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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** ***X-ray phase contrast and fluorescence analysis of metal distributions in bacteria***	**Experiment number**: EC-1066
Beamline: ID22NI	**Date of experiment**: from: 10/11/2011 to: 16/11/2011	**Date of report**: 26/03/2013
Shifts: 15	**Local contact(s)**: PhD Heikki SUHONEN	*Received at ESRF:*

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Report:

In the experiment “X-ray phase contrast and fluorescence analysis of metal distributions in bacteria” we aimed to study the microbial impact on arsenic water pollution in China and the role of potential role of microorganisms in the arsenic geochemical cycling in natural environment.
In former experiment EC-812, a natural population of mixed wild-type bacteria were collected from Inner Mongolia and the natural bacterial population was isolated, purified via separation of soil particles and grown in artificial DEV media containing 378 µg/L Arsenic (AsV). Afterwards the samples were embedded with epon and sliced to a thickness of 1 and 5 µm, characterized with the phase contrast holotomography and scanning fluorescence microscopy in beamline ID22-NI for morphology and chemical content investigation.

Based on the analyzed results from last beamtime, we scaled down from the natural population isolated from the sediments further to only 6 pure bacteria isolations, picked and cultivated from single colonies of the wild-type bacteria mixture, and sequenced to identify the species as following:

1. *Cellulosimicrobium sp.* 93%,
2. *Escherichia coli* 97%,
3. *Pseudomonas sp.* 97%,
4. *Enterobacteriaceae* 97%,
5. *Klebsiella sp.* 97%,
6. Not identified.

These isolations were prepared and embedded under the same protocol like before, and pre-characterized also with high magnification (3000x and 12000x) transmission electron microscopy(TEM) method. A successful sample preparation can be confirmed from the TEM images, see figure 1 and 2, and the difference in size and shape among various species can also be observed. In total, 12 different samples were prepared, with 2 samples for each species, slices from embedded epon blocks were fixed onto a silicon nitride windows to minimize the absorption and fluorescence signal interference.
During the beamtime at ID22NI, firstly energy was tuned to 17 keV to cover the K-emission lines of the heavy metals of interest, with measured focal spot size 75 × 7*1 (vertical × horizontal)* nm^2 *FWHM. Then* 2D x-ray fluorescence (XRF) analysis were performed with a step size of 50 nm and a dwell time of 0.1s in order to reveal the chemical content and distribution at cellular level. The high photon flux rendered a satisfying signal to noise ratio in a relatively short time. All 12 samples were measured with fluorescence mapping, for each sample three to five areas (10 x 15 µm^2 each) were chosen to acquire sufficient statistics. Also, a standard sample was measured for calibration of the XRF spectra in order to get quantitative results. Holotomography were performed along XRF measurements for a confirmation of morphological reliability and also a quantitative estimation of the fluorescence signal to volume ratio of a single bacteria.
The possibility to pre-align the sample by means of a built-in optical microscopy objective at the experimental station of ID22-NI combined with thorough pre-characterization of the samples severely facilitated sample alignment and thus significantly contributed to preventing loosing beam time and in turn making best use of the scheduled shifts. Furthermore, due to the outstanding stability of the synchrotron source, no beam time was lost due to beam loss or required re-calibration of the beamline.
First results (compare fig. 3) look very promising, as individual bacteria can be distinguished in the XRF maps and it can be observed that arsenic distribution is inhomogeneous and strongly species-dependent. More information were hoped to be found out between these trace elements and possible relation with arsenic mobilization within the bacteria species. Combined with additional micro- and molecular-biological techniques we thus want to shed more light on the possible pathways of drinking water pollution with heavy metals.

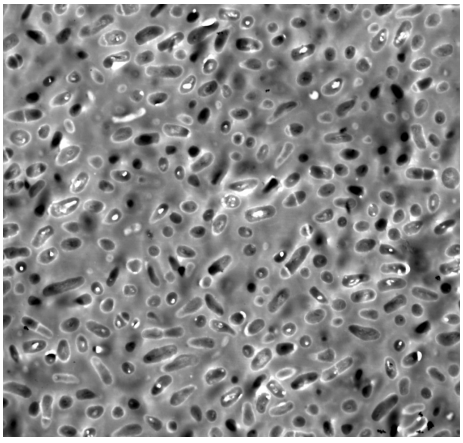

Figure 1: TEM image of the embedded bacteria. demonstating a successful embedding step. Magnification 3000x, field of view 14.03 µm. The isolation is identified to be *Cellulosimicrobium sp.*, with 93% confidence.

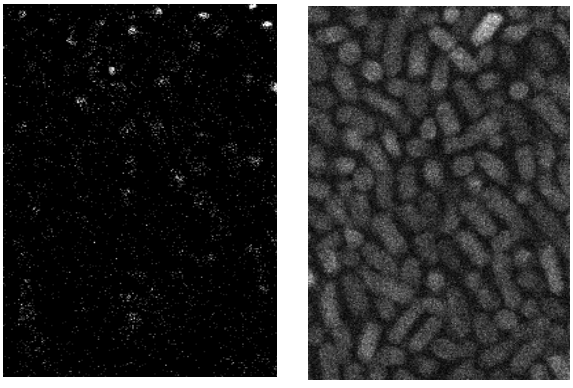

Figure 3: Elemental maps of Arsenic (left) and Zinc (right) in the isolation strain *Escherichia coli.* Due to the excellent beam conditions at ID22NI , individual bacteria can be detected. Obviously, the different elements are not homogeneously distributed among the different bacteria. Field of view 10x15 µm^2, step size 50nm, dwell time 0.1s.