EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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 via the User Portal:
https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"*),
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

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.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number 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:** Structural relationships between extracellular matrix biopolymers in Bacillus subtilis biofilms. An Xray scattering analysis.	**Experiment number**: LS-2995
Beamline: ID13	**Date of experiment**: from: 24.02.2021 to: 28.02.2021	**Date of report**: 18.04.2021
Shifts: 12	Local contact(s): BURGHAMMER Manfred LIU Jiliang	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

The experiment LS-2995 was performed on the microbranch of ID13 with a beamsize of (2x2) μm² - (10x10) μm² at an energy of 13 keV with exposure times between 20 ms and 1 s per point.
The aim of the project was to elucidate the XRD/XRF signatures of intact biofilms and to map them on the micron scale (within the radiation damage limits). This beamtime continued LS2951. In LS2995 we ellaborated the measurements with WT biofilms and mapped larger areas in three areas: biofilm periphery and two areas towards the middle of the biofilms. In this beamtime we corroborated the contribution of the ECM components to the WT XRD fingerprint, and we ruled out the contribution of cells and spores to this signal. In addition to XRD, we measured the XRF signal from WT and mutant biofilms. Finally, we managed to obtained a clean (background-free) signal from three prtoein (TasA) samples that were mounted between silicon nitride membranes.

Results

LS2951 focused on the XRD signal of WT biofilms. We previsously hypothesized that the WT doublet may originate from the TasA protein that is present in the biofilms' spores. An important control at LS2995 that was conducted at LS2995 shows that the spores cannot account for the doublet observed in biofilms, and that it is unique to biofilms (Figure 1). However, a peak at ~ 7.89 nm-1 (corresponding to d ~ 7.95 Å) appears both in biofilms and in the spore sample.
Further to LS2951, here we measured the XRF from WT biofilm colonies, and showed that in agreement with our previous ICP-MS studies (Ido Nir et al., PCCP, 2020), XRF confirms the accumulation of metal ions in biofilms. Surprisingly, the metals accumulate in the wrinkles area and they trace the morphology of the wrinkes in biofilms (Figure 2).
Finally, we managed to elucidate the XRD fingerprint of isolated protein fibers, and confirmed that they cannot account for the dominant structure in intact biofilms. Figure 3 shows the 2D and 1D XRD patterns measured from TasA protein 'chunks' in acid and in high salt concentrations, indicating that the protein itself cannot account for the doublet observed in WT biofilms. Figure 4 shows that isolated TasA fibers can bind metal ions (in particular Zn, Fe, Mn).

Figures.

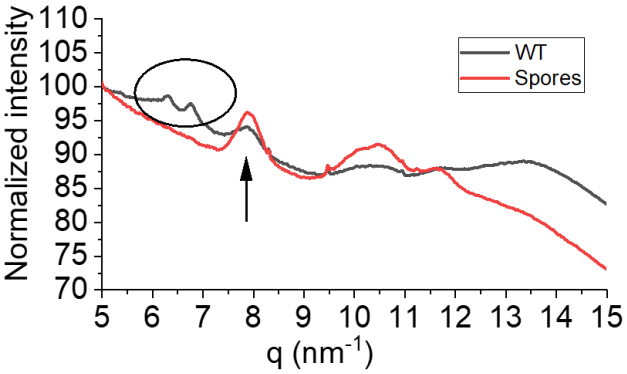

Figure 1. XRD measured from WT biofilm (black) and spores only (red). Data is normalized and zoomed into so that the doublet (marked with a circle) is observed. The doublet at ~ 6.2, 6.6 nm^{-1} appears in WT biofilms, but not in spores only. However, the peak at ~ 7.89 nm^{-1} (marked with an arrow) appears in both samples.

