ESRF	**Experiment title: Deciphering the distribution and long-distance metal transport mechanisms in synnemata, an original structure built by a multicellular bacteria**	**Experiment number**: LS3195
Beamline: ID16B	**Date of experiment**: from: 02/03/2023 to: 06/03/2023	**Date of report**: Sept 7th, 2023
Shifts: 12	**Local contact(s)**: Remi TUCOULOU TACHOUERES	*Received at ESRF:*

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Report:

The project aimed at describing elemental distribution in an original bacterial structure called synnemata (micron-scaled tree-like structure growing in the air). We tested different mounting apparatus so that we could easily center our samples (aluminum holders with one synnemata deposited on top, litholoop with one synnemata, or large piece of sample with multiple synnemata). We then were able to obtain data on different samples, using two different species (*A. mirum* and *A. pretiosum*):

- Air-dried samples, high resolution data (50 nm step size) on a portion of a synnemata (illustrated in Figure 1B)
- Air-dried samples, lower resolution (100-200 nm step size) on whole synnemata (>10 structures measured).
- Freeze-dried synnemata filaments deposited on Si_3N_4 membrane so that the filaments (aerial mycelium) are on a flat surface.

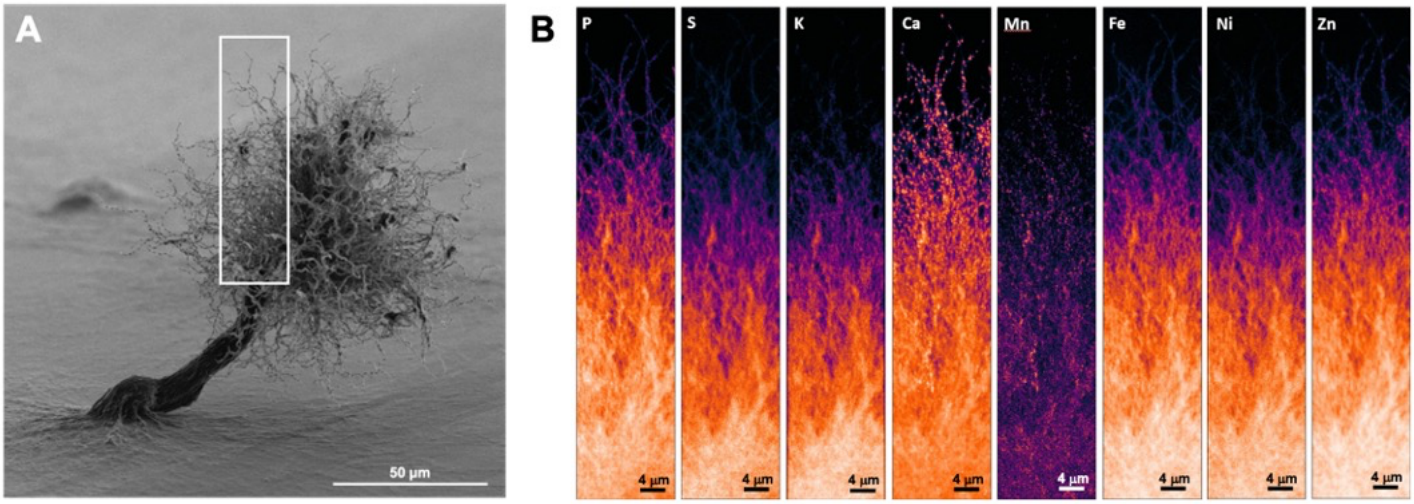

Figure 1: Synnemata of *A. mirum* and nanoXRF measurement. (**A**) Scanning electron microscopy image of synnemata formed by *A. mirum*. (**B**) preliminary XRF measurement of a part of a synnemata (white box in (A) shows example of region measured). This dataset was collected using 13 keV, 100 nm step and with a 50 ms dwell time.

The data are still being analyzed, though Figure 1 illustrates two of our main findings: 1/ elements are distributed all along the synnemata, and the cells at the tips of the aerial mycelium have the same elemental composition as the ones closer to the growth substrate. This indicates that there are efficient mechanisms of sharing oligoelements and metals between the cells that compose the synnemata. 2/ some elements are not

evenly distributed along a single filament, but are rather concentrated in specific places in a cell, which is clearly evidenced for calcium (Figure 2). Distances separating two consecutive puncta in a single filament indicates a pattern, with distances of roughly 2 µm (the length of a cell) alternating with distances of 0.4 µm (Figure 2B). Our preliminary analyses also suggest that calcium, iron, zinc and phosphate are colocalized, which might be an indication of the chemical nature of these structures, but this observation requires further investigation (Figure 2B). Our current model, based on Scanning Electron Microscopy and Transmission Electron Microscopy images combined with nanoXRF measurements, leads us to propose that metals accumulate at the poles of each cell (Figure 2C).

