Standard Project

Experimental Report template

Proposal title: Fate of Ag nanoparticles after interaction with bacteria and bacterial secretome				**Proposal number:** **30-02-1106**
Beamline: BM30B	**Date(s) of experiment:** from: feb 17, 2016	to:	feb 23, 2016	**Date of report:** April 6, 2016
Shifts: 18	**Local contact(s):** Olivier Proux			*Date of submission:*

Objective & expected results (less than 10 lines):

There is a need of better evaluating their toxicological and ecotoxical effect, after their release in the environment. This study is focused on a model bacteria present both in the rhizosphere and in the gastro intestinal tractus, *Bacillus subtilis*. The purpose is to evaluate the impact of the bacteria and of their secretome (molecules excreted in their medium) on the fate of the NPs. We wish to evaluate the changes in Ag speciation and to identify the molecules involved in Ag binding, both in the intra and extracellular compartments. Systems of increasing complexity were studied by Ag K-edge EXAFS spectroscopy: pristine Ag NPs, aged NPs (Ag_2S NPs) and ionic Ag after incubation (1) with single secreted molecules, (2) with the whole secretome, and (3) with *B. subtilis* culture. These spectroscopic data, combined with the identification of the secreted molecules, will provide a better understanding of the role of microorganisms in the fate of silver nanoparticles in the environment.

Results and the conclusions of the study (main part):

The exposure conditions and experimental procedures were performed as described in the proposal. Bulk Ag k-edge EXAFS spectra were recorded on frozen hydrated cells and samples, at 10°K.
The samples included the bacteria, the secretome (supernatant after culture), the growth medium before bacterial culture, poly gamma glutamate (PGA), surfactin, and ADN extracted from the bacteria and medium.
The three contaminants tested were Ag NPs, aged NPs (Ag_2S NPs) and ionic Ag (Ag lactate).
We already had a database of reference spectra. We recored only two new ones (amorphous Ag2S, and Ag2O) (Figure 1). A set of representative sample spectra is shown in figure 2. The whole set was treated by principal component analysis and linear combination fitting. Results suggested major changes for Ag lactate in contact with the purified molecules (PGA, surfactin, DNA), with the culture medium and secretome, including sulfidation and even reduction to elemental Ag. AgNPs were much less sensitive to the molecules, culture medium and secretome, but were significantly modified when in contact with bacteria. The formation of Ag-thiol and/or small Ag2S clusters was observed, in addition to crystalline Ag_2S. Our results contradict a recent paper, who identified Ag_2O after exposure of *B. subtilis* to Ag NPs (Hsue et al., 2015). The comparison with the AG-NPs exposed to the secretome only, and to isolated molecules of the secretome suggest that the living bacteria are necessary to transform Ag-NPs.For Ag_2S NPs, only the PGA, surfactin and DNA ligands were tested. Surfactin possibly induces a change in Ag speciation.

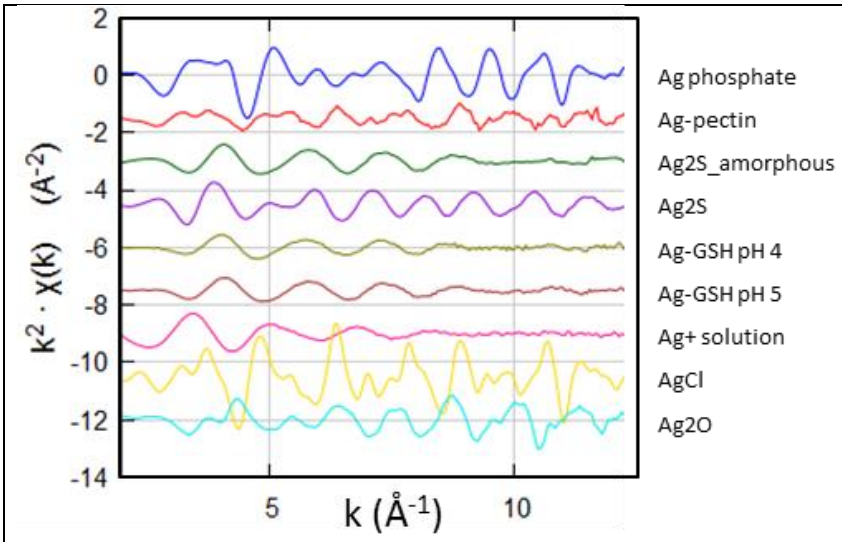

Figure 1: Ag K-edge EXAFS spectra for selected reference compounds

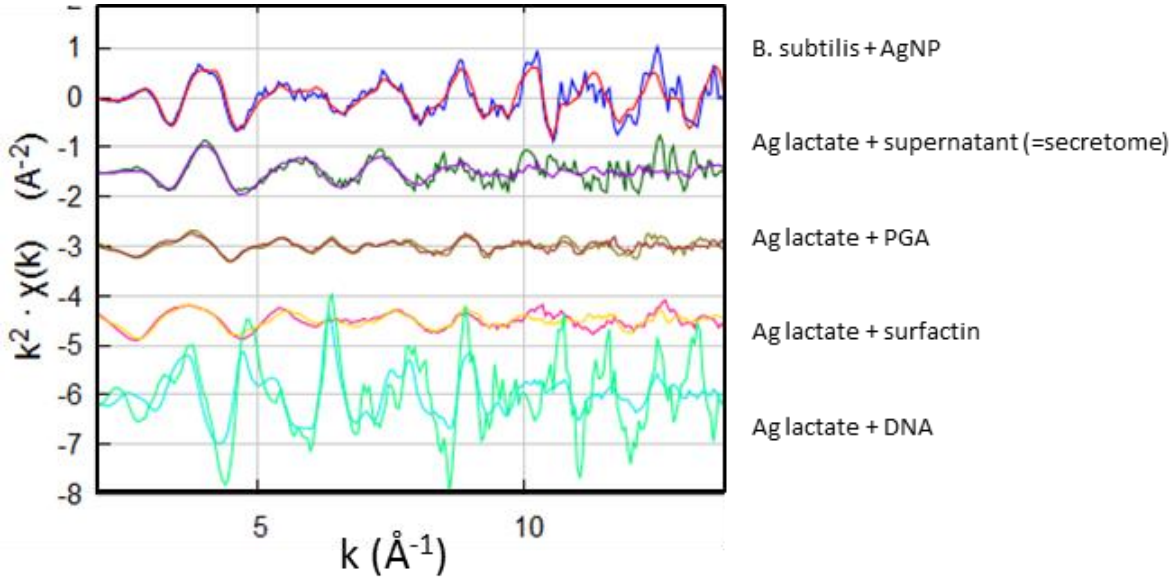

Figure 2: Ag K-edge EXAFS spectra for selected reference samples, and linear combinaiton fits

Justification and comments about the use of beam time (5 lines max.):

The experiment went perfectly well until the last day, during which the window of the cryostat was broken by a piece of kapton tape heading out of the sample holder. So a few hours were lost. Except this problem, the beamline was highly reliable and the support was great.

Publication(s):

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