geotraces 1). FB received funding by the Austrian Science Fund (FWF) project OCEANIDES (project number AP3430411/21). RL was supported by INTERACTOMICS (CTM2015-69936-P, MINECO, Spain). GJH was supported by grants of the Austrian Science Fund (FWF) project ARTEMIS (P28781-B21) and I486-B09 and by the European Research Council under the European Community's Seventh Framework Programme (FP7/2007-2013) / ERC grant agreement No. 268595 (MEDEA project). JMG was supported by project PID2019-110011RB-C32 (Spanish Ministry of Science and Innovation, Spanish State Research Agency, doi: 10.13039/501100011033).

Author contributions: FB and SEM performed sampling and experiments at RIS. SEM, RL, JMG performed nucleic acid extraction and library preparation for metagenomics and metatranscriptomics, respectively. TR collected samples and provided materials, CMP, CA, ZZ, RL, SRE, JMG and FB analyzed the data. FB and JMG wrote the manuscript with contributions from all co-authors.

Competing interests: The authors declare no competing interests.

Data and materials availability: The sequence data generated in this study have been deposited in the EMBL Nucleotide Sequence Database (ENA) database under Bioproject PRJEB35712 (metagenomic and metatranscriptomic raw reads, metagenomic and metatranscriptomic assemblies, metagenomic assembled genomes, and single-cell amplified genomes) and in the NCBI Sequence Read Archive (SRA) under Bioproject PRJNA593264 (16S rRNA amplicon reads).

Materials & Correspondence: Correspondence and requests for materials should be addressed to FB and JMG.

