Influence of the environmental conditions on the methylation rates and source of Hg species in microorganisms

ABSTRACT:
Mercury (Hg) is a persistent pollutant in the environment, highly volatile and able to be converted into highly toxic methylmercury (MeHg). MeHg is a serious threat as it is a neurotoxic compound, which is bioaccumulated and bioamplified in food webs. Microorganisms play a central role in MeHg conversion, either directly by controlling Hg methylation and MeHg degradation, either indirectly. Although hgcA and hgcB genes have been identified as necessary for Hg methylation, the methylation process cannot be fully explained. Environmental parameters, particularly the sulfur containing ligands, have been shown to play a role in the Hg uptake and methylation process. However, to date, little is known about the cellular and environmental mechanisms favouring MeHg production, and Hg methylation processes are far from being deciphered. In this work, the PhD student will focus on the environmental parameters (thiol containing ligands, Se, Cl...) involved in Hg uptake, methylation and demethylation.

Our team at IPREM lab aims to characterize Hg methylation at cellular level, from Hg recognition by the cell to Hg export, including methylation steps. We intend to decipher the role of Hg cell trafficking in the Hg methylation process in two Sulfate Reducing Bacteria (SRB) model strains, Desulfovibrio hydrargyri BerOc1, able to methylate Hg and demethylate MeHg and Desulfovibrio alaskensis G20, able to exclusively demethylate MeHg. The objective of the thesis is to investigate the molecular aspects of mercury biotransformations, including studies on the biomolecular speciation and the localization of Hg transformations at the subcellular level by combining chemical and physical analytical methods.

Keywords: mercury methylation, sulfate-reducing bacteria, Hg speciation, mass spectrometry, electron microscopy

WORKING CONDITIONS
Laboratoire: Institut des Sciences Analytiques et de Physico-chimie pour l'Environnement et les Matériaux (IPREM UMR 5254, CNRS and Université de Pau et des Pays de l'Adour, Pau, France)
Site web: https://iprem.univ-pau.fr/fr/index.html
PhD Director: Mathilde Monperrus
PhD co-Director: Marie-Pierre Isaure
In Collaboration with Marisol Goñi-Urriza
The proposed PhD is part of the project ‘GO-BEAM’ (Go inside a bacterial cell methylating Mercury) funded by E2S-UPPA from 2018 to 2021. GO-BEAM, selected as a ‘Key Scientific Challenges E2S-UPPA’ (http://e2s-uppa.eu/en/index.html) is a collaborative and transdisciplinary project involving genetic microbiology, analytical chemistry, imaging and spectroscopy. The objective of the project is to improve the understanding of the Hg methylation/demethylation processes at the cell level. 2 PhDs and 1 Post-Doctorate are funded for the GO-BEAM project: PhD1 on analytical chemistry and imaging (the present proposition), PhD2 on genetic microbiology and physiological studies, and the Post-Doc on both imaging and spectroscopy.
Scientific team: MP Isaure, M Goñi-Urriza, M Monperrus, B Khalfaoui-Hassani, R Guyoneaud, C. Gassie, 2 PhD students, 1 post-doc.
Place: IPREM, Pau, France
Start: November/December 2018  Duration: 3 years
Employer: Université de Pau et des Pays de l’Adour (UPPA)
Monthly salary before taxes: 1868 €  (doctoral contract UPPA, according to E2S Key scientific challenges project,
I. Scientific Context
Mercury (Hg) is one of the major contaminants at the global scale. It is persistent, highly volatile and is able to convert into highly toxic methylmercury (CH₃Hg or MeHg), a strong neurotoxic. The production of highly toxic methylmercury is mediated by microorganisms but little is known about the cellular and environmental mechanisms favoring MeHg production. Understanding the biotransformation processes of Hg by microorganisms is thus a key for Hg risk assessment in ecosystems and human health. A few years ago, Parks et al. (2013) identified two genes, hgcA and hgcB required for Hg methylation. However, strains carrying hgcAB genes produce methylmercury at different rates, partly depending on their physiological state and environmental parameters (Goñi-Urriza et al. 2015). For instance, mercury speciation in the extracellular medium affects the methylation (Ndu et al. 2012, Liu et al. 2016), and Hg uptake is also enhanced by some thiols such as cysteine (Ndu et al. 2012; Schaefer et al. 2014). Our preliminary studies also showed that Hg and MeHg were both associated to thiol ligands pointing out the crucial role of sulfur in Hg trafficking and methylation.

