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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:** The function of a bacterial synapse-like signalling complex	**Experiment number**: SC2474
Beamline: ID21	**Date of experiment**: from: 10/04/2008 to: 15/04/2008	**Date of report**: 06/09/2008
Shifts: 15	**Local contact(s)**: Dr. Murielle Salomé	*Received at ESRF:*

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Report:

Spore development begins with an asymmetric cell division that produces two dissimilar daughter cells. The smaller cell (forespore) differentiates into the spore while the large cell (mother cell) becomes a terminally differentiated cell that nurtures the developing forespore. During development the mother cell engulfs the prespore to produce a free protoplast within the mother cell cytoplasm surrounded by two bilayer membranes and isolated from the external medium [1,2, and references therein]. Activation of the late forespore-specific RNA polymerase sigma factor, σ^G, coincides with completion of engulfment and further requires the action of the conserved membrane protein insertase SpoIIIJ, and the expression of the *spoIIIA* operon in the mother cell [1,2]. Recently was proposed a model in which the *spoIIIA*-encoded proteins may form a synapse-like signaling complex, that following engulfment completion conveys a signal to the forespore that antagonizes the putative inhibitor of σ^G, there by releasing its activity [3]. Because some of the *spoIIIA*-encoded proteins show sequence similarity to ion permeases, we decided to monitor differential *spoIIIA*-dependent ion accumulation between the forespore and the mother cell.

We used fixed cells from wild type *B. subtilis* and from the congenic *spoIIIA* and *sigG* mutants collected just after completion of the engulfment process. The measurements were performed under vaccum in order to detect the light elements, the exciting energy was 7.2 keV and the energy flux was 3.8×10^9 ph/s at 163 mA. Before the synchrotron measurements were conducted, the cells were extensively washed with water

to remove any salts from their surface. This step proved to be critical in avoiding a background signal from the buffers used during fixation. The cells were next observed by X-ray microscopy in phase contrast and fluorescence mode. A global fluorescence spectrum collected for each sample have shown the presence of P, S, Cl, K, Ca, Mn and Fe in the samples. Interesting the mutant cells were found to accumulate more iron then the wild type cells. We then used phase-contrast X-ray image and the 2D elemental mapping analysis to localize the iron accumulation inside the cell. The results indicated that, and as expected from increased accumulation in the forespore compartment, the intensity of the signal increased close to the cell poles. This is the first clue ever for SpoIIIA-dependent substrate. However, this observation need to be further investigated. In any event, due to the chamber properties on ID21 beamline there is a low contrast for the iron signal, most likely due to the iron scattering inside of the chamber. Therefore for future work it may be necessary to perform those studies on a different station (ID22 or ID22N).

In an extension of these results, we have also looked at the accumulation of metals in intact spores. Bacterial spores are an extremely resistant resting cell type, in part because of their high degree of mineralization. We obtained fluorescence spectra, phase-contrast X-ray image and the 2D elemental mapping analysis from wild type purified spores. The results show that Ca^{2+}, Mn^{2+} and K^{+} are the main elements that accumulate in spores to high levels (Fig. 1).

