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Mercury methylation and transformations by the sulfate reducing bacterium *Pseudodesulfovibrio hydrargyri* combining synchrotron cryo-nano-XRF, XRF tomography and HERFD-XANES.

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Mercury (Hg) is a concerning pollutant on Earth, particularly because it converts into methylmercury (MeHg), a strong human neurotoxin. MeHg is mainly produced in the environment by sulfate-reducing bacteria (SRB), and is then biomagnified and bioaccumulated in the aquatic food web. Untangling the biotransformation processes of Hg by these microorganisms is thus a key to grasp the release of MeHg in ecosystems.

The occurrence of *hgcA* and *hgcB* genes is required for bacteria to methylate Hg[1], but the various Hg chemical forms involved in Hg methylation are still unknown. Our aim was to determine these Hg species as well as the Hg distribution in the cell to clarify the processes involved in Hg transformations. For that, we investigated an original Hg methylating SRB strain *Pseudodesulfovibrio hydrargyri* BerOc1 and its mutant deleted for *hgcB* (BerOc1*DhgcB*). They were exposed to various Hg concentrations (from 10 to 1000 ppb $HgCl_2$). Hg speciation was followed by High Energy Resolution Fluorescence Detected – X-ray Absorption Near Edge Structure spectroscopy (HERFD-XANES) at the Hg L_{III}-edge[2]. Hg localization in the cells was tackled in 2 and 3 dimensions by synchrotron cryo-nano-X-ray Fluorescence (nano-XRF) and XRF tomography, respectively. Hg methylation was also determined using isotopic dilution and Gaz Chromatography-ICP-MS (GC-ICP MS).

Results showed that mercury methylation potential decreased with increasing Hg concentration. HERFD-XANES identified a dominant tetracoordinated bHgS form and a tetracoordinated Hg-thiol species for BerOc1, BerOc1D*hgcB*, and in the medium spiked with $HgCl_2$ after bacterial growth and cell remove, indicating that these Hg species were not produced intracellularly. In contrast to BerOc1*DhgcB* and medium, digonal Hg-thiol was detected in BerOc1 and was predominant at low Hg exposure – presenting higher Hg methylation potentials- suggesting that this form could be related to the methylating activity. Cryo-nano-XRF and tomography revealed extracellular nano HgS particles and intracellular Hg associated to sulfur. Importantly, they evidenced various populations with Hg hyperaccumulating cells and low accumulating ones. The role of these hyperaccumulating cells is questioning for the functioning of the population and the cycle of mercury.

[1]Parks JM et al. (2013), Science, 339, 1332.

[2]Isaure MP et al. (2020), Frontiers Microbiol., 11, 2506.