ESRF	**Experiment title:** **Hg methylation by sulfate reducing bacteria and involvement of the metal efflux system.**	**Experiment number**: A16-1 807
Beamline: FAME-UHD	**Date of experiment**: from: June 8 2021 to: June 14 2021	**Date of report**:
Shifts: 18	**Local contact(s)**: Mauro Rovezzi	*Received at ESRF:*

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Report:

Introduction

Mercury (Hg) is a contaminant of concern particularly because methylmercury (MeHg) is highly toxic. MeHg is mainly produced by anaerobic microorganisms in the environment, and we have been studying a sulfate-reducing model strain *Pseudodesulfovibrio hydrargyri* (BerOc1) that methylates Hg and demethylates MeHg. We have produced a mutant *ΔSfx* impaired with both the metal efflux system and a two-component sensing system present at the membranes level that responds to environmental changes and allows communication between the periplasm and the cytoplasm (now called BerOc1 *Δ470-467*). The objective of the proposal was to determine Hg speciation in the mutant in comparison to the wildtype (BerOc1 WT) after various Hg exposures (10, 400 and 1000 ppb Hg). For that we used HERFD-XANES at Hg L_3-edge.

Material and methods

Sulfate reducing bacteria BerOc1 and *ΔSfx* BerOc1 were grown in anaerobic conditions in a synthetic medium and exposed to 10, 400 ppb and 1000 ppb of $HgCl_2$ during 4 h. At the end of the incubation, cultures were centrifuged, rapidly washed with medium without Hg and pure water, and prepared as frozen pellets to get the 'bulk' speciation (unwashed samples). Some bacteria were also washed with glutathione (GSH) and EDTA and filtered at 0.2 µm to remove potential small extracellular Hg particles and Hg adsorbed on the cell in order to measure intracellular Hg speciation (washed samples). Hg L_3-edge (12284 keV) HERFD-XANES was collected on SRB pellets, with the Crystal Analyzer Spectrometer (12 Si(111) crystals) using a He cryostat on FAME-UHD beamline.

During this run, 2 shifts were used for beam-aligment and 16 shifts were used to measure bacterial samples. For low exposures (10 and 400 ppb) collection of a high number of scans was necessary (up to 60 for 10 ppb), which was time consuming.

Results

The *ΔSfx* mutant was first exposed to 400 ppb HgCl exposure during 4h. Washed and unwashed cell were compared (Fig. 1). Results showed that spectra for washed and unwashed samples have global similar features with potential very subtle variations at the edge level (arrows), suggesting that Hg species change very moderately. Comparison with Hg references showed that Hg was mainly tetracoordinated with sulfur atoms. Features of the Hg edge suggest the occurence of tetracoordinated Hg-cysteine, but the oscillations with relative

high frequency after edge also suggest occurrence of a bHgS form. No MeHg was detected in these samples, suggesting that it was exported out of the cells.

Comparison of the *ΔSfx* mutant with BerOc1 wildtype for both washed and unwashed cells showed that the spectra have similar patterns with maybe, a slighlty different feature at the edge level for the unwashed *ΔSfx* mutant as mentionned above (Fig. 2). Thus the average intracellular Hg speciation is likely the same for the *ΔSfx* mutant and for the wildtype, suggesting that intracellular Hg species were not modified by the unability to export metal and the impairement of the two-component system.

Exposure to a higher (1000 pbb) Hg concentration did not change the intracellular Hg speciation compared to 400 ppb for both BerOc1 WT and *ΔSfx* mutant (Fig. 3). In contrast when we added 400 ppb Hg to the extracellular medium where bacteria were grown and then removed before the addition of Hg, we obtained a clearly different spectrum in comparison to the BerOc1Wt or *ΔSfx* mutant. The spectrum showed common features with βHgS species, indicating that βHgS can form in the extracellular level, likely due to the occurrence of sulfides produced by bacteria and/or extracellular biomolecules. In addition, spectrum from BerOc1 WT exposed to a low Hg exposure (10 ppb) showed a different edge than the spectra collected on bacteria exposed to higher Hg concentrations, suggesting different Hg species. Linear combination with our Hg references compounds (Isaure et al. 2020) are in progress.

