



	<b>Experiment title:</b> Mechanism of mutual detoxification of mercury and selenium by the bacteria <i>Ralstonia metallidurans</i> CH34	<b>Experiment number:</b> LS-2141
<b>Beamline:</b> BM 30B	<b>Date of experiment:</b> from: 05/02/2003 to: 10/02/2003	<b>Date of report:</b> 9/05/2003
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### Introduction:

The bacteria *Ralstonia metallidurans* CH34 is a microorganism characteristic of metal-contaminated biotopes, which can resist to a broad range of metals including Cd<sup>2+</sup>, Co<sup>2+</sup>, Zn<sup>2+</sup>, Tl<sup>2+</sup>, Cu<sup>2+</sup>, Pb<sup>2+</sup>, Ni<sup>2+</sup>, Hg<sup>2+</sup>, and CrO<sub>4</sub><sup>2-</sup>. In a previous experiment, we showed that it could also resist selenite (SeO<sub>3</sub><sup>2-</sup>) by reducing it into elemental selenium (monoclinic form), which was accumulated inside the cell (ROUX et al., 2001). However, the molecular mechanism of selenite bioreduction is still unknown.

A preliminary study of the bacteria in the presence of SeO<sub>3</sub><sup>2-</sup> and Hg<sup>2+</sup> suggested a possible mutual detoxification mechanism. So the original scope of this experiment was to study the reactions occurring in this ternary system (bacteria + Se + Hg), and to identify the final chemical forms of Se and Hg. However, in the following experiments we were not able to reproduce the phenomenon of detoxification. In consequence, we decided to further study the binary system (bacteria + Se), and particularly the kinetics of bioreduction of selenite and selenate by the bacteria *Ralstonia metallidurans*, and by the cyanobacteria *Synechosystis*. This second strain is also a good model for the study of metal resistance and bioreduction: It is representative of the phytoplankton, it is resistant to numerous metals, and its genome has been entirely sequenced.

### Experimental

The bacteria were grown in solution, and 2 mM SeO<sub>3</sub><sup>2-</sup> or SeO<sub>4</sub><sup>2-</sup> were added at t<sub>0</sub>. The systems were studied during 100 hours after the introduction of the contaminant, with an interval of 10h between each sampling for *R. metallidurans* CH34, and of 24h for *Synechosystis*. In addition, for *Ralstonia metallidurans* CH34, a sampling was realized at t<sub>0</sub>, t<sub>0</sub> + 30 min and t<sub>0</sub> + 2 hours. For each sampling, the bacteria and the solution were separated by centrifugation. Total Se concentrations in the supernatants and in the bacteria were measured by ICP-MS. Concentrations ranged from 1 to 250 mg Se g<sup>-1</sup> of protein for the bacteria, and from 1 to 2 mM for the solutions.

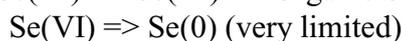
Se K-edge XANES spectra for the bacteria and for the supernatants were recorded in fluorescence mode using the 30-element Canberra detector. The spectra were calibrated, normalized, and simulated by linear

combinations using Se reference compound spectra (Fig. 1). Then, given the total Se concentration in each sample, the percentages of each Se species were converted into molar concentrations.

## Results

The study of the supernatants shows that for both bacteria and for both oxyanions studied, the reactions always take place in the bacteria, as not in the solution. For *R. metallidurans* CH34 exposed to selenite, the bioaccumulation and bioreduction is very fast, and no intermediate species was detected.

In the presence of selenate, *R. metallidurans* CH34 accumulates this oxyanion in much smaller amount ( $7 \text{ mg g}^{-1}$  Se per g or protein compared to 210 for selenite), and reduces it into organic selenium and selenite in various proportions as a function of the reaction time (Figures 1 and 2). Curiously, the selenite formed is not reduced into elemental selenium as in the previous system, but it is transformed into organic selenium. For instance, the selenite present in the bacteria at  $t = 36\text{h}$  (about  $1.8 \cdot 10^{-2}$  mM per g of protein) is totally transformed into organic selenium at  $t=48\text{h}$ . Thus, several reactions take place in the bacteria:



The direct reaction  $\text{Se(VI)} \Rightarrow \text{Organic Se}$  could also occur.

In order to better describe these reactions and their products, we plant to study the system on a longer time range, and using shorter sampling intervals at the beginning of the reaction. We will record XANES spectra as well as EXAFS spectra, as EXAFS is much more sensitive to organic species.

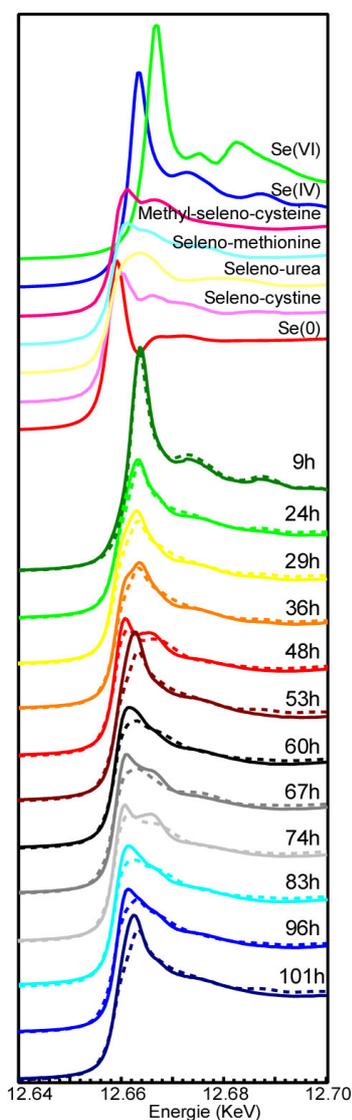


Fig. 1: XANES spectra for Se references and for *R. metallidurans* CH34 exposed to selenate. Dashed lines are the simulations using the reference spectra.

