EUROPEAN SYNCHROTRON RADIATION FACILITY

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Experiment Report Form

	Experiment title: Hg methylation processes by sulfate-reducing bacteria at the cell level	**Experiment number**: EV-389
Beamline: ID16-A	**Date of experiment**: from: January 27, 2021 to: February 1, 2021	**Date of report**:
Shifts: 15	**Local contact(s)**: Murielle Salomé/Sylvain Bohic	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists): **Marie-Pierre Isaure*, Maureen Le Bars, Marisol Goñi-Urriza, Université de Pau et des Pays de l'Adour, IPREM UMR5252**

** One experimentalist due to COVID restriction*

Context and objective

Mercury Hg is one of the most concerning contaminant on Earth, particularly because it is converted into methylmercury (MeHg), a strong neurotoxin. MeHg is mainly produced and released in the environment by sulfate-reducing bacteria (SRB) in anoxic sediments but the mechanisms of Hg methylation are still poorly understood, especially at the cell level. We investigated an original SRB Hg methylating model strain *Pseudodesulfovibrio hydrargyri* BerOc1 and its mutant *ΔhgcB BerOc1* unable to methylate Hg that we have produced in our laboratory. Our aim was to clarify the way of Hg accumulation at the cell level.

Experimental:

BerOc1 cells were grown in anaerobic conditions in culture medium and exposed to 0 or 400 ppb $HgCl_2$ during 4h. Cells were centrifuged, rapidly washed twice in culture medium and water, deposited on Si_3N_4 windows, blotted and immediately frozen in liquid ethane using a cryoplunge Leica EM GP. Frozen samples were transferred into the analysis chamber using a cryotransfert shuttle and analyzed at LN_2 temperature. 2D nanoXRF maps were collected at 17 keV with a flux of 1 10^{11} ph/s and the beam was focused on the sample with KB mirrors to a beamsize of 39 nm (V) x 63 nm (H). Fluorescence signal was collected with a 4-element detector and measurements were carried out with a dwell time of 100 ms and a step of 30 nm. An AXO standard was measured for calibration. Elemental maps were obtained using PYMCA software after spectral fitting. Some cells were selected for nano XRF tomography. In this case, the sample was rotated with a 6° angle and for each position, maps were collected with a step of 40 nm and a countng time of 50 ms to minimize beam radiation damage. The volume was then reconstructed using a Maximum Likelihood Expectation Maximization approach.

Result:

First, for the BerOc1 wildtype we observed a high heterogeneity between the cells in a same culture (Fig. 1). Some cells showed high amount of Hg, preferentially located at the periphery of the cell, while most of the cells contain undetectable level of Hg. We identified extracellular nano particles of Hg that colocalized with S. These nanoparticles probably correspond to βHgS particles that we previously observed (Isaure et al. 2020) and that could form due to cysteine degradation by the bacteria. Nano-XRF tomography performed on a Hg-enriched cell showed that Hg was preferentially distributed at the rim level, suggesting a sorption of Hg and/or sequestration ate the membranes/periplasm level (Fig. 2). We hypothesized that the Hg hyperaccumulating cells are dead cells that sorb mercury while the 'healthy' bacteria are able to methylate Hg and export it out of the cell (Isaure et al. 2020).

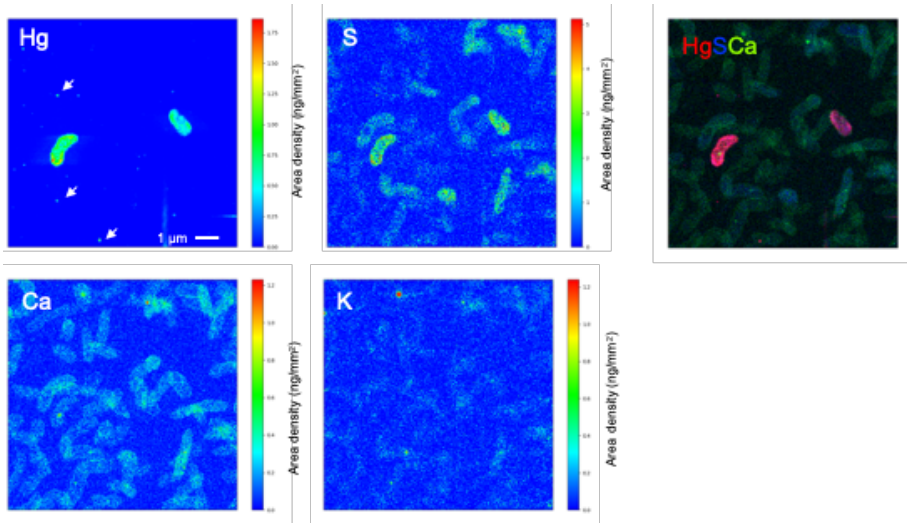

Fig.1: Hg, S, Ca and K maps of BerOc1 cells exposed to Hg (Left) and tricolor map showing Hg, S and Ca (Right)
We observe some few Hg enriched cells - while most of the bacteria do not contain Hg -and extracellular Hg/S nanoparticles (arrows).

