ESRF	**Experiment title:** **Hg methylation by sulfate reducing bacteria exposed to environmental levels of Hg**	**Experiment number**: EV 371
Beamline: BM16	**Date of experiment**: from: October 27th 2020 to: November 3rd 2020	**Date of report**: 28/02/2021
Shifts: 18	**Local contact(s)**: Mauro ROVEZZI, Isabelle KIEFFER	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Maureen LE BARS*, IPREM UMR 5254, Université de Pau et des Pays de l'Adour & CNRS (present on site)

Marie-Pierre ISAURE*, IPREM UMR 5254, Université de Pau et des Pays de l'Adour & CNRS (remotely due to Covid)

Marisol GONI-URRIZA, IPREM UMR 5254, Université de Pau et des Pays de l'Adour & CNRS

Report:

Context and objective :
Methylmercury (MeHg) is highly toxic and is mainly produced in the environment by sulfate-reducing bacteria (SRB). However, the mechanisms of Hg methylation by SRB are poorly understood. We study the Hg methylating model strain *Pseudodesulfovibrio hydrargyrii BerOc1* and our aim was to identify the various Hg species produced by the strain under various environmental Hg exposures. We also recently obtained a *BerOc1* mutant impaired with *hgcB* gene *(ΔhgcB*), unable to methylate Hg and our objective was to compare Hg structural environment in both strains to better understand Hg trafficking in the cells and the effect of the delation of the gene on Hg species. Hg L_3-edge High-Energy Resolution Fluorescence Detected -X-ray Absorption Near Edge Structure spectroscopy (HERFD-XANES) was applied.

Experimental :
Sulfate reducing bacteria *BerOc1* and *ΔhgcB BerOc1* were grown in anaerobic conditions in a synthetic medium containing either 0.1 mM cysteine either 0.1 mM sulfides, and exposed to 10 and 400 ppb $HgCl_2$ at 37°C in the dark. Bacteria were collected after 30 min and 4 h of Hg exposure to follow Hg incorporation. 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 (raw 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 and filtered 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. Calibration was done with a Se foil (12658 eV).

Results :

Comparison of HERFD-XANES spectra collected on BerOc1 exposed to 400 ppb $HgCl_2$ with sulfides during 30 min and prepared as raw sample or as washed and filtered sample showed small variations at 12288 eV, indicating that Hg species slightly differed between intracellular species and bulk species (Figure 1). Comparison of both spectra with Hg references discarded linear 2-coordinate Hg-S bonds, which exhibited a sharp and pronounced near-edge peak ($HgCys_2$) and suggested a tetrahedral four-coordinate Hg-S species such as βHgS. A deeper examination of the edge features and linear combination are in progress to quantify the various Hg coordinations in both samples, particularly the occurrence of MeHg species.

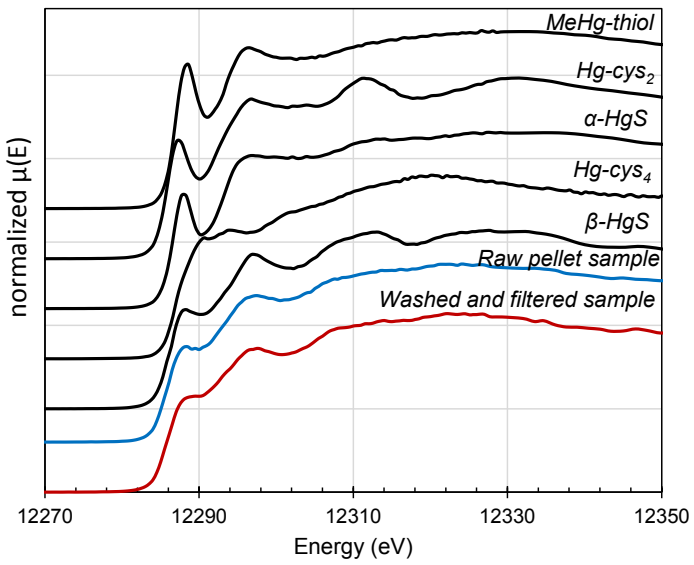

Figure 1 : Hg L_3-edge HERFD-XANES spectra of Hg references and BerOc1 exposed to 400 ppb $HgCl_2$ with 0.1 mM sulfides during 30 min and washed with water and centrifuged (in blue) or washed with EDTA+ GSH and filtered (in red). Both spectra resemble tetrahedral geometry of βHgS while small variations in the near-edge region are observed.

