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Prevalence of heterotrophic methylmercury detoxifying bacteria across oceanic regions

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ABSTRACT

Microbial reduction of inorganic divalent mercury (Hg^{2+}) and methylmercury (MeHg) demethylation is performed by the *mer* operon, specifically by *merA* and *merB* genes respectively, but little is known about the mercury tolerance capacity of marine microorganisms and its prevalence in the ocean. Combining culture-dependent analyses with metagenomic and metatranscriptomic analyses, here, we show that marine bacteria encoding *mer* genes are widespread and active in the global ocean. We explored the distribution of these genes in 290 marine heterotrophic bacteria (*Alteromonas* and *Marinobacter* spp.) isolated from different oceanographic regions and depths, and assessed their tolerance to diverse concentrations of Hg^{2+} and MeHg. In particular, *Alteromonas* sp. ISS312 strain presented the highest tolerance capacity and a degradation efficiency for MeHg of 98.2% in 24 h. Fragment recruitment analyses of *Alteromonas* sp. genomes (ISS312 strain and its associated reconstructed metagenome assembled genome MAG-0289) against microbial bathypelagic metagenomes confirm their prevalence in the deep ocean. Moreover, we retrieved 54 *merA* and 6 *merB* genes variants related to *Alteromonas* sp. ISS312 strain from global metagenomes and metatranscriptomes from *Tara* Oceans. Our findings highlights the biological reductive MeHg degradation as a relevant pathway of the ocean Hg biogeochemical cycle.

Keywords: mercury, methylmercury, marine bacteria, mercury resistant bacteria, *merA*, *merB*, minimum inhibitory concentration (MIC)

Synopsis

Active mercury resistance genes detected in marine cultured bacteria are widely distributed in the ocean, including the bathypelagic zone.

Introduction

Mercury (Hg) is one of the most toxic, widespread and worrisome contaminants[1,2], and is emitted to the atmosphere by natural sources, such as volcanoes and rock weathering, but particularly by anthropogenic activities. The rising Hg levels since the industrial era, estimated as an increase of 450 % in the atmosphere[3], makes the study of its biogeochemical cycle a major concern to the scientific community, and as a consequence, the Minamata convention, a global treaty to protect human, wildlife health and the environment from the adverse effects of mercury by e.g. reducing its atmospheric emissions, was hold in 2013 and entered into force in August 2017[4].

Emitted elemental (Hg^0) and inorganic divalent Hg (Hg^{2+}) can be deposited to land and oceans by wet and dry depositions[5,6]. Inorganic divalent Hg in the ocean can be then volatilized back again to the atmosphere as Hg^0 [7], or can be methylated[8–12] to methylmercury (MeHg), which bioaccumulates and biomagnifies in aquatic food webs[3,13,14]. As a consequence, humans are exposed to this neurotoxicant mainly through fish and seafood consumption[13,15,16]. Methylmercury levels in the oceans vary with depth, and usually, measures are being reported low in open ocean surface waters, maximal in intermediate layers, especially in regions of low-oxygen and near or below the thermoclines (up to 1000 m depth), and low and relatively constant in deeper waters (>1000 m depth)[13,17,18]. While Hg^{2+} methylation has been reported to occur in oxic and sub-oxic layers of the water column[8,17,19–21] mainly associated with the microbial remineralization of sinking particulate organic matter[17,18,22], much less is known about MeHg demethylation and Hg^{2+} reduction processes in the ocean water column. Although MeHg demethylation and Hg^{2+} reduction processes can be photochemically mediated[23–25], light penetration in the ocean water column is limited to 200 m[26], thereby biological MeHg degradation and Hg reduction processes likely govern in the ocean water column. Moreover, taking into account that different concentrations of MeHg can be found through the ocean water column [20,27–29], it would be plausible to find microorganisms with Hg detoxification capacity, especially in deep aphotic zones. However, very few studies have unveiled and demonstrated the role of microorganisms in MeHg degradation in the vast deep ocean.

Biological MeHg demethylation and Hg^{2+} reduction detoxification processes can be mediated by different biotic processes including reductive reactions mediated by the *mer* operon[30,31], but also oxidative reactions[32,33], as well as the recently described MeHg degradation performed by methanotrophic bacteria[34]. In this study, we will focus only in the study of bacteria carrying the *mer* operon (i.e. biological reductive MeHg degradation pathways**)**. While the operon can be composed by different sets of genes[30,31], the key genes are *merA* and *merB*. The first one

codifies a mercuric reductase and is responsible for the transformation of Hg^{2+} to the less harmful and volatile Hg^0 [30]. The *merB* gene encodes an organomercurial lyase enzyme that confers resistance to the organic MeHg form. It is the responsible for its demethylation releasing Hg^{2+} that will be then reduced to Hg^0 by *merA* gene[30]. These machineries have been found in numerous microorganisms including aerobic and anaerobic species, although demethylation appears to be predominantly accomplished by aerobic organisms[31,35]. To date, very few studies have showed the presence of *mer* genes in oceanic waters, with the exception of some studies in the North Pacific and Arctic Oceans[36] and a recent analysis in the global bathypelagic ocean. In order to build this gap in knowledge, the aim of this study was to demonstrate that marine bacteria encoding *mer* genes could degrade MeHg and to explore its prevalence in the open ocean including deep-ocean waters. For this, we took advantage of the MARINHET[37] culture collection, that includes marine bacterial strains from a wide variety of oceanographic regions and depths. We analyzed 290 marine heterotrophic bacteria in order to: (i) detect the presence of *merA* and *merB* genes in different oceanographic regions and depths, (ii) assess the tolerance of an important fraction of strains encoding those genes to different concentrations of divalent inorganic Hg (Hg^{2+}) and monomethylmercury (MeHg), (iii) describe the degradation potential for the most tolerant strains, and (iv) explore its prevalence and biogeography patterns across oceans and depths by retrieving *merA* and *merB* genes from marine microbial metagenomes and metatranscriptomes from *Tara* Oceans Expedition[38,39].

Identification of Hg resistant bacteria in contrasting aquatic ecosystems and the assessment of their tolerance to different concentrations of MeHg provide new opportunities to explore the ubiquity and prevalence of marine cultured bacteria with detoxification capacity in the open ocean (i.e. non-contaminated sites). On the other hand, this type of study sets the fundamentals for finding suitable microorganisms to be used for bioremediation strategies.

Materials and Methods

Primer design for *merA* and *merB* genes of *Alteromonas* and *Marinobacter*

Primers were designed in order to identify mercury resistant bacteria among *Alteromonas* and *Marinobacter* strains in a culture collection because: (i) these genera are well known to encode the *mer* operon[40–44] in their genomes, (ii) they are the most common culturable heterotrophic bacteria living in open marine waters all around the world, as they have been isolated from a wide variety of marine environments[42,45–50], and in the case of *Alteromonas* it is one of the most ubiquitous cultured taxa in the ocean[37], (iii) we have access to a large number of isolates thanks to the MARINHET culture collection (see Supplementary Information for more details about

the culture collection). For all these reasons, these two genera were a suitable target to prove that *mer* genes can be found across different oceanographic regions and depths but also demonstrate the MeHg degrading capability of several strains.

Specific primer pairs were designed separately for: (i) *merA* of *Alteromonas*, (ii) *merA* of *Marinobacter*, (iii) *merA* + *merB* of *Alteromonas*, and (iv) *merA* + *merB* of *Marinobacter* based on reference sequences downloaded (in 2016) from the from Integrated Microbial Genomes (IMG) database of the Joint Genome Institute (JGI). See Supplementary Information, **Supplementary Figure S1** and **Supplementary Table S1** for detailed information. Noteworthy, the primers design in this study are useful for detecting exclusively *Alteromonas* and *Marinobacter* and might not be suitable for other taxa.

DNA extraction and PCR conditions

The primers previously designed were used for the screening of *merA* and *merAB* Hg resistance genes in all *Alteromonas* and *Marinobacter* strains (n=290) available at the MARINHET culture collection in 2016 (n=1313). DNA of all the strains was extracted from 48 h liquid cultures grown in Zobell broth medium (i.e. 5 g peptone, 1 g yeast extract in 750 ml of 30 kDa filtered seawater and 250 ml of Milli-Q water) using the DNeasy Blood & Tissue kit (Qiagen) following the manufacturer's recommendations. Detailed PCR conditions are described in Supplementary Information. The PCR products were verified and quantified by agarose gel electrophoresis with a standard low DNA mass ladder (Invitrogen). Purification and OneShot Sanger sequencing of *merA* and *merAB* gene products was performed by Genoscreen (Lille, France) with both forward and reverse primers. Geneious software v.11.0.5[51] was used for manual cleaning and quality control of the sequences.

