EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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. The effect of hydration and metal ions distribution.	**Experiment number**: SC-5186
Beamline: ID13	**Date of experiment**: from: 01.12.2021 to: 03.12.2021	**Date of report**: 28.02.2023
Shifts: 12	**Local contact(s)**: **BURGHAMMER Manfred**	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Liraz Chai
Institute of Chemistry
The Hebrew University of Jerusalem
Jerusalem 91904- Israel

Report:

The experiment SC-5186 was performed on the microbranch of ID13 with beamsize of (2x2) μm^2 – (10x10) μm^2 at energy of 13keV with expusre time 20 ms per point. The aim of the project was to study the effect of enviromental hydration on the structural features and the distribution of metal ions in biofilms at different time points of biofilm formation. In SC-5186 we corroborated the contribution of spores to the XRD fingerprint of WT biofilms, and measured the effect of humidity on isolated spores. In addition, we compated the XRD fingetprint of biofilms mutants as a functionof biofilm age. Finally, we maped the distripution of metal ions on WT and mutant biofilms both on top view (2D) orientation and cross-sections (3D).

Results:

In this beamtime we performed control experiments to better support our previous findings as well as performed additional experiments to ellaborate on our previous results.

1. we measured the XRD from two important controls: isolated spores and a mutant that cannot form spores ($\Delta SigF$), and showed that the WT doublet originate from spores in the biofilms (Figure 1A). We also discovered that hydrating isolated spored lead to a shift in the doublet peaks toward lower q values (larger d spacing), indicating structural changes in the spores due to water absorbtion (Figure 1B).
2. We evaluated the effect of time (biofilm age) on XRD signals of WT and matrix mutants: ΔEPS (lack of polysacchride) and $\Delta TasA$ (lack of major protein). In WT biofilms, the doublet peak (attributed to spores) appeares only after 48 h (Figure 2A), corroborating our previosly published results. Interestingly, in the lack of the polysaccharide EPS, matrix signal (at ~6 $nm^{-1}$and 14 nm^{-1}) is detained and only appears at 24h (Figure 2B); The lack of the protein TasA seems to be leff substantial to the matrix XRD signature (Figure 2C).
3. We measured XRF signals from cross sections of young (24 h) and old (72 h) WT biofilms and observed a gradient in the signal of metal ions (e.g., Ca and Mn) from bottom to the top of the young biofilm; These gradients were less dominant in older (72h) biofilms (Figure 3B). We also show here 1D XRD profiles of cross sections. The thickness of these cross sections will need to optimized in the next beamtime.

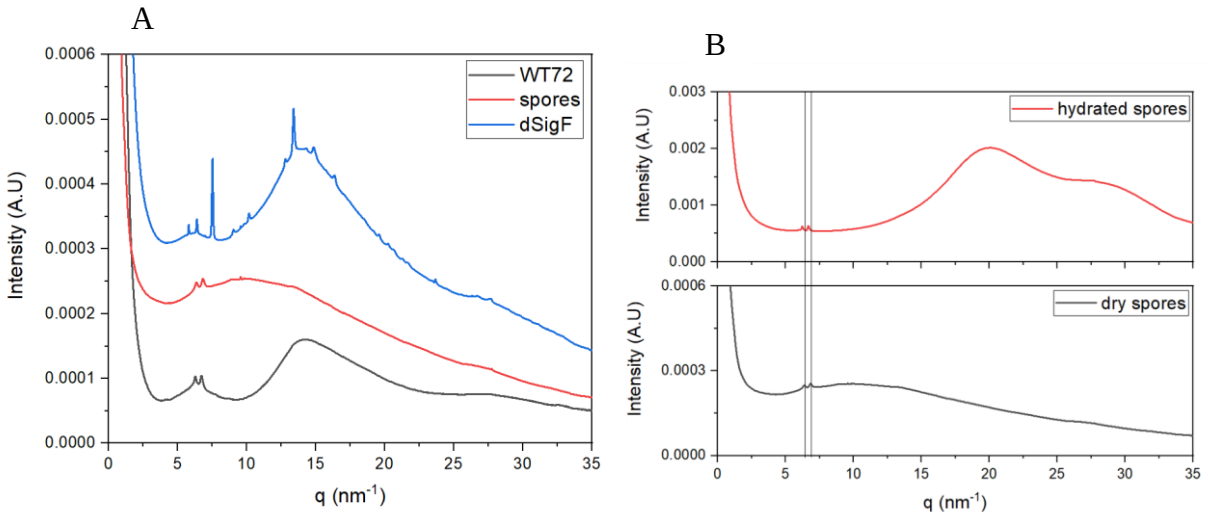

Figure 1. Spores XRD signal in biofilms and in isolated spores. (A) XRD measured from WT biofilm (black), isolated spore sample (red) and ΔSigF mutant (blue). The doublet at ~6.2,6.6 nm^{-1} appears in WT biofilm and pure spore but not in dSigF mutant. however the peak at ~14 nm^{-1} appears in all samples. (B) XRD signals measured from dry and wet isolated spores. Data shows slight shift in doublet peak toward lower q values and water broad peak ~19 nm^{-1}, 26 nm^{-1} become dominant in wet samples.