Comparison of spectra collected after 30 min and 4h of exposure did not show significant difference (Figure 2), indicating that the main Hg species form rapidly in the culture.

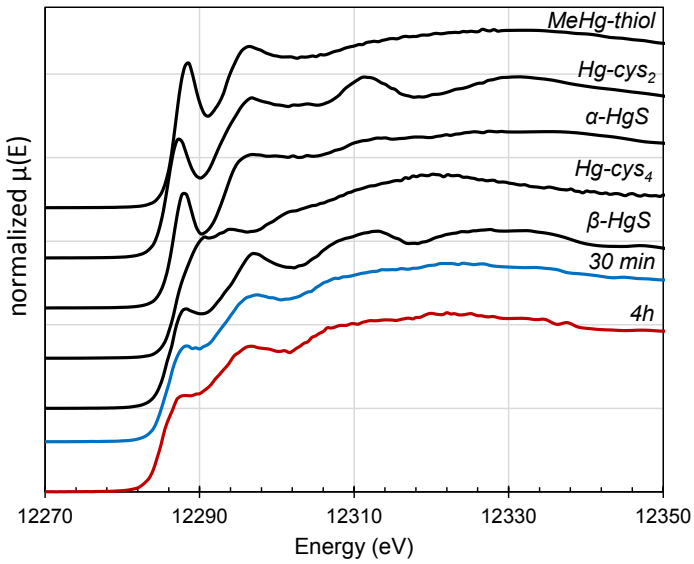

Figure 2 : Hg L_3-edge HERFD-XANES spectra of Hg references and BerOc1 exposed to 400 ppb $HgCl_2$ with 0.1 mM sulfides during 30 min (in blue) and 4h (in red) and washed with water and centrifuged.

We showed that bacteria grown with 400 ppb $HgCl_2$ and sulfides produced more methylmercury than bacteria grown with cystein (manuscript in preparation). Spectra were thus collected on both pellets to evaluate the change of Hg species depending on the source of sulfur. Again, spectral features of both samples resemble those of tetracoordinated βHgS (Figure 3). Small variations in the near-edge structure were observed on the spectra and suggested a not identical Hg speciation depending on the sulfur source.

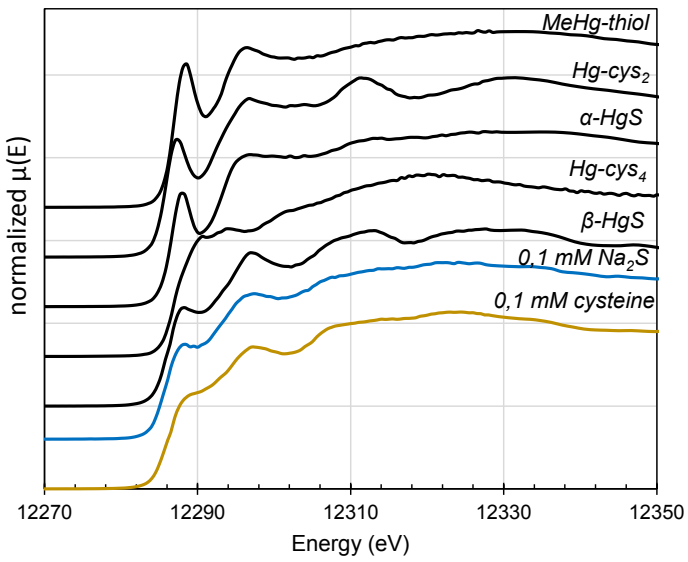

Figure 3 : Hg L_3-edge HERFD-XANES spectra of Hg references and BerOc1 exposed to 400 ppb $HgCl_2$ with 0.1 mM sulfides (in blue) or 0.1 mM cysteine (in yellow) during 30 min and washed with water and centrifuged. Both spectra resemble tetrahedral geometry of βHgS but small variations in the near-edge region suggest an effect of the source of sulfur in the Hg species (or at least in the proportion of the various species).