Minimum inhibitory concentration experiments

A total of 73 strains from the 290 isolates previously screened by PCR were subjected to Minimum inhibitory concentration (MIC) assays, including then all strains with positive results for *merA* and/or *merAB* presence except one that could not grow again from the cryostock. MIC assays were designed based on previous studies[44,52] in order to assess the tolerance of the marine strains to different concentrations of inorganic Hg (as mercury(II) chloride, $HgCl_2$) and organic Hg (as methylmercury chloride, CH_3HgCl) and thus, to test the activity of *merA* and *merB* genes respectively. A stock solution of $HgCl_2$ was prepared at 500 µM with autoclaved Milli-Q water. Liquid cultures of the strains growing in Zobell broth with an optical density (O.D. at 600nm) of 0.1 were placed in 24-well plates and inoculated with 5 µM, 10 µM, 20 µM, 25 µM and 50 µM

$HgCl_2$. In specific cases growth was observed in all $HgCl_2$ concentrations and further MIC assays were done increasing the final concentrations to 50 µM, 60 µM, 70 µM, 80 µM, 90 µM and 100 µM. The tolerance to CH_3HgCl was also tested for the most tolerant strains to $HgCl_2$. In these case, 24-well plates were inoculated with the stock solution to reach final concentrations at 2.5 µM, 5 µM, 10 µM, 15 µM and 20 µM. In all plates a positive control (liquid culture of the strains not amended with CH_3HgCl or $HgCl_2$) and a negative control (broth media without bacteria in order to check for possible environmental contamination) were included in the assays. Plates were sealed with parafilm and incubated at room temperature (RT, ~20 ºC) and in the dark for 72 h. Visual examination and O.D. measurements at 600 nm were done in a 24 h period using an automatic plate reader (Infinite ® M200, Tecan) and data was collected using the Magellan™ Data Analysis Software (Tecan Diagnostics®).

Growth curves

Growth curves were performed to characterize the growth rates of the most tolerant strain (ISS312) to different concentrations of CH_3HgCl. We prepared 200 ml of liquid cultures in Zobell broth supplemented with CH_3HgCl at final concentrations of 0 µM (positive control), 1 µM, 2.5 µM and 5 µM in triplicates. The initial O.D. at 600 nm of the cultures was 0.05 in order to assure enough concentration of cells for growing. Samples for O.D. measurements and for bacterial cell counts were taken approximately every two hours. O.D. was measured at 600 nm with a spectrophotometer (Varian Cary® 100 UV-Vis) and cells were stained with 4',6-diamidino-2-phenylindole (DAPI) and counted with an automated microscope Zeiss Axio Imager Z2M[53,54] using the automated image analysis software ACME Tool (www.technobiology.ch). Predicted growth curves based on O.D. observations and kinetics values including growth rates (μ_{max}), carrying capacity (k) and lag phase time, were calculated with R package *growthcurver v*.0.3.0[55] and GrowthRates v.4.3 software[56]. For graphical representation, replicates of the different growth curves experiments at several CH_3HgCl concentrations were averaged. Hence, mean O.D. and standard deviation was calculated for each time point of the curves.

From the 1 µM and 5 µM growth curves 2 ml samples were also taken for characterizing CH_3HgCl degradation rates at time 0, 6, 12, 24 and 48 h. Besides, in order to check the possibility that CH_3HgCl was being abiotically removed, we also measured the CH_3HgCl concentrations from samples taken from multiwell plates experiments including different controls (more details in Supplementary Information). Description of Hg species concentrations measurements are also explained in Supplementary Information.

Phylogenetic analyses

In order to show that MeHg degradation capacity is strain specific, a phylogeny of the isolates screened by PCR for *merA* and *merAB* genes was inferred from their partial 16S rRNA sequences in order to detect a possible clustering between all the positive strains. The closest sequence to each isolate 16S rRNA gene was obtained by BLASTn[57] against SILVA v.132 database. Alignment of the isolates and reference sequences was performed with MUSCLE from the Geneious software v.11.0.5[51]. The alignment was trimmed to the common 16S rRNA gene fragment covered by both sets of sequences. Phylogeny was constructed using maximum-likelihood inference with RAXML-NG 0.9.0[58] and the GTR evolutionary model with optimization in the among-site rate heterogeneity model and the proportion of invariant sites (GTR+G+I), and 100 bootstrap replicates. In the same way a phylogenetic tree was constructed with the partial 16S rRNA sequences of the positive isolates only. In this tree the closest match in SILVA v.132 database was also included. Presence of *merA* and *merAB* genes, origin of the strains, plus their tolerance to $HgCl_2$ and MeHg were added with Interactive Tree of Life (ITOL)[59]. Supplementary phylogenetic trees were also constructed using aminoacid sequences of *merA* and *merB* genes (see Supplementary Information for details).

Fragment recruitment analysis of the genome of strain ISS312 and MAG-0289 in bathypelagic metagenomes

The abundance of ISS312 across the global bathypelagic ocean was assessed thanks to the sequencing of its complete genome (Supplementary Information). In addition, thanks to a previous study[60] we could reconstruct a metagenome assembled genome (MAG-0289) also affiliating to *Alteromonas mediterranea* species and encoding *merA* and *merB* genes. Fragment Recruitment Analysis (FRA) of the ISS312 strain and the MAG-0289 was performed by mapping the metagenomic reads of 58 bathypelagic microbial metagenomes from 32 stations[60,61] from Malaspina Expedition, including free-living (0.2-0.8 μm) and particle-attached (0.8-20 μm) bacterial communities. Analysis were done with BLASTn v2.7.1+[57]. Details of FRA are explained in Supplementary Information.

Detection of merA and merB genes in global metagenomes and metatranscriptomes from Tara Oceans expedition

BLASTp analyses were performed using a conservative *e-vale* (>1E-100) with the *merA* and *merB* sequences of the ISS312 genomes against global prokaryotic metagenomes (metaG) and metatranscriptomes (metaT) available in the Ocean Microbial Reference Gene catalogue V2 (OMRGC.v2)[39] from *Tara* Oceans Expedition covering surface, deep chlorophyll maximum (DCM) and mesopelagic layers across oceanographic regions. The generation and annotation of the OMRGC.v2, the profiling of taxonomic of metagenomic and metatranscriptomic composition, and the normalization of metagenomic and metatranscriptomic profiles used for the extraction of the abundances of the *merA* and *merB* gene homologous had been thoroughly explained in Salazar et al.[39]. Analyses could be performed thanks to the Ocean Gene Atlas web resource[38] and abundance results from metagenomes and metatranscriptomes were normalized based on the percentage of mapped reads.

Statistical analyses

ANOVA tests from the *stats* package of the R Statistical Software [62] was applied in order to observe if the presence of *merA* and/or *merAB* genes in the studied strains was linked to specific oceanographic locations. Further, non-parametric Kruskal-Wallis test, from the *stats* package of the R Statistical Software[62], was applied followed by the post hoc pairwise Wilcox test to see the differences between FRA results in different oceanographic regions and between free-living and particle-attached bacterial communities. To assess significance, all the statistical analyses were set to an alpha value of 0.05.

Nucleotide accession numbers

Mercury detoxification genes (*merA* and *merAB*) detected in this study through PCR were deposited in GenBank under accession numbers MW273028 - MW273125. *Alteromonas mediterranea* ISS312 genome was deposited in ENA under study accession number PRJEB46669.

Results and Discussion

Presence of merA and merB genes among Alteromonas and Marinobacter strains

Among the 290 strains of the MARINHET bacterial culture collection[37], 244 were taxonomically classified as *Alteromonas sp.* and 46 strains to *Marinobacter sp.* (**Supplementary Table S2**). These strains were isolated from different depths, including the surface, the deep chlorophyll maximum (DCM) and the bathypelagic zone. The strains here

studied for *merAB* genes were isolated from different oceanic regions such as the North Western Mediterranean Sea (89), South (101) and North Atlantic (42), Indian (44), Arctic (7) and Southern (7) Oceans and included isolates from photic (160) and aphotic (130) layers (**Table 1**; **Supplementary Figure S2 and Supplementary Tables S3-4**).

The functional screening of the *merA* and *merB* genes from the 244 *Alteromonas* and 46 *Marinobacter* strains revealed that 13.5 % (32 out of 244) and 89.1 % (41 out of 46) of the strains presented only *merA,* respectively, while only 1.6 % (4 out of 244), and 47.8 % (22 out of 46) presented both *merA* and *merB* genes (*merAB*) (**Table 1**). No significant differences were found between depths neither between oceans (ANOVA, P-value > 0.05) but in general, we found a higher proportion of positive strains coming from waters of the Southern Ocean (71 % despite the lower number of strains tested) and the South Atlantic Ocean (48 %), followed by those retrieved from the North Atlantic (17 %) and North Western Mediterranean Sea (13 %).

