Transcriptomic evidence for versatile metabolic activities of mercury cycling microorganisms in brackish microbial mats
Adrien Vigneron, Perrine Cruaud, Johanne Aubé, Remy Guyoneaud, Marisol Goñi

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Methylmercury, biomagnifying through food chains, is highly toxic for aquatic life. Its production and degradation are largely driven by microbial transformations; however, diversity and metabolic activity of mercury transformers, resulting in methylmercury concentrations in environments, remain poorly understood. Microbial mats are thick biofilms where oxic and anoxic metabolisms cooccur, providing opportunities to investigate the complexity of the microbial mercury transformations over contrasted redox conditions. Here, we conducted a genome-resolved metagenomic and metatranscriptomic analysis to identify putative activity of mercury reducers, methylators and demethylators in microbial mats strongly contaminated by mercury. Our transcriptomic results revealed the major role of rare microorganisms in mercury cycling. Mercury methylators, mainly related to Desulfobacterota, expressed a large panel of metabolic activities in sulfur, iron, nitrogen, and halogen compound transformations, extending known activities of mercury methyliators under suboxic to anoxic conditions. Methylmercury detoxification processes were dissociated in the microbial mats with methylmercury cleavage being carried out by sulfide-oxidizing Thiotrichaceae and Rhodobacteraceae populations, whereas mercury reducers included members of the Verrucomicrobia, Bacteroidetes, Gammaproteobacteria, and different populations of Rhodobacteraceae. However, most of the mercury reduction was potentially carried out anaerobically by sulfur- and iron-reducing Desulfuromonadaceae, revising our understanding of mercury transformers ecophysiology.

INTRODUCTION

Mercury (Hg) is a toxic and widespread heavy metal of natural and anthropogenic origins that accumulates in soils and sediments. The methylmercury (MeHg), largely produced from methylation of inorganic mercury by anaerobic microorganisms, is of special concern since this compound is highly toxic and biomagnifies in aquatic food webs, generating a health hazard to wild animals and humans. Proteins associated with this metabolic capacity are coded by the gene pair hgcAB, initially detected in Geobacter and Desulfovibrio strains and now identified in genomes of few iron reducers, sulfate reducers, and methanogens. Metagenomic mining and recovery of metagenome-assembled genomes (MAGs) of uncultured microbial populations also extended the phylogenetic and metabolic diversity of hgcAB-bearing lineages in various environments, suggesting that the list of known methylmercury producers is far from complete. However, the mercury methylation activity of these uncultured lineages remains to be confirmed. Parameters driving hgcAB gene expression remain unknown. The characterization of cultured mercury methylators revealed that the expression of those genes is not inducible by mercury, and no link have been observed between their expression and the methylation potential. The expression of hgcA has been proposed to be constitutive but recent metatranscriptomic analysis of environmental samples challenged this hypothesis. Therefore, further work is required to understand the in situ expression of hgcAB and, ultimately, the toxic mercury production.

Microbial activities that directly or indirectly affect Hg(II) methylation or MeHg degradation play a critical role in modulating mercury toxicity. The prokaryotic mercury resistance mechanism, encoded by the mer operon, plays a key role in this paradigm. Within the mer operon, merA gene encodes the mercuric reductase, reducing Hg(II) to gaseous mercury Hg (0), while merB gene encodes an alkylmercury lyase, cleaving organomercury compounds, including methylmercury, allowing the complete detoxification of a broad spectrum of mercury compounds, providing a major decontamination mechanism for various microbial lineages in mercury contaminated environments. The taxonomic and functional diversity of these microorganisms remains unclear, blurred by numerous horizontal gene transfer events that frequently fractionated the mer operon in microbial genomes, but could be limited to aerobic microorganisms. Furthermore, although the genetic capabilities of mercury cycling microorganisms have been previously investigated, their gene expression profiles in situ remain largely unexplored, leaving their activities in natural habitat poorly understood.