Supplementary materials

Materials and methods

References

Ext. Figures S1-S7

Ext. Table S1-S4

Figures

Fig. 1

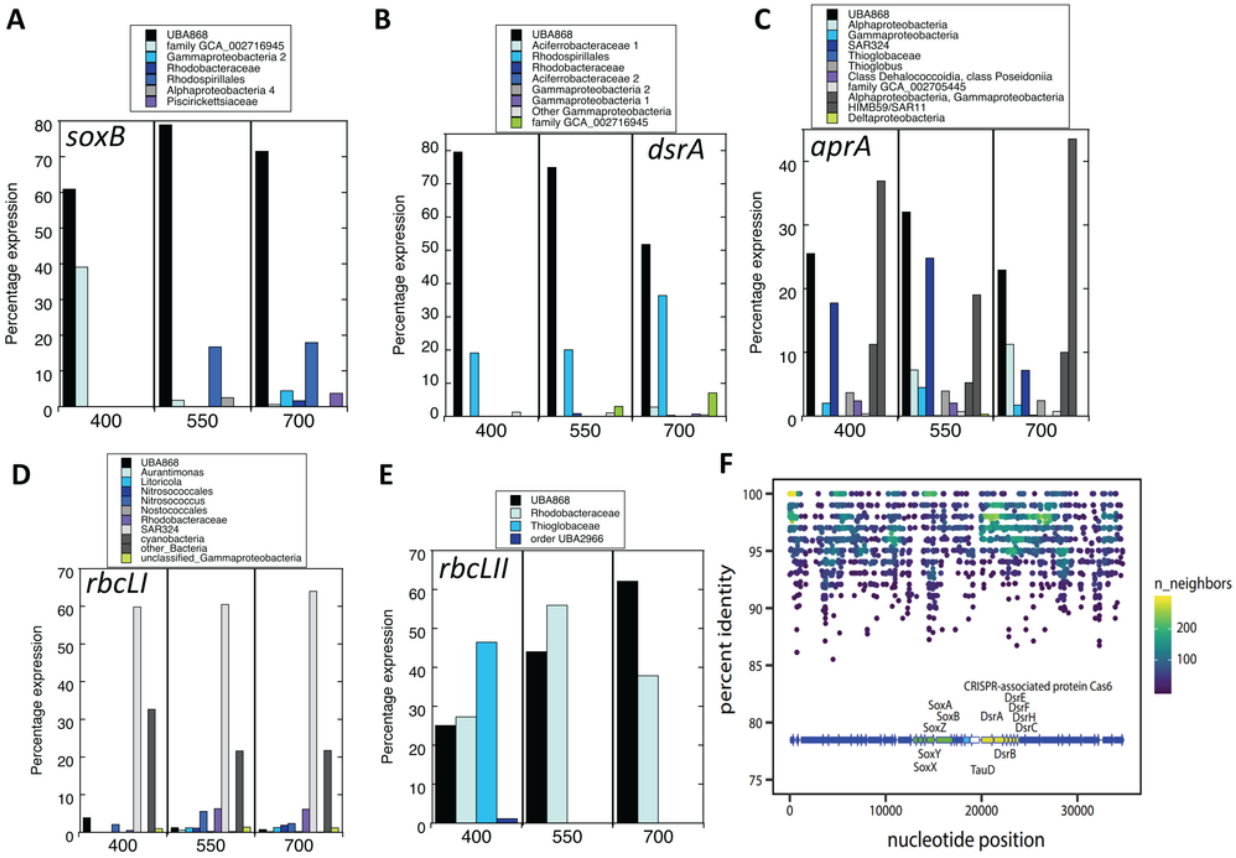

Figure 1

Key role of UBA868 in the ocean under the Ross Ice Shelf. Relative contribution of UBA868 to transcript abundances of (A) the *soxB*, (B) *rdsrA*, (C) *aprA*, (D) *rbcL* type I, and (E) *rbcL* type II genes at 400 m, 550 m and 700 m depth. The taxonomic assignments of the clusters are described in Materials and Methods. (F) Metatranscriptome reads of a sample collected at 550 m depth were aligned against the genome of RIS SAG16, a SAG genome assembled from the metagenome obtained from a sample taken under the Ross Sea ice. Both sets of sequences are from Martínez-Pérez et al. [21]. The inset figure highlights a gene arrangement conserved across the UBA868 family. Gene products are shown. *sox* and *rdsr* genes are involved in the oxidation of thiosulfate to sulfate presumably to obtain energy for CO_2 fixation in the dark. The product of *tauD* is involved in the hydroxylation of taurine to release sulfite, which could be further

oxidized to sulfate. The *cas6* endoribonuclease gene is also conserved in this region of the UBA868 genome. Individual sequence reads are colored by the number of neighbors as indicated in the color scale.

