EUROPEAN SYNCHROTRON RADIATION FACILITY

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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:** Correlated high spatial resolution infrared and x-ray microfluorescence analysis of fungal hyphae	**Experiment number**: EC 448
Beamline: ID21	**Date of experiment**: from: 01/07/2009 to: 07/07/2009	**Date of report**: 08/31/2009
Shifts: 24	**Local contact(s)**: **Murielle Salome**	*Received at ESRF:*

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Report:

We undertook a correlative spectromicroscopy study using the infrared (FTIR) and x-ray fluorescence (XRF) spectroscopy capabilities available at the ID21 beamline, on two fungal models. Fungi play a crucial role in the environment, biochemistry, and plant and animal diseases. They form filamentous structures called hyphae that grow from the tip, exploring the environment for nutrients. Their relationship with plants is especially complex, as they are a major agricultural pathogen responsible for crop loss. However, many fungi engage in symbiotic relationship with plants, which allow them to grow in otherwise intolerable conditions of high temperature or salt, low nutrients or heavy metal contamination.

Fungi are important organisms in biology, due to their short growth cycles, their many interactions with plants, and their use in biotechnology. Our group has wide-ranging experience with IR of fungi, which allows the organic components such as proteins and cell-wall sugars to be studied. However, this was the first high-spatial resolution (sub-micrometer) study of the inorganic distribution of elements in filamentous fungal hyphae, including P, S, Cl, K, Ca, Mn and Fe.

Following test experiments during in-house research time, we were able to choose the most suitable sample preparation methods and sample substrates, which allowed both IR and XRF to be applied to the same sample. These were silicon nitride windows, which are transparent to IR and X-rays, as well as specially-designed sample holders used to stretch a thin layer of Ultralene® foil. We grow cells with normal morphology so that they extend away from their growth medium, which otherwise would cause interference.

We studied two fungal systems. *Aspergillus nidulans* is a leading experimental model system for cell biology. *Curvularia protuberata* is a plant endosymbiont that confers host tolerance to growth on high temperature soils.

We obtained x-ray and IR maps of spores, hyphae and spore-forming structures of wild-type strain of *A. nidulans*; these are critical stages in its asexual life cycle. Figure I shows typical elemental maps of a conidiophore, which is the structure producing the spores by which the fungus forms new ornanism. As can be seen, all the elements shown show similar spatial resolutions.

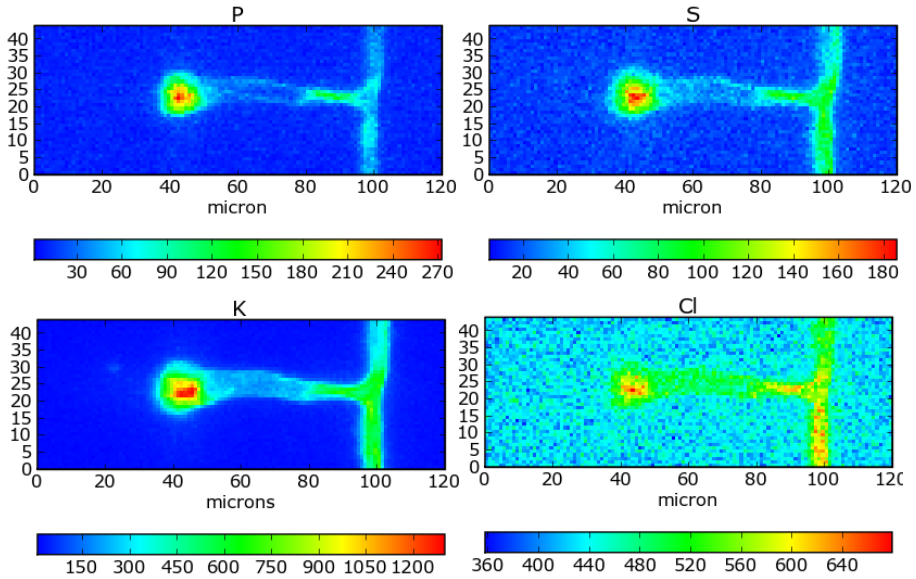

Figure 1: Wild-type *Aspergillus nidulans* conidiophore (spore forming structure), showing the distribution of several elements.

We also studied an *A. nidulans* single-gene deletion strain, *ugmA*Δ, which has aberrant wall formation (Figure 2). Differences in the distribution of inorganic elements in the *ugmA*Δ strain compared to its near-isogenic parental strain, are consistent with defects in cell wall formation shown by electron microscopy [El-Ganiny et al 2008 Fung Genet Biol 45:1533-1542]. Both P (a proxy for nucleotides) and S (a proxy for proteins) were found in the cytoplasm as expected (Fig 2 left). The *ugmA*Δ wall also had substantial S and K content (Figure 2, right). The similar distribution of S and K was unexpected, but is consistent with results for *C. protuberata*, and will be pursued. As the genetics of A. *nidulans* are well understood, this organism has considerable potential to explore the effects of gene expression on the morphology and function of living organisms. The preliminary results on the plant endosymbiont *C. protuberata* also proved promising, but until the complete data analysisis completed, it is difficult to make further conclusions. The data analysis is currently on its way and we hope to have a manuscript out shortly. We would like to perform further experiments on genetic mutants of this model organism with both xrf and IR microspectroscopies.

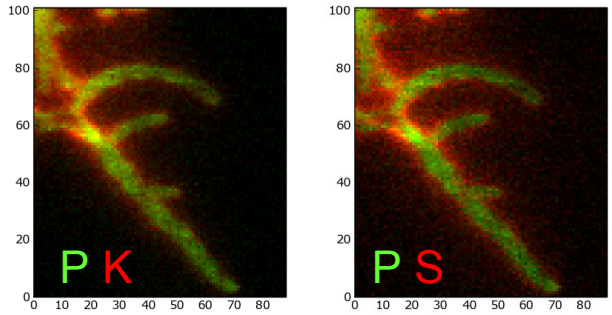

Figure 2: *Aspergillus nidulans* strain deleted for *ugmA*, which affects cell wall formation. The element distribution in the *ugmAΔ* strain is unlike that of wild-type hyphae.