The Etang de Berre in the south of France is the second largest European brackish lagoon covering 155.3 km². The edge of the lagoon has a strong history of petroleum industry activity, leading locally to hydrocarbon pollution and regionally to mercury atmospheric deposition. Shallow sediments of the lagoon are colonized by photosynthetic microbial mats. These microbial ecosystems are thick biofilms with a complex structural organization with filamentous cyanobacteria, autotrophic and heterotrophic prokaryotic lineages, protists, fungi, metazoa, and viruses distributed across the thickness and the step gradients of light, oxygen, nitrogen and sulfur compounds, which are characteristic of these biofilms. Microbial mats play critical roles in many environments as hot spots of biological production, carbon mineralization, organic matter degradation, sediment stabilization, and decontamination of various pollutants including hydrocarbons or heavy metals. The multiple environmental niches of these stratified biofilms gather a large variety of aerobic...
Mercury cycling populations were identified, resulting in the recovery of 407 high- to medium-quality MAGs. This study extends our knowledge on the physiology, the activities than usually presumed for mercury cycling microorganisms in coastal environmental niches, and the involvement in biogeochemical cycles. Geochemistry of the microbial mats was investigated in Mediterranean Sea brackish lagoons, located at Berre l’Etang (EDB) and Salins du Lion (SL) (Fig. 1) were sampled in the Salins du Lion bird reserve. Chemical and physical parameters of the microbial mats during the sampling events. Alkanes include C11 to C38. PAH polycyclic aromatic hydrocarbons. Additional and more detailed dataset is available in Aubé et al. (2016, 2020)15,19.

RESULTS

Geochimistry of the microbial mats

The taxonomic and genetic composition as well as the expression profile of two coastal microbial mats developing in neighboring brackish lagoons, located at Berre l’Etang (EDB) and Salins du Lion (SL) (Fig. 1) were investigated in fine detail. Detailed chemical and physical parameters of both microbial mats during the sampling events have been previously analyzed15,19. As expected, both mats have high sulfate and sulfide concentrations while oxygen concentration and redox potential measurements indicated that both aerobic and anoxic niches likely cooccur in the microbial mats. Presence of alkanes and polycyclic aromatic hydrocarbons, coming from the oil industries, is the main environmental feature that distinguishes the two microbial mats (Fig. 1).

Microbial community composition

The microbial community composition was determined using all 16S rRNA genes recovered from the metagenomic datasets (2.1 ± 0.9 × 10^4 16S rRNA genes per sample; Fig. 2). The microbial community structure of these mats have been previously analyzed15,19, microbial mercury transformations throughout the environmental gradients of the biofilm remain unexplored.

In this study, we applied a genome-resolved metagenomics and metatranscriptomics approach to determine the taxonomy, functional potential, and putative activity of microbial mercury transformers in two microbial mats recovered from the Etang de Berre area, which are strongly contaminated by mercury (17.8 ± 4.1 μg of Hg per gram, Fig. 1) due to atmospheric deposition from neighboring industries and bioaccumulation of mercury by the microbial mats15. A total of 30 metagenomic datasets, covering the spatial and temporal variability of the microbial mats, was generated, leading to 389 GB of sequences, then co-assembled, resulting in the recovery of 407 high- to medium-quality MAGs. Mercury cycling populations were identified and protein sequences of these microbial MAGs were compared to 300 GB of rRNA-depleted metatranscriptomic sequences produced from the same samples. The expression profiles of hgcA, merA, merB genes, and energetical metabolic pathways were investigated at the population level, revealing a larger variety of metabolic activities than usually presumed for mercury cycling microorganisms. This study extends our knowledge on the physiology, the environmental niches, and the involvement in biogeochemical cycling of mercury-transforming microorganisms in coastal sediments.

Metagenome assembled genomes of the microbial mat and mercury cycling populations

Binning of the contigs obtained after coassembly of all metagenomic reads resulted in the recovery of 407 high- to medium-quality MAGs (completeness > 50%, contamination < 5%). Taxonomic affiliation of these MAGs, inferred from 16S rRNA genes and ribosomal genes phylogenetic analysis (Fig. 3) indicated that MAGs from both dominant and rare lineages of the microbial mats were reconstructed. Bacteroidetes (72 MAGs), Cyanobacteria (25 MAGs), Alphaproteobacteria (42 MAGs), Gammaproteobacteria (68 MAGs), Desulfovibrio (26 MAGs), Patescibacteria (19 MAGs), and Verrucomicrobia (14 MAGs) were the most represented phyla (Fig. 2). In addition, members of Chloroflexi (4.26%), Campylbacteriales (1.54%), Patescibacteria (3.40%), and Verrucomicrobia phyyla (2.52%) were also detected in all samples but in minor proportions (Fig. 2).

Fig. 1 Description of the study sites. EDB and SL microbial mats, separated by 5 km, are both localized in the Etang de Berre area, South of France. EDB mat was collected near an oil industry wastewater discharge whereas SL mat was sampled in the Salins du Lion bird reserve. Additional and more detailed dataset is available in Aubé et al. (2016, 2020)15,19.
another one to the Aminicenantes phyla (Table 1 and Figs. 3 and 4). All of the binned hgcA genes were followed on their contigs by the hgcB gene. Unbinned hgcA sequences without binned references were related to archaeal methanogen (1 sequence), Geobacter (1 sequence), and Desulfovibrio (3 sequences) groups (Fig. 4).