High variability of mercury tolerance within marine bacteria

It is unknown whether mercury tolerance is a conservative trait within marine bacterial strains of the same genera. To prove so, MIC experiments were done in all *Alteromonas* and *Marinobacter* isolates (73 strains) presenting *merA* and/or *merAB* detected by PCR except one isolate that was not able to grow again from the cryostock. First, we tested the tolerance for inorganic mercury ($HgCl_2$) and we observed that 32 *Alteromonas* and 41 *Marinobacter* strains displayed different levels of tolerance. MIC values ranged generally from 5 to 50 µM. Around 50 % of the *Alteromonas* and *Marinobacter* strains tested presented a MIC of 20 µM and one of the isolates stood out as it presented a tolerance to $HgCl_2$ up to 70 µM (**Supplementary Table S5**). Besides, we tested the tolerance to MeHg amended in form of CH_3HgCl for those strains already presenting a tolerance to inorganic mercury above 20 µM and encoding the *merB* gene. For *Alteromonas*, three strains taxonomically classified as *Alteromonas mediterranea* presented a high tolerance to CH_3HgCl, growing at concentrations up to 10 µM (**Figure 1**), but all the tested *Marinobacter* sp. strains did not show a substantial growth above 2.5 µM of CH_3HgCl (**Figure 1**).

If we take a look into the phylogenetic trees constructed with the 16S rRNA sequences **(Figure 1)** of the strains presenting *merA* and/or *merAB* genes different tolerances were found within the same phylogenetic cluster. For example, within the cluster of *Alteromonas mediterranea* some strains presented a MIC of 20 µM, while the isolate that presented the highest tolerance (70 µM, ISS312) also belonged to the same *Alteromonas* species (**Figure 1**). The same occurred among *Marinobacter* isolates, where members of the *Marinobacter hydrocarbonoclasticus* cluster

presented MIC values ranging from 10 to 50 µM (**Supplementary Table S5 and Figure 1**). The same pattern can be observed in the *merA* and *merAB* aminoacids phylogenies **(Supplementary Information and Supplementary Figures S3 and S4).**

The MIC values heterogeneity within strains belonging to the same phylogenetic cluster, suggested that the level of Hg resistance was strain specific, and we probably retrieved different ecotypes within a same species with different tolerances to Hg. Despite these differences between strains, the tolerances found for inorganic Hg were similar to those found in other studies where *Alteromonas*[44,63–65] and *Marinobacter*[66] genera were also isolated from different marine ecosystems such as hydrothermal vents, estuaries or contaminated sediments. However, to the best of our knowledge, this is the first study addressing the tolerance of *Marinobacter spp.* and *Alteromonas mediterranea* isolated from the ocean to MeHg. Hence, we found out that a strain affiliating to *Alteromonas mediterranea* (ISS312) presented a MIC to inorganic Hg higher than other strains already published, up to 70 µM, and we also determined that it was able to grow in the presence of MeHg, presenting a MIC up to 10 µM, representing a good candidate for future bioremediation studies in highly contaminated areas with both organic and inorganic mercuric compounds.

Description of the highly tolerant Alteromonas sp. strain ISS312

Strain ISS312, isolated from South Atlantic bathypelagic waters at 4000 m and classified as *Alteromonas mediterranea,* displayed the highest tolerance to both $HgCl_2$ (70 µM) and MeHg (10 µM). The growth rates of this strain at different concentrations of MeHg were assessed and included: a control without MeHg (0 µM), 1 µM, 2.5 µM and 5 µM MeHg. Growth curves at 0 µM and 1 µM were very similar, as well as between 2.5 µM and 5 µM (**Figure 2A**). We observed that the major difference between growth curves was the length of the lag phase, where bacteria adapt themselves to the growth conditions. This phenomenon seems to be a common trait for Hg resistant strains in the presence of the toxic compound, as this behavior has been repeatedly observed in different species of *Pseudomonas* sp., *Alcaligenes* sp. or *Bacillus* sp.[67–69]. However, once the cultures started to grow, their growth rates (μ_{max}) were very similar independently of their initial MeHg concentrations, ranging from 0.10 h^{-1} in the control to 0.09 h^{-1} at 5 µM. Stationary phase was reached in all concentrations at 80 h, even though at this time cultures at higher concentrations of MeHg seemed to be only entering to the plateau (**Figure 2A**). In addition, their carrying capacity (k), i.e. the maximum population size of a species, was between 1.6 and 1.9 based in O.D. measures, revealing very similar values between tested concentrations, an observation also recurrently reported[68,70]. Transmission electron microscopy (TEM) observations of the ISS312 cultures growing at 0 µM and 5 µM of MeHg also showed similar morphology and ultrastructure of the cells **(Figure 2A).**

MeHg concentrations were measured in the isolate exposed to 5 μM at different incubation times. MeHg concentrations were reduced by 36 % (2.6 μM) and 72 % (1.1 μM) (**Figure 2B**) during the lag phase at 6 h and 12 h, respectively. After 24 h, when almost all MeHg was removed (removal of 98.2 %), the microorganism began the exponential growth phase. After 48 h, MeHg could not be detected (**Figure 2B Supplementary Table S6**). It is important to highlight that we detected a certain level of abiotic MeHg degradation (up to 25%) in the medium exposed without bacteria and in the killed control (**Supplementary Table S7**).

Global distribution of ISS312 strain and MAG-0289 in the bathypelagic ocean

The biogeographic and size fraction distribution of ISS312 genome was assessed in all available bathypelagic metagenomes of the Malaspina Expedition[60] since strain ISS312 was originally retrieved from bathypelagic waters of the South Atlantic Ocean. We found that this strain belonging to *Alteromonas mediterranea* species was distributed across all the temperate bathypelagic waters, including the Atlantic, the Pacific and the Indian Oceans (**Figure 3**). Its abundances, according to the data from the fragment recruitment analyses (FRA), varied across ocean basins and we found significant differences between the Pacific and the Brazil basins (P-value= 0.019), and between the Pacific and the Canary basins (P-value: 0.011), suggesting a higher abundance of this bacterium in the Atlantic Ocean. Despite finding these differences between oceans, we did not find significant differences between plankton size fractions, indicating that the isolate could be present in both the free-living (0.2-0.8 μm) and particle-attached (0.8-20 μm) bacterial communities (**Figure 3**). Moreover, by using bathypelagic metagenomes a Metagenome Assembled Genome (MAG) was reconstructed, the MAG-0289, which genome aligned at 99 % with the *Alteromonas* sp. ISS312 strain genome and it has a 99.34 % of average nucleotide identity (ANI). The FRA of MAG-0289 and ISS312 strain against the bathypelagic metagenomes displayed identical biogeographical patterns **(Supplementary Figure S5)**, indicating that the MAG-0289 is a good representative of the ISS312 strain.

Our results confirm the prevalence and wide distribution for *Alteromonas mediterranea* species carrying Hg resistance genes across the global bathypelagic ocean but also shows its occurrence in both plankton size fractions analyzed. To the best of our knowledge the detection and characterization of *mer* genes from non-contaminated open ocean waters at a large scale has not been previously reported.

Recovery of merA and merB homologous gene variants of ISS312 from marine microbial metagenomes and metatranscriptomes

A remaining question is if *merA* and *merB* genes are active under natural ambient Hg concentrations. For answering this question, we have analyzed available metatranscriptomes (metaT) of the Ocean Microbial Reference Gene catalogue V2 (OMRGC.v2)[39] from *Tara* Oceans Expedition through the Ocean Gene Atlas resource[38] covering surface, DCM and mesopelagic layers across oceanographic regions. BLASTp search using the *merA* and *merB* genes from the ISS312 strain as input allowed us to extract 54 *merA* and 6 *merB* homologous (*e-value* 1E-100) that ranged from 64 to 99.3 % identities in their aminoacids sequences and belonged to gammaproteobacterial lineages mainly from *Alteromonadales* order and *Halomonadaceae* family (**Supplementary Table S8**). These *merA* and *merB* transcripts were prevalent across oceans and depths (see **Figure 4, Supplementary Figures S6 and S7**). We found that *merB* genes were more transcribed (**Figure 4**) in the mesopelagic of the North Indian and Eastern South Pacific Oceans, which are well known regions of oxygen minimum zone areas, and also in some stations from surface South Atlantic waters. It is known that MeHg production presents its maximum concentrations usually near the thermocline and in regions with low oxygen concentrations[20,71] as those oxygen minimum zone areas, and perhaps bacteria present in those regions encode and transcribe the Hg resistance genes in order to cope with those more elevated MeHg concentrations.

Outlook

A combination of culture-dependent analyses with metagenomic and metatranscriptomic analyses from the global photic and aphotic ocean unveiled that biological reductive MeHg degradation capacities were widely distributed and active in the open ocean, especially in the mesopelagic regions. In particular, this study has uncovered that heterotrophic isolates containing *mer* genes are present in the open ocean from different oceanographic regions, depths but also plankton size fractions. Also, this study reveals that strains closely related phylogenetically presented contrasting minimum inhibitory concentration values, indicating that the level of Hg resistance is strain specific. In particular we show that ISS312 strain isolated from bathypelagic waters of the South Atlantic Ocean presented a strong and fast capacity to degrade MeHg in 24 h. Moreover, the ISS312 strain genome and lineages related harboring *merA* and *merB* genes are present and transcribed globally in marine samples across oceans and depths including polar regions. This outcome has important implications in the biogeochemical cycle of Hg as it provides new understanding on the main players driving MeHg levels in the

ocean and can ultimately help to improve current frameworks for marine food webs and human exposure to MeHg.

