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## Research Paper

# Revisiting the mercury cycle in marine sediments: A potential multifaceted role for Desulfobacterota



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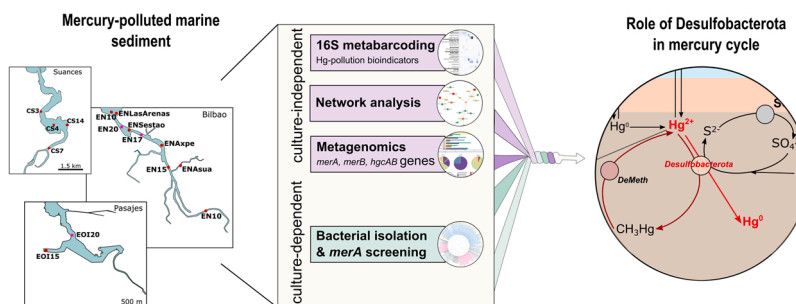
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## HIGHLIGHTS

- Key microbial players in mercury cycling in sediments are identified by culture dependent and independent methods
- Genes involved in mercury reduction (*merA*) are highly abundant in anoxic marine sediments
- Desulfobacterota potentially have a predominant role in both mercury methylation and reduction in anoxic sediments

## GRAPHICAL ABSTRACT



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## ABSTRACT

Marine sediments impacted by urban and industrial pollutants are typically exposed to reducing conditions and represent major reservoirs of toxic mercury species. Mercury methylation mediated by anaerobic microorganisms is favored under such conditions, yet little is known about potential microbial mechanisms for mercury detoxification. We used culture-independent (metagenomics, metabarcoding) and culture-dependent approaches in anoxic marine sediments to identify microbial indicators of mercury pollution and analyze the distribution of genes involved in mercury reduction (*merA*) and demethylation (*merB*). While none of the isolates featured *merB* genes, 52 isolates, predominantly affiliated with Gammaproteobacteria, were *merA* positive. In contrast, *merA*

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genes detected in metagenomes were assigned to different phyla, including Desulfobacterota, Actinomycetota, Gemmatimonadota, Nitrospirota, and Pseudomonadota. This indicates a widespread capacity for mercury reduction in anoxic sediment microbiomes. Notably, *merA* genes were predominately identified in Desulfobacterota, a phylum previously associated only with mercury methylation. Marker genes involved in the latter process (*hgcAB*) were also mainly assigned to Desulfobacterota, implying a potential central and multifaceted role of this phylum in the mercury cycle. Network analysis revealed that Desulfobacterota were associated with anaerobic fermenters, methanogens and sulfur-oxidizers, indicating potential interactions between key players of the carbon, sulfur and mercury cycling in anoxic marine sediments.

## 1. Introduction

Mercury (Hg) is a hazardous heavy metal pollutant with a major impact on aquatic and terrestrial ecosystems. Hg naturally enters water bodies through volcanic activity and weathering of minerals, but over the past century, anthropogenic activities have drastically increased its concentration. Hg is a byproduct of many industrial processes including chloralkali-based production of chlorine and caustic soda, iron and steel production, fossil fuel combustion and gold mining. Additionally, the volatility of elemental Hg and its long atmospheric half-life facilitates the spread of Hg contamination at the global scale [22], posing a significant health-threat to wildlife and humans.

Mercury exists as different chemical species, mainly as elemental mercury ( $\text{Hg}^0$ ), oxidized mercury or mercuric ion ( $\text{Hg}^{2+}$ ), and methylmercury (MeHg). The redox cycle between  $\text{Hg}^0$  and  $\text{Hg}^{2+}$  is mediated abiotically via (photo)-chemical reactions and biotically by microorganisms [48,10]. In general, sediments represent a major Hg sink [72], as in this environment, Hg can accumulate by co-precipitating with sulfide and forming cinnabar ( $\text{HgS}$ ), or by binding to different chelators [28,29]. Under anoxic conditions, Hg can be methylated to MeHg by a range of sulfate-reducing, iron-reducing and methanogenic microorganisms, mainly Desulfobacterota, Bacillota, Chloroflexota and Euryarchaeota [11,46]. Being less mobile than other forms of Hg, MeHg easily enters the food chain, magnifying its concentration in upper trophic levels by bioaccumulation (e. g. [41]).

Microorganisms play a key role not only in the formation of more toxic Hg species such as MeHg, but also in detoxification of the

environment [29]. Volatilization of inorganic Hg by the action of the Hg reductase enzyme, encoded by the *merA* gene, is considered a key mechanism for microbial mercury resistance and has also a great value in bioremediation approaches (e.g. [52,56,20]). *merA* is part of the *mer* operon, which in some cases includes the *merB* gene, involved in the demethylation of MeHg to  $\text{Hg}^{2+}$ . Microorganisms carrying *merA* genes are widely distributed in the environment and across the prokaryotic tree of life [16]. While mercury reduction was formerly thought to operate only under aerobic conditions, *merA* genes have also been identified in facultative and strict anaerobic microorganisms such as *Clostridium* sp., *Shewanella* sp., *Escherichia* sp., and certain members of the phylum Desulfobacterota [16,20,49,52,69]. Despite the global relevance of marine sediments as repositories of Hg pollution, there is limited information available on mercury reduction in these systems. The few published studies reporting *merA* positive bacteria from sediments have identified Firmicutes (currently Bacillota), Gammaproteobacteria, and some Archaea [16,19,49,52,7], but their diversity, ecology and involvement in the Hg cycle remain largely hidden.

