Standard Project

Experimental Report template

Proposal title: Deciphering Hg methylation by sulfate-reducing bacteria.		**Proposal number:** 20151061
Beamline: FAME	**Date(s) of experiment:** from: June 9 2016 to: June 13 2016	**Date of report:** 1 February 2017
Shifts: 12	**Local contact(s): Isabelle Kieffer/ Denis Testemale**	*Date of submission:*

Objective & expected results (less than 10 lines):

Methylmercury (MMHg) is mainly produced by sulfate-reducing bacteria (SRB) in aquatic systems and is biomagnified in the food chain, thus constituting a risk to human health. Little is known regarding the mechanism of inorganic Hg (IHg) uptake and methylation by these organisms as well as the demethylation process. Our general objective was to determine the Hg speciation in two SRB strains with contrasted methylation capacities to progress in the understanding of the involved metabolic pathways. We started this study one year before and completed our measurements during this experiment. Using Hg L_{III}-edge High Resolution X-ray Absorption Near Edge Structure spectroscopy (HR-XANES) carried out with the five Si(111)-crystal analyser spectrometer (CAS) available on FAME beamline, we expected to pinpoint different pathways of methylation and demethylation in both strains depending on the Hg concentration.

Results and the conclusions of the study (main part):

Two anaerobic strains were studied: strain *BerOc1* (*Desulfovibrio dechloroacetivorans*) able to both methylate IHg and demethylate MMHg and strain *G200* (*Desulfovibrio desulfuricans*) only able to demethylate MMHg. Bacteria were grown in anaerobic conditions and in fumarate medium *i.e.* without sulfate to avoid HgS precipitation. They were spiked with 0, 0.1, 1 and 10 ppm $Hg(II)Cl_2$ (IHg) and CH_3HgCl (MMHg) and incubated for 24 h. SRB cells were centrifuged, rapidly washed and prepared as frozen pressed pellets. Hg-references were also synthesized and analyzed as solid pellets or aqueous solutions. Samples were positioned in the He cryostat operating at 12K. Hg L_{III}-edge HR-XANES spectra were collected with the CAS system, selecting the Hg Lα1 fluorescence (L3-M5, 9988.8 eV) line and the diffracted intensity was collected with a one Si detector.

In our previous experiment (see report 20141413), we showed that *BerOc1* exposed to the 0.1, 1 and 10 ppm IHg concentration was not linearly coordinated and we hypothesized that it was tetracoordinated as βHgS. Spectra had also some slighly different features suggesting some variation in Hg species. Here, we confirmed that βHgS HR-XANES had similar features than *BerOc1*, particularly for the 10 ppm exposure (Fig. 1 and 2). The *BerOc1* 0.1 and 1 ppm IHg spectra displayed a near-edge peak slightly shifted to a higher value compared to βHgS, suggesting that a methyl Hg form could be present as minor compound. *G200* exposed to 10 ppm IHg was similar to *BerOc1* 10 ppm IHg: βHgS was likely the predominant Hg species. The lower *G200* exposures showed different patterns compared to *BerOc1*, particularly at the near-edge level: the shift to higher energy values observed for *BerOc1* was not present for *G200*, in agreement with an absence of MMHg.
When exposed to any concentration of MMHg, spectra for both *BerOc1* and *G200* drastically differed from IHg exposure. All of them had a pronouced peak at 12.288 keV typically observed for methyl species e.g. MMHg-thiol resin, CH_3HgOH, or CH_3Hg-cysteine. These results suggest that the demethylation did not occur within the cells and/or that demethylated Hg was readily exported from the cell.
A fingerprint approach by linear combination fitting is in progress. In *BerOc1* exposed to IHg the proportion of βHgS was at least 70% for the lower IHg exposure (0.1 ppm) and increased for higher ones.

CH_3Hg-cysteine was identified as the best second component with the highest proportion found for the 0.1 ppm exposure (20%). For *G200*, we obtained a higher proportion of βHgS with values above 90%. Data treatment is still in progress.

To conclude, we found that βHgS was detected as the main Hg species for the both SRB strains exposed to IHg although these strains were not grown under sulfato reduction. An hypothesis is that mercury sulfide could nucleate from other forms, possibly organic thiol-containing molecules produced by bacteria, as observed for natural organic matter (Manceau et al., 2015, *Environ. Sci. Technol.*). Mercury methylation was detected for the lowest IHg exposure suggesting that this process was inhibited for high concentrations. Results on demethylation also suggest that the demethylation did not occur within the bacteria or that demethylated Hg was rapidly exported out of the cell.

