	Experiment title: **Fate of Ag nanoparticles after interaction with bacteria and bacterial secretome**	**Experiment number**: EV216
Beamline: ID16B	**Date of experiment**: from: 26 to: 31 Oct 2016	**Date of report**: 21 Fev 2017
Shifts: 15	**Local contact(s)**: Vanessa Tardillo Suarez	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Geraldine Sarret, ISTerre
Elise Eymard Vernain, ISTerre & CEA/LCBM
Ana Elena Pradas del Real, ESRF & ISTerre

Report:

Silver nanoparticles are used in a variety of consumer products, from which they are easily leached. There is a need of better evaluating their toxicity and their fate in the environment. This study is focused on the impact of bacteria and of their secretome (molecules excreted in their medium) on the fate of Ag-NPs. We chose a model bacteria present both in the rhizosphere and in the gastro intestinal tractus, *Bacillus subtilis*. Bacteria were exposed to pristine Ag-NPs, aged NPs (Ag_2S NPs) and ionic Ag at realistic concentrations (1 mg L^{-1}). TEM-EDX analyses on cryosubstituted samples showed that Ag-NPs remained essentially outside the cells. However, this technique is not sensitive to diffuse concentrations, and it was not possible to determine whether a small fractino of Ag was internalized or sorbed on cell walls. Bulk EXAFS analyses on frozen hydrated cells evidenced major changes in Ag speciation. The purpose of this experiment was to perform nanoXRF and nanoXANES on the same sections as studied by TEM, in order to search for possible diffuse Ag concentrations inside the cells or in the cell wall, and to localize the various Ag species identified by bulk EXAFS. The ultrathin sections were treated with OsO4 in order to visualize the bacterial cells by nanoXRF.
The whole experiment was done in monochromatic mode, since it would be too time consuming to switch from PINK mode for nanoXRF to monochromatic mode for nanoXANES. For nanoXANES, a full XANES mapping mode was used: a stack of maps at each energy step of the XANES spectum was recorded. Then all maps were aligned to correct for drifts, and finally the

nanoXANES spectra were extracted. They were then treated by linear combination fits using a database of Ag K-edge reference spectra.
Representative nanoXRF maps obtained for each condition are shown in Figure 1. The bacteria were very easily visualized with Os. For all conditions, no Ag was detected inside cells or in the cell wall. Based on the analysis of standard samples in the same configuration, we can conclude that Ag content in these compartments is lower than 200 µg g-1.
Good quality NanoXANES spectra were recorded (Figure 1 left). They were compared with bulk XANES spectra for samples and references, and fitted by linear combinations. Several Ag species were identified, in varying proportions depending on the treatment. In order to test for possible artefacts due to cryosubstitution, we compared thin sections and samples deposited on ultralene film and dried. We did evidence slight differences in speciation between the two treatments.

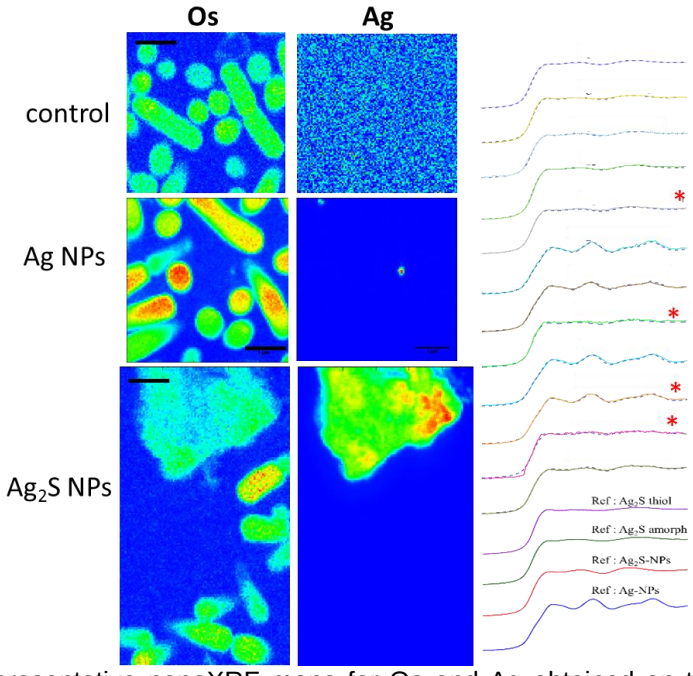

Figure 1: left: representative nanoXRF maps for Os and Ag obtained on the ultrathin sections. Right: representative nanoXANES spectra (noted with a red star) compared with bulk XANES spectra for samples and references, and fitted by linear combination (dashed lines).

Overall, the experiment was very successful, and the beamtime was used efficiently. We are currently finalizing the data treatment and writing the article related to this work.