ASSOCIATED CONTENT

Supporting Information

Supplementary Materials and Methods section including detailed description of some extra analyses and methodologies used in the study. Supplementary Results describe first exploration in the MARINHET collection and phylogenetic analyses with the 16S rRNA genes of all 290 tested strains and the *merA* and *merAB* phylogenies. Supplementary Figures including description of primers used, extra phylogenetic trees and results from metagenomes and metatranscriptome analyses for detecting presence and activity of *merA* and *merB* in natural conditions. Supplementary Tables excel file for a more detailed description of the origin of tested strains, primers construction, PCR screening analyses, physiological characterization of strains and taxonomic classification of detected *merA* and *merB* genes in metagenomes and metatranscriptomes of Tara Oceans data.

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Author Contributions

The study was conceptualized by: ISS, SGA, AGB and OS; experimental procedures were performed by: ISS, CPG, LT, MPiF, MC, RCRM-D; data analyses conducted by: ISS, PS, SGA and writing by: ISS, AGB, SGA, OS. * These authors contributed equally. All authors have given approval to the final version of the manuscript.

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References

(1) Miller, M. W.; Clarkson, T. W. *Mercury, Mercurial, and Mercaptans.*; Thomas, Springfield, ILL., 1973. https://doi.org/https://doi.org/10.1016/s0015-6264(75)80173-8.

(2) Clarkson, T. W. Mercury: Major Issues in Environmental Health. *Environ. Health Perspect.* **1993**, *100*, 31–38. https://doi.org/10.1289/ehp.9310031.

(3) UN Environment. *Global Mercury Assesment 2018.*; UN Environment Programme, Chemicals and Health Branch Geneva, Switzerland, 2019.

(4) Eriksen, H. H.; Perrez, F. X. The Minamata Convention: A Comprehensive Response to a Global Problem. *Rev. Eur. Comp. Int. Environ. Law* **2014**, *23* (2), 195–210. https://doi.org/10.1111/reel.12079.

(5) Saiz-Lopez, A.; Sitkiewicz, S. P.; Roca-Sanjuán, D.; Oliva-Enrich, J. M.; Dávalos, J. Z.; Notario, R.; Jiskra, M.; Xu, Y.; Wang, F.; Thackray, C. P.; Sunderland, E. M.; Jacob, D. J.; Travnikov, O.; Cuevas, C. A.; Acuña, A. U.; Rivero, D.; Plane, J. M. C.; Kinnison, D. E.; Sonke, J. E. Photoreduction of Gaseous Oxidized Mercury Changes Global Atmospheric Mercury Speciation, Transport and Deposition. *Nat. Commun.* **2018**, *9* (1), 4796. https://doi.org/10.1038/s41467-018-07075-3.

(6) Enrico, M.; Roux, G. Le; Marusczak, N.; Heimbürger, L.-E.; Claustres, A.; Fu, X.; Sun, R.; Sonke, J. E. Atmospheric Mercury Transfer to Peat Bogs Dominated by Gaseous Elemental Mercury Dry Deposition. *Environ. Sci. Technol.* **2016**, *50* (5), 2405–2412. https://doi.org/10.1021/acs.est.5b06058.

(7) Mason, R. P.; Sheu, G.-R. Role of the Ocean in the Global Mercury Cycle. *Global Biogeochem. Cycles* **2002**, *16* (4), 1093. https://doi.org/10.1029/2001GB001440.

(8) Lehnherr, I.; St. Louis, V. L.; Hintelmann, H.; Kirk, J. L. Methylation of Inorganic Mercury in Polar Marine Waters. *Nat. Geosci.* **2011**, *4* (5), 298–302. https://doi.org/10.1038/ngeo1134.

(9) Munson, K. M.; Lamborg, C. H.; Boiteau, R. M.; Saito, M. A. Dynamic Mercury Methylation and Demethylation in Oligotrophic Marine Water. *Biogeosciences* **2018**, *15*, 6451–6460. https://doi.org/10.5194/bg-15-6451-2018.

(10) Monperrus, M.; Tessier, E.; Amouroux, D.; Leynaert, A.; Huonnic, P.; Donard, O. F. X. Mercury Methylation, Demethylation and Reduction Rates in Coastal and Marine Surface Waters of the Mediterranean Sea. *Mar. Chem.* **2007**, *107* (1), 49–63. https://doi.org/10.1016/j.marchem.2007.01.018.

(11) Podar, M.; Gilmour, C. C.; Brandt, C. C.; Soren, A.; Brown, S. D.; Crable, B. R.; Palumbo, A. V.; Somenahally, A. C.; Elias, D. A. Global Prevalence and Distribution of Genes and Microorganisms Involved in Mercury Methylation. *Sci. Adv.* **2015**, *1* (9), e1500675. https://doi.org/10.1126/sciadv.1500675.

(12) Gionfriddo, C. M.; Tate, M. T.; Wick, R. R.; Schultz, M. B.; Zemla, A.; Thelen, M. P.; Schofield, R.; Krabbenhoft, D. P.; Holt, K. E.; Moreau, J. W. Microbial Mercury Methylation in Antarctic Sea Ice. *Nat. Microbiol.* **2016**, *1* (10), 16127. https://doi.org/10.1038/nmicrobiol.2016.127.

(13) Mason, R. P.; Choi, A. L.; Fitzgerald, W. F.; Hammerschmidt, C. R.; Lamborg, C. H.; Soerensen, A. L.; Sunderland, E. M. Mercury Biogeochemical Cycling in the Ocean and Policy Implications. *Environ. Res.* **2012**, *119*, 101–117. https://doi.org/10.1016/j.envres.2012.03.013.

(14) Harding, G.; Dalziel, J.; Vass, P. Bioaccumulation of Methylmercury within the Marine Food Web of the Outer Bay of Fundy, Gulf of Maine. *PLoS One* **2018**, *13* (7), e0197220. https://doi.org/10.1371/journal.pone.0197220.

(15) Mergler, D.; Anderson, H. A.; Chan, L. H. M.; Mahaffey, K. R.; Murray, M.; Sakamoto, M.; Stern, A. H. Methylmercury Exposure and Health Effects in Humans: A Worldwide Concern. *Ambio* **2007**, *36* (1), 3–11. https://doi.org/10.1579/0044-7447(2007)36[3:MEAHEI]2.0.CO;2.

(16) Karagas, M. R.; Choi, A. L.; Oken, E.; Horvat, M.; Schoeny, R.; Kamai, E.; Cowell, W.; Grandjean, P.; Korrick, S. Evidence on the Human Health Effects of Low-Level