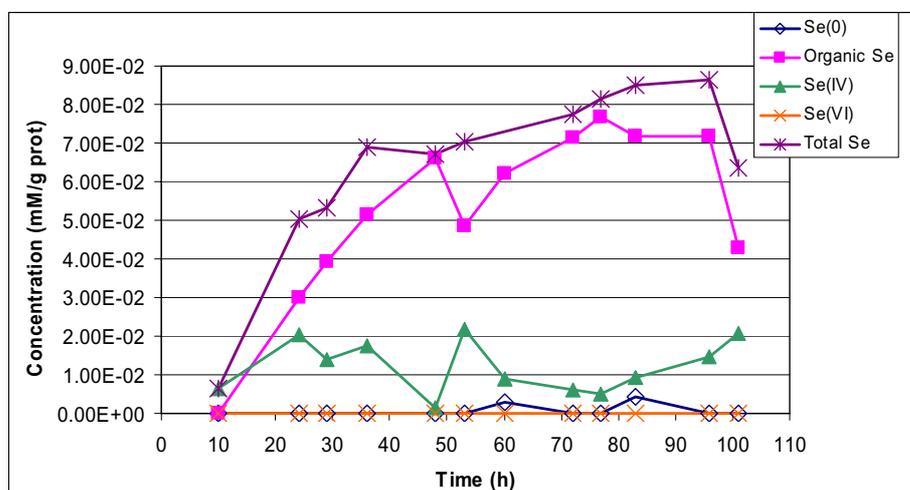


Fig. 2: Concentrations of the different chemical forms of Se in *R. metallidurans* CH34 exposed to selenate, obtained by simulation of XANES spectra and measurement of total Se by ICP-MS.

The bacteria *Synechosystis* also accumulates selenite and selenate. When exposed to selenite, this bacteria reduces it into organic Se (detected during the first 24h of the reaction) and then into elemental selenium (after 48h). Thus, the reaction scheme is:



When exposed to selenate, *Synechosystis* reduces it into organic selenium and elemental selenium in a small proportion (about 10%) during the first 100h of the reaction.

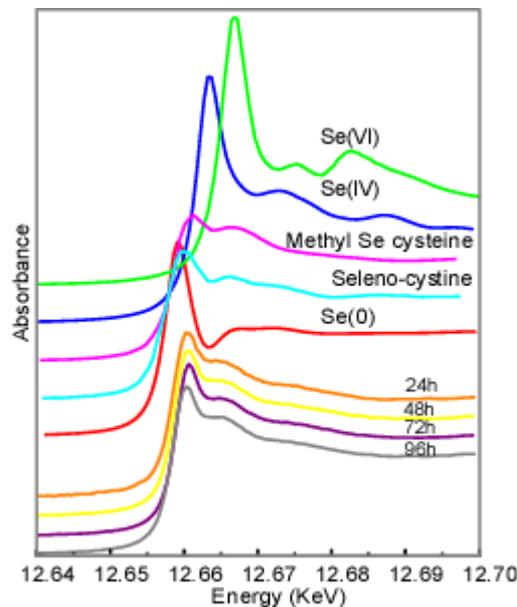


Fig. 3: XANES spectra for the bacteria *Synechosystis* in the presence of selenate. The chemical form of Se is identical in the four samples, i.e., organic Se and a minor proportion (about 10%) of elemental Se.

Thus, this bacteria is able to reduce organic Se into elemental Se when Se is supplied as selenite, but not when Se is supplied as selenate. We plan to follow this system on a longer time range to see whether organic Se is reduced into elemental Se later.

## Conclusions

This experiment brought several new findings on the bioreduction capabilities of the two bacterial strains investigated, and on the mechanisms and kinetics of the reactions: Selenite is accumulated and immediately reduced into elemental selenium by *R. metallidurans* CH34, whereas the bioreduction of selenite goes through an organic intermediate for *Synechosystis*. In the case of selenate, the oxyanion is accumulated and reduced into selenite (minor species) and organic selenium (major species) in the case of *R. metallidurans* CH34, and into organic selenium and then elemental Se in the case of *Synechosystis*.

These different behaviors as a function of the bacterial strain and of the initial form of Se data raise several questions:

- Why is selenite formed in *R. metallidurans* CH34 not reduced into elemental selenium, but transformed into organic Se ?
- Can this organic Se species be then reduced into elemental Se, as shown for *Synechosystis* in the presence of selenite?
- Why is *Synechosystis* able to reduce organic Se into elemental Se when Se is supplied as selenite, but not when Se is supplied as selenate ?
- What is the exact nature of the organic Se species found in the bacteria ?

To answer to these questions, further experiments are planned, in which the various systems will be studied at appropriate contact times. EXAFS spectra will be recorded in addition to XANES spectra, as it allows the distinction of the various organic forms of Se. In addition, some genetically transformed strains of *R. metallidurans* and of *Synechosystis*, which are hyper-resistant or hyper-sensitive to the oxyanions, will be studied. The differences in the expression of the metabolic ways involved in Se tolerance, accumulation and bioreduction might help to better delineate these pathways.

## **Bibliography**

Roux M., Sarret G., Pignot-Paintrand I., Fontecave M., and Covès J. (2001) Mobilization of Selenite by *Ralstonia metallidurans* CH34. *Appl. Environ. Microbiol.* **67**(2), 769-773.