After phylogenetic analysis of the merA genes recovered from the metagenomes, a total of 20 putative merA genes (10 merA and 10 merA-like) were identified, whereas 16 sequences were found to be misannotated by the bioinformatic pipelines (uncharacterized pndr and dld genes in Fig. 5). Furthermore, analysis of the amino acid sequences confirmed the presence of essential cysteines in MerA protein sequences whereas these amino acid signatures were not detected in MerA-like protein sequences. Nine of the 10 merA genes and 1 of 10 merA-like genes were found in the recovered MAGs. These MAGs were affiliated to Gammaproteobacteria, Rhodobacteraceae (Alphaproteobacteria), Desulfuro- musa (Desulfobacterota), Verrucomicrobiae, and Bacteroidetes (Balneolaceae and Rubrivirga) (Table 1). In addition, unbinned merA-like genes without binned close representative were affiliated to Cyanobacteria and Peregrinibacteria (Fig. 5).

Only two merB genes were identified in the metagenomic dataset. These genes were identified in one Thiotrichaceae (Gammmaproteobacteria) MAG (bin660) and one Rhodobacteraceae MAG (bin246), but none of these MAGs also included the merA gene (Fig. 5).

Coverage of the MAGs was used as a proxy for the relative abundances of the corresponding populations (Table 1). Overall, the average coverage of the 407 recovered MAGs was 3.5 reads per base in the contig, with a median value of 1.62 and a maximum of 203.12 for a cyanobacterial MAG. Most of the MAGs with hgcA, merA, or merB genes (62.5% of the MAGs) were recovered with a coverage lower than the average coverage (Table 1). Nonetheless, bin805 and bin387 with hgcAB genes, bin785, bin674, bin345, bin55, and bin72 with merA gene, and bin246 with merB gene were recovered with a coverage above the average coverages of all recovered MAGs (Table 1). In addition, coverage of unbinned contigs with hgcAB or merA genes was lower or similar to the binned contigs with coverage <1.5 and 4.5 reads per base for unbinned contigs with hgcAB and merA respectively.
Expression profile of mercury transformation genes

A MAG-centric metatranscriptomic approach was carried out to evaluate expression of mercury transformation genes (hgcA, merA and merB) in the microbial mats (Fig. 6). No mercury cycling gene was found to be expressed in all sampling dates and no clear pattern of expression could be identified between fall and spring periods or day and night, despite the decrease of oxygen concentration and redox potential during the night due to oxygen consumption and reduced photosynthetic activity (Fig. 1).

Transcripts of hgcA genes were detected in both microbial mats (Fig. 6a). Except for the Aminicenantes, expression of hgcA was detected for all populations in at least one sample. hgcA was strongly expressed (number of hgcA transcripts > number of rpoB transcripts) by the Desulfatiglans (bin165) population in the EDB samples whereas a strong expression of hgcA by a Desulfobacteraceae (bin762) population was identified in most of the SL samples (Fig. 6a).

Transcripts of merA gene were identified in both EDB and SL microbial mats (Fig. 6b). Transcription of merA was identified for four populations: Gammaproteobacteria bin761, Rhodobacteraceae bin785, Verrucomicrobia bin674, and Desulfuromusa bin93. Only the Desulfuromusa bin93 population expressed merA at higher level than the housekeeping gene rpoB. Finally, although merA gene was detected in Balneolaceae, no merA transcript of Balneolaceae was identified in the metatranscriptomic dataset (Fig. 6b).

Transcription of merB genes was only detected in SL samples. All merB transcripts were assigned to the Thiotrichaeae bin660 and the Rhodobacteraceae bin246 populations (Fig. 6b). When detected, the transcription level of merB gene in bin660 was higher or similar to the expression level of rpoB gene while expression of merB in bin246 was lower or similar to rpoB expression.

Metabolic pathways expressed by mercury cycling populations

Genes expression profile of mercury cycling populations was investigated to determine their putative metabolic activities within the microbial mats (Fig. 7 and Supplementary Data 1). Although the expression profile of each bin depended on its completeness, an average of 2409 ± 1470 genes per bin was found to be expressed over the different seasons and daytime of the study (Table 1).