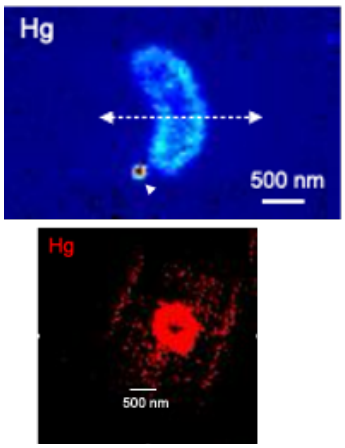

Fig.2: Top image: Hg map of a Hg enriched BerOc1 cell and extracellular nano Hg/S particle (arrow). The dashed line indicates the position of the reconstructed virtual slice shown at the bottom.
Bottom image: Virtual slice extracted from nano-XRF tomography.

Similar heterogeneity was observed for the *ΔhgcB* BerOc1 mutant with Hg accumulating cells and most of the cells containing undetectable Hg (Fig.3). Extracellular nano particles were also identified. We conclude that the unability to methylate Hg does not change the location of Hg in the cell and does not result in a higher Hg accumulation in the cells.

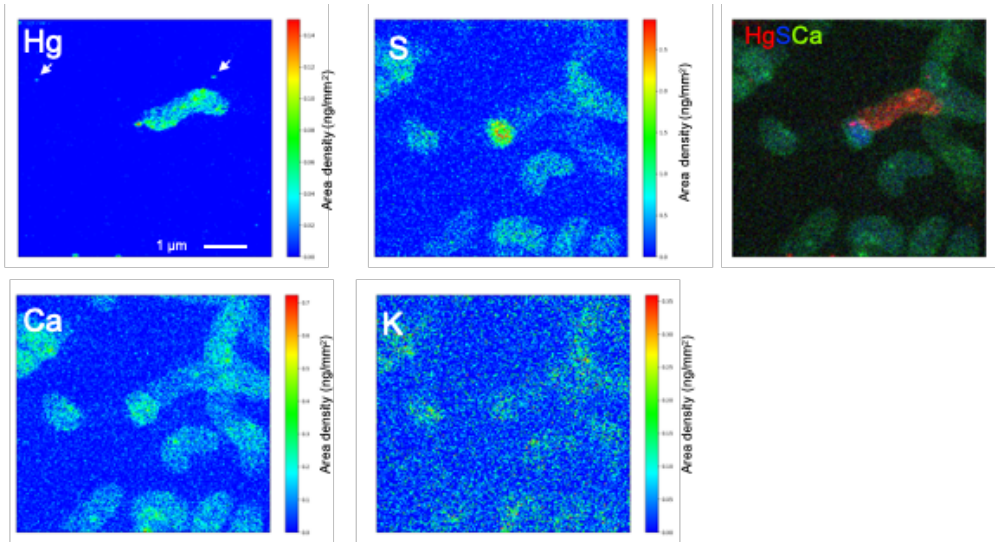

Fig.3: Hg, S, Ca and K maps of *ΔhgcB* BerOc1 mutant exposed to Hg (Left) and tricolor map showing Hg, S and Ca (Right)
As for the wild type, we observe some few Hg enriched cells - while most of the bacteria do not contain Hg -and extracellular Hg/S nanoparticles (arrows).

Reference and published work:

- Isaure MP, Albertelli M, Kieffer I, Tucoulou R, Petrel M, Gontier E, Tessier E, Monperrus M, Goni-Urriza M. 2020. Relationship between Hg speciation and Hg methylation/demethylation processes in the sulfate-reducing bacterium Pseudodesulfovibrio hydrargyri: evidences from HERFD- XANES and nano-XRF. Frontiers in Microbiology, 11, 584715.

- Le Bars M, Barrouilhet S, Monperrus M, Goñi Urriza M, Tessier E, Isaure MP. 2021. Subcellular localization and imaging of mercury species during bacterial methylation process. *Goldschmidt Conference*, July 4-9th 2021, Lyon, France (Oral presentation in virtual mode).