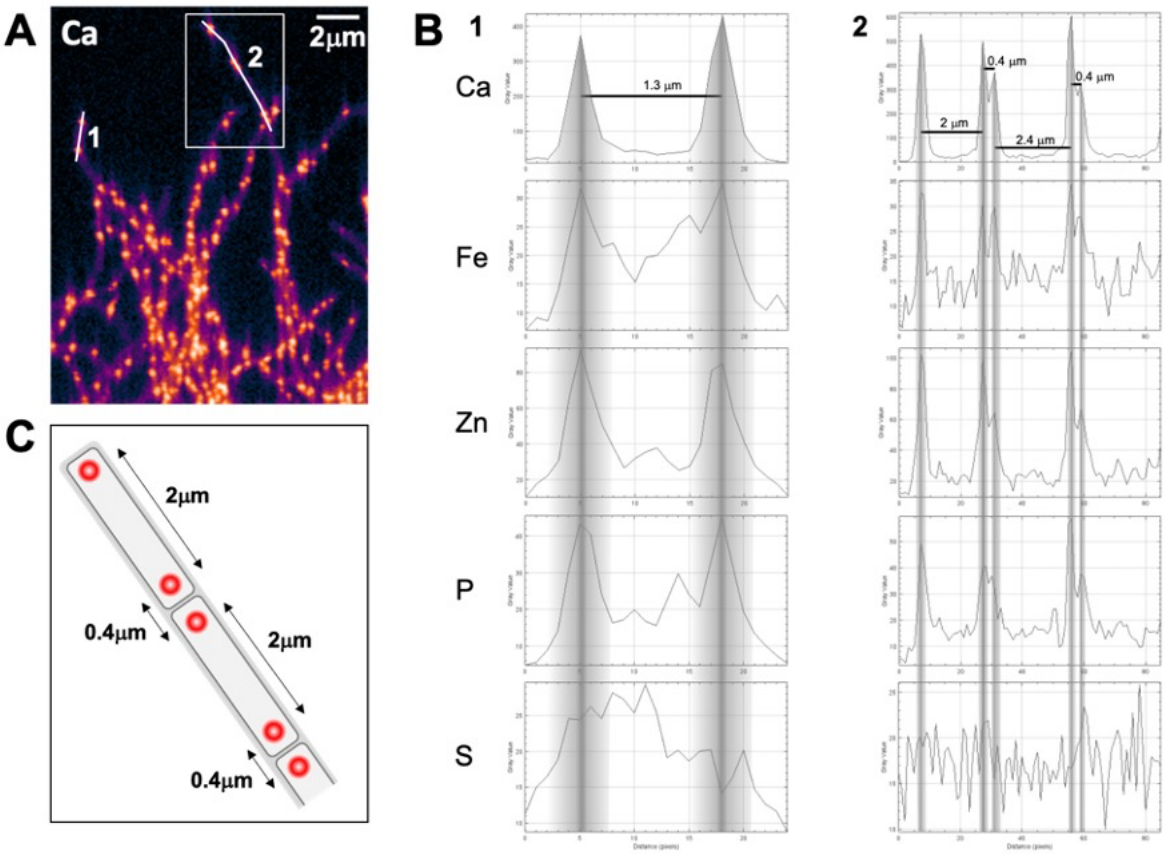

Figure 2: nanoXRF measurements suggest metal accumulation at the poles of each bacterial cells. (**A**) Zoom on the top part of the nanoXRF measurements shown in Figure 1. (**B**) Profile of elemental distribution along two lines (1, 2), suggesting that metal accumulation follows a pattern that could be explained by metal accumulation at the poles of each cell (**C**).

Our initial hypothesis was that metal transporters would be highly expressed at the tip of synnemata, ensuring an efficient uptake from the bottom of the synnemata where the sources of metals are found (agar substrate), up to the tips of the aerial mycelium. The nanoXRF measurements obtained on ID16B are now invaluable to our updated hypothesis on how essential metals are transported over long distances. Indeed, our additional unpublished data (promoter-GFP fusions, proteomic and transcriptomic data) all indicate that metal transporters are expressed at a low basal level, clearly challenging our initial model. Reconciling these two observations (low expression level of metal transporters and patterned repartition of high metal concentration) our updated working hypothesis is that the cell junctions are crucial points of exchange between adjacent cells (Figure 2C). In this current model, there would be no need to express metal transporters over the entire membrane of a cell, but rather a need to localize these transporters at the poles. Accumulation of metals close to the poles would further ensure that the transporters have access to their substrates. If this is repeated over the entire synnemata, this organization would ensure that there is no traffic jam at cell junctions, which would ensure an efficient metal transport over long distances.