Targeting mercury bioremediation of marine sediments by using *omics* and culture-driven approaches

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Introduction:
Marine sediments impacted by urban and industrial pollutants are major reservoirs of toxic mercury species, like inorganic mercury (Hg^{2+}) and monomethylmercury (CH_3Hg^+). Some microorganisms inhabiting marine sediments play a key role in the transformation of these mercury species. Mercury reduction and demethylation driven by the mercury reductase (MerA) and the organomercurial lyase (MerB) enzymes are mechanisms of resistance to this metal and hold great potential for bioremediation applications. In the context of the European project MER-CLUB we have performed a large-scale screening of marine sediment samples to identify and isolate environmental bacteria or microbial consortia able to carry out these key processes. The aim of this project is to advance our understanding of mercury biogeochemistry in marine sediments and design novel bioremediation solutions for this complex environmental matrix.

Methods:
Sediments from three different basins (Atlantic, Mediterranean and Baltic Sea) impacted by mercury releases were collected and used in parallel for environmental DNA analysis and the isolation of bacteria and microbial consortia. Marine bacteria were grown on selective media and a soil-substrate membrane system (SSMS; [1]) to try to increase the genetic diversity of the isolates. Consortia were obtained by cell sorting or the dilution-to-extinction approach. The taxonomic identification of isolated strains and consortia was performed via 16S rRNA gene amplification and metagenomic analysis, respectively. A screening of *merA* and *merB* genes was subsequently performed to identify potential mercury detoxifiers among the isolates and consortia. DNA was extracted in more than 30 sediment samples and used for metabarcoding and metagenomic analysis to retrieve the taxonomic composition of microbial communities and specifically, those taxa containing *merA* and *merB* genes in the sediments.

Results: Environmental DNA analysis of mercury polluted sediments revealed that bacteria were dominated by sulfide-oxidizing bacteria, sulfate-reducing bacteria and heterotrophic bacteria commonly found in marine environments. From a set of samples, a culture collection was obtained containing more than 1000 isolates and 400 consortia. The composition of the isolated bacteria was dominated by Proteobacteria and also included other clades such as Bacteroidetes, Firmicutes, and Actinobacteria. The screening of *mer* genes in both the culture collection and the metagenomic dataset resulted in contrasting results, with Proteobacteria and Desulfobacterales dominating the bacteria harbouring *merA* genes in the cultures and *in situ*, respectively.

Discussion: Our results unveil a new role of Desulfobacterota in mercury transformations in marine sediment. In addition to the participation of some members of this group in mercury methylation [2], our results highlight the crucial role of this taxa in its detoxification via mercury reduction. Due to their anoxic lifestyle and metabolic requirements, we were not able to retrieve Desulfobacterota by our culturing approach. However, we have obtained a large diversity of bacterial isolates with *merA* genes, able to detoxify mercury and adapted to the sediment environment, which can be potentially used for bioremediation purposes.

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