Methylmercury Exposure. *Environ. Health Perspect.* **2012**, *120* (6), 799–806. https://doi.org/10.1289/ehp.1104494.
(17) Cossa, D.; Averty, B.; Pirrone, N. The Origin of Methylmercury in Open Mediterranean Waters. *Limnol. Oceanogr.* **2009**, *54* (3), 837–844. https://doi.org/10.4319/lo.2009.54.3.0837.
(18) Sunderland, E. M.; Krabbenhoft, D. P.; Moreau, J. W.; Strode, S. A.; Landing, W. M. Mercury Sources, Distribution, and Bioavailability in the North Pacific Ocean: Insights from Data and Models. *Global Biogeochem. Cycles* **2009**, *23* (2), GB2010. https://doi.org/10.1029/2008GB003425.
(19) Blum, J. D.; Popp, B. N.; Drazen, J. C.; Anela Choy, C.; Johnson, M. W. Methylmercury Production below the Mixed Layer in the North Pacific Ocean. *Nat. Geosci.* **2013**, *6* (10), 879–884. https://doi.org/10.1038/ngeo1918.
(20) Hammerschmidt, C. R.; Bowman, K. L. Vertical Methylmercury Distribution in the Subtropical North Pacific Ocean. *Mar. Chem.* **2012**, *132–133*, 77–82. https://doi.org/10.1016/j.marchem.2012.02.005.
(21) Malcolm, E. G.; Schaefer, J. K.; Ekstrom, E. B.; Tuit, C. B.; Jayakumar, A.; Park, H.; Ward, B. B.; Morel, F. M. M. Mercury Methylation in Oxygen Deficient Zones of the Oceans: No Evidence for the Predominance of Anaerobes. *Mar. Chem.* **2010**, *122* (1–4), 11–19. https://doi.org/10.1016/J.MARCHEM.2010.08.004.
(22) Lamborg, C. H.; Hammerschmidt, C. R.; Bowman, K. L. An Examination of the Role of Particles in Oceanic Mercury Cycling. *Philos. Trans. R. Soc. A* **2016**, *374* (2081), 20150297. https://doi.org/10.1098/rsta.2015.0297.
(23) Zhang., T.; Hsu-Kim, H. Photolytic Degradation of Methylmercury Enhanced by Binding to Natural Organic Ligands Tong. *Nat. Geosci.* **2010**, *3* (7), 473–476. https://doi.org/10.1038/ngeo892.
(24) Seller, P.; Kelly, C. A.; Rudd, J. W. M.; Mac Hutchon, A. R. Photodegradation of Methylmercury in Lakes. *Nature* **1996**, *380* (6576), 694–697. https://doi.org/10.1038/380694a0.
(25) Costa, M.; Liss, P. S. Photoreduction of Mercury in Sea Water and Its Possible Implications for Hg0 Air-Sea Fluxes. *Mar. Chem.* **1999**, *68* (1–2), 87–95. https://doi.org/10.1016/S0304-4203(99)00067-5.
(26) Stal, L. J.; Cretoiu, M. S. What Is so Special about Marine Microorganisms? Introduction to the Marine Microbiome - from Diversity to Biotechnological Applications. In *The Marine Microbiome*; Stal, L. J., Cretoiu, M. S., Eds.; Springer, Switzerland, 2016; pp 3–20. https://doi.org/10.1007/978-3-319-33000-6.
(27) Wang, F.; Macdonald, R. W.; Armstrong, D. A.; Stern, G. A. Total and Methylated Mercury in the Beaufort Sea: The Role of Local and Recent Organic Remineralization. *Environmetal Sci. Technol.* **2012**, *46*, 11821–11828. https://doi.org/10.1021/es302882d.
(28) Heimbürger, L.-E.; Cossa, D.; Marty, J.-C.; Migon, C.; Averty, B.; Dufour, A.; Ras, J. Methyl Mercury Distributions in Relation to the Presence of Nano- and Picophytoplankton in an Oceanic Water Column (Ligurian Sea, North-Western Mediterranean). *Geochim. Cosmochim. Acta* **2010**, *74* (19), 5549–5559. https://doi.org/10.1016/J.GCA.2010.06.036.
(29) Gworek, B.; Bemowska-Kałabun, O.; Kijeńska, M.; Wrzosek-Jakubowska, J. Mercury in Marine and Oceanic Waters—a Review. *Water, Air, Soil Pollut.* **2016**, *227* (10), 371. https://doi.org/10.1007/s11270-016-3060-3.
(30) Barkay, T.; Miller, S. M.; Summers, A. O. Bacterial Mercury Resistance from Atoms to Ecosystems. *FEMS Microbiol. Rev.* **2003**, *27* (2–3), 355–384. https://doi.org/10.1016/S0168-6445(03)00046-9.
(31) Boyd, E. S.; Barkay, T. The Mercury Resistance Operon: From an Origin in a Geothermal Environment to an Efficient Detoxification Machine. *Front. Microbiol.* **2012**, *3* (October), 349. https://doi.org/10.3389/fmicb.2012.00349.
(32) Oremland, R. S.; Miller, L. G.; Dowdle, P.; Connell, T.; Barkay, T. Methylmercury Oxidative Degradation Potentials in Contaminated and Pristine Sediments of the Carson River, Nevada. *Appl. Environ. Microbiol.* **1995**, *61* (7), 2745–2753.

https://doi.org/10.1128/AEM.61.7.2745-2753.1995.
(33) Oremland, R. S.; Culbertson, C. W.; Winfrey, M. R. Methylmercury Decomposition in Sediments and Bacterial Cultures: Involvement of Methanogens and Sulfate Reducers in Oxidative Demethylation. *Appl. Environ. Microbiol.* **1991**, *57* (1), 130–137. https://doi.org/10.1128/AEM.57.1.130-137.1991.
(34) Barkay, T.; Gu, B. Demethylation─The Other Side of the Mercury Methylation Coin: A Critical Review. *ACS Environ. Au* **2021**. https://doi.org/10.1021/acsenvironau.1c00022.
(35) Oremland, R. S.; Culbertson, C. W.; Winfrey, M. R. Methylmercury Decomposition in Sediments and Bacterial Cultures: Involvement of Methanogens and Sulfate Reducers in Oxidative Demethylation. *Appl. Environ. Microbiol.* **1991**, *57* (1), 130–137. https://doi.org/10.1128/AEM.57.1.130-137.1991.
(36) Bowman, K. L.; Collins, R. E.; Agather, A. M.; Lamborg, C. H.; Hammerschmidt, C. R.; Kaul, D.; Dupont, C. L.; Christensen, G. A.; Elias, D. A. Distribution of Mercury-cycling Genes in the Arctic and Equatorial Pacific Oceans and Their Relationship to Mercury Speciation. *Limnol. Oceanogr.* **2019**, *65* (S1), S310–S320. https://doi.org/10.1002/lno.11310.
(37) Sanz-Sáez, I.; Salazar, G.; Sánchez, P.; Lara, E.; Royo-Llonch, M.; Sà, E. L.; Lucena, T.; Pujalte, M. J.; Vaqué, D.; Duarte, C. M.; Gasol, J. M.; Pedrós-Alió, C.; Sánchez, O.; Acinas, S. G. Diversity and Distribution of Marine Heterotrophic Bacteria from a Large Culture Collection. *BMC Microbiol.* **2020**, *20* (1), 207. https://doi.org/10.1186/s12866-020-01884-7.
(38) Villar, E.; Vannier, T.; Vernette, C.; Lescot, M.; Cuenca, M.; Alexandre, A.; Bachelerie, P.; Rosnet, T.; Pelletier, E.; Sunagawa, S.; Hingamp, P. The Ocean Gene Atlas: Exploring the Biogeography of Plankton Genes Online. *Nucleic Acids Res.* **2018**, *46* (W1), W289–W295. https://doi.org/10.1093/nar/gky376.
(39) Salazar, G.; Paoli, L.; Alberti, A.; Huerta-Cepas, J.; Ruscheweyh, H. J.; Cuenca, M.; Field, C. M.; Coelho, L. P.; Cruaud, C.; Engelen, S.; Gregory, A. C.; Labadie, K.; Marec, C.; Pelletier, E.; Royo-Llonch, M.; Roux, S.; Sánchez, P.; Uehara, H.; Zayed, A. A.; Zeller, G.; Carmichael, M.; Dimier, C.; Ferland, J.; Kandels, S.; Picheral, M.; Pisarev, S.; Poulain, J.; Acinas, S. G.; Babin, M.; Bork, P.; Boss, E.; Bowler, C.; Cochrane, G.; de Vargas, C.; Follows, M.; Gorsky, G.; Grimsley, N.; Guidi, L.; Hingamp, P.; Iudicone, D.; Jaillon, O.; Kandels-Lewis, S.; Karp-Boss, L.; Karsenti, E.; Not, F.; Ogata, H.; Pesant, S.; Poulton, N.; Raes, J.; Sardet, C.; Speich, S.; Stemmann, L.; Sullivan, M. B.; Sunagawa, S.; Wincker, P. Gene Expression Changes and Community Turnover Differentially Shape the Global Ocean Metatranscriptome. *Cell* **2019**, *179* (5), 1068-1083.e21. https://doi.org/10.1016/j.cell.2019.10.014.
(40) Singer, E.; Webb, E. A.; Nelson, W. C.; Heidelberg, J. F.; Ivanova, N.; Pati, A.; Edwards, K. J. Genomic Potential of Marinobacter Aquaeolei, a Biogeochemical "Opportunitroph." *Appl. Environ. Microbiol.* **2011**, *77* (8), 2763–2771. https://doi.org/10.1128/AEM.01866-10.
(41) López-Pérez, M.; Gonzaga, A.; Martin-Cuadrado, A.-B.; Onyshchenko, O.; Ghavidel, A.; Ghai, R.; Rodriguez-Valera, F. Genomes of Surface Isolates of Alteromonas Macleodii: The Life of a Widespread Marine Opportunistic Copiotroph. *Sci. Rep.* **2012**, *2*, 696. https://doi.org/10.1038/srep00696.
(42) Handley, K. M.; Lloyd, J. R. Biogeochemical Implications of the Ubiquitous Colonization of Marine Habitats and Redox Gradients by Marinobacter Species. *Front. Microbiol.* **2013**, *4*, 136. https://doi.org/10.3389/fmicb.2013.00136.
(43) Fontanez, K. M.; Eppley, J. M.; Samo, T. J.; Karl, D. M.; DeLong, E. F. Microbial Community Structure and Function on Sinking Particles in the North Pacific Subtropical Gyre. *Front. Microbiol.* **2015**, *6*, 469. https://doi.org/10.3389/fmicb.2015.00469.
(44) Ivars-Martinez, E.; Martin-Cuadrado, A.-B.; D'Auria, G.; Mira, A.; Ferriera, S.; Johnson, J.; Friedman, R.; Rodriguez-Valera, F. Comparative Genomics of Two Ecotypes of the Marine Planktonic Copiotroph Alteromonas Macleodii Suggests Alternative Lifestyles Associated with Different Kinds of Particulate Organic Matter. *ISME J.* **2008**, *2* (12), 1194–1212. https://doi.org/10.1038/ismej.2008.74.

