ESRF	**Experiment title:** **Kinetics of Hg incorporation and methylation in the sulfate-reducing bacteria *Desulfovibrio dechloroacetivorans***	**Experiment number**: EV 183
Beamline: ID16B	**Date of experiment**: from: 23 June 2016 to: 28 June 2016	**Date of report**: 8 September
Shifts: 15	**Local contact(s)**: Rémi Tucoulou	*Received at ESRF:*

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Report:

Scientific background and objectives:

Methylmercury (MeHg) is a human neurotoxic wich is biomagnified through the aquatic food web. It is mainly produced by sulfate-reducing bacteria (SRB) like *Desulfovibrio* strains from inorganic mercury (IHg), and understanding this first step should shed light on this global contamination. Despite recent significant advances coming from the genetic field, little is known about the Hg metabolic pathway at the microbe level. Our objective was to follow Hg incorporation at the cellular level to clarify its uptake and transformations. For that, chemical imaging was performed using nano X-ray fluorescence. We expected to determine first, if Hg distribution changed with time, and then, to identify wich microbe compartment could be involved in Hg fate. The initial idea was to operate in cryogenic conditions, but the cryostage was not stable, and thus we moved to measurements at ambient temperature.

Experimental:

The SRB strain *Desulfovibrio dechloracetivorans BerOc1*, isolated from Etang de Berre in France, able to both methylate Hg(II) and demethylate MeHg was investigated. Based on growth cell and toxicity results, microbes were grown in anaerobic conditions and exposed to 1 $mg.l^{-1}$ $HgCl_2$ (5 μM) . They were collected 10 min, 1h, 4h and 24h after spiking to follow Hg incorporation, since Hg methylation in another SRB strain was found to reach a plateau 3h after spiking (Graham et al., 2012). Cells were centrifuged, rapidly washed twice in water, blotted on Si_3N_4 membranes, and immediately frozen in liquid ethane using the cryoplunge Leica EM GP. As the cryostage did not give satisfactory results (see report EV157) and could not be improved, bacteria deposited on Si_3N_4 membranes were freeze-dried using a protocol developped for thin biological samples (several steps to progressively warm up from -120°C to 25°C). Membranes were transferred to the beamline and analyzed at ambient temperature in pink beam mode (flux of photons : 5.6 10^{11} ph/s). Fluorescence signal was collected with a 7-element silicon drift detector. XRF maps were performed at 17.5 keV, and the beamsize was 59 nm (H) x 52 nm (V). Fluorescence signal was deconvoluated using PYMCA software to obtain elemental maps.

Results:

Hg was located as pluri-nanometer-sized hot spots during the whole kinetics (Figure). After 10 min and 1h, Hg was found in the bacteria, and started to be present at the tip also at T1h. This location at the tip was confirmed at T4h, and the metal was extracellular at T24h, thus suggesting an excretion process with time. This is a new finding. Hg was always colocated with S, suggesting an association with thiol complexes or a HgS form. A colocation with Zn was clearly observed at T4h but this was less obvious at T24h, and was not observed at T10min and T1h. It can be hypothesized that the Hg excretion process is related to Zn pathway but more investigations are necessary to verify this point. Fe always occurred as intense spots in the bacteria. These intense Fe accumulations were also observed using transmission electron microscopy in these conditions and in control bacteria not exposed to Hg, and are unidentified to date. Ca was homogeneously distributed in the bacteria and was used as 'cell marker'.