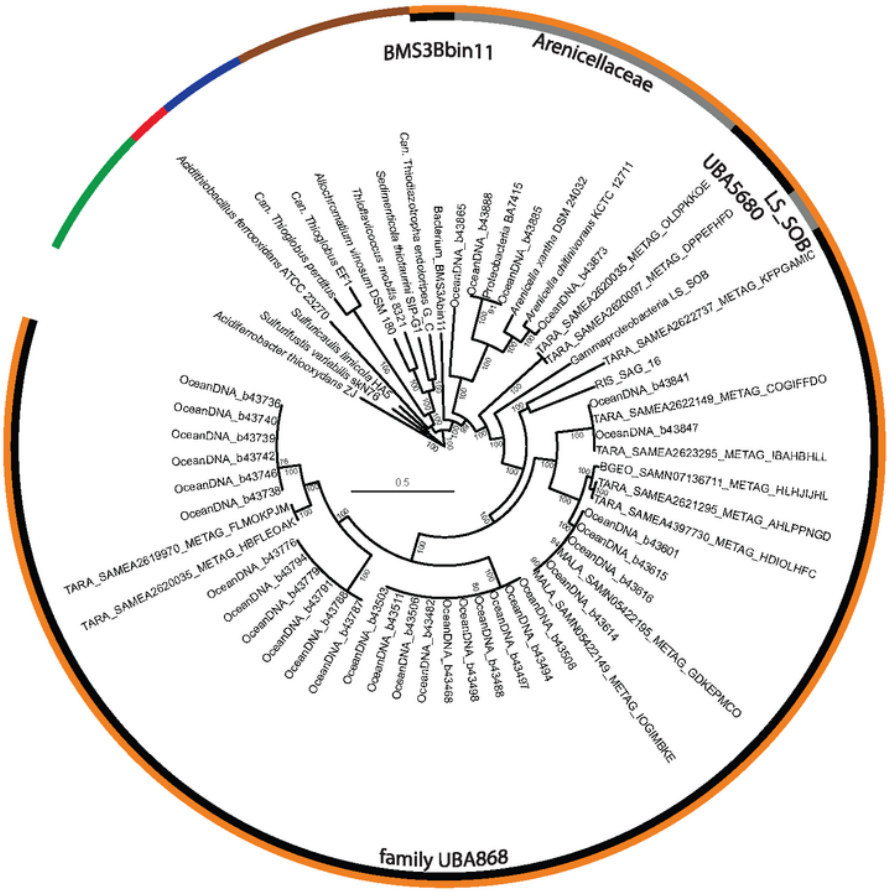

Fig. 2

Figure 2

Phylogenomics of UBA868. Maximum likelihood phylogenetic tree based on 72 concatenated peptides in a selection of genomes that share the greatest number of single copy genes with the genomes in closely related orders represented in the figure, as well as “RIS SAG16”, a SAG from under the Ross Sea ice. Colors in the outer ring indicate the order (orange, *Arenicellales*; red, *Acidithiobacillales*; green, *Acidiferrobacterales*; blue, order PS1; and brown, *Chromatiales*) and family names are shown for the inner circle. The phylogenetic reconstruction suggests that UBA868 belongs to an order different from that of *Arenicella*, its type genus. There is no representative in the family UBA868 that has been isolated yet. Number at nodes show bootstrap values > 70% (100 replicates). Scale bar indicates number of substitutions per site.

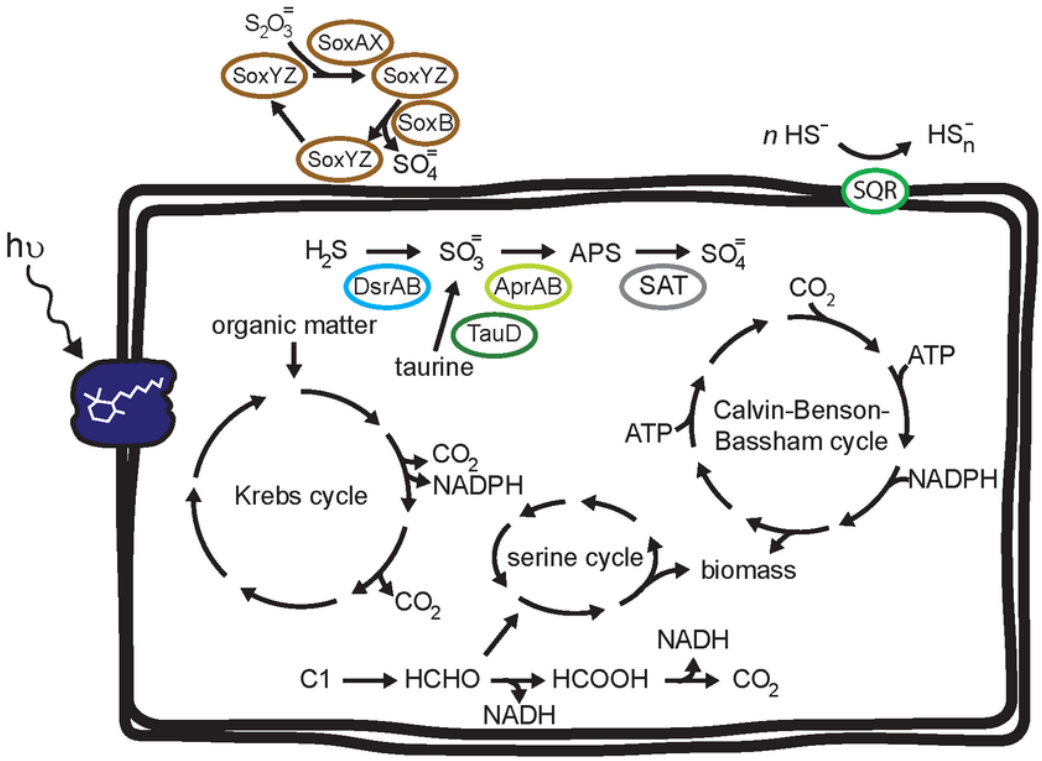

Fig. 3

Figure 3

Simplified overview of major components constituting sulfur oxidation and carbon processing pathways in UBA868. The bacterium is predicted to obtain energy from the oxidation compounds such as thiosulfate, elemental sulfur, sulfide and sulfite with sulfate as the end-product, to fix CO_2. C1 compounds of the type methanesulfonate, methanethiol or trimethylamine could fuel the serine pathway for growth. Taurine and other organic compounds could be a source of reduced sulfur as well. A number of UBA868 genomes harbor one or two copies of the proteorhodopsin gene. Sulfate adenylyltransferase (SAT) releases sulfate from adenylyl sulfate (APS).