(45) Baumann, L.; Baumann, P.; Mandel, M.; Allen, R. D. Taxonomy of Aerobic Marine Eubacteria. *J. Bacteriol.* **1972**, *110* (1), 402–429.
(46) Eilers, H.; Pernthaler, J.; Glöckner, F. O.; Amann, R. Culturability and in Situ Abundance of Pelagic Bacteria from the North Sea. *Appl. Environ. Microbiol.* **2000**, *66* (7), 3044–3051. https://doi.org/10.1128/AEM.66.7.3044-3051.2000.
(47) Floyd, M. M.; Tang, J.; Kane, M.; Emerson, D. Captured Diversity in a Culture Collection: Case Study of the Geographic and Habitat Distributions of Environmental Isolates Held at the American Type Culture Collection. *Appl. Environ. Microbiol.* **2005**, *71* (6), 2813–2823. https://doi.org/10.1128/AEM.71.6.2813-2823.2005.
(48) Gärtner, A.; Blümel, M.; Wiese, J.; Imhoff, J. F. Isolation and Characterisation of Bacteria from the Eastern Mediterranean Deep Sea. *Antonie van Leeuwenhoek, Int. J. Gen. Mol. Microbiol.* **2011**, *100*, 421–435. https://doi.org/10.1007/s10482-011-9599-5.
(49) Lekunberri, I.; Gasol, J. M.; Acinas, S. G.; Gómez-Consarnau, L.; Crespo, B. G.; Casamayor, E. O.; Massana, R.; Pedrós-Alió, C.; Pinhassi, J. The Phylogenetic and Ecological Context of Cultured and Whole Genome-Sequenced Planktonic Bacteria from the Coastal NW Mediterranean Sea. *Syst. Appl. Microbiol.* **2014**, *37* (3), 216–228. https://doi.org/10.1016/j.syapm.2013.11.005.
(50) Kai, W.; Peisheng, Y.; Rui, M.; Wenwen, J.; Zongze, S. Diversity of Culturable Bacteria in Deep-Sea Water from the South Atlantic Ocean. *Bioengineered* **2017**, *8* (5), 572–584. https://doi.org/10.1080/21655979.2017.1284711.
(51) Kearse, M.; Moir, R.; Wilson, A.; Stones-Havas, S.; Cheung, M.; Sturrock, S.; Buxton, S.; Cooper, S.; Markowitz, S.; Duran, C.; Thierer, T.; Ashton, B.; Meinties, P.; Drummond, A. Geneious Basic: An Integrated and Extendable Desktop Software Platform for the Organization and Analysis of Sequence Data. *Bioinformatics* **2012**, *28* (12), 1647–1649.
(52) Wiegand, I.; Hilpert, K.; Hancock, R. E. W. Agar and Broth Dilution Methods to Determine the Minimal Inhibitory Concentration (MIC) of Antimicrobial Substances. *Nat. Protoc.* **2008**, *3* (2), 163–175. https://doi.org/10.1038/nprot.2007.521.
(53) Zeder, M.; Ellrott, A.; Amann, R. Automated Sample Area Definition for High-Throughput Microscopy. *Cytom. Part A* **2011**, *79A* (4), 306–310. https://doi.org/10.1002/cyto.a.21034.
(54) Zeder, M.; Pernthaler, J. Multispot Live-Image Autofocusing for High-Throughput Microscopy of Fluorescently Stained Bacteria. *Cytom. Part A* **2009**, *75A* (9), 781–788. https://doi.org/10.1002/cyto.a.20770.
(55) Sprouffske, K.; Wagner, A. Growthcurver: An R Package for Obtaining Interpretable Metrics from Microbial Growth Curves. *BMC Bioinformatics* **2016**, *17* (1), 172. https://doi.org/10.1186/s12859-016-1016-7.
(56) Hall, B. G.; Acar, H.; Nandipati, A.; Barlow, M. Growth Rates Made Easy. *Mol. Biol. Evol* **2014**, No. 31, 232–238. https://doi.org/doi: 10.1093/molbev/mst187.
(57) Altschul, S. F.; Gish, W.; Miller, W.; Myers, E. W.; Lipman, D. J. Basic Local Alignment Search Tool. *J. Mol. Biol.* **1990**, *215* (3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.
(58) Kozlov, A. M.; Darriba, D.; Flouri, T.; Morel, B.; Stamatakis, A. RAxML-NG: A Fast, Scalable and User-Friendly Tool for Maximum Likelihood Phylogenetic Inference. *Bioinformatics* **2019**, *35* (21), 4453–4455. https://doi.org/10.1093/bioinformatics/btz305.
(59) Letunic, I.; Bork, P. Interactive Tree Of Life (ITOL) v4: Recent Updates and New Developments. *Nucleic Acids Res.* **2019**, *47* (W1), W256–W259. https://doi.org/10.1093/nar/gkz239.
(60) Acinas, S. G.; Sánchez, P.; Salazar, G.; Cornejo-Castillo, F. M.; Sebastián, M.; Logares, R.; Royo-Llonch, M.; Paoli, L.; Sunagawa, S.; Hingamp, P.; Ogata, H.; Lima-Mendez, G.; Roux, S.; González, J. M.; Arrieta, J. M.; Alam, I. S.; Kamau, A.; Bowler, C.; Raes, J.; Pesant, S.; Bork, P.; Agustí, S.; Gojobori, T.; Vaqué, D.; Sullivan, M. B.; Pedrós-Alió, C.; Massana, R.; Duarte, C. M.; Gasol, J. M. Deep Ocean Metagenomes Provide Insight into the Metabolic Architecture of Bathypelagic Microbial Communities. *Commun. Biol.* **2021**, *4* (1), 604. https://doi.org/10.1038/s42003-021-02112-2.

(61) Duarte, C. M. Seafaring in the 21St Century: The Malaspina 2010 Circumnavigation Expedition. *Limnol. Oceanogr. Bull.* **2015**, *24* (1), 11–14. https://doi.org/10.1002/lob.10008.
(62) R core team. R Core Team. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria Https://Www.R-Project. Org/. R Foundation for Statistical Computing: Vienna, Austria 2017.
(63) Chiu, H.-H.; Shieh, W. Y.; Lin, S. Y.; Tseng, C.-M.; Chiang, P.-W.; Wagner-Dö Bler, I. Alteromonas Tagae Sp. Nov. and Alteromonas Simiduii Sp. Nov., Mercury-Resistant Bacteria Isolated from a Taiwanese Estuary. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 1209–1216. https://doi.org/10.1099/ijs.0.64762-0.
(64) Math, R. K.; Jin, H. M.; Kim, J. M.; Hahn, Y.; Park, W.; Madsen, E. L.; Jeon, C. O. Comparative Genomics Reveals Adaptation by Alteromonas Sp. SN2 to Marine Tidal-Flat Conditions: Cold Tolerance and Aromatic Hydrocarbon Metabolism. *PLoS One* **2012**, *7* (4), e35784. https://doi.org/10.1371/journal.pone.0035784.
(65) Morishita, K.; Nakamura, K.; Tuchiya, K.; Nishimura, K.; Iwahara, M.; Yagi, O. Removal of Methylmercury from a Fish Broth by Alteromonas Macledii Isolated from Minamata Bay. *Japanese J. Water Treat. Biol.* **2006**, *42* (2), 45–51. https://doi.org/10.2521/jswtb.42.45.
(66) Vetriani, C.; Chew, Y. S.; Miller, S. M.; Yagi, J.; Coombs, J.; Lutz, R. A.; Barkay, T. Mercury Adaptation among Bacteria from a Deep-Sea Hydrothermal Vent. *Appl. Environ. Microbiol.* **2005**, *71* (1), 220–226. https://doi.org/10.1128/AEM.71.1.220-226.2005.
(67) De, J.; Ramaiah, N.; Mesquita, A.; Verlekar, X. N. Tolerance to Various Toxicants by Marine Bacteria Highly Resistant to Mercury. *Mar. Biotechnol.* **2003**, *5* (2), 185–193. https://doi.org/10.1007/s10126-002-0061-6.
(68) De, J.; Ramaiah, N. Characterization of Marine Bacteria Highly Resistant to Mercury Exhibiting Multiple Resistances to Toxic Chemicals. *Ecol. Indic.* **2007**, *7* (3), 511–520. https://doi.org/10.1016/J.ECOLIND.2006.05.002.
(69) Zheng, R.; Wu, S.; Ma, N.; Sun, C. Genetic and Physiological Adaptations of Marine Bacterium Pseudomonas Stutzeri 273 to Mercury Stress. *Front. Microbiol.* **2018**, *9*, 682. https://doi.org/10.3389/fmicb.2018.00682.
(70) Robinson, J. B.; Tuovinen, O. H. Mechanisms of Microbial Resistance and Detoxification of Mercury and Organomercury Compounds: Physiological, Biochemical, and Genetic Analyses. *Microbiol. Rev.* **1984**, *48* (2), 95–124.
(71) Lamborg, C.; Bowman, K.; Hammerschmidt, C.; Gilmour, C.; Munson, K.; Selin, N.; Tseng, C. M. Mercury in the Anthropocene Ocean. *Oceanography* **2014**, *27* (1), 76–87. https://doi.org/10.5670/oceanog.2014.11.