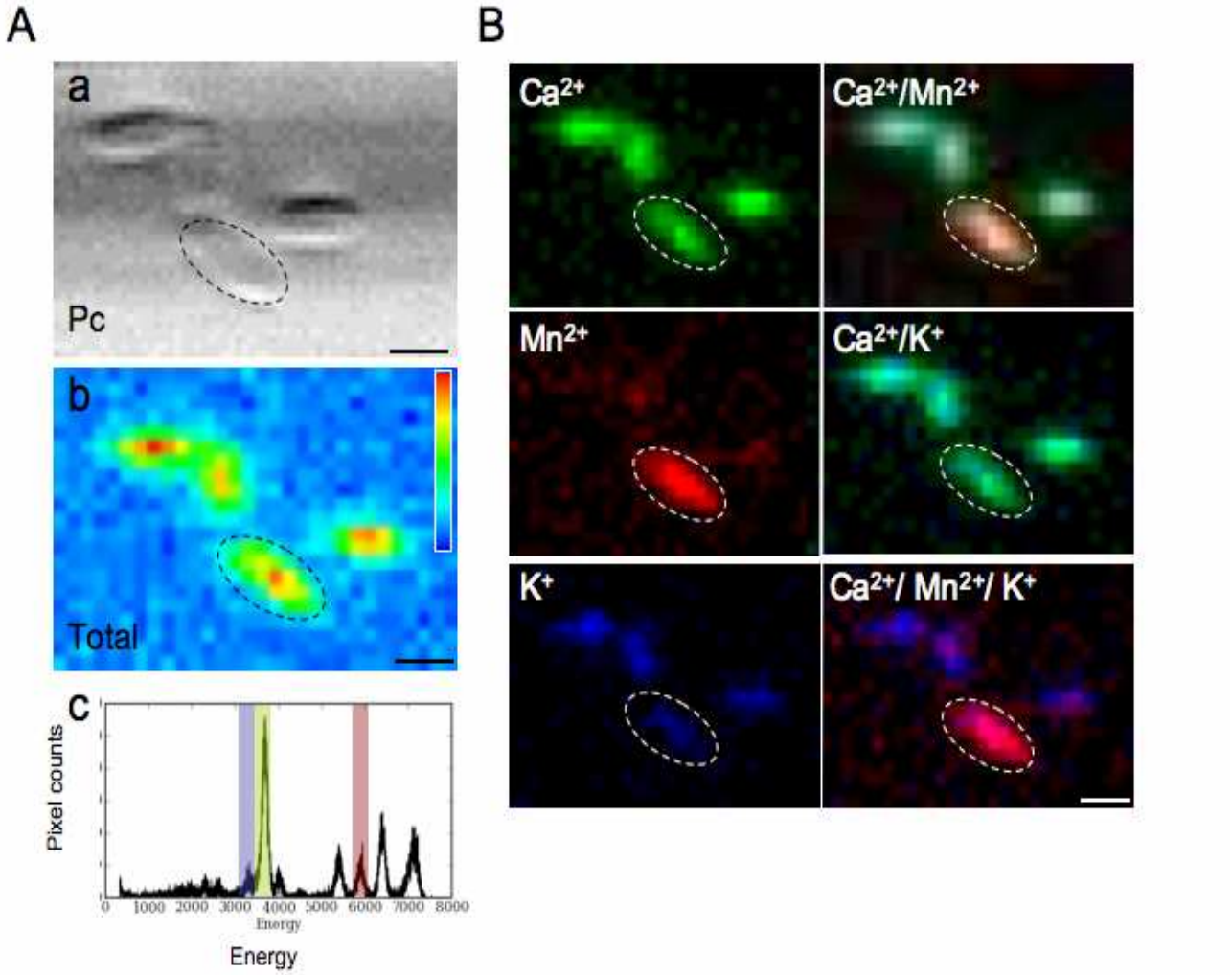

Fig. 1. Purified wild type spores shown by X-ray microscopy in transmission and fluorescence modes. **A.** Phase contrast (Pc) (a); total two-dimensional elemental distribution (b); and fluorescence spectrum (c) of the same field blue corresponds to the potassium K-edge peak, green – calcium K-edge peak, red – manganese K-edge peak. **B.** two-dimensional elemental distribution for each indicated elements.

In conclusion, the results obtained using the ID21 X-Ray Microscopy beamline revealed some features concerning the localization of manganese, iron, calcium and potassium on wild type *B. subtilis* cells and from the congenic *spoIIIA* and *sigG* mutants and also in spores. More experiments need to be performed to study of mutants involved in the accumulation of these elements in the spore. These studies will be of the uttermost importance, as they will allow the identification of the main determinants of spore heat resistant. They will also have the additional motivation of defining strategies for the rapid identification of spores at the species levels (*e.g.*, spores of pathogenic organisms in samples from patients, hospital settings or the environment), as well as establishing novel methods for spore inactivation.

References:

1. **Serrano, M., L. Côrte, J. Opdyke, C. P. Moran Jr., and A. O. Henriques.** 2003. Expression of *spoIIIJ* in the prespore is sufficient for the activation of s^G and for sporulation in *Bacillus subtilis*. J. Bacteriol. **185**: 3905-3917.
2. **Serrano, M., A. Neves, C. M. Soares, C. P. Moran Jr., and A. O. Henriques.** 2004. Role of the anti-sigma SpoIIAB in regulation of s^G during *Bacillus subtilis* sporulation. J. Bacteriol. **186**: 4000-4013.
3. **Meisner, J., X. Wang, M. Serrano, A. O. Henriques, and C. P. Moran Jr.** 2008. A channel connecting the mother cell and forespore during bacterial endospore formation. Proc. Natl. Acad. Sci. USA., in press.