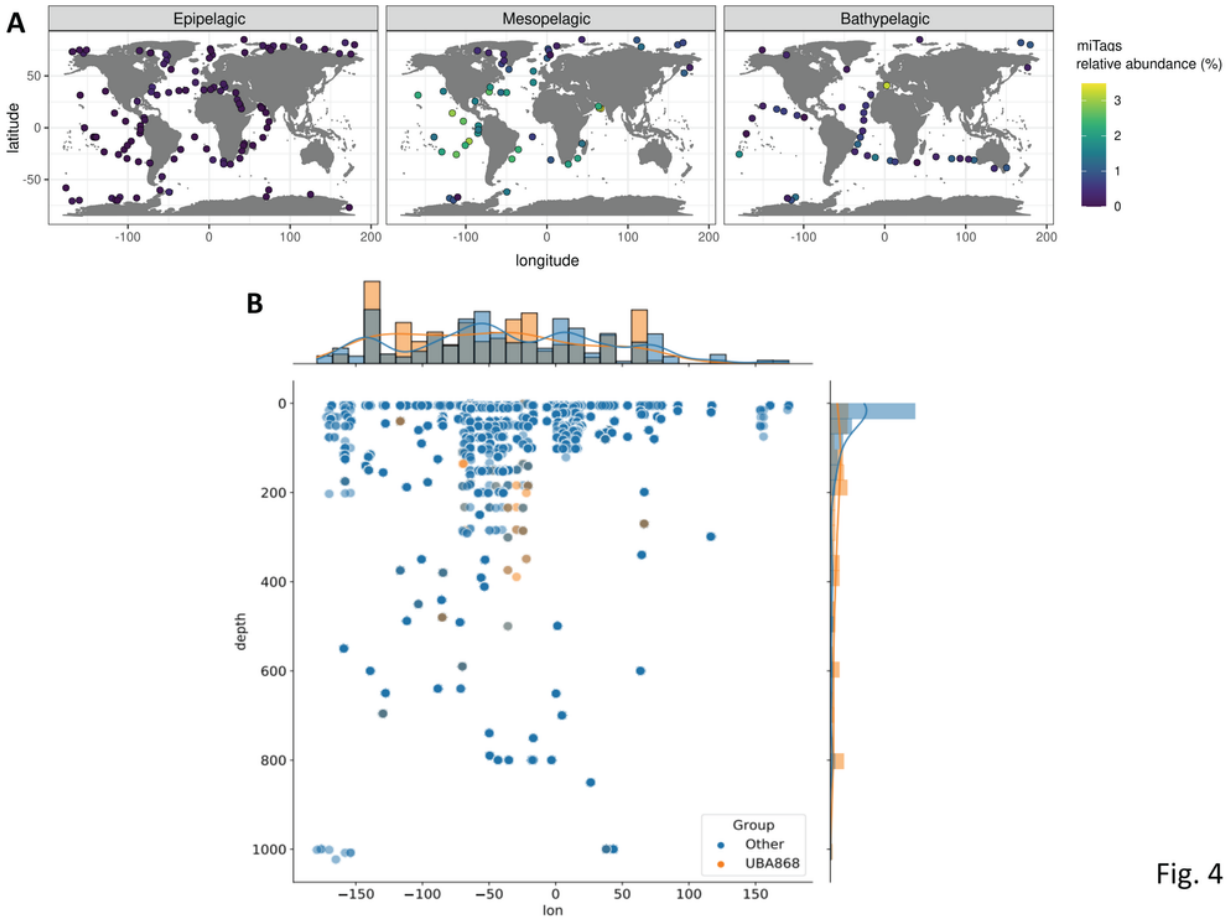

Fig. 4

Figure 4

Distribution of UBA868 in the global ocean. (A) Percentage of UBA868 marine group-associated 16S rDNA fragments derived from Illumina-sequenced environmental metagenomes (miTags) of global metagenomic datasets (*Tara* Oceans and Malaspina). Epipelagic: 0-200 m; Mesopelagic: 200-1000m; Bathypelagic: 1000-4000 m. (B) Depth distribution for UBA868 and non-UBA868 genomes across longitude. Genomes classified as UBA868 (depicted in orange) mainly retrieved from samples at greater depths than other genomes (blue) across longitudes (Mann-Whitney-Wilcoxon test, *p* value = 2.5×10^{-36}). Specifically, UBA868 shows a median depth value of 284 m, while non-UBA868 samples display a median depth of 34 m. Genomes are those from Paoli et al. [30].