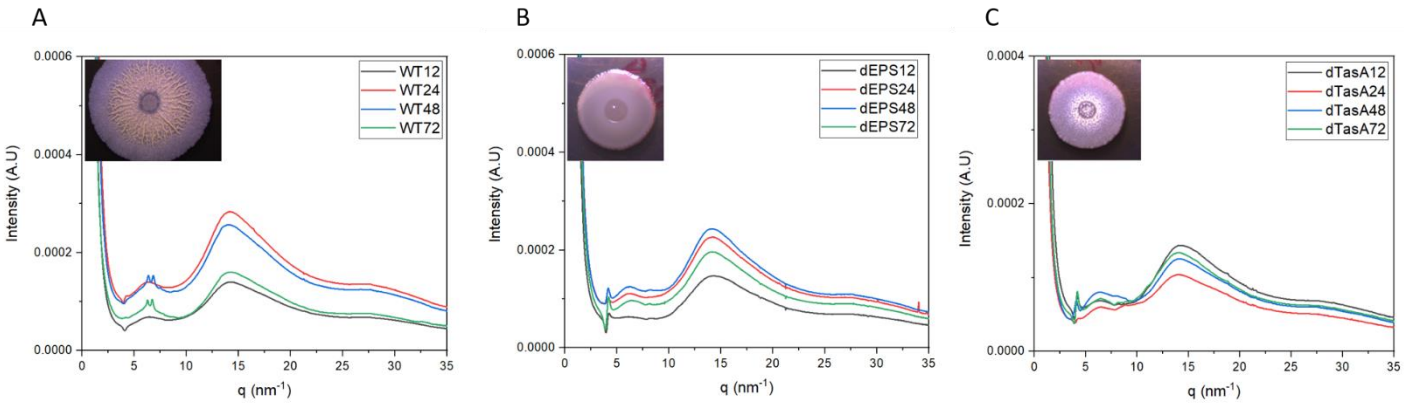

Figure 2. XRD signals from WT biofilm (A), ΔEPS mutant (B), and ΔTasA mutant (C) at different ages: 12 h (black), 24 h (red), 48 h (blue) and 72 h (green). Data shows that doublet peak at ~ 6.2,6.6nm^{-1} is observed only for WT samples and only for old biofilms (48 h and 72 h). The spore-attributed doublet disappears in both ΔTasA and ΔEPS mutants but only EPS affects matrix formation at early time points (12h).

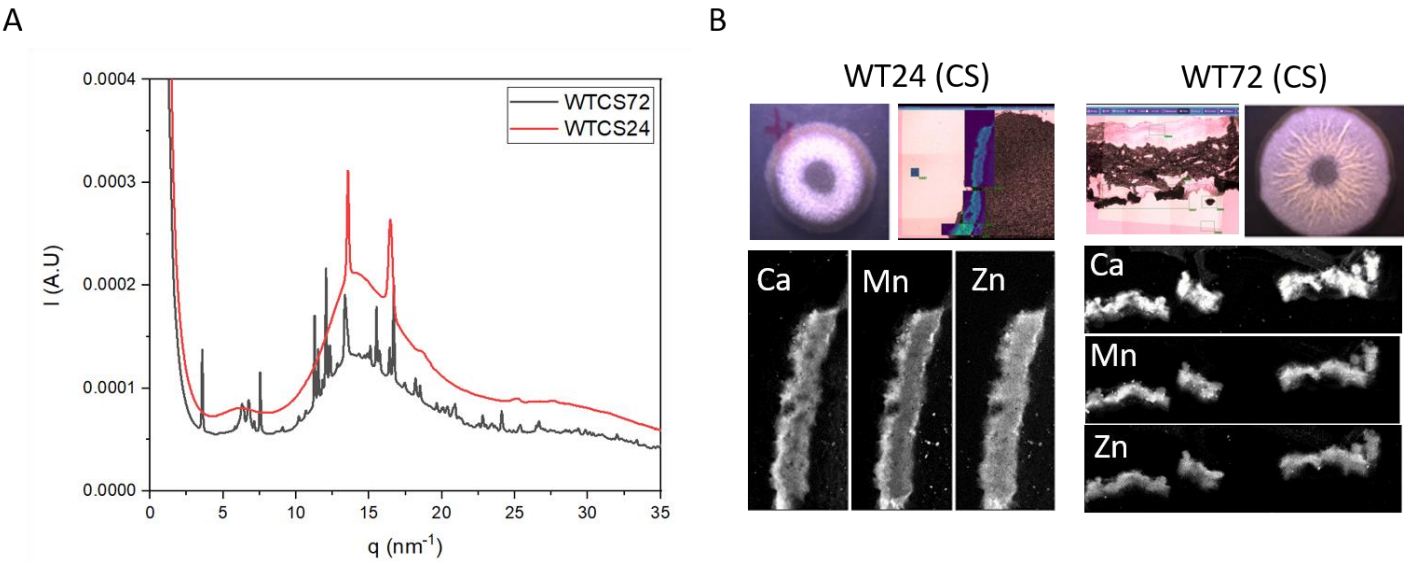

Figure 3. XRD and XRF signals measured from cross-sections of WT biofilm at 24 h and 72 h. (A) XRD signals of WT24 (CS) in red and WT72 (CS) in black salt peaks overlapped with biofilm signal. (B) XRF maps of Ca, Mn, and Zn. A concentration gradient of Ca and Mn is observed from bottom to top of young biofilms (24 h), while Zn is homogenous across the biofilm cross-section. This gradient is less clear in cross sections of mature (72 h) biofilms.