In this study, we analyzed marine sediments from three estuaries located in the Bay of Biscay which are all highly impacted by mercury pollution due to industrial activities such as the exploitation of local iron ores, ferrous metallurgy and chloralkali plants [26,6]. By combining culture-dependent (microbial isolation) and culture-independent approaches (i.e., metabarcoding and metagenomics), we (i) identified microbial Hg-pollution indicators, (ii) analyzed bacterial taxa potentially involved in mercury transformation (i.e. harboring *merA*, *merB* and *hgcAB* genes), and (iii) explored their potential microbial community

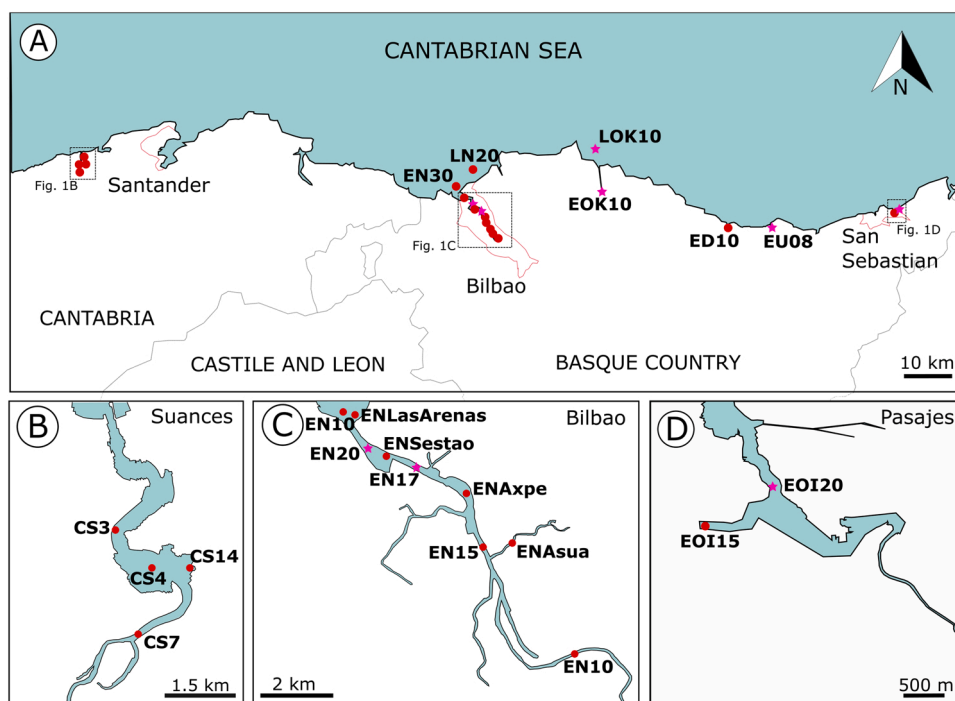


Fig. 1. Location of sampling sites in the Bay of Biscay. Stations where metagenomic samples have been analyzed are highlighted with a star symbol.

interactions by network analysis. Our aim was to shed new light on the key players of mercury cycling in this highly relevant, yet poorly characterized environment.

## 2. Materials and methods

### 2.1. Sample collection and mercury measurements

Sediment samples ( $n = 64$ , including 14 samples with replicates, Table S1) used for analysis in this study were collected from 2013 to 2019 in different estuaries and 3 marine coastal sites (EN30, LN20 and LOK10) along the coast of the Bay of Biscay (Northern Spain, Fig. 1) as part of an environmental monitoring program in this area impacted by industrial pollution (Table S1, see [39] for methodological details on ancillary parameters). Here, we additionally included 8 new samples collected in winter 2020 for performing the microbial isolation work from the estuary of the Nervión river (ENAxpe, ENLasArenas, EN15 and EN17; Fig. 1, C) and the estuary of the Saja river (CS3, CS4, CS7 and CS14; Fig. 1, B), as these sites have been shown to be highly impacted by Hg pollution. Determination of total mercury concentration [THg] analysis in the latter samples was carried out by thermo-desorption atomic absorption spectrometry (AMA-254, Altec). Matrix dependent calibration and accuracy were performed with reference material for total Hg concentration, IAEA 433 and IAEA 405 (Hg and trace elements in marine and estuarine sediments).

### 2.2. Amplicon sequencing of 16 S rRNA genes from sediments

Total environmental DNA was extracted using the RNEasy PowerSoil Total RNA kit followed by the PowerSoil DNA Elution kit (Qiagen), following the manufacturer's protocol. All nucleic acid extracts were visualized by gel electrophoresis to assess DNA integrity. Approximate yield and purity of DNA extracts were measured using a Nanodrop spectrophotometer. Libraries were prepared by amplifying a 253 bp fragment of the prokaryotic small subunit ribosomal RNA (SSU rRNA) gene using primers 519 F (5'-CAGCMGCCGCGGTAA-3') adapted from Øvreås et al. [54] and 806 R (5'-GGACTACNVGGGTWCTAAT-3') [2], both with overhanging Illumina adapters. Amplicons were cleaned-up using Ampure XP (Beckman-Coulter) according to the manufacturer's description and then subjected to a second PCR, using adapter-binding primers with individual indices, as described previously by Aylagas et al. [4]. Technical replicates ( $n = 3$ ) were mixed in equimolar amounts for sequencing. Libraries were sequenced using Illumina MiSeq platform with paired-end v3 chemistry (300 bp paired-end reads).

Read-pair overlapping and initial sequence data filtering was carried out using vsearch [59] and clustering into sequence variants (SVs) using SWARM v2 [44], followed by removal of likely chimeric sequences and singletons, and finally curation using LULU [25], as previously described by Lanzén et al. (2020). Curated SVs were classified using CREST [40] v3.2.2 with SilvaModPR2 v138 as reference database (<https://github.com/lanzen/CREST>). The resulting SV contingency tables were further processed in R using the package vegan v2.5 [53]. Relative abundances were calculated using the *vegan* function *decostand* (with mode="total"). All eukaryotic and unclassified SVs were removed, as well as rare SVs with an abundance consistently below a detection limit set to 0.03% (corresponding to 2.5 reads in the sample with the lowest number of reads). Only SVs present in at least 25% of all samples (i.e. 16 of 64 samples, including replicates) were retained for further statistical analyses. CREST classification was used to agglomerate remaining SV relative abundances with identical annotation into classification-based Operational Taxonomic Units (OTUs).