Fig. 5

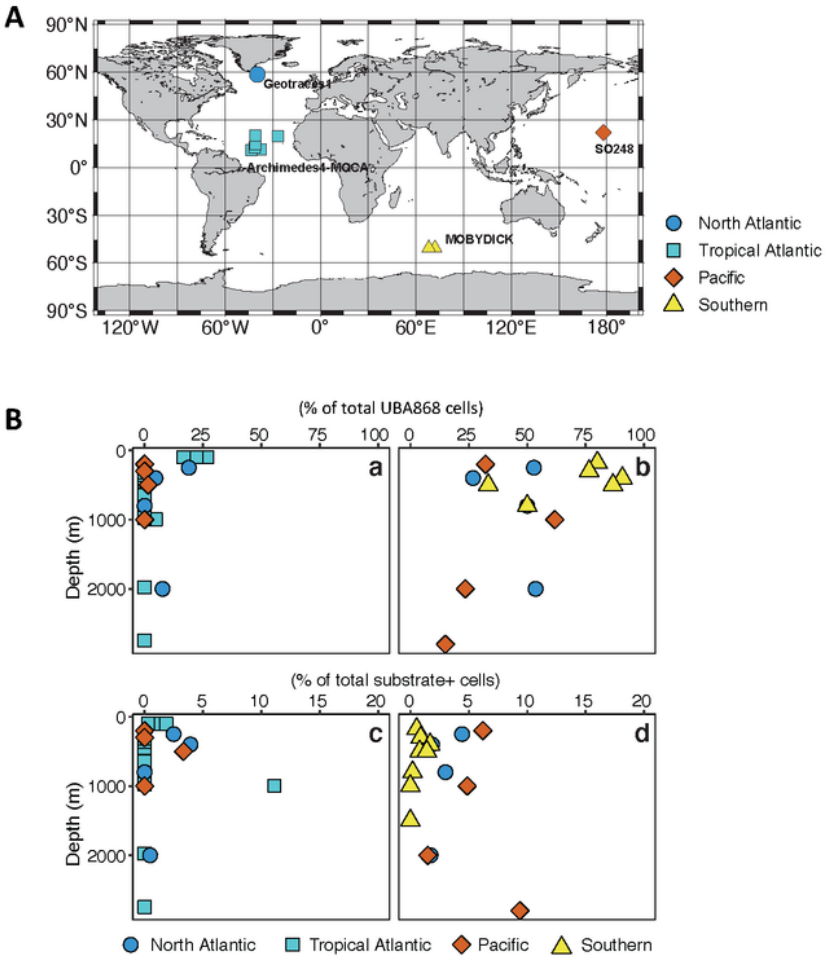

Figure 5

Single-cell heterotrophic and chemoautotrophic activity of UBA868. (A) Location of sampling stations occupied during the cruises in the different oceanic regions: Geotraces-1 in the North Atlantic (May 2010), Archimedes-4-MOCA (Oct 2010) in the tropical Atlantic, SO248 (May 2016) in the Pacific and MOBYDICK (Mar 2018) in the Southern Ocean. (B) Relative abundance of UBA868 taking up ^{14}C-bicarbonate (a, c) and ^{3}H-leucine (b, d), shown as percentage of total UBA868 cell abundance (a, b) and percentage of total

substrate-positive cells (c, d) determined by MICRO-CARD-FISH. Sampling locations are indicated in different colors and shapes.