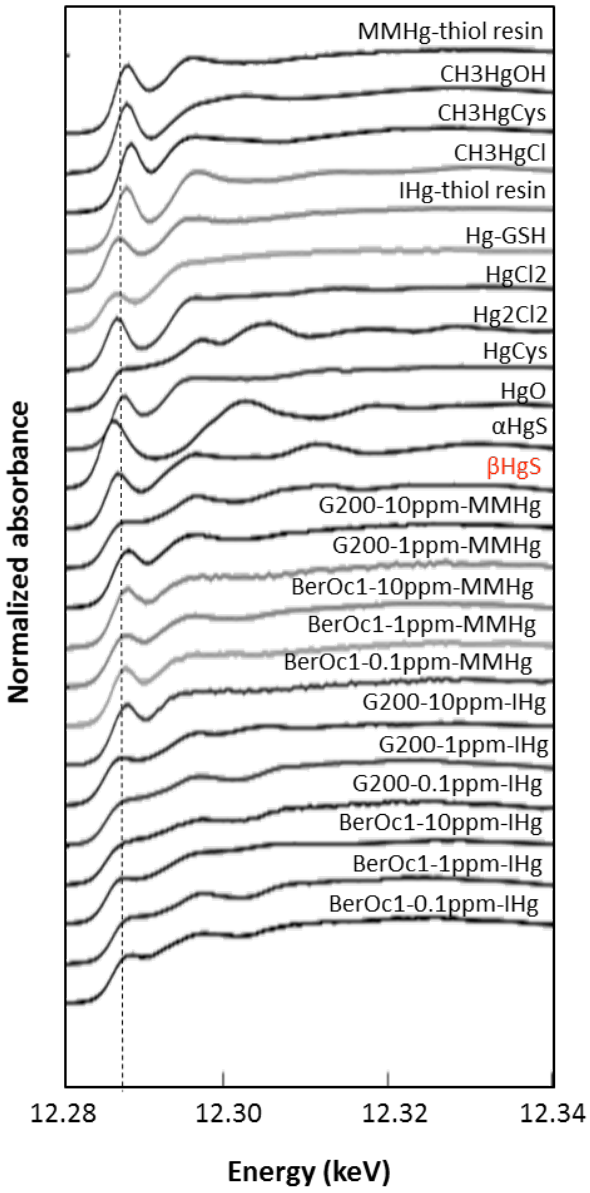

Fig. 1 : Hg L_{III}-edge HR-XANES spectra of *BerOc1* and *G200* exposed to 0.1, 1 and 10 ppm of IHg and MMHg compared to Hg references.

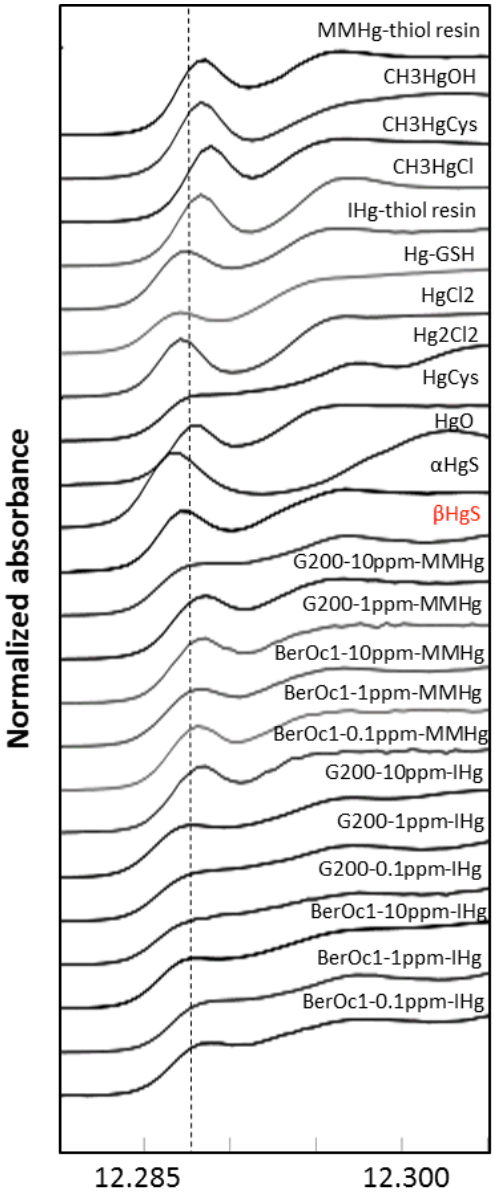

Fig. 2 : Zoom of the near-edge region of *BerOc1*, *G200* and Hg references HR XANES spectra (between 12.28 and 12.305 keV in energy).

Justification and comments about the use of beam time (5 lines max.):

The beamline worked very well and no beamlost occurred. One shift was used for the alignment of the CAS. One shift was also used for the change of samples since this step is time consuming due to the one-position holder of the crysotat. Ten shifts were finally used to measure bacteria samples and Hg-references.

Publication(s):

- Albertelli M, Isaure MP, Kieffer I, Tucoulou R, Tessier E, Monperrus M, Goñi-Urriza M. Understanding the mercury transformation in sulfate-reducing bacteria cells. Submitted to the *13th International Conference on Mercury as a Global Pollutant (ICMGP)*, July 16-21 2017, Providence, Rhode Island, USA.

- This work is part of Marine Albertelli PhD work. Publications are in progress.