### 2.3. Identification of Hg-pollution indicators

To identify potential indicator taxa of Hg pollution in the 16 S rRNA gene amplicon sequencing dataset, we used Threshold Indicator Taxa

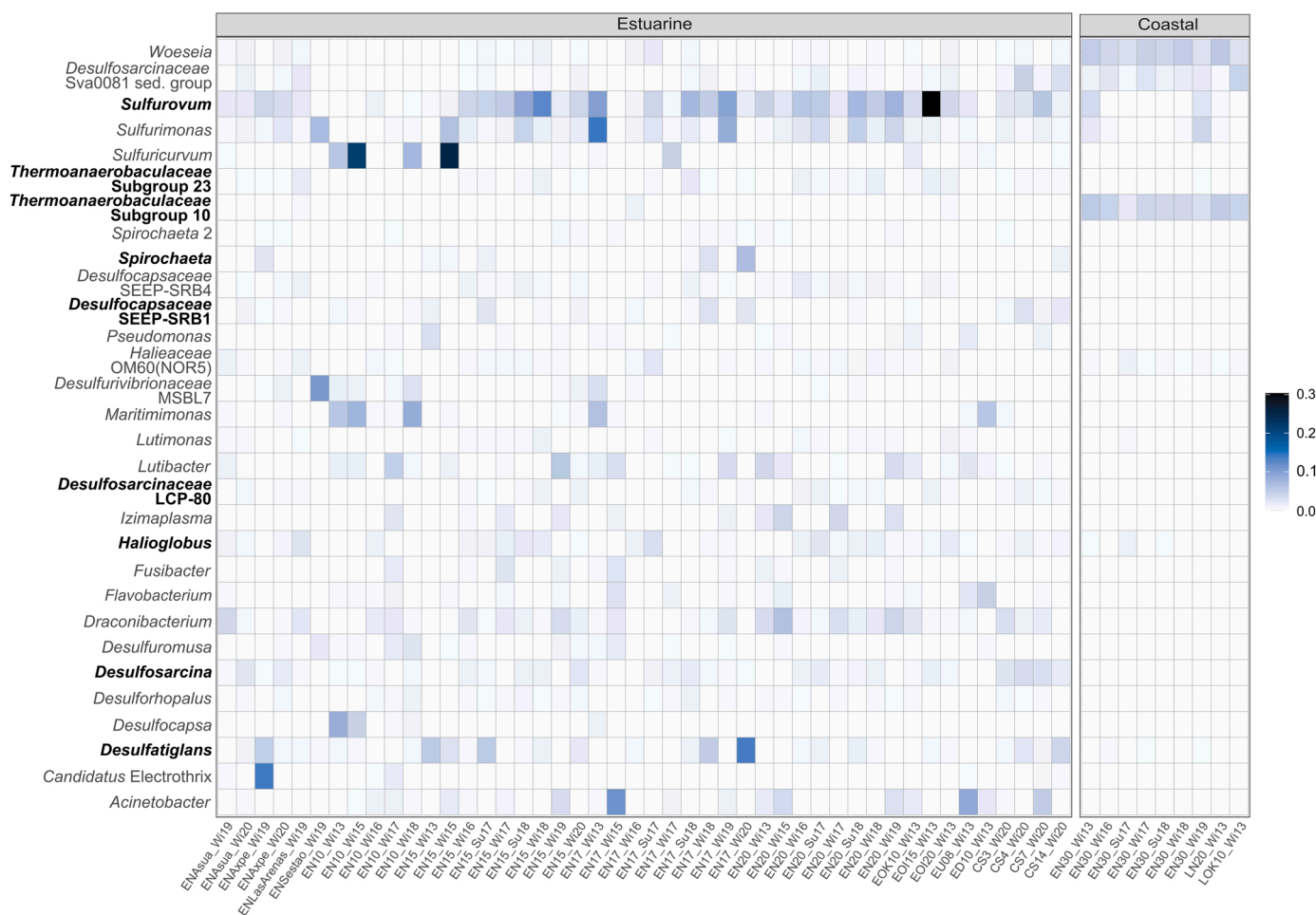
Analysis as implemented in TITAN2 v2.1 [5]. Average relative abundances were used for this analysis to pool biological replicates lacking replicated measurements of Hg concentration. This analysis was based on a custom Mercury Pressure Index (Hg PI) derived from measurements of total Hg concentrations of each sample and transformed according to the ecotoxicological Threshold Effect Limit (TEL) of Hg in sediments (0.13 ppm as established by the National Oceanic and Atmospheric Administration [50]). For details on pressure index calculation see [8]. Taxa significantly associated with high Hg PI ( $p < 0.01$ ) in all samples were considered as potential Hg-pollution bioindicator taxa.

### 2.4. Screening of *merA*, *merB* and *hgcAB* genes in metagenomic contigs and taxonomic affiliation

Six sediment samples collected in winter 2013 from area of study (EN17, EN20, EOK10, LOK10, EOI20 and EU08) and characterized by different levels of environmental impact [39], were used for metagenomic analysis. Metagenomes were obtained by shotgun sequencing with 150 bp paired-end sequencing on the Illumina HiSeq Platform, in the National Center for Genomic Analysis (CNAG) in Barcelona. Metagenomic reads were quality controlled using FastQC [11], and sequencing adaptors trimmed using Cutadapt v1.15 [45]. Resulting sequence data was subjected to serial co-assembly as described in Tully et al. [68]. Briefly, sample-wise assemblies were first carried out using Megahit [42]. All resulting contigs across assemblies with a size of 2000 bp or larger were then concatenated and subjected to co-assembly using Minimus2 [63]. To determine the normalized relative abundance of each contig (Reads Per Kilobase per Million mapped reads, RPKM), all reads were mapped to contigs using Bowtie2 v.2.1.0 [38] and resulting counts divided by the total number of mapped reads from each dataset, as well as the length of the corresponding contig.