II. Objectives
The objective of this PhD thesis is to contribute to understanding the environmental parameters involved in Hg methylation/demethylation and on the identification of Hg biomolecules produced by sulfato reducing bacteria. For that, the identification of the Hg species, particularly thiols-complexes suspected to play an important role, is thus crucial, and the PhD student will carry out a combination of hyphenated mass spectrometry (GC-Chromatography – Inductively Coupled Plasma Mass Spectrometry GC-ICPMS, and High Performance Liquid Chromatography- Electrospray Ionization – Mass Spectrometry HPLC-ESI-MS/MS). Results will be compared to speciation using modelling approach. An objective is also to track the origin of sulfur groups binding Hg and MeHg and for that, the PhD student will design experiments with NanoSIMS to follow sulfur. The impact of Hg and MeHg on the bacterial cell will finally be followed using transmission electron microscopy.

III. Work plan
The PhD student will focus her/his work on the understanding of the environmental parameters involved in Hg methylation/demethylation and on the identification of Hg biomolecules.
- He/she will be in charge of the bacterial cultures in various conditions and various ligands.
- He/she will have to model Hg speciation using (bio)geochemical software (VMinteq).
- He/she will perform incubations using isotopically enriched mercury species followed by GC-ICPMS analysis to establish simultaneously methylation and demethylation rates and quantities of net methyl mercury production rates.
- He/she will be strongly involved in the identification of thiols ligands by HPLS-ESI-MS/MS.
- He/she will also set experiments with labeled stable S isotopes to track source of S in Hg-S containing compounds, particularly using nanoSIMS imaging.
- He/she will also implement transmission electron microscopy combined to energy dispersive spectroscopy (TEM-EDS, HRSTEM-EDS) to examine cell structures (membranes, periplasm, cytoplasm). Correlative imaging with NanoSIMS will be also performed. This will be done in collaboration with the Bordeaux Imaging Center.
- He/she will work with another PhD student involved in microbial genetics and physiology and a post-doc, involved in X-ray imaging and X-ray Absorption Spectroscopies techniques. The role of candidate genes in Hg methylation will be assessed through gene deletion and complementation in D. hydrargyi Ber Oc1. Applying X-ray imaging on the wild type and mutants, the Hg methylation potentials and localization will be characterized in order to decipher the role of the genes on Hg pathways.

The PhD student will also participate to teaching activities at the undergraduate level (96h/3 years)

IV. Literature References


**REQUIRED COMPETENCES**

Skills in speciation, mass spectrometry, separative techniques, (GC, HPLC), electron microscopy

The candidate should have a strong predilection for laboratory work, analytical chemistry and modeling for speciation using speciation software (VMinteq...).

The ideal candidate has a master degree in analytical chemistry. He/She is rigorous and highly motivated. He/she must have a good English level and the capacity to work autonomously.

French spoken will be a plus (teaching activities).

**CRITERIA USED TO SELECT CANDIDATE**

Two steps selection process:

1st step:
- Evaluation of the applicants’ cv
- The candidates selected after this first step, will be interviewed then.

2nd step: interview
- Candidates will have 5 min to present their CV, 5 min to present their Master2 thesis and 5 min to present the phD subject
- This presentation will be followed by questions and discussion.

Criteria used in selection of the candidate:
- The candidate’s motivation, scientific maturity and curiosity.
- Candidate’s knowledge.
- Candidate’s marks and rankings in Licence/undergraduate, M1 and M2.
- English proficiency
- Candidate’s ability to present his/her work
- Professional experience of internship (s) in laboratory or other; any research work already carried out (reports, publications).

**REQUIRED DOSSIER, DATE**

Application should be send by e-mail. The application should contain:
- CV
- Cover letter detailing candidate's motivations
- Candidate’s Licence and MSc marks and ranking
- Reference letters
- Contact details (for 2 referees)

Limiting date: 10/10/2018

**CONTACTS**

Name: Mathilde Monperrus (mathilde.monperrus@univ-pau.fr), Marie-Pierre Isaure (marie-pierre.isaure@univ-pau.fr), Marisol Goñi (marisol.goni@univ-pau.fr)