ESRF	**Experiment title:** Quantitative mapping of selenium at nanoscale spatial resolution in *Stenotrophomonas maltophilia* SeITE02 cells exposed to selenite and in the biosynthesized Se nanoparticles	**Experiment number**: LS2707
Beamline:	**Date of experiment**: from: 25/1/2018 to: 30/1/2018	**Date of report**: 5/3/2018
Shifts: 15	**Local contact(s)**: Yang Yang	*Received at ESRF:*

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Report:

Our objective was to shed light into the still not fully understood mechanism by which bacterial cells of *Stenotrophomonas maltophilia* SeITE02 reduce toxic selenite (oxidation state 4+) into non-toxic elemental Selenium and how the subsequent formation of Selenium bio-nano-particles (SeNPs, from 100 to 400 nm diameter) occurs, by applying for the first time 2D X-ray fluorescence at nano-scale spatial resolution (XRFM), also combined with nano-tomography, to obtain a map of Selenium distribution in the frozen-hydrated bacterial cells of *Stenotrophomonas maltophilia* SeITE02. The main open research questions are:
RQ1: Which are the biochemical reactions involved in this process? Which compartments of the cells are involved in this process?
RQ2: Which is the mechansim of formation of the SeNPs? Which is the mechanism of the release of Selenium from inside to outside the cells? Are the SeNPs formed inside the cells or do they entirely form after the release of Selenium in the extracellular space?
RQ3: How is Selenium distributed inside the SeNPs (which are known, from our XAFS data and other previous different spectroscopic studies, to contain a major component of organic nature)?

Sample preparation and measurements at the ID16N1 beamline
There is no literature for the specific task of preparing frozen-hydrated samples of bacterial cells of the dimensions of *Stenotrophomonas maltophilia* SeITE02 (2 µm x 3 µm). In view of the experiment, we made at ESRF a preliminary study (one week before the experiment) aimed at optimizing, by visual analysis through optical microscopy, the concentration of the cells as well as of the SeNPs in the liquid suspension before deposition on Silicon Nitride membranes (size 1.5 mm x 1.5 mm, thickness 500 nm), manual wiping and subsequent freezing.
The first fresh samples for XRFM measurements were prepared at EMBL, then deposited on the Si_3N_4 membranes, manually wiped and finally frozen using the Leica EM GP automatic plunge freezer available at the ID16N1 beamline, in the first two days immediately before the experiment. The frozen-hydrated samples were cryo-transferred using a Leica VCT500 system and directly mounted in the sample compartment of the beamline. Only at this stage they could be visually analyzed by optical microscopy before measurement. A similar procedure was applied also to the samples of the extracted nano-particles. This procedure implied that, actually, the protocol for sample preparation had to be refined during the days of the experiment, because the final concentration of cells and of the extracted nanoparticles on the membrane for the first prepared samples

resulted to be quite low, requiring to spend a lot of time in searching for the cells (and the nanoparticles) to be measured.
As regards the measurements, we lost almost 1 hour of beamtime due to a machine problem. Morever, the first night of measurements (8 hours) was lost due to an unexpected irreproducibility of the pre-programmed micro-positioning of the sample holder. For each acquisition, at 20 nm spatial resolution, the order of magnitude of the covered sample area was typically of 1 µm x 2 µm up to about 10 µm x 10 µm. The intensity of the nano-beam was adjusted so as to avoid sample damage due to excessive heating.

Preliminary results

2D X-ray fluorescence maps were collected on the extracted SeNPs formed after 48 hours of incubation (30 maps resulting in about 10 useful SeNPs maps); on bacterial cells after 3 (T3, 3 cells measured), 12 (T12, about 10 cells measured) and 24 (T24, 6 cells measured) hours exposure to 0.5 mM of sodium selenite; on bacterial cells not exposed to selenite and grown for 6 hours taken as control (5 cells measured). During the last night a X-ray nano-tomography was collected on a T12 bacterial cell with a nanoparticle, for which data analysis is still in progress. As regards RQ3, although a more accurate data analysis is required, our preliminary images suggest that Selenium distribution is uniform inside the nanoparticles. As regards RQ2, no selenium was detected in the T3 sample (Fig.1), while the SeNPs where detected extracellularly in the T12 sample (Fig.2) together with the presence of selenium inside the cells. As regards RQ1, interestingly a non-uniform Selenium distribution is seen in the cells during the reduction process, especially in the T24 sample (Fig.3), while the distribution of biochemically relevant elements such as Potassium and Phosphorous appears to be different among the investigated samples. Here we present only few figures of our preliminary analysis still lacking of quantification, which is in progress.

