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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:** ***Investigation of trace element distribution in different physiological states in pseudomonas aeruginosa with X-ray phase contrast and fluorescence analysis***	**Experiment number**: EC-1067
Beamline: ID22NI	**Date of experiment**: from: 07/11/2012 to: 10/11/2012	**Date of report**: 22/03/2013
Shifts: 15	**Local contact(s)**: PhD Heikki SUHONEN	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. **HEINE Ruth**
Laboratory Karlsruhe Institute of Technology Institute of Photon Science and Synchrotron Radiation (IPS) Hermann-von-Helmholtz-Platz 1 D - 76344 EGGENSTEIN-LEOPOLDSHAFEN, GERMANY

Dr. **XU Feng** *
Laboratory Karlsruhe Institute of Technology Institute of Photon Science and Synchrotron Radiation (IPS) Hermann-von-Helmholtz-Platz 1 D - 76344 EGGENSTEIN-LEOPOLDSHAFEN, GERMANY

Dr. **SCHWARTZ Thomas**
Laboratory Karlsruhe Institute of Technology Institute of Functional Interfaces (IFG) Hermann-von-Helmholtz-Platz 1 D - 76344 EGGENSTEIN-LEOPOLDSHAFEN, GERMANY

Dr. rer. nat. **WACKER-SCHRÖDER Irene**
Laboratory Karlsruhe Institute of Technology Institute for Biological Interfaces (IBG) Hermann-von-Helmholtz-Platz 1 D - 76344 EGGENSTEIN-LEOPOLDSHAFEN, GERMANY

Dr. **HELFEN Lukas***
Laboratory Karlsruhe Institute of Technology Institute of Photon Science and Synchrotron Radiation (IPS) Hermann-von-Helmholtz-Platz 1 D - 76344 EGGENSTEIN-LEOPOLDSHAFEN, GERMANY

Ms. **YANG Yang***
Laboratory Karlsruhe Institute of Technology Institute of Photon Science and Synchrotron Radiation (IPS) Hermann-von-Helmholtz-Platz 1 D - 76344 EGGENSTEIN-LEOPOLDSHAFEN, GERMANY

Dr. **SCHLECHER Nina***
Laboratory Karlsruhe Institute of Technology Institute of Mineralogy and Geochemistry (IMG) Hermann-von-Helmholtz-Platz 1 D - 76344 EGGENSTEIN-LEOPOLDSHAFEN, GERMANY

Dr. **ROSENHAHN Axel**
Laboratory Karlsruhe Institute of Technology Institute of Functional Interfaces (IFG) Hermann-von-Helmholtz-Platz 1 D - 76344 EGGENSTEIN-LEOPOLDSHAFEN, GERMANY

Prof. **GRUNZE Michael**
Laboratory Universitaet Heidelberg Angewandte Physikalische Chemie Im Neuenheimer Feld 253 D - 69120 HEIDELBERG, GERMANY

Report:

Pseudomonas aeruginosa is a Gram-negative rod bacterium, representing a critical causative agents for infections of the human organs, wounds blood, etc., thus was of special interest to be studied as an ideal model for the comparison and characterization of elemental distribution under various growth states. Three typical physiological states out of four (see figure.1): lag phase, log phase, and stationary phase were chosen and were correspondingly cultured. Furthermore, *Pseudomonas aeruginosa* was exposed to 10 µM copper sulfate in tap water in order to induce a special state called “viable but non-culturable” (VBNC) state. Bacteria entering the VBNC state do not propagate, but exhibit higher stress tolerance. VBNC bacteria cannot be detected by conventional culture methods, but they can regain culturability under specific conditions. Therefore, it is of great interest to study the influence of copper stress on *Pseudomonas aeruginosa* and its role as a chemical inducer to VBNC states by investigating the chemical content variations under changed growth states.

In total, 8 different samples were prepared, with 2 samples for each growth states, 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* × 71 *(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 eight 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.
The possibility to pre-align the sample by means of a built-in optical microscopy objective at the experimental station of ID22NI 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.
Preliminary results (figure.2) have already uncovered differences in chemical contents and distributions among the four physiological states. Zn distribution can be detected within single bacteria cells, prominent variations in shape and size were clearly observed. Quantitative elemental concentration and correlation analysis will be done to further study the nature of this microbial mediation with trace elements. Combined with additional micro- and molecular-biological techniques the results will help developing new strategies to target *Pseudomonas aeruginosa* during infection and devising new concepts to avoid and treat infections.

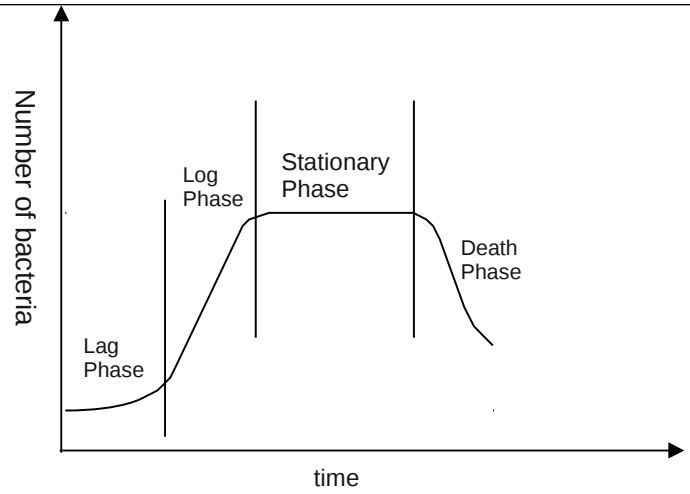

Figure 1: Bacteria growth curve. Bacterial population growth will proceed through these four stages: lag phase, log phase, stationary phase, and death phase.

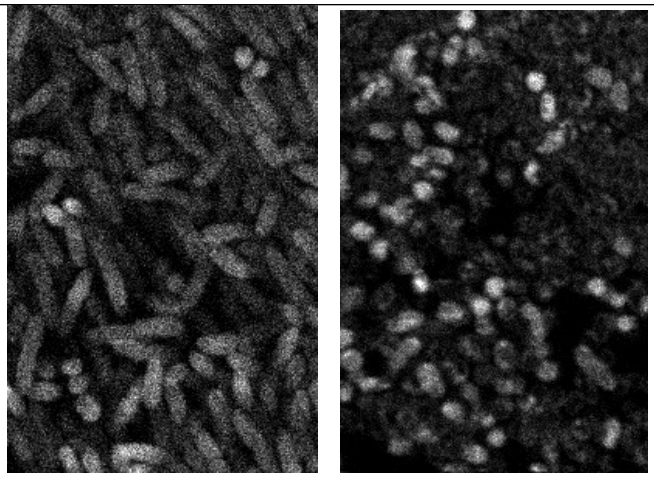

Figure 2: Elemental distribution of Zn in the embedded bacteria slices under growth state Lag phase (left) and VBNC state (right), single bacteria can be distinguished, and different morphology was observed.