The Desulfobacterota populations with hgcAB genes expressed numerous metabolic pathways including sulfate (sat, aprAB, dsrABC), tetrathionate (otr), thiosulfate/polysulfide (psh-psrABC), and sulfur reduction (dsrE) genes. Transcription of nitrate, nitrite, and nitric oxide reduction (narG, mrfA, and norQ) and nitrogen fixation genes was also largely detected in Desulfobacterota.
populations (Fig. 7). Transcripts of iron reduction genes \(\text{omcA/mtrC}\) were detected in two populations (bin805 and bin387) whereas sulfur oxidation (SQR) and cytochrome C oxidase genes were expressed by the \textit{Desulforhopalus} (\textit{Desulfobulbaceae}) bin752 population that also expressed nitrate and nitrite reduction genes. Expression of key enzymes of the Wood–Ljungdahl pathway and subsequent acetate and pyruvate metabolism was also detected for all \textit{Desulfobacterota} populations. Genes coding hydrogenases and malate, lactate, alcohols, and formate dehydrogenases were also strongly expressed among \textit{Desulfobacterota} populations. In addition, transcripts of 2-haloacid dehalogenase and trimethylamine-N-oxide reductases (\textit{yedYZ} and \textit{torC}) were also detected for half of the \textit{Desulfobacterota} populations. Hydrocarbon degradation genes (\textit{assA}, \textit{hssA}, \textit{badA}) were expressed in all \textit{Desulfobacterota} populations recovered from EDB. The \textit{Roseimarinus} (bin731) population with \textit{hgcAB} genes expressed genes of tetrathionate/nitrate and nitrite reducing cytochromes (\textit{otr}, cytochrome c-552, and \textit{nrfAH}), bacteriochlorophyll synthesis (\textit{bhsC}), and pyruvate metabolism (Fig. 7). The \textit{Aminicenantes} (bin506) population with \textit{hgcAB} genes also expressed nitrate and nitrite reduction genes (\textit{napA}, \textit{otr}, and \textit{nrfAH}) as well as alcohol dehydrogenase and pyruvate metabolism genes. Alkyl succinate synthase gene (\textit{assA}) involved in hydrocarbon degradation was identified in bin506 but not expressed (Fig. 7).

The microbial populations with \textit{merA} gene expressed a large variety of metabolic pathways including light utilization for members of the \textit{Bacteroidetes}, \textit{Alpha-} and \textit{Gammaproteobacteria}, sulfur (\textit{DsrE}), sulfide (SQR), and thiosulfate oxidation (Sox operon) for the \textit{Alpha-} and \textit{Gammaproteobacteria}, thiosulfate, nitrate (\textit{narG}), and nitrite (\textit{nrfA}) reduction for \textit{Desulfobacterota}. Iron reduction encoding genes (\textit{omcA/mtrC}, and predicted CXXCH cytochrome) were also expressed by the \textit{Desulfobacterota} and the \textit{Gammaproteobacteria} bin761 populations. \textit{Gammaproteobacteria} bin72, which lacked light utilization genes, expressed the \textit{Calvin–Benson–Bassham} cycle, a complex sulfur oxidation pathway (\textit{sox}, \textit{SQR}, \textit{dsrE}, \textit{fcaA}), as well as nitrite reductase and nitrogen fixation genes (\textit{nif}). Genes coding for nitrate (\textit{narG}) and arsenate reductases (\textit{assA}), hydrogenase, halogen, urea, and ethanolamine utilization were also expressed by a large fraction of the \textit{merA}-bearing populations (Fig. 7).

For the \textit{merB}-bearing \textit{Thiotrichaeae} (\textit{Gammaproteobacteria}) bin660 population, a strong expression of the Rubisco, \textit{cbb-3} type cytochromes, \textit{sox} operon, and the oxidative-type \textit{dsrAB} genes was detected. Expression of nitrate reducing genes (\textit{narG} and \textit{otr})
and hydrogrenases was also observed. The *Rhodobacteraceae (Alphaproteobacteria)* bin246 population with *merB* gene strongly expressed light-harvesting complex and photosynthetic reaction center genes (*pufLM*), nitrate reductase genes (*narABG*), and the Sox operon. The lower level of transcription was also detected for dehalogenase and arsenate reductase genes (Fig. 7).

**DISCUSSION**

To better understand the fate and transformations of mercury compounds in coastal environments, the microbial community composition, function, and potential activity of two shallow microbial mats exposed to strong mercury contamination were investigated in detail. The full thickness of the mats was collected, including the oxic and anoxic layers, allowing the simultaneous investigation of diversity in these coastal microbial mats. However, the large majority of the *hgcA* transcripts were found in the metagenomic dataset (Fig. 4) was similar to *Roseimarinus* (Bacteroidetes) and the uncultivated phyla of *Aminicenantes*, supporting previous metagenomic mining in marine environments. However, phylogenetic analysis of *hgcA* gene and transcripts as well as taxonomy of the *hgcAB*-bearing MAGs indicated that members of the *Desulfobacterota* represented most of the mercury methylator diversity in these coastal microbial mats (Figs. 4 and 6a), which is consistent with the sulfate and sulfide rich conditions of the mats (Fig. 1).