Open reading frames (ORFs) were predicted from all resulting co-assembled contigs (including "singleton" contigs, i.e. original sample-specific contigs) and translated to amino acid sequences using Prodigal [32]. The resulting ORFs were screened for *merA*, *merB* and *hgcAB* genes. Custom Hidden Markov Models (HMM) for *merA* and *merB* were built querying UniProtKB for the KEGG orthologs (KO) identifier K00520 and K00221, respectively (BioStudies accession number S-BSST1154). Confirmed sequences for each gene (377 and 55, respectively) were aligned in mafft v7.402 with default parameters [37]. In the case of *hgc* genes, we used the procedure described in Capo et al. [13]. Briefly, HMM profiles of *hgcA* and *hgcB* genes derived from the Hg-MATE database v1.01142021 [27] were used to develop a HMM using *hmmbuild* from HMMER v3.2.1 [24]. We considered genes with E-values  $< 10^{-3}$  as significant hits. To decrease false positives, we then used the stringency cutoff defined by Capo et al. [13], to verify the presence of the conserved motifs from *hgcA* (NV(I)WCA(A/G/S)GK) and *hgcB* genes (C(M/I)EC(G/S)(A/G)C), and thereby performed a manual inspection of the presence of *hgcA* and *hgcB* genes in our dataset. Certain *hgcA* genes were found side-by-side with *hgcB* genes on the same contig.

Each putative *merA* and *merB* gene retrieved from the metagenomes was taxonomically assigned by mapping them to UniRef90 (release 2021\_03 from 9 of June 2021), with mmseqs2 development version, commit 13-45111, with the taxonomy workflow options "-max-accept 100 -tax-lineage 1 -e 1E-5 -v 3 -a" and converted to table with *mmseqs createtsv*. All ranks out of domain, phylum, class, order, family, genus or species were stripped out from classification and missing fields were marked as "unclassified". The lowest common ancestor for each sequence was also recorded. In the case of *hgc* genes, phylogenetic analyses were done with the *pplacer* approach as part of the marky-coco pipeline [13]. The software MEGA11 [66] was used for performing the alignment of *merA* genes extracted from the metagenomes and amplified from the isolates (see above) using the UPGMA method, and for building the phylogenetic tree (ML method).



**Fig. 2.** Heatmap of the relative abundance of most representative genera in all sediment samples analyzed in this study. Hg-pollution bioindicators as identified by TITAN analysis are highlighted in bold.

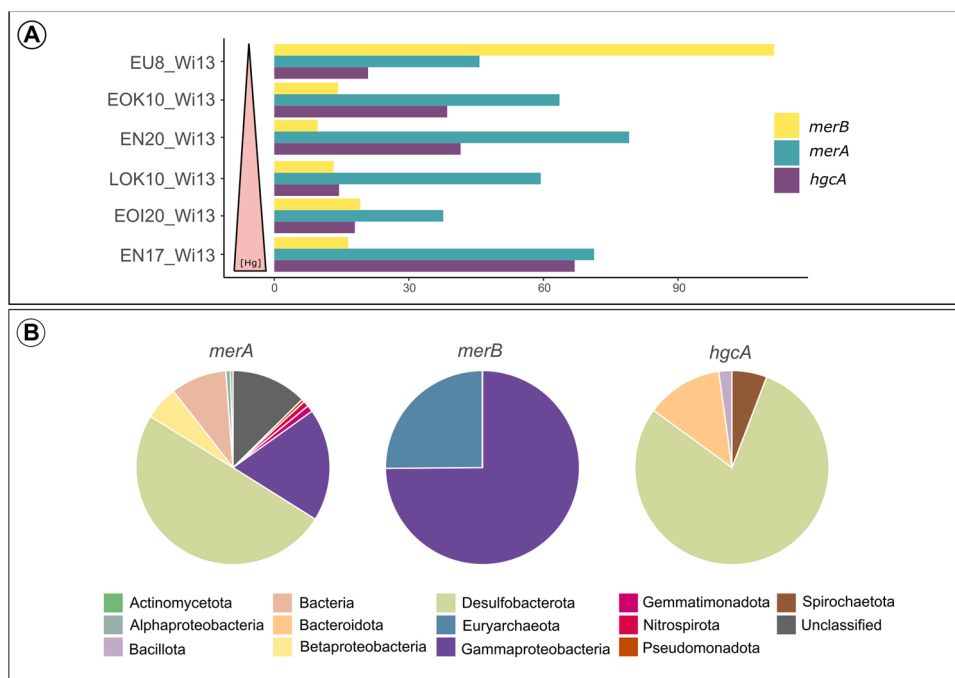
### 2.5. Screening of Metagenome-Assembled Genomes (MAGs) affiliated with Desulfobacterota

A collection of metagenome-assembled-genomes built from the same sediment samples was screened to search for mercury cycling genes. Each metagenome was assembled individually following the SqueezeMeta v1.6.0 pipeline [65]. Binning was done using MaxBin2 [71] and Metabat2 [35] and the combination of binning results was done using DAS Tool [62]. Bin statistics were computed using CheckM [55]. Binning results were filtered for MAGs assigned to Deltaproteobacteria (using a taxonomy predating the phylum Desulfobacterota) via the LCA algorithm as implemented in SqueezeMeta. Only MAGs with > 70% completeness and < =10.1% contamination according to CheckM results were retained (following a common quality threshold as reported in [9]). Taxonomic classification was later confirmed with GTDB-Tk v2.1.1 ([15]; GTDB release 207\_v2). Binned contigs were annotated with prokka v1.14.6 [60] to get gene prediction and a basal functional annotation (gene name, COG, E.C. number). Additionally, predicted coding DNA sequences were annotated with PFAM (PFAM release 34.0) in HMMER v3.3, and KEGG KOs (v98.0) in kofamscan v1.3.0 (options *-format detail -E 0.01*; [3]). Detection of *merA*, *merB* and *hcgAB* genes was carried out by combining the analysis of the results from the MAG annotation pipeline with a further screening step with the custom HMMs developed for *hgc* genes described above for metagenomic contigs.

