Resolving the dynamics of spore germination using phase-contrast and fluorescent microscopy

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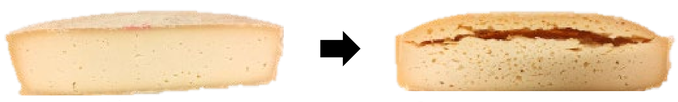
Significance

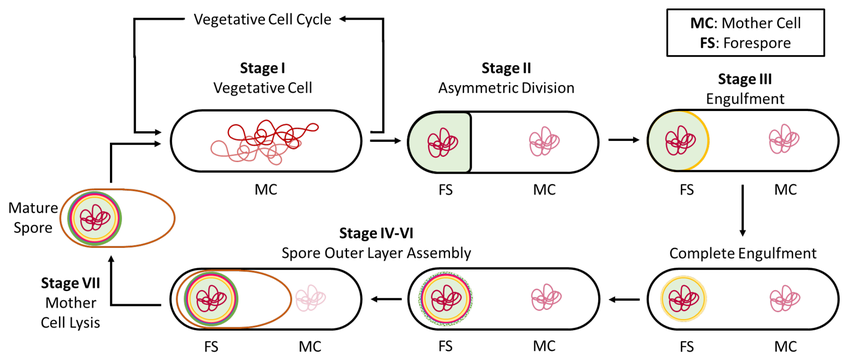
Clostridium sporogenes is an anaerobic, spore-forming, gram-positive bacteria that causes a significant amount of spoilage in the food industry. *C. sporogenes* is also used as a surrogate to study *Clostridium botulinum*, the main cause of Botulism. Under conditions of stress, *C. sporogenes* cells produce spores, which are dormant but extremely robust. Spores can remain viable for many thousands of years and can survive in harsh conditions, including exposure to aerobic environments, unlike their vegetative cell counterparts. Spores are also crucial for human transmission during *C. botulinum* infection. Thus, it is important to understand both how the architecture of spores protects them and the processes that facilitate spore germination.

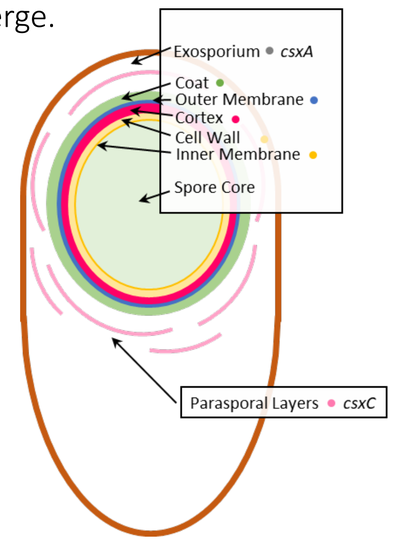


Cheese → *C. sporoge*cheese

Sporulation and Germination Cycle

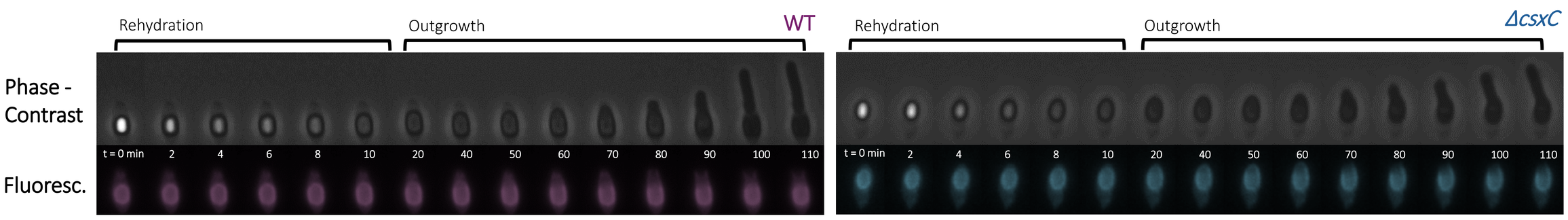
When *C. sporogenes* cells become stressed, they sporulate. They build many layers around the core for several purposes. Sometime later, when conditions are favourable, this spore will begin to germinate, and a vegetative cell will emerge.