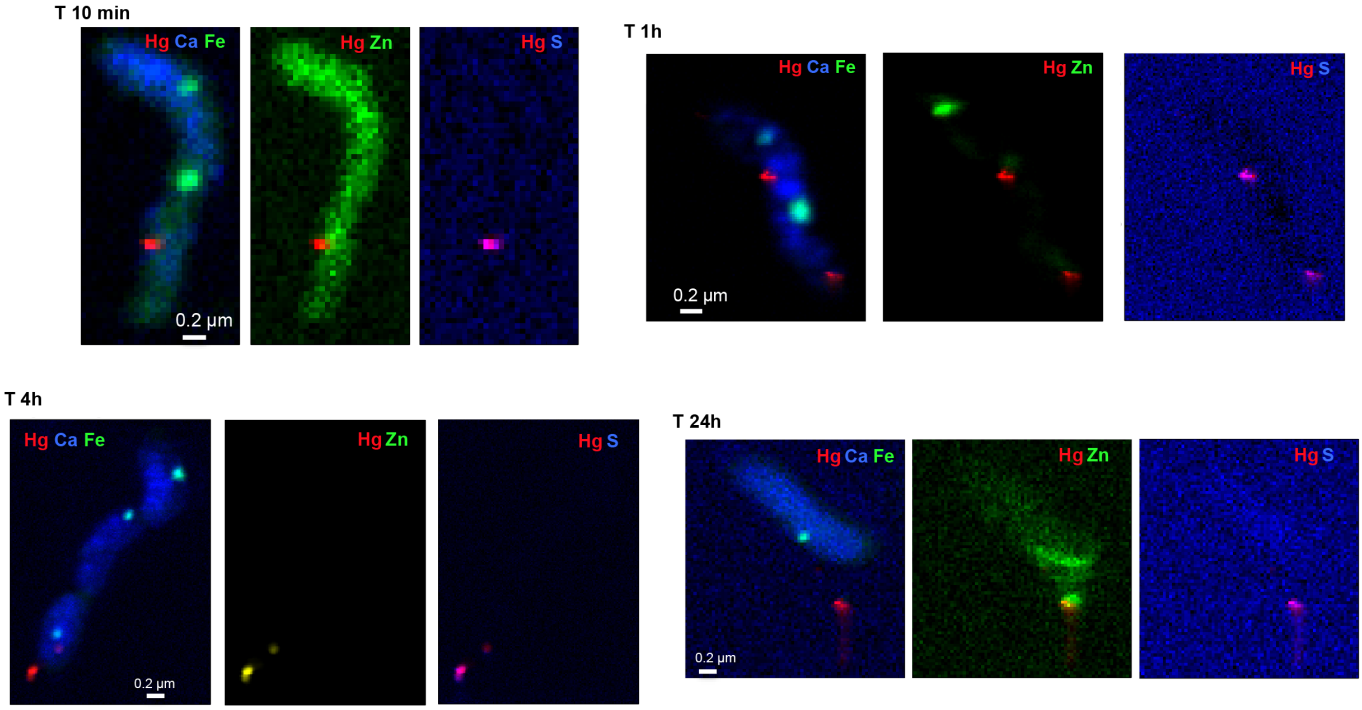

Figure:Tricolor and bicolor maps showing the distribution of Hg, Ca, Fe, Zn and S in *BerOc1* exposed to 1mg.l^{-1} $HgCl_2$ during 10 min, 1h, 4h and 24h. E: 17.5 keV, Beamzise: 59 nm (H) x 52 nm (V). Step size: 50 nm for 10 min and 24h and 25 nm for 1h and 4h. Counting: 100 ms/pt for 10 min and 24h and 200 ms/pt for 1h and 4h.

Conclusion:

This kinetics showed that Hg was first incorpored in the bacteria and was excreted after a few hours of Hg exposure, probably as thiol-complexes or as a form of HgS. A part of this excreted mercury could be also methylated, which would be in line with a methylation in the cytosol and excretion as proposed by Gilmour et al. (2011). HR-XANES measurements are in progress to discriminate the various forms of mercury. The observation of this Hg excretion is of high input. Importantly, these findings should be validated by cryo-imaging to rule out Hg redistribution potentially coming from freeze-drying and beam exposure at ambient temperature.

References:

- Gilmour et al. 2011. Sulfate-reducing bacterium Desulfovibrio desulfuricans ND132 as a model for understanding bacterial mercury methylation. *Appl. and Environ. Microbiol.* 77: 3938-3951.
- Graham et al. 2012. Detailed Assessment of the Kinetics of Hg-Cell Association, Hg Methylation, and Methylmercury Degradation in Several Desulfovibrio Species. *Appl. and Environ. Microbiol.* 78: 7337–7346.