### 2.6. Isolation and identification of marine sediment bacteria with *merA* genes

Cultures from sediment samples recovered in winter 2020 (see above) were obtained using different selective media. Briefly, 100 µl of sediment-PBS mix (1:10) were inoculated on Marine Agar 2216, 10% Zobell Agar, and in parallel on a soil substrate membrane system (SSMS; [56]), both of which were then incubated at room temperature for 7 days under oxic conditions. Membranes from the SSMS method were used to inoculate additional Marine Agar 2216 and 10% Zobell Agar plates and incubated for 4 days. From agar plates with a number of colonies between 10 and 70, colonies from the half-left side of the plate were individually picked and isolated. These isolates were purified and stored at - 80 °C in glycerol stocks while aliquots were also retained for DNA extraction and 16S rRNA based identification.

An initial taxonomic identification of isolated strains was performed via partial 16 S rRNA gene amplification, using universal primers 519 F and 1492 R. Then, we screened isolates for presence of *merA* by using the primer set1 NsfF (5'-ATCCGCAAGTNGCVACBGTTGG-3') and NsfR (5'-CGCYGCRAGCTTYAAYCYTCRRCCATYGT-3') [70]. Positive strains were identified as those producing 300 bp PCR products as visualized on an agarose gel. All amplicons were purified using Cytiva Illustra ExoProStar™ (Cytiva, Barcelona, Spain) and sequences were obtained by Sanger sequencing (Stabvida, Lisbon, Portugal).



**Fig. 3.** Normalized abundances (RPKM) of *merA*, *merB* and *hgcA*-carrying contigs in each sample (A) and its taxonomic assignment (B), retrieved from metagenomic analyses in the six samples analyzed. The gradient of mercury concentration in the samples (represented by a triangle in panel A) ranged from 108 to 2100 ng/g in dry weight (see **Supplementary Table S1**). The label of the samples in the same panel includes the sample location (see **Fig. 1**) and the time of collection (Wi13: Winter 2013).

## 2.7. Association networks of *Desulfobacterota* taxa

For reconstruction of association networks of *Desulfobacterota* in the sediment samples, we used FlashWeave v1.4 [64] with default parameters on the 16S rRNA gene amplicon sequencing dataset. The network was constructed based on OTUs resulting from merging all SVs with the same taxonomic grouping. The redox potential and total Hg concentration were included as parameters and submitted to FlashWeave in addition to OTU abundance tables. Associations with  $p > 0.01\%$  were discarded. Negative associations (edges) were also discarded to simplify the network structure and minimize potential bias introduced by differences in community composition associated with contrasting environmental conditions unrelated to Hg concentration. The resulting networks were inspected and illustrated using Cytoscape v3.9.1 [61], which was also used to derive a sub-network of *Desulfobacterota* and associated nodes (OTUs) up to two steps away in the network graph.

## 3. Results

### 3.1. Microbial community composition and identification of potential Hg-pollution bioindicators

Environmental 16S rRNA gene analysis revealed that most abundant genera in the sediment samples were affiliated with sulfide-oxidizing Campylobacterota (e.g. *Sulfurovum* sp., *Sulfuricurvum* sp.), sulfate-reducing bacteria (e.g. *Desulfuromusa* sp., *Desulfosarcina* sp., *Desulforhopalus* sp., *Fusibacter* sp.) and heterotrophic or fermentative bacteria commonly found in marine environments (e.g. *Acinetobacter* sp., *Flavobacterium* sp., *Lutibacter* sp., *Draconibacterium* sp., *Woeseia* sp.) (**Fig. 2**). Samples from coastal stations (EN30, LN20 and LOK10), were clearly different from the estuarine samples (**Fig. 2**; **Fig. S1**). According to TITAN2, some abundant OTUs were identified as Hg-pollution bioindicators (highlighted in bold in **Fig. 2**), including *Thermoanaerobaculaceae* Subgroup 23, *Spirochaeta* sp., *Halioglobus* sp., and several *Desulfobacterota* (e.g., *Desulfocapsaceae* SEEP-SRB1, *Desulfosarcinaceae* LCP-80, and *Desulfosarcina* sp.).

### 3.2. Screening of *merA*, *merB* and *hgcA* genes in sediment metagenomes and MAGs

From the analysis of the collection of marine sediment metagenomes analyzed here, a total of 103 contigs contained *merA*, *merB* or *hgcA* genes (61, 10 and 32 contigs respectively, supplementary **Table S2**). Among the 32 *hgcA* genes, 18 were found side-by-side with *hgcB*. The normalized abundance of contigs (calculated as RPKM) was generally highest for *merA* among the three genes, and similar across samples regardless of their mercury concentration (**Fig. 3, A**). In contrast, the highest normalized abundance of *hgcA* was found in the sample with the highest total Hg (2100 ng/g dw; EN17, supplementary **Table S1**). The normalized abundance of *merB* genes was highest in a single sediment sample collected in the estuary of Urola which had a very low Hg concentration (EU08, **Fig. 1, Fig. 3, A**).

For the metagenomes, *merB* was mainly identified in contigs affiliated to Gammaproteobacteria and Euryarchaeota, while contigs carrying *merA* or *hgcA* genes were mainly associated with *Desulfobacterota* (50% and 80% of contigs, respectively, **Fig. 3, B**; supplementary **Table S2**). Additionally, other contigs containing *merA* genes were related to Actinomycetota, Nitrospirota, Pseudomonadota, and other Proteobacteria, while in the case of *hgcA* genes they were related to Bacteroidota, Bacillota and Spirochaetota.