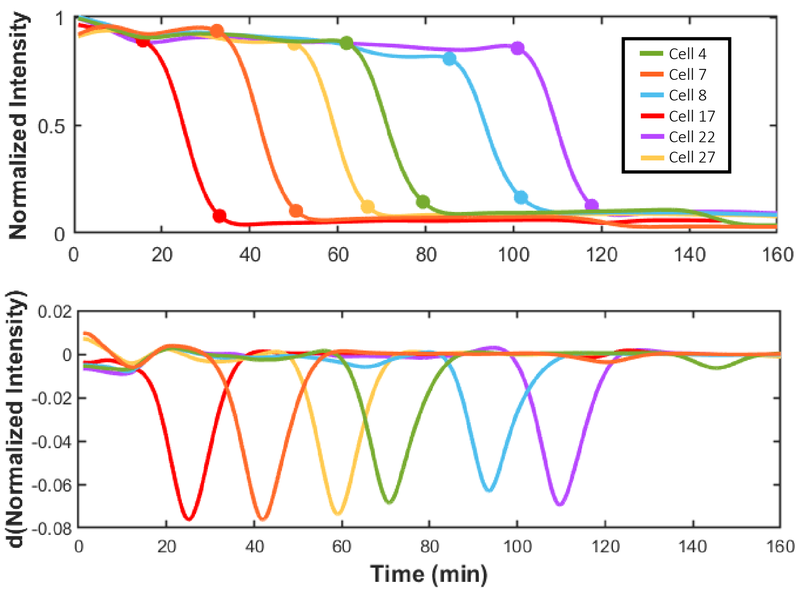


Sporulation and germination cycle and outer layer diagram adapted from the doctoral thesis of Dr. Hannah Fisher (2023).

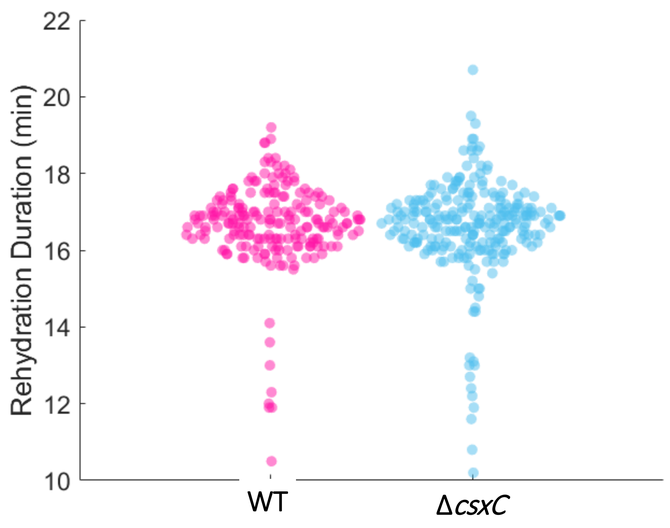
Visible Stages of Germination



Rehydration Analysis

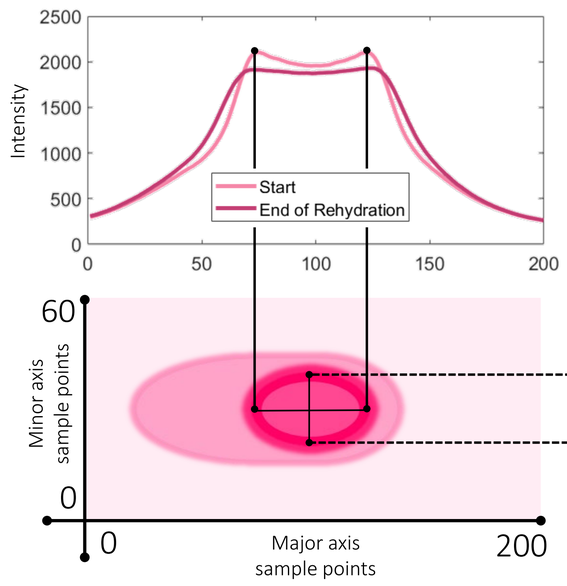


When rehydration begins, pixel intensity undergoes a steep drop due to a transition from phase bright to phase dark. Using the minimum of the first derivative of intensity over time, we can extract the time at which rehydration rate peaks. From this, we can use derivative thresholds on either side of this minimum to determine the start and end of rehydration.