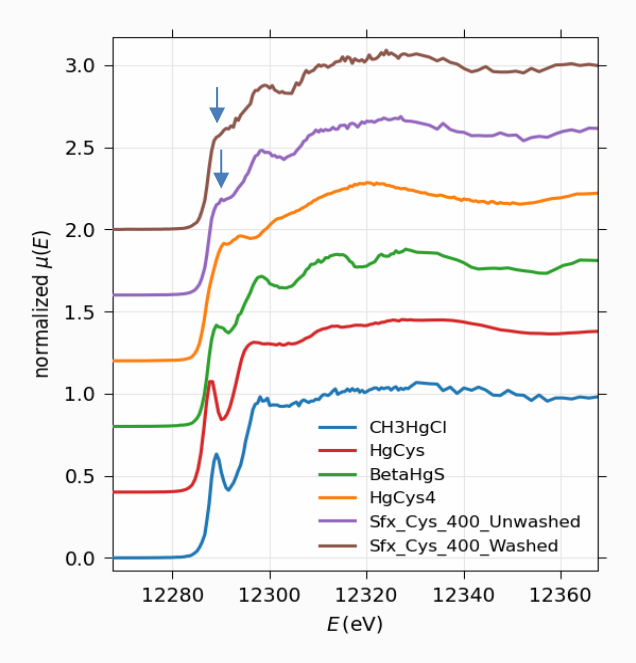

Fig.1 : Hg L_3-edge HERFD-XANES spectra of *ΔSfx* BerOc1 mutant exposed to 400 ppb Hg. Cells were unwashed or washed to remove extracellular and adorbed Hg. Spectra were compared to four Hg model compounds, tetracoordinated HgS i.e. βHgS, tetracoordinated Hg-cysteine (HgCys4), digonal cysteine (HgCys) and C H_3HgCl (MeHg form).

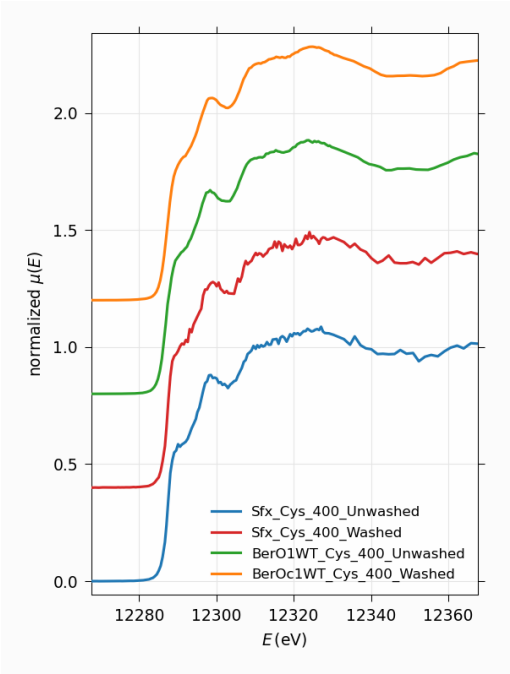

Fig.2 : Comparison of unwashed and washed BerOc1 WT and *ΔSfx* BerOc1 mutant exposed to 400 ppb Hg

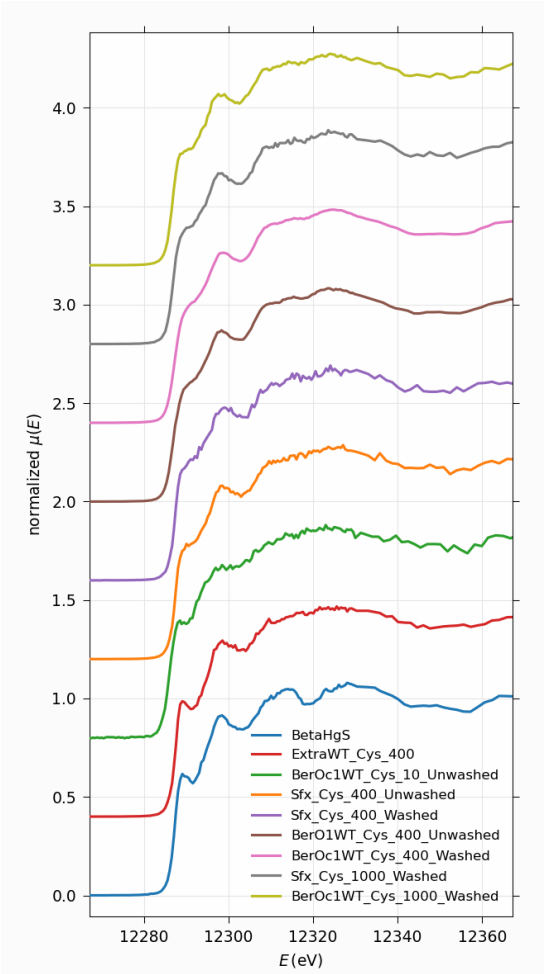

Fig.3 : Hg L3-edge HERFD Xanes spectra of unwashed and washed BerOc1 WT and *ΔSfx* BerOc1 mutant exposed to various Hg concentrations (1000, 400 and 10 ppb), of extracellular Hg from a BerOc1 WT culture (ExtraWT_Cys_400) compared to βHgS

Conclusion

As a conclusion, intracellular Hg in both BerOc1 and *ΔSfx* BerOc1 mutant occured as a mixture of βHgS and Hg-cysteine4. The deletion of the metal efflux system and the two-component sensing system together did not impact the intracellular Hg speciation. MeHg was likely exported out of the cells for both the wildtype and the mutant. In the extracellular medium, addition of Hg (where bacteria were not present anymore) induced the formation of tetracoordinated βHgS. We thus infer that growing Hg methylating cells are able to uptake this form (or directly of partly dissolved) and modify it. Importantly it is necessary to differentiate the metal efflux system from the two-component one in the future.

- 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.