To evaluate mercury methylation in the microbial mats, *hgcA* genes were investigated. The frequency of *hgcA* genes identified in the metagenomic dataset (Fig. 4) was similar to previous reports on marine polluted sediments, indicating a strong potential for methylation of mercury in microbial mats. All *hgcA* genes identified in genomic bins were followed by *hgcB* gene in the contigs, supporting the metabolic potential for mercury methylation. Mercury methylation genes were identified in genome of populations related to *Roseimarinus* and *Desulfobacterota* genes identified in genome of populations related to *Roseimarinus* and *Desulfobacterota*. The lower level of transcription was also detected for dehalogenase and arsenate reductase genes (Fig. 7).

**Table 1.** Detailed description of the MAGs with mercury transformation genes recovered in the microbial mats.

| Bin ID | Taxonomy             | Complet. (%) | Conta. (%) | 16S rRNA gene | Relative abundance (av. coverage) | MerA cycling gene | Number of ORF with predicted protein | Number of gene expressed |
|--------|----------------------|--------------|------------|---------------|-----------------------------------|------------------|--------------------------------------|--------------------------|
| Bin731 | *Roseimarinus*       | 87.28        | 5          | Yes           | 1.56                              | *hgcAB*          | 3827                                 | 484                      | 2830                     |
| Bin506 | *Aminicenantes*      | 57.45        | 2.65       | Yes           | 1.03                              | *hgcAB*          | 4462                                 | 447                      | 1753                     |
| Bin752 | *Desulfurhopalus*    | 86.41        | 1.52       | Yes           | 1.16                              | *hgcAB*          | 3543                                 | 526                      | 2810                     |
| Bin27  | *Desulfobulbaceae*   | 65.67        | 2.11       | Yes           | 0.98                              | *hgcAB*          | 2613                                 | 350                      | 1975                     |
| Bin165 | *Desulfatifilans*    | 98.06        | 2.6        | Yes           | 1.12                              | *hgcAB*          | 3733                                 | 385                      | 3428                     |
| Bin456 | *Desulfobacteraceae* | 66.32        | 2.82       | Yes           | 0.99                              | *hgcAB*          | 2566                                 | 323                      | 1935                     |
| Bin805 | *Desulfobacteraceae* | 91.61        | 4.27       | Yes           | 4.20                              | *hgcAB*          | 8173                                 | 1058                     | 7361                     |
| Bin727 | *Desulfobacteraceae* | 88.42        | 4.2        | Yes           | 1.10                              | *hgcAB*          | 5617                                 | 780                      | 4529                     |
| Bin387 | *Desulfobacteraceae* | 54.08        | 1.61       | Yes           | 1.10                              | *hgcAB*          | 4064                                 | 424                      | 2886                     |
| Bin762 | *Desulfobacteraceae* | 53.75        | 1.79       | Yes           | 1.13                              | *hgcAB*          | 3546                                 | 502                      | 2713                     |
| Bin761 | *Gammaproteobacteria*| 89.00        | 3.05       | Yes           | 1.34                              | *merA*           | 3563                                 | 424                      | 2986                     |
| Bin495 | *Rhodobacteraceae*   | 50.23        | 4.85       | Yes           | 1.29                              | *merA*           | 1966                                 | 273                      | 1149                     |
| Bin785 | *Rhodobacteraceae*   | 93.64        | 2.7        | Yes           | 17.82                             | *merA*           | 3600                                 | 487                      | 1072                     |
| Bin416 | *Rhodobacteraceae*   | 50.01        | 1.08       | Yes           | 0.80                              | *merA*           | 1791                                 | 215                      | 855                      |
| Bin93  | *Desulfuromusa*      | 83.87        | 2.1        | Yes           | 0.79                              | *merA*           | 2580                                 | 337                      | 2356                     |
| Bin674 | *Verrucomicrobiaceae*| 94.76        | 1.16       | Yes           | 5.25                              | *merA*           | 3036                                 | 277                      | 2223                     |
| Bin299 | *Verrucomicrobiaceae*| 52.00        | 0.2        | Yes           | 1.98                              | *merA*           | 2124                                 | 178                      | 111                      |
| Bin759 | *Verrucomicrobiaceae*| 97.47        | 4.76       | Yes           | 2.48                              | *merA*           | 4343                                 | 438                      | 2489                     |
| Bin345 | *Balneolaceae*       | 90.16        | 2.55       | Yes           | 19.83                             | *merA*           | 2935                                 | 327                      | 2843                     |
| Bin55  | *Rubrivirga*         | 97.27        | 1.37       | Yes           | 33.86                             | *merA*           | 3708                                 | 348                      | 399                      |
| Bin72  | *Gammaproteobacteria*| 96.88        | 1.16       | Yes           | 3.76                              | *merA*           | 3027                                 | 302                      | 2509                     |
| Bin660 | *Thiotrichaceae*     | 96.89        | 2.93       | Yes           | 1.71                              | *merB*           | 3124                                 | 340                      | 2371                     |
| Bin246 | *Rhodobacteraceae*   | 52.53        | 4.55       | Yes           | 5.63                              | *merB*           | 2507                                 | 399                      | 1824                     |
environmental microniches. As expected from previous results based on samples of similar environmental conditions, the dissimilatory sulfate reduction pathway was one of the most expressed energetical pathway in mercury methylator populations\textsuperscript{2,26–28}. However, we also detected transcripts of tetrathionate and thiosulfate/polysulfide reductases in 60% of \textit{Desulfobacteraceae} populations.\textsuperscript{2,26–28}
Interestingly, transcripts of cytochrome c oxidase and sulfide quinone oxidoreductase (SQR) enzymes were identified in the Desulfobulbaceae population, supporting a sulfide/sulfur oxidation metabolism, potentially coupled to nitrate reduction through the expression of narG gene, as previously proposed. This result suggests that mercury methylation might not be limited to low redox and anaerobic conditions, but could also be carried out in oxic to suboxic conditions by some Desulfobulbaceae populations, potentially extending the distribution of environmental niches prone to methylmercury production.

