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).

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?

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.

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.

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 Technologies for the Future as Slimfield microscopy allows us to explore the foundations of life at the molecular level.

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

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

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

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.

Figure 6A (left): Copy number plot mGFP-Q. Figure 6B (right): Copy number plot mCherry-AH.