We then evaluated the effect of Hg concentration on bulk Hg species by comparing 400 ppb $HgCl_2$ exposure and 10 ppb $HgCl_2$ exposure ('low environmental concencentration'). Results showed a more intense near-edge peak around 12288 eV and less structured oscillations after edge for 10 ppb in comparison to 400 ppb (Figure 4). Thus Hg species differed for both exposures, and data treatment is in progress to identify these spectral variations.

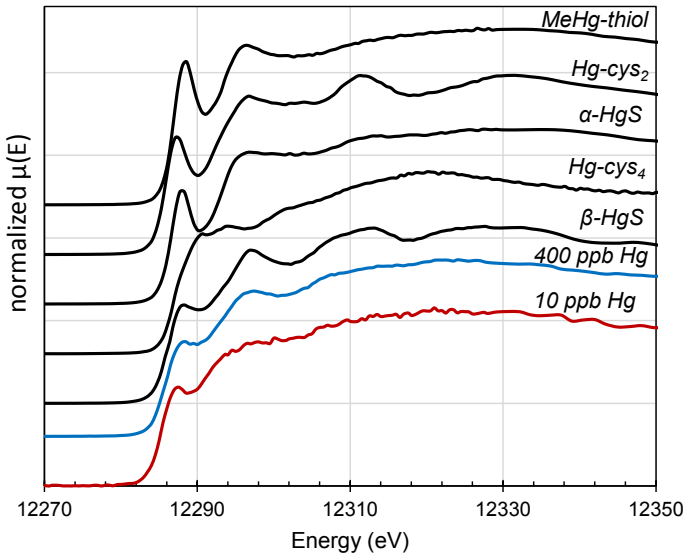

Figure 4 : Hg L_3-edge HERFD-XANES spectra of Hg references and BerOc1 exposed to 400 ppb (in blue) and 10 ppb (in red) $HgCl_2$ with 0.1 mM sulfides during 30 min and washed with water and centrifuged.

Finally, HERFD-XANES spectra were collected on *ΔhgcB* mutant, unable to methylate Hg, exposed to 400 ppb $HgCl_2$ and sulfides during 4 h (Figure 5). Spectra from BerOc1 and from the mutant also slighlty differed in the near-edge region. We suspect that the more pronounced peak for BerOc1 results from the occurrence of methylmercury. In-progress data treatment should specify this point.

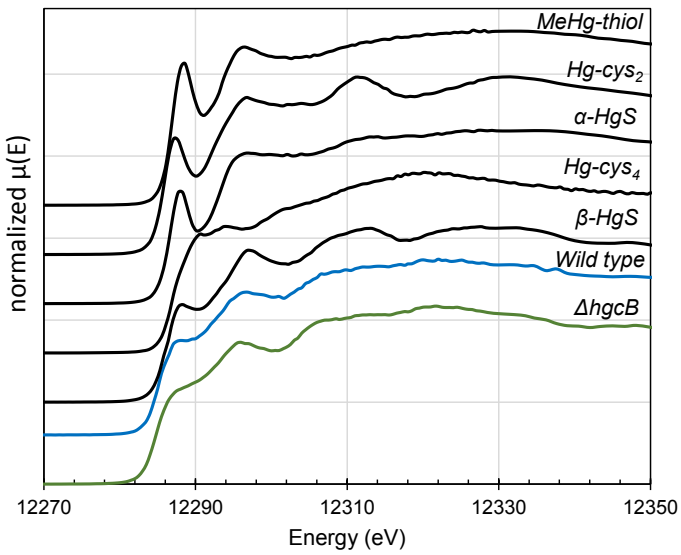

Figure 5 : Hg L_3-edge HERFD-XANES spectra of Hg references and BerOc1 (in blue) and its mutant *ΔhgcB* (in green) exposed to 400 ppb $HgCl_2$ with 0.1 mM sulfides during 4h min and washed with water and centrifuged.

During this run, 2 shifts were used for beam-aligment and 16 shifts were used to measure bacterial samples and references.