Transcripts analysis revealed a major involvement of mercury methylators in nitrogen cycling with expression of nitrate reduction and/or denitrification genes for all recovered populations (Fig. 7), as previously proposed. Expression of genes involved in iron, halogenated and methylated compounds utilization and various fermentation was also detected, suggesting that some methymercury producers might thrive on numerous additional metabolism than previously suspected. In addition, genes associated with syntrophic metabolism (hydrogenases, formate dehydrogenases) were also expressed by Desulfobacterota populations, supporting a potential role of syntrophic interactions in mercury methylation. Together these results expanded the environmental niches and the known metabolic flexibility of mercury methylators.

As toxic methylmercury and mercury accumulation might be mitigated by demethylation and reduction, through the activity of organomercury lyase (MerB) and mercury reductase (MerA) enzymes respectively, diversity of merB and merA genes were also assessed. Based on merA genes phylogeny and taxonomy and coverage of the MAGs, the diversity (number of different lineages) and relative abundance (average coverage) of mercury reducers were larger than those of mercury methylators (Fig. 3 and Table 1). Mercury reducers detected in the microbial mats included known lineages of mercury reducers such as members of the Gammaproteobacteria, Rhodobacteraceae, and Bacteroidetes. Rhodobacteraceae and Bacteroidetes populations were identified as dominant members of the microbial mats (Fig. 2) but no or very little expression of merA was detected for these lineages (Fig. 6). Most of the merA transcripts were assigned to Gammaproteobacteria (bin761) and Desulfuromusa (bin93) populations with low coverage (Table 1), suggesting that mercury detoxification activity of abundant microorganisms might be lower than those of rare lineages.

Genetic composition and expression profile of the Desulfuromusa bin93 population (Fig. 7) was consistent with the strict anaerobic metabolism of the Desulfuromonadales members, suggesting that although merA is generally identified in aerobic lineages, most of the mercury reduction might be carried out by anaerobic lineages in the anoxic layers of microbial mats and sediments. This results suggest a potential cycle of mercury methylation, demethylation, and reduction in anoxic part of the microbial mat, as previously suspected in other anoxic environments. Interestingly, merA gene was also identified in three Verrucomicrobiaceae populations with the genetic composition and the expression profile consistent with an anaerobic metabolism based on fermentation (Fig. 7). The merA gene of these...
populations branched at the base of the phylogenetic tree without known references but in proximity with Desulfurobacterium sequences (Fig. 5), indicating that these Verrucomicrobiae populations may represent a new group of mercury reducers under anoxic conditions. Expression of merA gene was detected for one of the Verrucomicrobiae population (Fig. 6), supporting their role in mercury detoxification. Together, detected activities of mercury reducer populations were diverse, spanning sulfur, nitrogen and iron cycles and phototrophic, autotrophic and heterotrophic lifestyles, indicating a large metabolic flexibility in aerobic and anaerobic mercury detoxifying populations.