Fig. 6

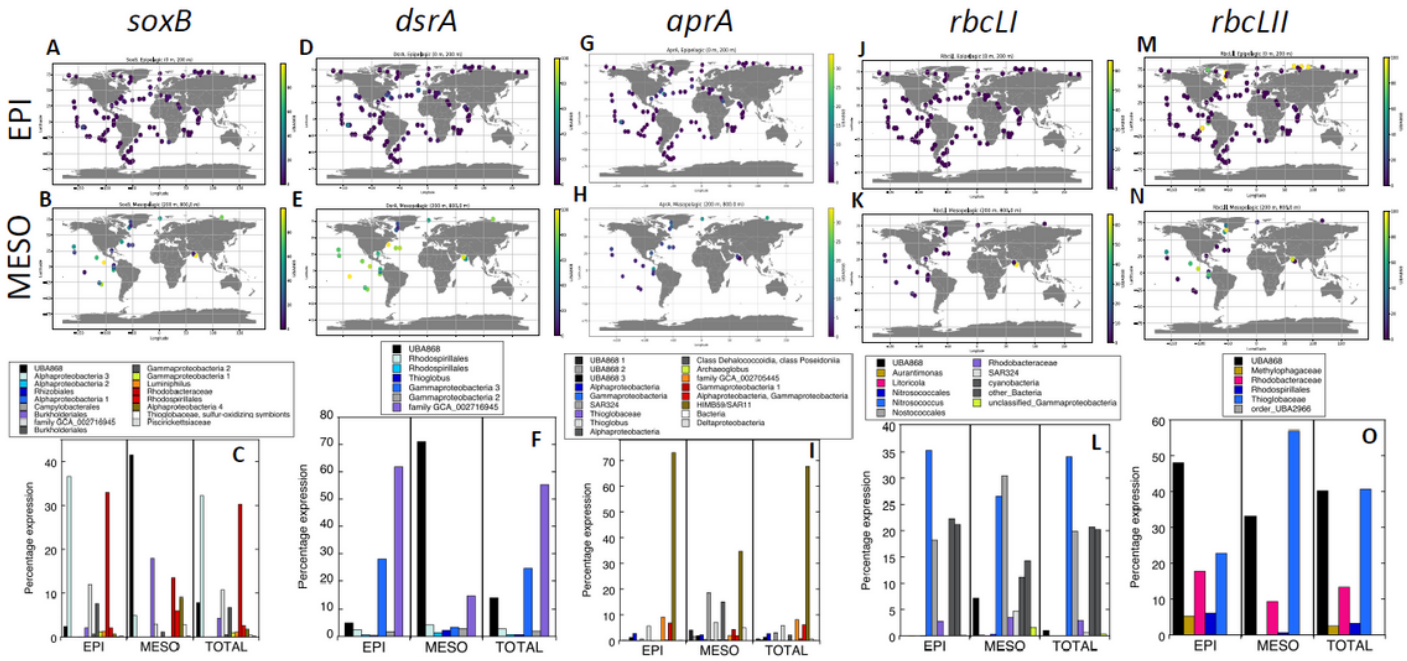

Figure 6

Distribution of UBA868 sulfur oxidation genes in the global ocean. Distribution of UBA868 metatranscriptomic sequence reads of *soxB* (A, B, C), *rdsrA* (D, E, F), *aprA* (G, H, I), *rcbL* type I (J, K, L), and *rcbL* type II (M, N, O). The percentages of metatranscriptomic reads assigned to UBA868 (relative to total) are shown at epipelagic (0-200 m) (A, D, G, J, M) and mesopelagic (200-1000 m) (B, E, H, K, N) depths. Percentage of *soxB* (C), *rdsrA* (F), *aprA* (I), *rcbL* type I (L), and *rcbL* type II (O) transcripts assigned to UBA868 as compared to those assigned to the other major sulfur oxidizing groups identified in the phylogenetic analyses of *soxB* (Fig. S3), *rdsrA* (Fig. S4), *aprA* (Fig. S5), type I *rcbL* (Fig. S6), and type II *rcbL* (Fig. S7).

Supplementary Files

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- TableS1.xlsx
- TableS2.xlsx

- TableS3.xlsx
- TableS4.xlsx
- SupplementaryMaterial.docx