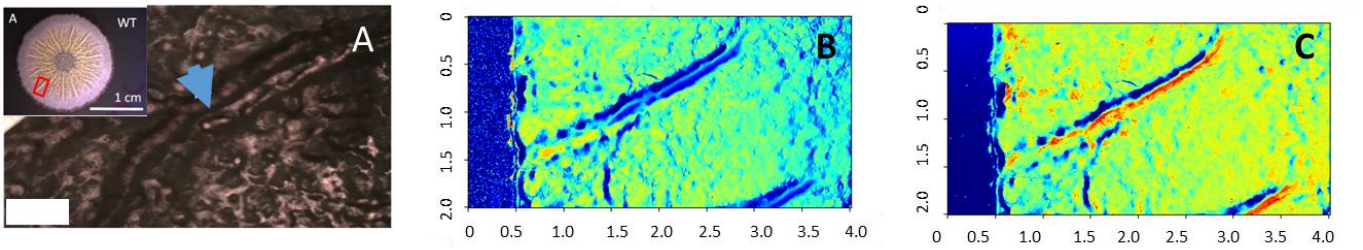

Figure 2. XRF measured from whole WT biofilm. WT biofilm image and a wrinkle marked in a red rectangle (A, insert, red rectangle), together with a bright light microscope image (main image, A, an arrow points at a wrinkle). scale bar is 300 μm. XRF map measured in the location shown in (A) is shown in (B) for calcium and (C) for iron. x,y axes on (B,C specify distance in milimeters. Remarkably, the XRF map follows the morphology of the wrinkle with great detail, owing to the high resolution at ID13.

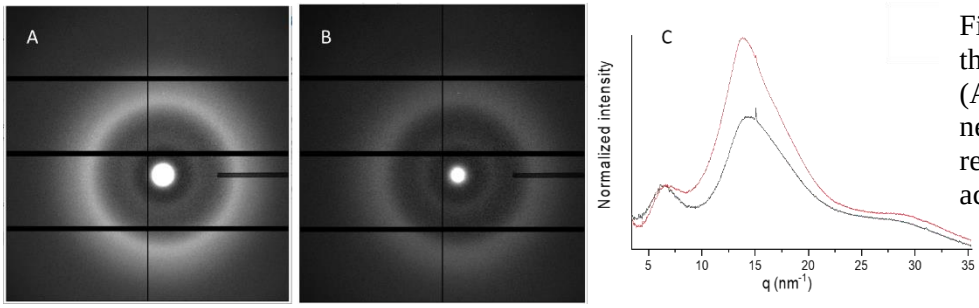

Figure 3. XRD measured from TasA fibers that formed in two different environments. (A), (B) show 2D XRD from acidic and neutral with high salt concentration, respectively. (C) shows a 1D integration in acid (red) and high salt concentration (black).

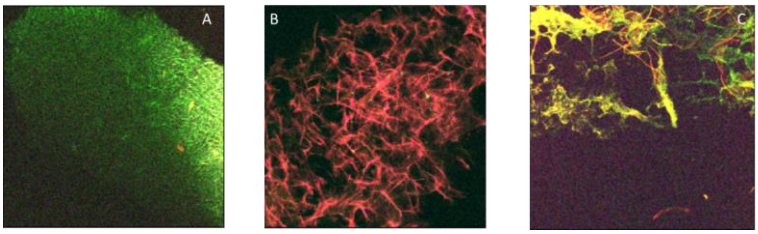

Figure 4. XRF overlay maps of Zinc (green), iron (red) and Manganese (blue), measured from TasA protein 'chunks', prepared in different ways. The images are 5X5 mm^2. (A) is a sample prepared in high concentation of salt without gel filtration, pH 7, (B) was prepared at pH 3, and (C) was prepared at high salt concentration with gel filtration, pH 7. The XRF maps expose differences in fibrilar morphology, that matches the differences in metal ion binding. Specifically, in fibers formed in a salt environment (C), iron (red) binding is observed within distinctively straight fibers, while iron and zinc appears to be distributed in larger areas where the fibers assemble into a mesh (red and green appearing yellow). In fibers formed in an acidic environment (B), iron binding dominates over zinc binding, that is observed as speckles in the background of the XRF map, and at high salt concentrations (protein was not cleaned with gel filtration) (A), zinc is dominating.