Structure and Composition of an Intercellular Channel in Sporulating *Bacillus subtilis*

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Introduction

Sporulation

Spore forming bacteria like ***Bacillus subtilis*** are tougher than the average microscopic unicellular organism. These species can surround themselves with **durable coats of protein**, allowing them to **survive harsh conditions**. As spores, bacteria can remain **dormant** for years. When revived, however, these bacteria cause diseases including **botulism**, **anthrax**, **tetanus** and **food poisoning**. In spore formation, the bacterial cell divides to give a **larger mother cell** and **smaller forespore**. The mother cell then **"engulfs"** the forespore and nurtures it, before lysing and releasing it into the environment (Figure 1)

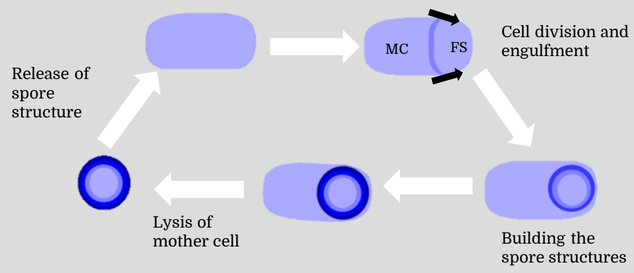


Figure 1. The process of sporulation. MC= mother cell. FS = forespore

Project Aims

SpoIIQ-SpoIIIAH Channel

The engulfment of the forespore by the mother cell is a universal feature of sporulation. In *Bacillus subtilis*, the **forespore protein SpoIIQ (Q)** and **mother cell protein SpoIIIAH (AH)** are believed to form a **channel** through which the **developing forespore** is **nurtured**. My project focuses on this channel complex shown in Figure 2. The aims are:

1. Do spoIIQ-spoIIIAH co-localize in live cells?
2. How many Qs and how many AHs are there per cell?
3. Is there a Q-AH complex and what is its stoichiometry?
4. Is the subunit stoichiometry of Q in the forespore same as AH in the mother cell?
5. How is the localization of one protein affected by the partner protein?

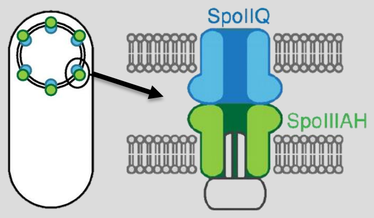
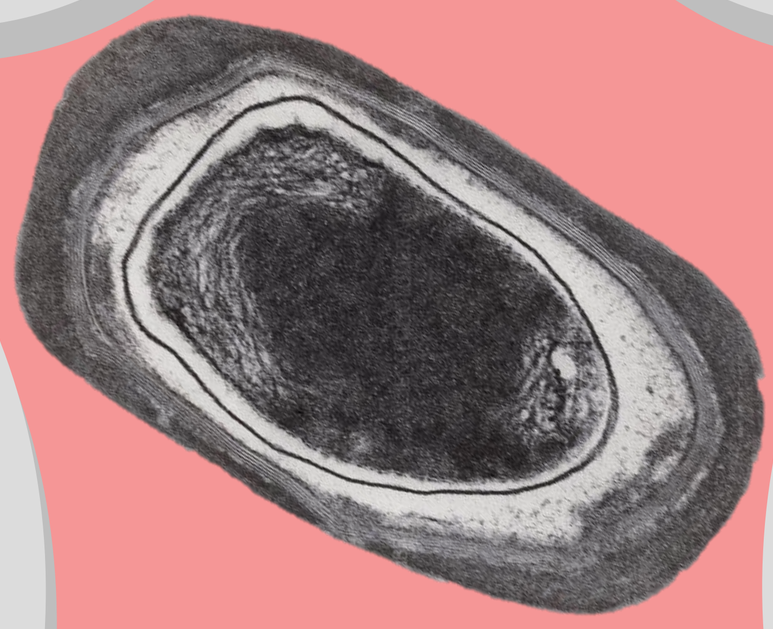


Figure 2. Q-AH hybrid transport system during endospore formation



Methodology

Slimfield Microscopy

My research uses novel cutting-edge **super resolution microscopy** and tools from the **physical sciences** to tackle open questions in the **life sciences**. Slimfield microscopy is used in my project as it allows **observation of single molecules** in **live cells** over a **millisecond** time scale.

Importance of Fluorescence

To bring my proteins to life, I **tag** them with **fluorescent proteins** derived from jellyfish. Q and AH are tagged to proteins with **different fluorescent characteristics** so I can observe them **independently** in the **same experiment**. By measuring the **intensity** of the "spots" on my image, I can quantify the **number of each species present**.

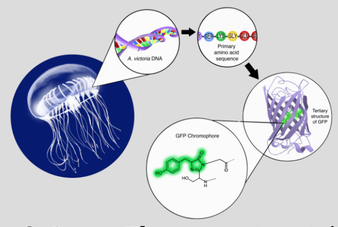


Figure 3. Green Fluorescent protein (GFP) from the jellyfish Aequorea Victoria

Conclusion

Data Analysis

The Slimfield images suggest that the localization of AH depends on its **partner protein Q**. This is because **AH** is **delocalized** in the **absence of Q** (Figure 5). The localization of Q is not affected by the absence of AH (Figure 4).

A typical Bacillus subtilis cell is **2µm** long. By using Slimfield microscopy I can determine how many Q and AH proteins are **present in a single cell**. Figure 6 shows that although an average cell contains around **200 copies** of **Q** and **AH**, there is variation as not all cells are identical. My research aligns with the research theme T**echnologies for the Future** as Slimfield microscopy allows us to explore the foundations of life at the molecular level.

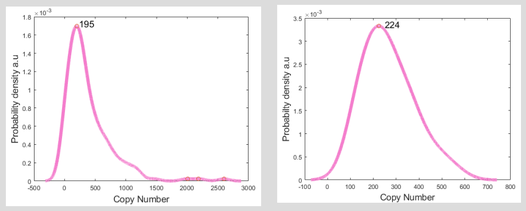


Figure 6A) Copy number plot mGFP-Q
Figure 6B) Copy number plot mCherry AH

Results

SpoIIQ SpoIIIAH Slimfield Imaging

Both spoIIQ and spoIIIAH were labelled with fluorescent proteins. **SpoIIQ** was labelled with **mGFP** to give **mGFP-spoIIQ**. **SpoIIIAH** was labelled with **mCherry** to give **mCherry-spoIIIAH**. Both proteins were imaged in wildtype and mutant backgrounds as shown in Figures 4 & 5.

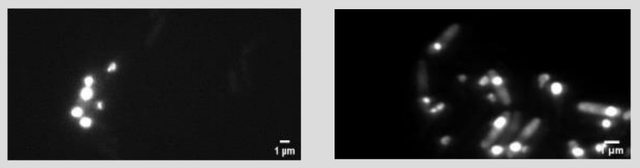


Figure 4A (left): mGFP-spoIIQ in a wildtype AH background.
Figure 4B (right): mGFP-spoIIQ in a mutant AH background.

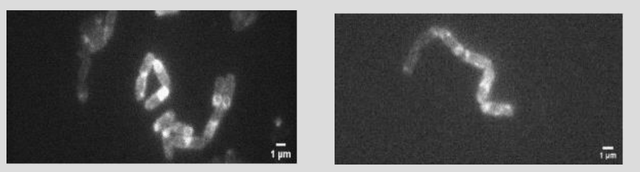


Figure 5A (left): mCherry-spoIIIAH in a wildtype Q background.
Figure 5B (right): mCherry-spoIIIAH in a mutant Q background.