In addition to the contigs, we analyzed three high-quality Deltaproteobacteria MAGs reconstructed from the sediment metagenomes containing *merA* genes to assess whether *merB* or *hgcAB* genes co-occurred in the same populations. The first MAG (namely EN17maxbin.002), which was taxonomically assigned to Desulfurivibrionaceae, contained a putative *merA* gene and a *hgcA* gene. However, *hgcB* was not found side-by-side to *hgcA* in this MAG. The other two MAGs (EN20maxbin.003 and LOK10maxbin.002) were also assigned to *Desulfobacterota* (*Desulfuromonadia* and *Synthrophobacteria*, respectively). One of these MAGs, MAG EN20maxbin.03, contained *merA* and *hgcAB* genes, indicating a potential to carry out both mercury reduction and methylation (Supplementary **Table S3**). This MAG featured 10.05% contamination as estimated by the software checkM, based on the

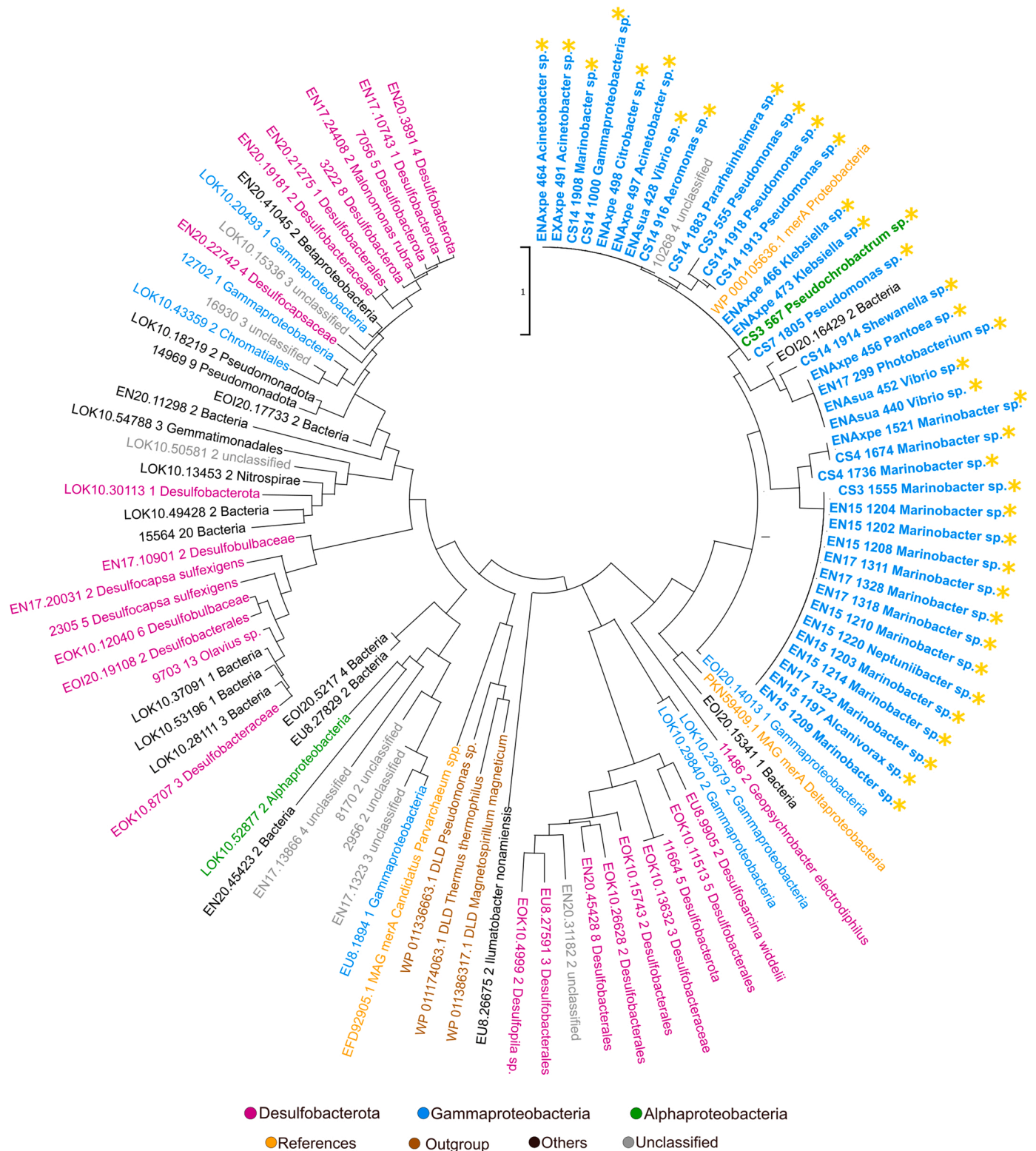


Fig. 4. Phylogenetic tree of *merA* genes retrieved from isolates and metagenomes from marine sediments. Sequences retrieved from isolates are highlighted in bold and with a yellow asterisk (\*). Some *merA* sequences publicly available in NCBI have been included for reference.

analysis of single-copy genes [55]. This contamination value is virtually the standard cutoff considered for obtaining medium quality MAGs (i.e., 10%, [9]). While we cannot rule out the possibility that *merA* and *hgcAB* genes from this MAG came from different taxa, the high value of strain heterogeneity in MAG EN20maxbin.03 (82.61%, Supplementary Table S3) indicates that the contamination was mostly from very closely

related organisms, likely from the pangenome of the species being considered [55].

### 3.3. Screening of *merA* genes in sediment bacterial isolates

A collection of altogether 449 bacterial isolates retrieved from the

sediment samples was screened to identify strains with *mer* genes. We found a total of 52 *merA* positive isolates. These were mainly affiliated with Gammaproteobacteria, including different genera such as *Marinobacter* sp. (17 isolates), *Pseudomonas* sp. (7 isolates), *Aeromonas* sp. (5 isolates), *Vibrio* sp. (5 isolates), *Acinetobacter* sp. (3 isolates), *Citrobacter* sp. (2 isolates), and *Klebsiella* sp. (2 isolates). Eight other genera with *merA* genes were represented with one isolate each (Fig. 4; supplementary Table S4). In general, *merA* sequences found in the metagenomes and in the cultures did not cluster together, with the exception of two metagenomic contigs (EOI20.16429 and 10268), which contained *merA* genes which were similar to those of different isolated Gammaproteobacteria.

