Determining the Mechanism of Action of the FtsA(P144L) Protein in *Bacillus subtilis*

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Background

- The FtsA protein is essential to the bacterial cell division process
- Among other functions, FtsA recruits proteins required for later stages of cell division – such as FtsW – to the division apparatus (Z ring)
- Wild type *Bacillus subtilis* bacteria expressing the viral protein gp56 lose their ability to recruit late cell division proteins to the Z ring
- A strain of *B. subtilis* with the mutant FtsA(P144L) protein retains its ability to divide even when expressing gp56

Objective

- Answer this question: How does the altered FtsA(P144L) protein make *B. subtilis* bacteria resistant to gp56?

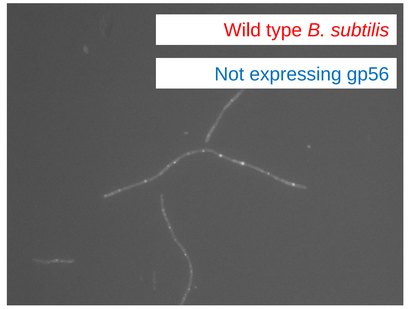
Hypotheses

- Hypothesis 1: FtsA(P144L) possesses enhanced capabilities to recruit late cell division proteins that wild type *B. subtilis* cannot recruit when expressing gp56
- Hypothesis 2: FtsA(P144L) performs the functions of these late cell division proteins, and *B. subtilis* expressing FtsA(P144L) can divide even in the absence of such proteins

Experimental Plan

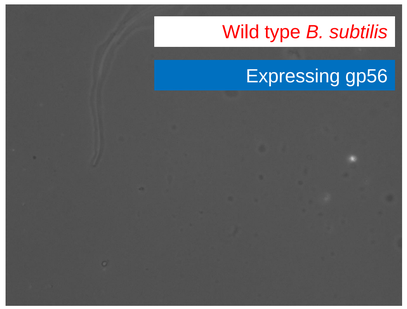
- Starting from a single wild type background, we used gene transformation to create four strains of *B. subtilis* cells
- Cells in Strain 1 expressed wild type FtsA and fluorescently-tagged FtsW
- Cells in Strain 2 expressed wild type FtsA, fluorescently-tagged FtsW, and gp56
- Cells in Strain 3 expressed FtsA(P144L) and fluorescently-tagged FtsW
- Cells in Strain 4 expressed FtsA(P144L), fluorescently-tagged FtsW, and gp56
- FtsW is one of the last proteins to localize to the Z ring in *B. subtilis* cell division, and we assumed that it could serve as a proxy for that species' late cell division proteins
- Hypothesis 1 could have been supported if cells in Strains 3 and 4 had shown similar levels of FtsW localization to the Z ring
- Hypothesis 2 could have been supported if cells in Strains 2 and 4 had shown similar levels of FtsW localization to the Z ring

Sample of Experimental Data



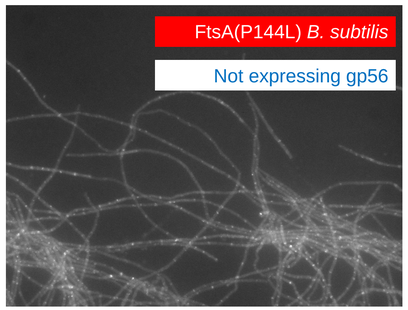
Strain 1

Control level of FtsW localization



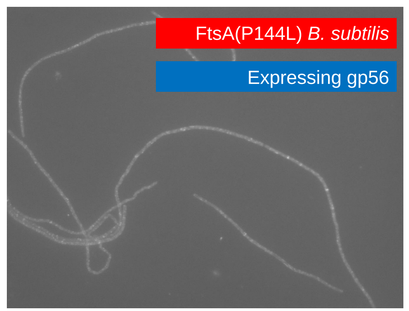
Strain 2

Low level of FtsW localization



Strain 3

High level of FtsW localization



Strain 4

Intermediate level of FtsW localization

Discussion of Results

Our results could provide support for both of our hypotheses:

Possible Support for Hypothesis 1

- Cells in Strains 2 and 4 both expressed gp56, but those in Strain 4 appear to show more FtsW localization to the Z ring
- Cells in Strain 2 expressed wild type FtsA, but those in Strain 4 expressed FtsA(P144L)
- This could suggest that FtsA(P144L) is able to recruit FtsW to the Z ring despite interference from gp56

Possible Support for Hypothesis 2

- Cells in Strains 3 and 4 both expressed FtsA(P144L), but those in Strain 4 appear to show less FtsW localization to the Z ring
- Cells in Strain 3 did not express gp56, but those in Strain 4 expressed gp56
- This could suggest that even if Hypothesis 1 is correct, the process by which FtsA(P144L) recruits FtsW to the Z ring may be hindered by gp56
- If the cells in Strain 4 were dividing normally, the above finding could suggest that these cells did not require FtsW for their division process

Conclusions

- Only additional data could allow us to determine which hypothesis more accurately describes the action of FtsA(P144L)

Additional Work

- We intend to measure the lengths of individual cells in future iterations of our experiment
  - Our current results could support Hypothesis 2 only if the cells in Strain 4 were dividing normally
  - If the cells in Strain 4 had been measured to be significantly longer than those in Strain 3, this would have indicated that the Strain 4 cells were not dividing as frequently as those in Strain 3
  - This in turn could have suggested that the Strain 4 cells' deficiency in FtsW was impeding their cell division, contradicting Hypothesis 2

Future Applications

- Understanding how FtsA(P144L) blocks gp56 could provide insights into how gp56 interferes with bacterial cell division
  - This could lead to the development of novel antibacterial drugs that mimic the action of gp56