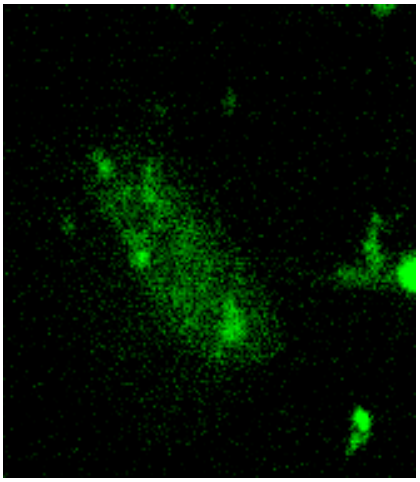

Fig.1 T3 (K, green)

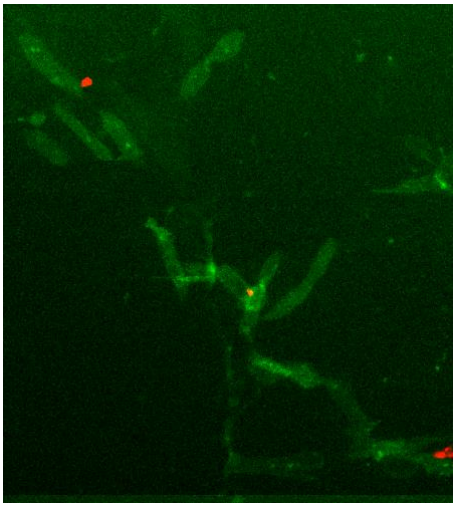

Fig.2 T12 (K (green) and Se (red))

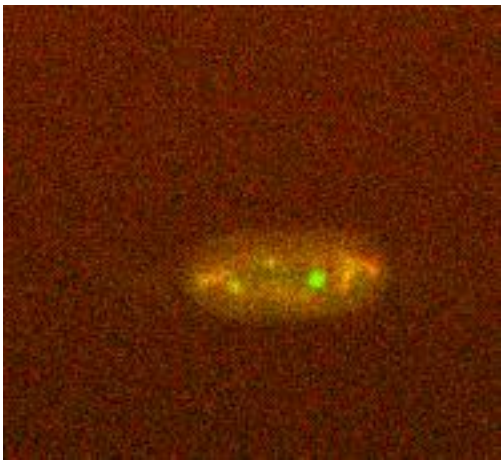

Fig.3 T24 (K (green) and Se (red))

Conclusions and need for future work

We have demonstrated that Synchrotron-based X-ray Fluorescence Microscopy (XRFM) allows to obtain the distribution of Se and other biochemically meaningful elements (such as K and P) in bacterial cells of *Stenotrophomonas maltophilia* SeITE02 after suitable sample preparation.
As for RQ3, our preliminary data analysis indicates that we got interesting results concerning the internal structure of the SeNPs. As for RQ2, comparison of the obtained preliminary T3 and T12 maps indicates that it would be important to map the Selenium distribution inside the cells from 3 to 12 hrs of incubation (i.e. after 6 (T6) and 9 (T9) hours of exposure to selenite) to investigate if the SeNPs are formed extracellularly or if they are formed intracellularly and then released by the cells. To this aim, acquisition of a 3D fluorescence map could also be of great help whenever an indication of a possible presence of intracellular SeNPs were seen in the 2D maps. As for RQ1, our preliminary results suggest that an increased statistics and deeper investigation on control cells and on bacterial cells at different times after exposure to selenite (namely, again T3, T6, T9 as well as T12) could really allow to establish a putative correlation between selenium and K and/or P localization in SeITE02 bacterial cells sheding light on the biochemical mechanism involved in the reduction process. To complete this very promising study, we plan to ask for a second experiment