ESRF	Experiment title: **Speciation of cobalt in resistant bacteria potentially usable for bioremediation**	**Experiment number**: 30-02 1083
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Shifts:	**Local contact(s)**: Olivier Proux	*Received at ESRF:*

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Report:

The aim of this project was to address the chemical speciation of Co in two bacteria, *Escherichia coli* (*E. coli*) and the magnetotactic bacterium *MSR1*, engineered to express a Co chelator derived from the nicotianamine which increases Co accumulation in bacteria. *MSR1* being a magnetotactic bacteria, easily removed from any liquid with a magnet, this engineered strain is a good candidate for bioremediation of Co-polluted environments.

Experimental details

We addressed the speciation of Co in several bacterial constructs which were :
-wild-type *E. coli*
-*E. coli* expressing NAS and DUF2338 enzymes from *Pseudomonas aeruginosa*, thanks to the transfection of nas and duf genes cloned in a pET-DUET plasmid
-*E. coli* expressing NAS and DUF2338 enzymes from *Pseudomonas aeruginosa*, thanks to the transfection of nas and duf genes cloned in a pBBR plasmid
-*E. coli* expressing EPI, NAS and DUF2338 enzymes from *Pseudomonas aeruginosa*, thanks to the transfection of Epi, Nas and Duf genes cloned in a pBBR plasmid
-wild-type *MSR1*
-*MSR1* expressing NAS and DUF2338 enzymes from *Pseudomonas aeruginosa*

These bacteria were exposed to either 5 µM of $CoCl_2$ (wild-type bacteria) or 20 and 50 µM of $CoCl_2$ (transfected bacteria). They were prepared in a liquid medium containing 20%

glycerol, and directly deposited and frozen on the sample-holders of FAME. Samples were prepared in our laboratories and measured at 15 K in the cryostat of FAME.
Co concentration in *E. coli* samples was high enough to obtain Co XANES spectra with a good quality, thanks to the 30-element fluorescence detector of FAME. Conversely, even if Co accumulation in *MSR1* bacteria was higher than in *E. coli*, we could not obtain good quality spectra because *MSR1* contains iron granules which pertub the detection of Co absorption. We also analyzed reference samples: Co(II)-nicotianamine, Co(II)-histidine, Co(II)-nitrate, Co(II)-phosphate, Co(II)-acetate, Co(III)-acethylacetonate, Co-Fe_20_4, Co-vitamin B12. Total integration time was adjusted in order to provide 10^6 fluorescence counts above the absorption edge on each sample.

Results:
The normalized XANES spectra for the bacterial samples are presented in Fig 1. In all the bacterial samples, Co is in the Co(II) oxidation state, i.e. is not reduced during its accumulation in bacteria. Moreover, the features of XANES spectra in *E. coli* suggest that the coordination sphere of Co is composed of O, N and/or C (Fig 1A), as previously described in higher vegetals (Collins et al., 2010) and as also observed in the Co-nicotianamine reference compound (Fig 1A, Ref Co_NA 15K). However the features of XANES spectra recorded on the E.coli pBBR sample (Fig 1A Ecoli pBBRpaND) differs from those recorded on E. coli pET (Fig 1A Ecoli pETpaND), suggesting the contribution of another Co species in the E.coli pBBR sample. We are currently fitting these spectra with the model that we previously published (Collins et al., 2010).

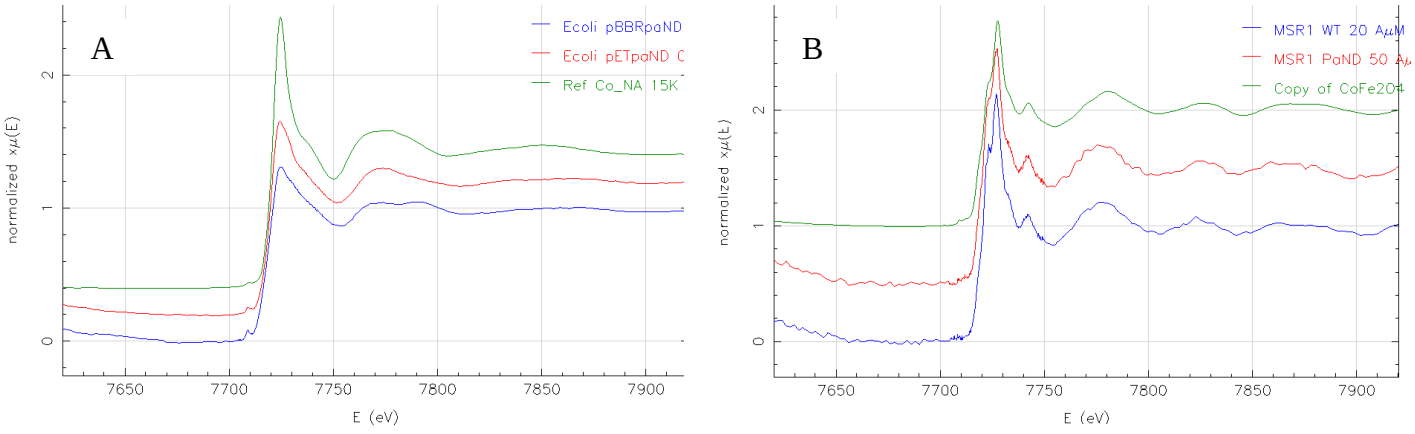

Figure 1. XANES spectra recorded on E. coli (A) and MSR1 (B) bacteria exposed to $CoCl_2$.

In the *MSR1* magnetotactic bacteria, the signal-to-noise ratio avoids advanced analysis of the EXAFS oscillations. However, linear combination fits of XANES spectra suggest that Co would be incorporated in the crystal lattice of magnetosomes. This incorporation does not modify the magnetotactic property of the bacteria, confirming that it is a good candidate for bioremediation.

Conclusions and perspectives:
The allocated beamtime was fully exploited, and the experiment provided satisfactory results, particularly on *E. coli* bacterial strain. Since the global objective of this project is to use bacteria for bioremediation, it would be important to now address the speciation of Co in the

magnetotactic bacteria. For this purpose, the use of a high definition spectrometer, such as the crystal analyzer spectrometer of FAME would be perfectly adapted, as previously published by Llorens et al. (Llorens et al., 2012). Thanks to this spectrometer, the fluorescence of Fe would not perturb the detection of Co absorption, and signal-to-noise ratio in EXAFS spectra would be much higher. Consequently, we would certainly be able to address Co speciation in magnetotactic bacteria. Moreover, since these preliminary results suggest that Co would be incorporated in magnetosomes, it would be important to directly analyze the purified magnetosomes by EXAFS. Finally we are currently constructing another magnetotactic bacteria based on another strain, *AMB1*, which accumulate more Co than *MSR1*. This strain would also be a good candidate for bioremediation, and our future study will aim at also addressing Co speciation in this bacterium.