AFM to probe structures involved in *Clostridium sporogenes* germination

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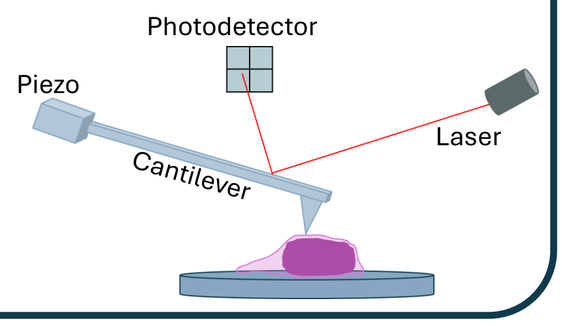
Background

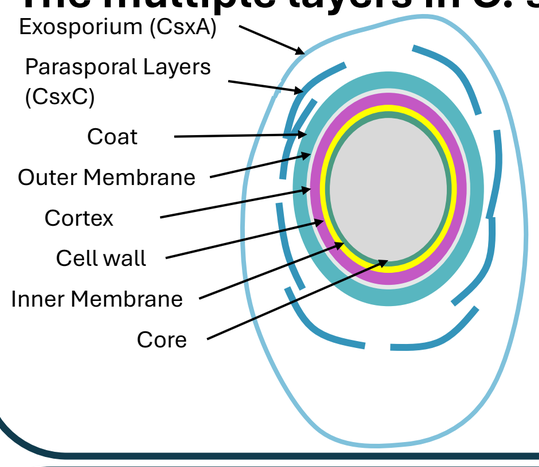
- Sporulating bacteria, including *Clostridium sporogenes*, can exist as dormant and highly environmentally resistant spores
- Germination is the process by which the bacterial spores release vegetative cells. Throughout this process, spores lose the features that give them high levels of environmental resistance
- Understanding germination could lead to the development of more targeted antibiotics

Atomic Force Microscopy

- AFM is a type of scanning probe microscopy that uses force sensing to reconstruct images of samples
- AFM can probe:
  - Topography of the whole spore/cell
  - Structures of individual protein layers

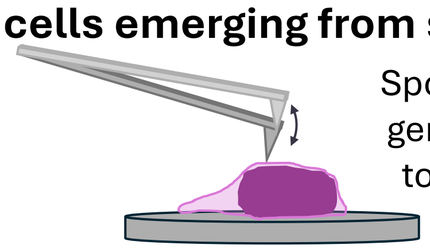


The multiple layers in *C. sporogenes* spores

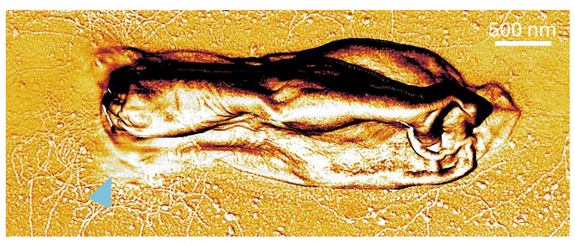


Spores consist of distinct layers. The outermost layers contain multiple cysteine rich proteins. The nomenclature of these proteins includes a Csx prefix.

AFM shows the detailed topography of vegetative cells emerging from spores

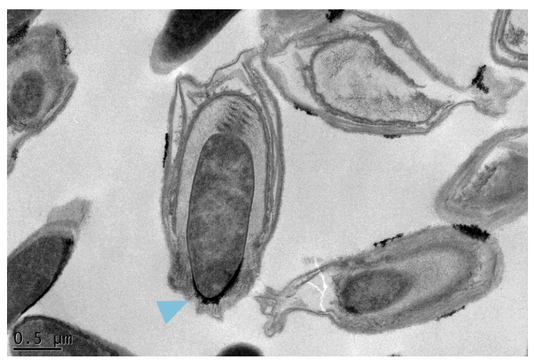


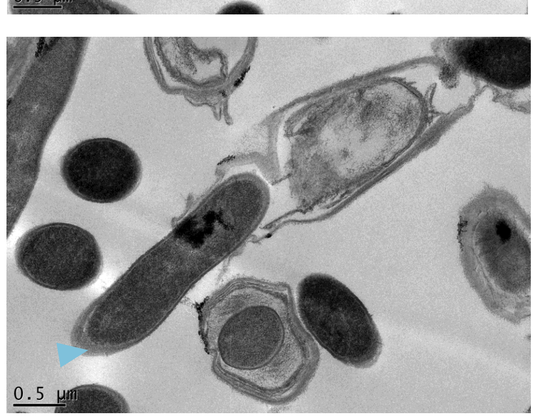
Spores can be fixed at different time points during germination and imaged with AFM (tapping in air) to observe the detailed topographical changes.