680 **Tables**

681

682 **Table 1. Summary of the PCR screening results for *merA* and *merAB* in *Alteromonas* and**
683 ***Marinobacter* strains**. Photic includes surface and deep chlorophyll maximum (DCM) isolates,
684 while aphotic includes bathypelagic isolates. NW Mediterranean: North Western Mediterranean.
685

Genus	**Nº of tested strains**	**Layer**	**Ocean**	**Positives PCR for**		**Total strains with *merA* and/or *merAB***
				merA	***merAB***	
Alteromonas	127	Photic	*Southern Ocean*	1	0	33 (13.5%)
			Indian Ocean	0	1	
			NW Mediterranean	2	0	
			North Atlantic Ocean	5	0	
			South Atlantic Ocean	4	0	
	117	**Aphotic**	*South Atlantic Ocean*	18	3	
Marinobacter	33	Photic	*Southern Ocean*	4	4	41 (89.1%)
			North Atlantic Ocean	1	0	
			South Atlantic Ocean	26	16	
	13	**Aphotic**	*NW Mediterranean*	9	2	
			North Atlantic Ocean	1	0	
			South Atlantic Ocean	1	0	

686

Figures

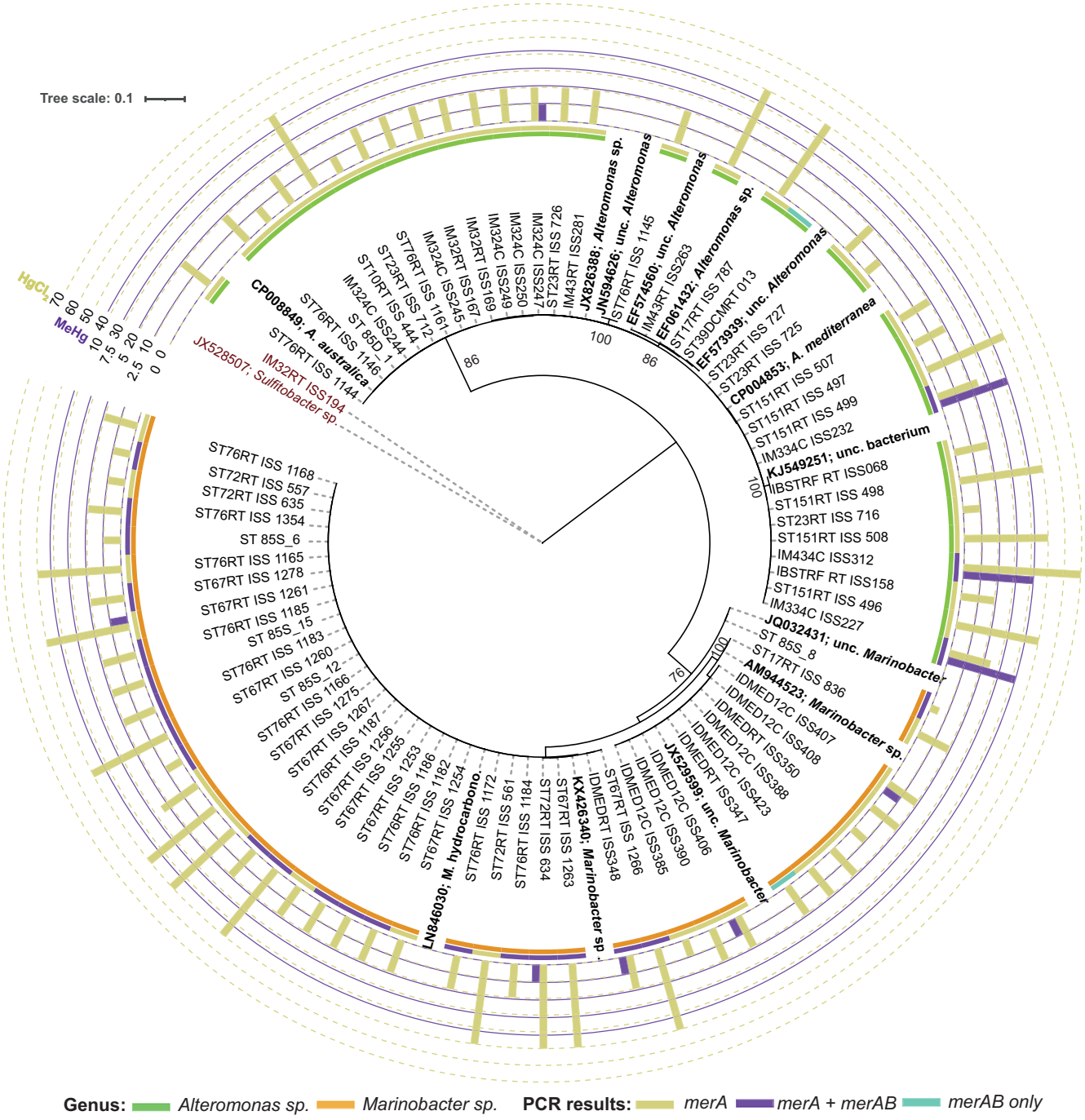
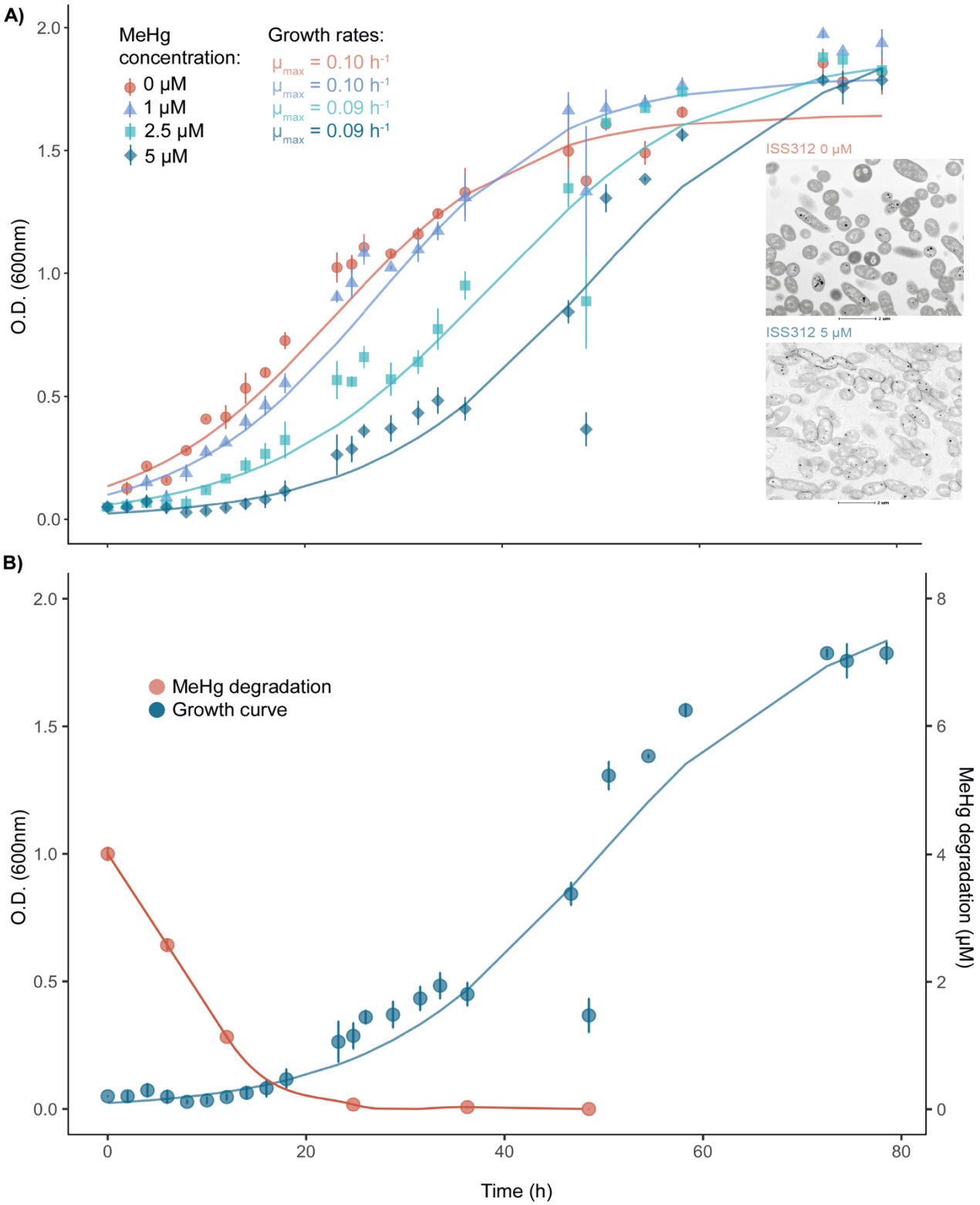


Figure 1. Phylogeny of the 16S rRNA gene of *Alteromonas* and *Marinobacter* positive strains for *merA* and/or *merAB* genes screening. First inner colored strip indicates genus of the strain. Second colored strip indicates presence or absence of genes based on PCR results. Bars indicate results from the MIC experiments: yellow, $HgCl_2$; purple, MeHg. Tolerance values are in µM. JX52807, *Sulfitobacter* and IM32RT_ISS194 are outgroups of the tree. The numbers in the nodes represent bootstrap percentages > 75%. Names in bold indicate reference sequences: *A. australica, Alteromonas australica; A. mediterranea, Alteromonas mediterranea;* unc., uncultured; *M. hydrocarbono., Marinobacter hydrocarbonoclasticus.*



699 **Figure 2. Growth effect of MeHg in strain ISS312. (A)** Growth kinetics of *Alteromonas*
700 *mediterranea* strain ISS312 in Zobell broth containing MeHg (control (0 µM), 1 µM, 2.5 µM
701 and 5 µM). μ_{max} indicates the maximum growth rate for each MeHg concentration.
702 Transmission electron microscopy (TEM) images of the strain growing at 0 µM and at 5 µM are
703 shown in the right side of the plot. Details for preparation and observation of samples for TEM
704 are explained in Supplementary Information. **(B)** MeHg removal by strain ISS312 during the
705 growth curve experiment at 5 µM. Mean and standard deviation from three replicates samples
706 are shown in both graphs.