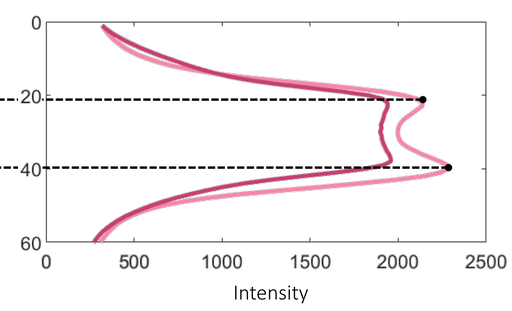


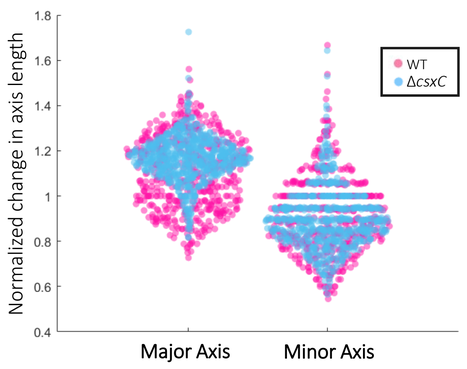
Fluorescent dyes allow us to investigate timings of WT along with mutants in co-culture. Here, we see no difference in rehydration duration between WT and Δ*csxC*

Fluorescent spore coat and exosporium analysis

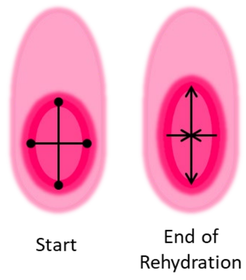


Using the fluorescent profiles shown, we can quantify the swelling of the spore coat. We define lines alone the major and minor axes and sample the fluorescent intensities at 200 and 60 points, respectively, along these lines.

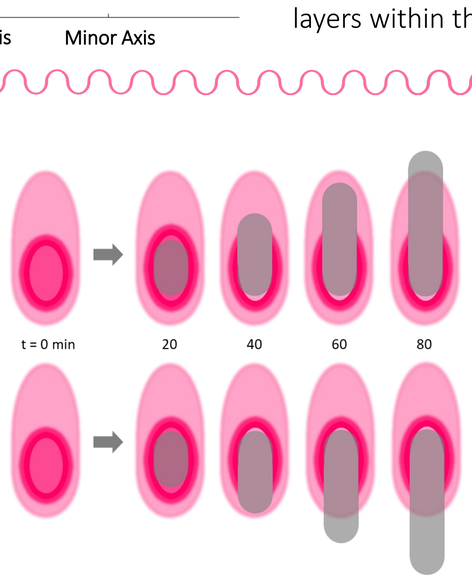


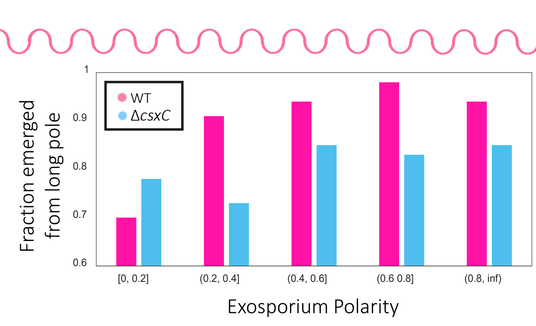


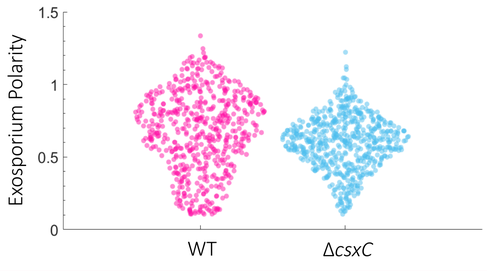
We see similar shrinking of the minor axis length in both WT and Δ*csxC,* however, in the major axis length, we see bimodal swelling in the WT and unimodal swelling in Δ*csxC.* This may have to do with a change in the cell's physical properties when lacking parasporal layers within the exosporium.



In *C. sporogenes*, it can be seen by eye that one pole of most exosporiums is longer than the other. Here, we quantify this polarity using mean intensities from the above fluorescent profiles and quantify the percent cells which emerge from this long pole.







Future Work

We are currently working to perform and process co-culture experiments of WT with outer layer mutants (Δ*csxA*, Δ*csxB*, Δ*csxC,* Δ*bclA),* all of which have been previously characterized using EM within our lab.

Note that all spores in this work are prepared within a Don Whitley scientific anaerobic workstation.

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