None of the recovered MAGs with merA gene also included the organomercury lyase gene (merB). However, merB gene was identified in two MAGs with high completeness (96.89%), detected exclusively in the Salins du Lion mats, and affiliated to members of the Rhodobacteraceae and Thiobacillusaceae with sulfur-oxidizing activity. Although this might be due to the incompleteness of our MAGs, this result is consistent with the low frequency of merA and merB gene cooccurrence in public microbial genomes [1].

Furthermore, mining of the available genomes in IMG database for merA and merB genes highlighted that over the 4530 genomes with merB gene, only 34% (1545) also include merA, supporting the occurrence of merA alone in our MAGs and recent observations [20]. Both Rhodobacteraceae and Thiobacillusaceae populations were found to express merB (Fig. 6), indicating an active methylmercury detoxification process in SL microbial mat. Together our results suggest that methylmercury cleavage and mercury reduction were dissociated in the microbial mats, with each reaction being carried out by taxonomically different lineages. The accumulation of mercury is detrimental for the cells, therefore additional merA-independent mechanism for mercury export or detoxification should occur in these populations. Various biotic and abiotic merA-independent detoxification process have been proposed [20,37], with notably the reduction of HgII to Hg0 in photomixotrophic microorganisms, providing a plausible mechanism of mercury detoxification for the Rhodobacteraceae bin246 population that strongly expressed phototrophic and mixotrophic genes.

The presence of a long-term hydrocarbon contamination in Etang de Berre is the main environmental feature that distinguishes the two habitats sampled in this study [15] (Fig. 1). Our 16S rRNA gene analysis indicated that the microbial community composition at the genus level was similar in the two habitats. However, the genome-centric approach indicated that up to 162 different populations (40% of the MAGs, Fig. 3) were recovered from only one habitat, suggesting a modification of the microbial community composition at the population level rather than at the genus level. Consistent with the alkane and PAH contamination in Etang de Berre, all MAGs with the potential for mercury methylation identified in this (bin165, bin506, bin727, bin805) were found to encode and express anaerobic hydrocarbon degradation genes (Benzyl-CoA reductase or Arylbenzylsuccinate synthase, annotated 88 and 89 in Fig. 7) except for the Aminicentantes bin506 for which no transcripts were identified (Fig. 7). By contrast, none of the MAGs from Salins du Lion encoded such genes. This result might indicate that hydrocarbon could structure the mercury methylator community by selecting lineages with hydrocarbon degrading metabolism. Although additional experiments are required, the absence of methylmercury degraders in EDB could also be a consequence of the hydrocarbon pollution in this habitat. By affecting both methylmercury producer and degrader diversity, the presence of hydrocarbons might have critical consequences for the methylmercury fate in hydrocarbons polluted environments.

The microbial mats analyzed in detail in this study provided an outstanding opportunity to investigate microbial populations controlling mercury toxicity at the foundation of the food chain. Our genetic and transcriptomic characterization of involved microbial populations extended the taxonomic and functional diversity of mercury cycle lineages, and emphasize the major roles of minority and rare mercury transformers in sulfur but also carbon, nitrogen, and iron biogeochemical cycles. The simultaneous detection of genes and transcripts of both mercury methylation and detoxification pathways indicated that mercury transformations are entangled in brackish biofilms, with potential cycles of methylation and demethylation under both anoxic and suboxic conditions.

**METHODS**

### Sample collection and nucleic acid extraction

Two coastal microbial mats developing in neighboring brackish lagoons, located at Berre l’Étang (EDB) and Salins du Lion (SL), a bird reserve located 5 km away from EDB were investigated (Fig. 1). Physical (temperature, pH, salinity, irradiance) and chemical (hydrocarbon content, metal, sulfate, H2S, dissolved oxygen, total and organic carbon and total nitrogen concentrations, redox potential) parameters have been previously published for both microbial mats and underlying sediments [15,19]. Sediments under both microbial mats are strongly contaminated by heavy metals, with mercury concentration averaging 1.7 × 10^6 μg of Hg per gram [21]. The major environmental parameter that distinguishes the two microbial mats is the presence of hydrocarbons, including alkanes and polycyclic aromatic hydrocarbons, caused by oil refinery contaminated rainwater discharges in EDB [15]. Microbial mat samples were collected in triplicate in September 2011, April 2012, and September 2012. In September and April 2012 microbial mats were sampled during both daytime (4 PM) and night-time (4 AM), leading to a total of 16 individual mat samples. Samples were stored in cryotubes and immediately submerged in liquid nitrogen for freeze-freezing. Back in laboratory, samples were stored at −80 °C until nucleic acids extraction. Nucleic acids (DNA and RNA) were extracted from all samples using the RNA PowerSoil™ Total RNA Isolation Kit (Qiagen, Hilden, Germany) coupled with the Allprep DNA/RNA mini kit and the RNaseFree DNase Set (Qiagen). RNA quality was checked using RNA nanochip on a Bioanalyzer 2100 (Agilent, Santa Clara, CA, USA) and absence of DNA contamination within RNA samples was confirmed by PCR amplification using RNA as a template. mRNA were enriched using Ribo-Zero™ Magnetic Kit (Bacteria), following the manufacturer’s instructions. The RNA was then immediately converted to cDNA using M-MLV Reverse Transcriptase and RNA OUT™ (Invitrogen, Carlsbad, CA, USA) with 1 μl of purified mRNA according to the manufacturer’s instructions.