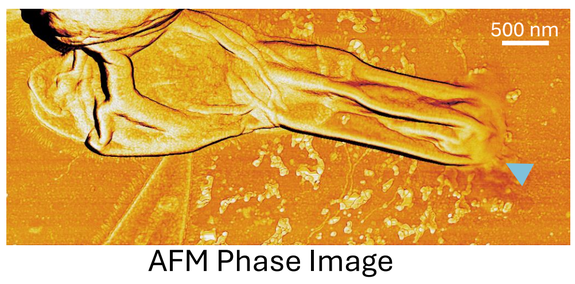
AFM Phase Image

Dark to bright variation is16^0





EM images provided by Dr Hannah Fisher



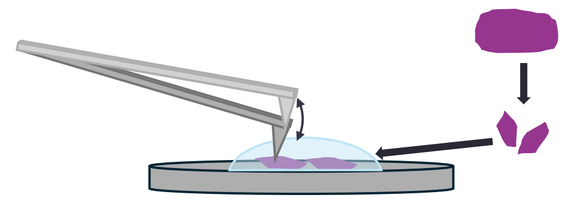
AFM Phase Image

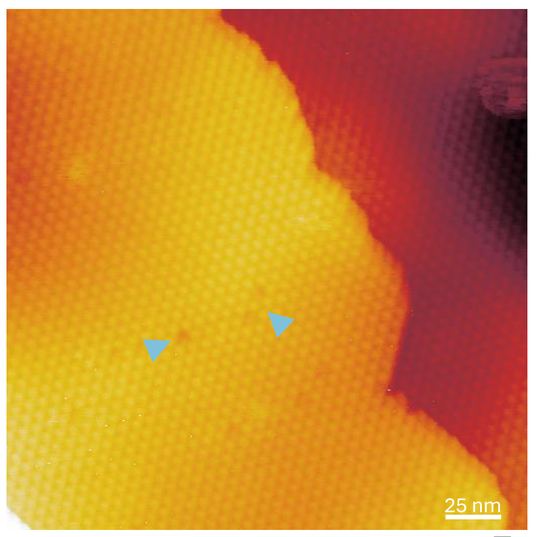
Dark to bright variation is 28^0

Similarities between Electron Microscopy (EM) thin sections and AFM phase images show vegetative cells emerge with debris on their leading end (blue arrows).

Parasporal layer fragments isolated from ΔcsxA *C. sporogenes* spores have a distinct crystal lattice

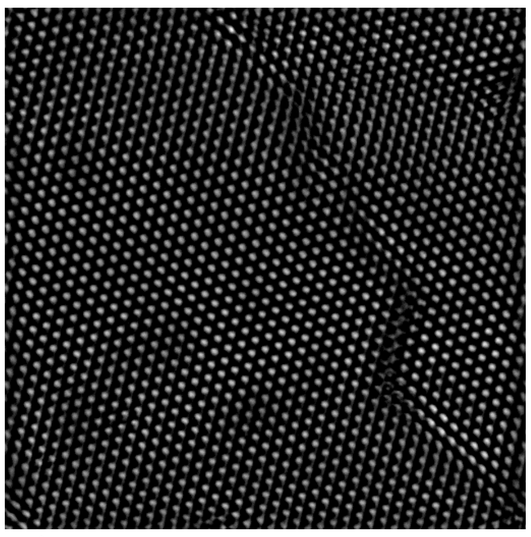
ΔcsxA spores lack an exosporium, allowing the parasporal layers to be isolated. The fragments of parasporal layers display a distinct crystal lattice when imaged with AFM tapping in liquid.