### 3.4. Association network related to Desulfobacterota phylum

Due to the predominance of Desulfobacterota among *merA*-containing microorganisms in marine sediment metagenomes, we analyzed bacterial community-level association networks for OTUs of this phylum in order to unveil potential interactions with other taxa. From a total network including 491 OTUs (taxa) and 509 significant associations (edges), we identified 218 OTUs belonging to the Desulfobacterota phylum or being connected to such OTUs directly or via one intermediate node (OTU) (Fig. S2). This sub-network included 56 Desulfobacterota OTUs distributed over 19 modules, with one large module connecting almost half of the OTUs (101 out of 218). The latter included 10 taxa that were also identified as indicative of high total Hg concentration. In general, we found that Desulfobacterota OTUs were associated with obligate or facultative anaerobes (e.g. Bathyarchaeota, *Draconibacterium* sp., Anaerolineacea, *Fusibacter* sp., *Acetobacterium* sp.), including taxa associated with methane metabolism (*Methanobacterium* sp., and anaerobic methanotrophic archaea ANME-3), but also with aerobic OTUs such as *Gallinoellaceae*, methylotrophic *Methylothera*, ammonia oxidizing TACK Archaea or sulfur-oxidizing bacteria (*Sulfurovum*, Fig. S2).

## 4. Discussion

Mercury reduction is a key step in the environmental mercury cycle. The marker gene of this process (*merA*) has been previously detected in isolate genomes, MAGs and/or single-cell amplified genomes of contaminated soil, freshwater environments, polar ice and geothermal springs (reviewed in [16]), and predominately affiliated with Gammaproteobacteria, Bacillota and Actinomycetota. In the case of marine sediments, the few reported genomes and MAGs containing *merA* genes affiliate mostly with Gammaproteobacteria and Alphaproteobacteria (e.g., [36,47,73]), but available data is still scarce. In accordance with these earlier findings, our isolation-based approach retrieved mostly Gammaproteobacteria as *merA*-containing marine sediment bacteria. Yet, in contrast with the results obtained by culturing, our metagenomic analysis unveiled a predominance of *merA* genes in Desulfobacterota (Figs. 3–4), with several OTUs of this phylum also identified as Hg-pollution bioindicators (Fig. 2). Our isolation approach was initially designed to target mercury reducers, which were expected to be dominant among aerobic or facultative anaerobic microorganisms. While alternative isolation approaches (i.e., SMSS; [23]) were used to minimize the classical bias from isolation on traditional agar plates, the fact that these procedures were carried out under oxic conditions clearly precluded the retrieval of anaerobic microorganisms in culture, such as Desulfobacterota.

Only a few members of Desulfobacterota have previously been found to carry *merA* and/or *merB* genes in isolate genomes and MAGs from acid mine drainage [67], a metal-contaminated aquifer [31], a microbial mat from a brackish coastal lagoon [69] and in an isolate genome from a sediment sample collected in Nankai Trough (*Halodesulfovibrio* sp.; [33]). Despite these observations, the potential relevance of Desulfobacterota as mercury detoxifiers in marine sediments and other oxygen deficient

environments has been largely overlooked, as this taxon has mainly been associated with mercury methylation [12,14]. The analysis of MAGs retrieved from our marine samples suggests that, indeed, some Desulfobacterota have the potential to carry out both processes, as at least one population carried both *hgcAB* and *merA* genes (EN20maxbin.003; supplementary Table S3).

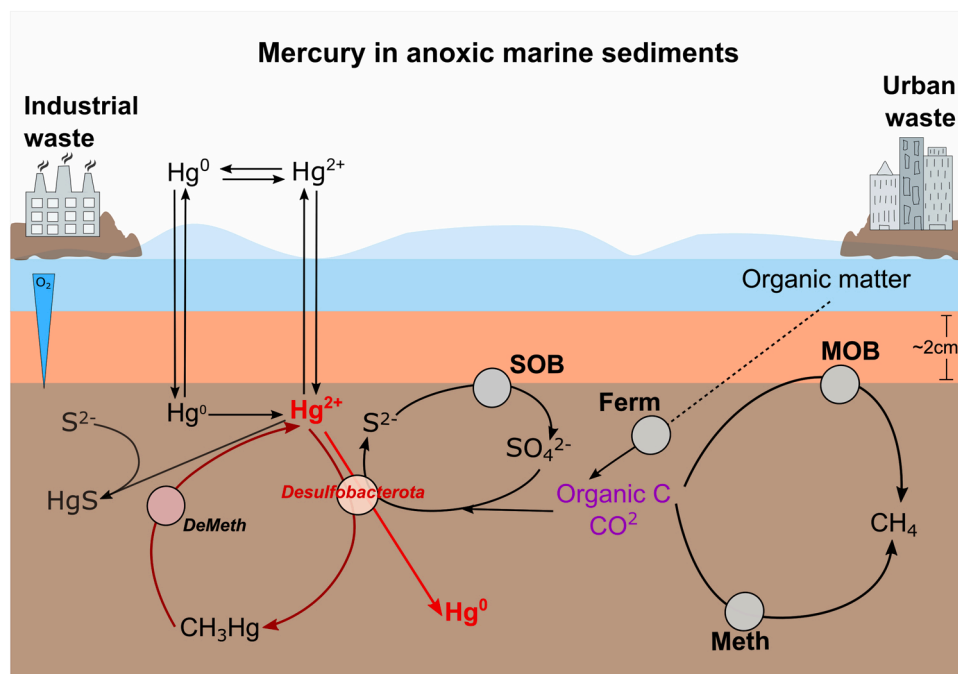
An intriguing result of our study is that *merA* genes were generally found in higher abundance than mercury methylation genes (*hgcA*) in anoxic sediments (Fig. 3, A; supplementary Table S1), even if mercury reduction is typically believed to be restricted to oxic conditions. Elemental mercury ( $Hg^0$ ) is expected to be stable in anoxic sediments due to relatively high  $Hg^{2+}/Hg^0$  redox potential [18]. Availability of oxidized mercury ( $Hg^{2+}$ ) for its reduction in the sediments is likely provided by (i) passive mercury oxidation via binding of  $Hg^0$  to thiol groups (-SH) from organic matter like humic acids and/or the surface of bacterial cell walls [17,30]; (ii) via direct input of oxidized species from the industrial waste like chloralkali plants and iron and steel production; or (iii) by previous mercury oxidation in the atmosphere, water column or oxic zone of the sediment abiotically or via active microbial activity [29,48]. Remarkably, the other common predominant Hg species, methylmercury, was below detection limit in the set of sediment samples analyzed here (sediment samples collected in 2020, results not shown). This implies that either mercury methylation is not active at these coastal sediments, or that some members of the microbial communities are able to rapidly demethylate methylmercury after its production. However, the generally low abundance of *merB* genes in the sediment samples suggests that mercury demethylation, at least via this operon, was not an important process in the area of study.