### Metagenomic library preparation, sequencing, and analysis

Metagenomes were constructed for all 30 samples using the BioScientific PCR-free kit following the manufacturer’s protocol while metatranscriptomes of each sample were prepared using the TruSeq Stranded mRNA sample prep kit (Illumina, San Diego, CA, USA) following the manufacturer’s instructions. Metagenomes and metatranscriptomes were sequenced using Illumina HiSeq (2 × 100 bp) platform at the Genotoul sequencing facility leading to a total 670 Gb of sequences (370 GB of metagenomes and 300 GB of rRNA-depleted metatranscriptomes). Datasets were quality filtered using the Trimomatic v.0.39 [8], keeping both R1 and R2 reads when reads overlapped. The 16S rRNA reads were isolated from the metagenomic reads using REAGO 1.1 (ref. 39), and taxonomic assignments were performed with Mothur [40] using RDP classifier (cut-off: 80) against Silva database release 138 as reference [41]. Since metagenomic 16S rRNA reads were only 100 bp long and spanned various regions of the 16S rRNA gene, taxonomic assignments were limited to the genus level and above.

### Binning and functional characterization

For MAGs reconstruction, all quality filtered sequences were pooled and co-assembled using MEGAHIT [50]; then coassembly was uploaded to IMG/ MER platform for gene annotation [43]. Read coverage of the contigs was carried out using bwa-mem (http://bio-bwa.sourceforge.net), followed by the binning of the contigs longer than 2000 bp by MetaBAT-2 (ref. 44). The completeness and contamination level of the MAGs were then evaluated using CheckM [40]. Only MAGs with a contamination level under 5% and completeness above 50% were analyzed. Open reading frames (ORFs) were identified using Prodigal [45] and compared against COG, Pfam, TIGRfam, and KEGG databases on IMG/MER platform, leading to 5.7 × 10^6 genes with product name (58.96% of the genes). Since hgcA gene is poorly
In the absence of the amino acid signature the gene were dephylogenetic trees were generated using Fasttree 2 ([ref.49]). Phylogenetic presence of cysteine pairs that are potentially essential for MerA activity20. Available in the NCBI Genbank database under Bioproject accession number SRP063590.

Expression profile analysis
To determine gene expression levels of each gene identified in MAGs, all metatranscriptomic reads passing quality filtration were mapped against all open reading frames identified in MAGs using bbam-mem. Due to rRNA depletion step malfunction, the September 2012 metatranscriptomes of EDB microbial mats were removed from the analysis. For each MAGs, the coverage value of genes of interest was normalized to the coverage of the rRNA polymerase subunit B (rpoB) gene to discriminate between low and high expression level compared to housekeeping genes. Results were represented in heatmaps using the software environment R (v.4.0.3) and R Studio (v.1.3.1093).

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this article.

DATA AVAILABILITY
Assembled metagenome data are available in IMG/MR (https://img.jgi.doe.gov/me/) under the following accession numbers: 3300040774–3300040799. Coassembl is also available on IMG/MR under accession number 3300040801. Bins with mercury cycling genes were deposited in Figshare (https://doi.org/10.6084/m9.figshare.1501530v2). Environmental metadata were previously published [35]. Nucleotide sequences are available in the NCBI Genbank database under Bioproject accession number SRP063590.

CODE AVAILABILITY
The merB gene HMM profile is available in Figshare (https://doi.org/10.6084/m9.figshare.1501530v2). Software version: REAGO 1.1, Mothur 1.44.3, Trimmomatic v0.39, Megahit 1.2.9, bwa 0.7.17, CheckM v.1.1.2, R 4.0.3 and R Studio 1.3.1093.

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AUTHOR CONTRIBUTIONS
R.G. and M.G. design the study, J.A. and M.G. collected the samples. J.A. generated the dataset. A.V. and P.C. analyzed the data and A.V. wrote the manuscript with contributions from P.C., J.A., R.G., and M.G.

COMPETING INTERESTS
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to Adrien Vigneron.

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