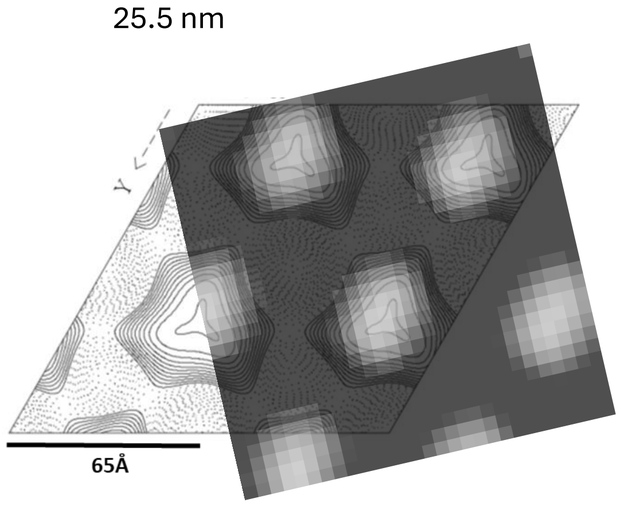


AFM Height Image

Dark to bright variation is 25.5 nm



Fourier reconstruction calculated from peaks up to the second order

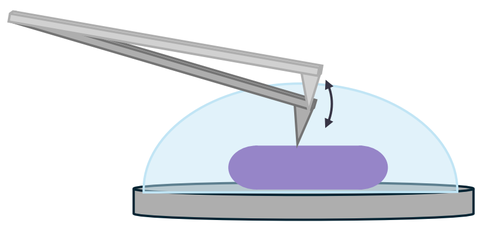


EM projection map provided by Dr Hannah Fisher

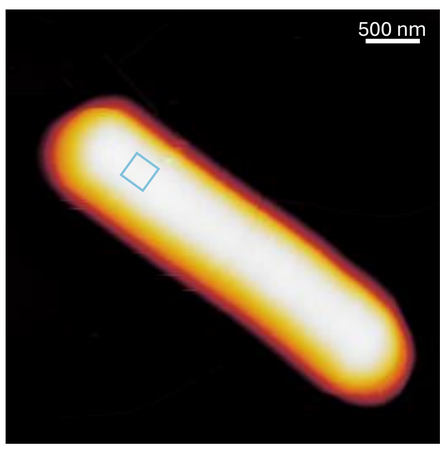
The lattice unit cell distance is 6.15 ± 0.33 nm and fits onto the EM projection map for the structure of the CsxC protein crystal.

Point defects were also visible in the AFM image of the lattice (blue arrows).

S-layer can be resolved on the surface of intact, fixed vegetative cells



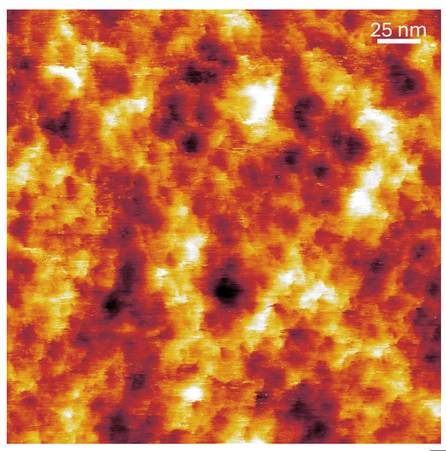
Fixed vegetative cells can be imaged via tapping mode in liquid. The whole cell can be imaged along with the detailed crystalline S-layer on the surface.



AFM Height Image

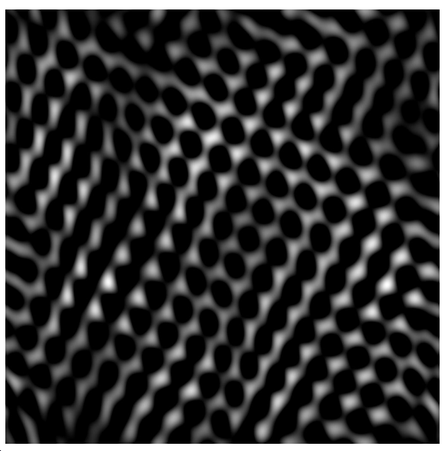
Dark to bright variation is 650 nm

Zoom



AFM Height Image

Dark to bright variation is 16 nm



Fourier reconstruction calculated from peaks up to the second order

Conclusions:

- AFM can be used for:
  - Analysis of individual protein layers, resolving crystal subunits
  - Resolving the topography of spores undergoing germination
  - Resolving crystalline layers on intact cells

Future Work:

- Imaging the surface of emerging cells in liquid to determine if the S-layer is present on emerging cells
- Imaging live spores and following germination in real time, collecting information on topography and mechanical properties

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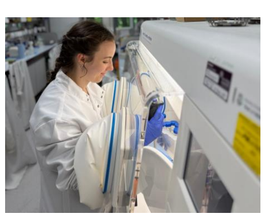

All cultures for this work were grown in a Whitley A35 Workstation.

don whitley scientific culturing innovation

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