The normalized abundance of mercury-related genes (i.e. *merA*, *hgcAB*) was not significantly correlated to the abundance of total mercury in the sediments (Spearman correlation,  $p > 0.05$ ). The relatively low number of samples available for this correlation analysis, and the fact that the presence of these genes cannot be directly extrapolated to an active transformation of Hg, likely limits our ability to detect significant correlations. Alternatively, estimates of total mercury may not be a good proxy of bioavailable mercury in the sediments, as previously reported [51,57]. In a previous study in polluted sediments from the Croatia coast, heavy metal pollution was found to influence the abundance of some metal resistance genes, but the effect of nutrients (organic carbon and nitrogen) had a stronger impact on microbial community composition [21]. Besides heavy metal pollution, most of the marine sediments analyzed here were also rich in organic matter (5–7% dw, reaching 8% dw, Table S1), mainly due to urban wastewater discharge. Under anoxic conditions, facultative as well as obligate anaerobes involved in decomposition of organic matter are expected to be active at these sites, first performing mainly fermentation, followed by sulfate reduction and methanogenesis (e.g., [43,34]). The network analysis revealed significant associations between Desulfobacterota with taxa known to drive some of the latter processes, including fermentative and methanogenic bacteria. The software used here to model the association networks intends to minimize indirect associations due to shared niches through the incorporation of environmental parameters [64]; however, it is possible that some of these associations still represent indirect, rather than direct metabolic interactions. On the other hand, Desulfobacterota were also associated with sulfide oxidizers, which likely represents metabolic associations in the context of sulfur cycling.

## 5. Conclusion

Our results indicate that the current known diversity of environmental mercury reducers is highly biased by the fact that a large fraction of them originate from oxic culturing approaches. Our metagenome-based identification of *merA* genes in members of Desulfobacterota, Gemmatimonadota, Betaproteobacteria, Nitrospirota, and Actinomycetota greatly expands the known diversity of potential mercury reducers in marine sediments (Figs. 3, B and 4). Our results also challenge





**Fig. 5.** Simplified scheme of some of the main metabolisms known to be operating in anoxic marine sediments in the context of the carbon, sulfur and mercury cycles, highlighting the potential multifaceted role of *Desulfobacterota* in some of these pathways. **SOB**: sulfide-oxidizing bacteria; **DeMeth**: mercury demethylating bacteria; **Meth**: methanogenic archaea; **MOB**: methane-oxidizing bacteria; **Ferm**: fermentative microorganisms.

the current view of mercury transformations in anoxic marine sediments, which has been traditionally regarded as a predominant niche for mercury methylation. The potential multifaceted involvement of sulfur-reducing *Desulfobacterota* in both mercury methylation and reduction may give them a unique role as main modulators of mercury speciation in marine sediments (Fig. 5). Interestingly, in a recent study in a brackish coastal lagoon, a single MAG affiliated with anaerobic *Desulfuromonadaceae* was found to contain *merA* (out of 11 *merA* positive MAGs in total), but it clearly dominated *merA* gene expression [69]. Additionally, previous studies have measured mercury reduction under anoxic conditions without detecting *merA*, indicating that other *mer*-independent mechanisms may also be operating [30,48,58]. We propose that the current view of mercury reduction in marine sediments, including key players and mechanisms involved, should be revisited and extended to other marine anoxic environments.

### Environmental Implications

Marine sediments impacted by mercury contamination represent a threat to ecosystem health due to its high toxicity. Mercury biomagnification through aquatic food webs is also harmful to human health. We targeted microbial mechanisms of mercury detoxification in sediments under anoxic conditions, which have been poorly explored. We uncovered a high abundance of genes involved in mercury reduction in marine sediments predominately affiliated with *Desulfobacterota*, a phylum previously known to methylate inorganic mercury. Our results shed new light on our understanding of the complex interplay between microbial communities and hazardous elements in marine sediments, which is key for informed mitigation strategies.

### CRedit authorship contribution statement

**BR-T**: Investigation, Data curation, Methodology, Writing – original draft. **AL**: Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – review & editing. **PS**: Data curation, Formal analysis, Methodology, Software, Writing – review & editing. **ME, EB, DR, IM**: Investigation. **CP-C**: Investigation, Writing – review &

editing. **IS-S**: Investigation, Methodology, Writing – review & editing. **MF**: Software, Formal analysis. **EC and IA-R**: Software, Formal analysis, Investigation, Writing – review & editing. **DA, SB, OS, SGA**: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **LAS**: Conceptualization, Funding acquisition, Supervision, Data curation, Investigation, Writing – original draft.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data Availability

We have shared the data/code in the manuscript with their accession numbers. Sequence reads and derived MAGs are available from the Sequence Read Archive of the INSDBC via EBI WebIn (BioProject accessions PRJEB65319 and PRJEB62120). HMM profiles for *merA* and *merB* genes can be found in BioStudies under accession number S-BSS1154.3. *merA* sequences obtained by Sanger sequencing have been deposited in NCBI under accession numbers OR672480-OR672517.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2023.133120](https://doi.org/10.1016/j.jhazmat.2023.133120).

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