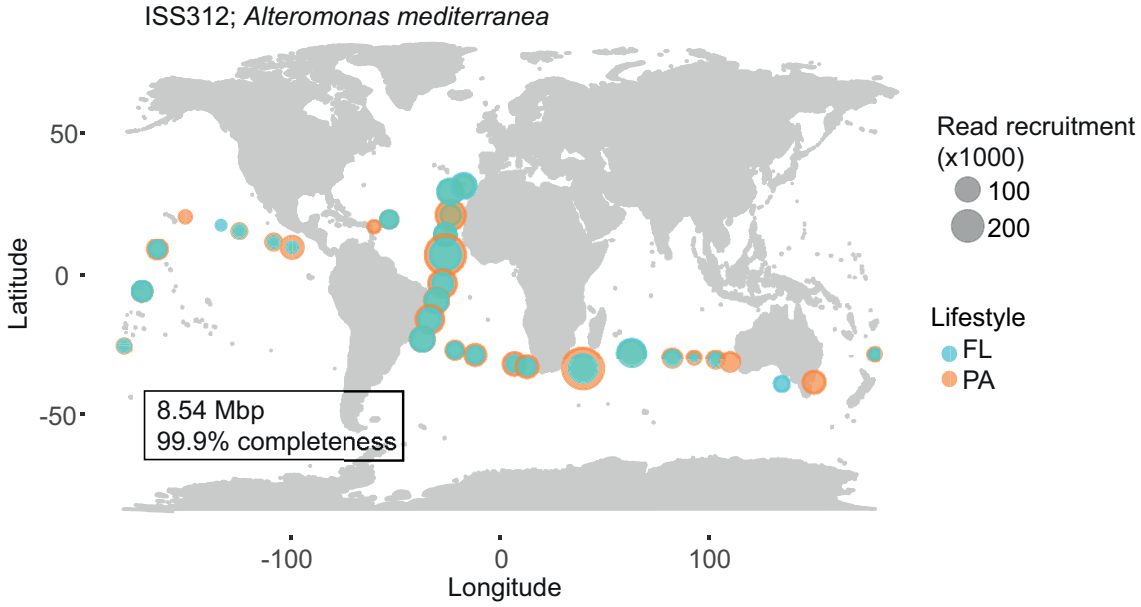


Figure 3. World map showing the distribution of *Alteromonas mediterranea* strain ISS312. Size of the dots indicate number of reads (x1000) and color indicate if the reads were recruited in the free-living (FL, 0.2-0.8 µm) or in the particle-attached (PA, 0.8-20 µm) bacterial communities of the bathypelagic samples.

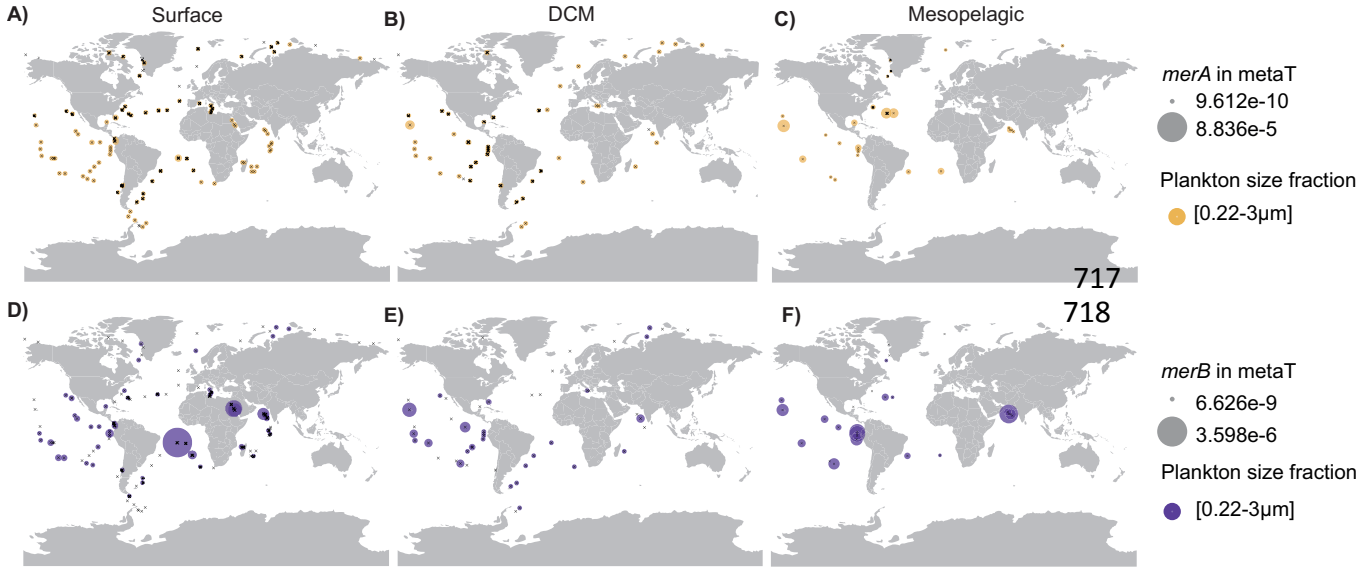


Figure 4. Biogeography of the *merA* and *merB* transcripts across oceanic regions and depths from microbial metatranscriptomes (metaT) from *Tara* Oceans Expedition. (A,B,C). Abundance of the *merA* transcripts found in the Surface, deep chlorophyll maximum (DCM), and Mesopelagic samples. **(D,E,F).** Abundance of *merB* transcripts found in the Surface, DCM and Mesopelagic samples.

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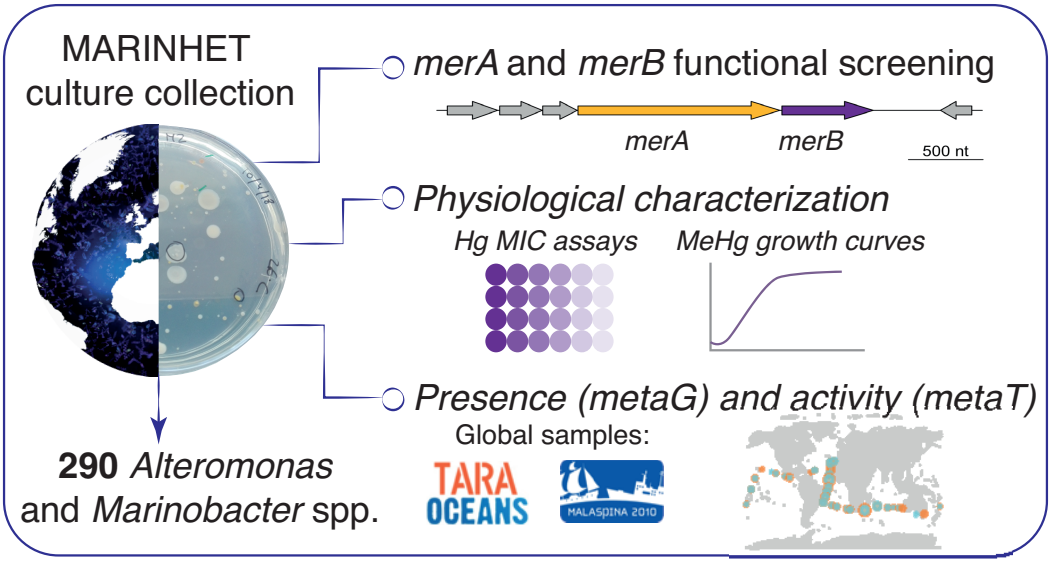
MARINHET
culture collection
merA and merB functional screening
merA
merB
500 nt
Physiological characterization
Hg MIC assays
MeHg growth curves
Presence (metaG) and activity (metaT)
Global samples:
TARA
OCEANS
MALASPINA 2010
290 Alteromonas
and Marinobacter spp.