Targeted engineering of phages to understand infection mechanisms

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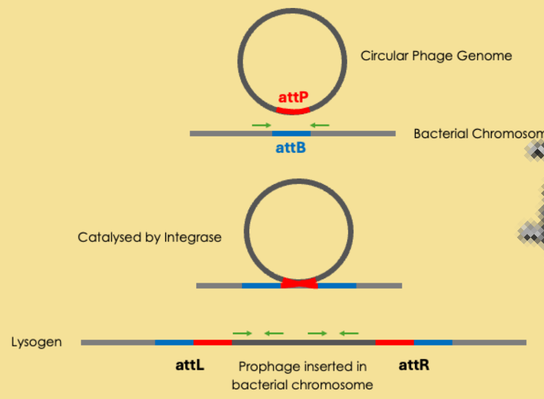
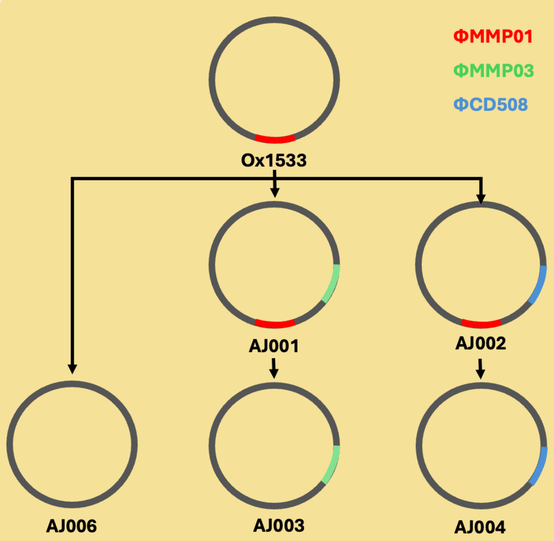
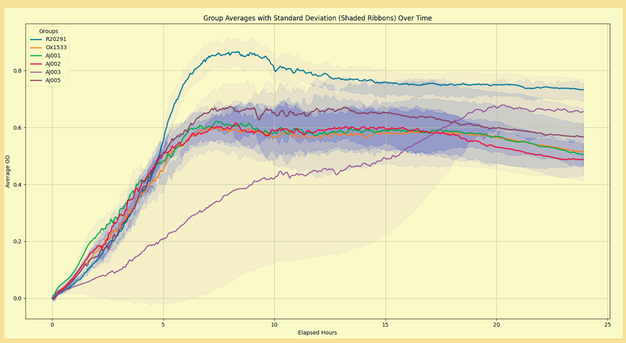
Introduction

- There has been a lack of *C. difficile* phage studies due to their difficult molecular biology.
- We made *C. difficile* lysogens in order to be able to mutate them.
- Using the structure of phages, we discovered two enzymes on the needle tip of a phage that potentially help the phage in infection.
- Phages and bacteria were cultured in Whitley DG250 Workstation to ensure optimum growth conditions for infections.

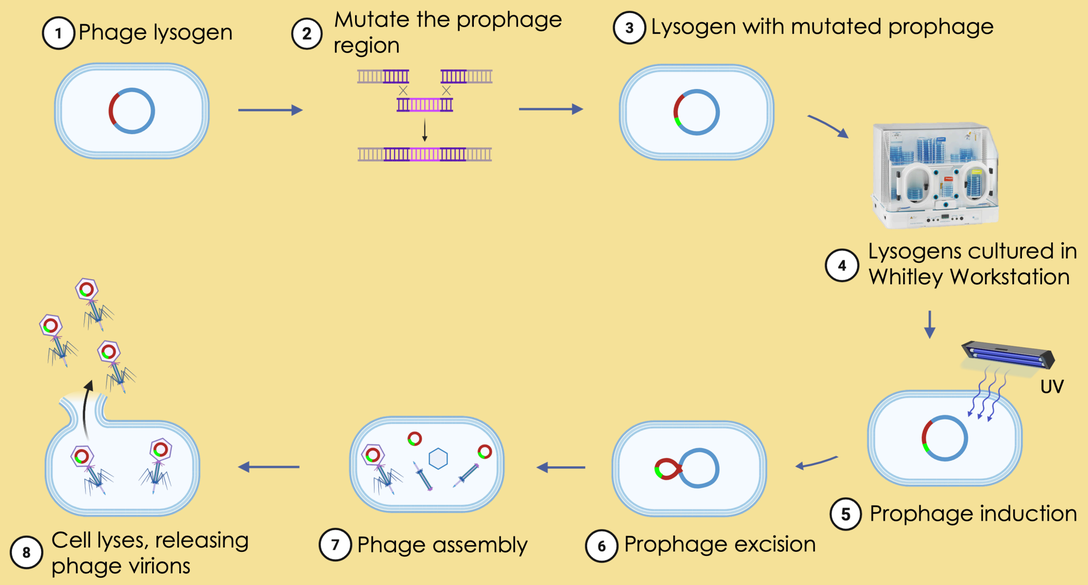


Strain Background

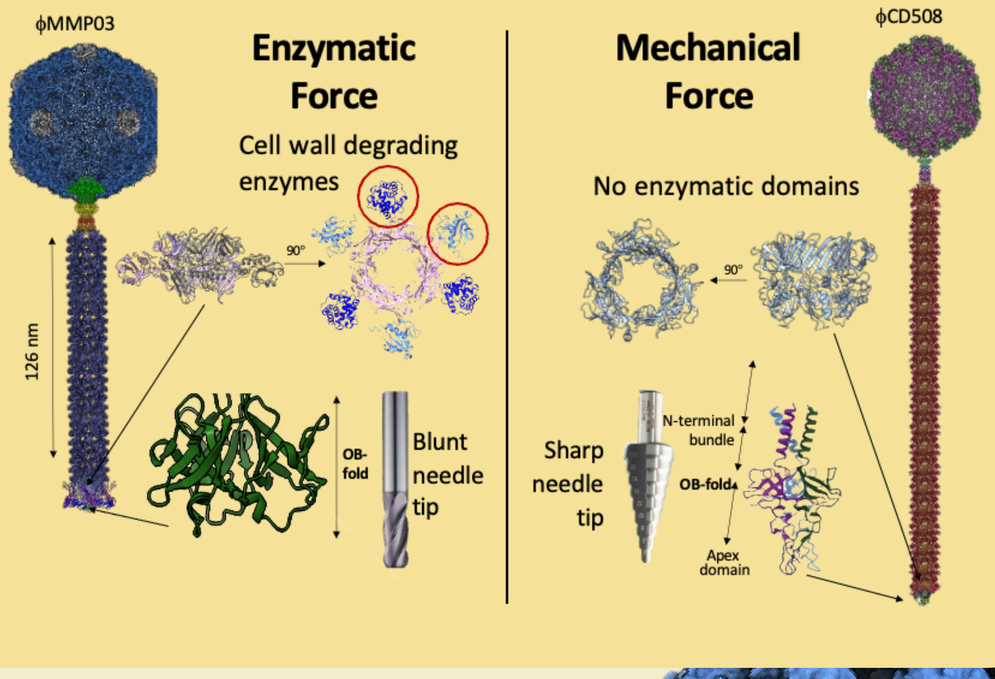




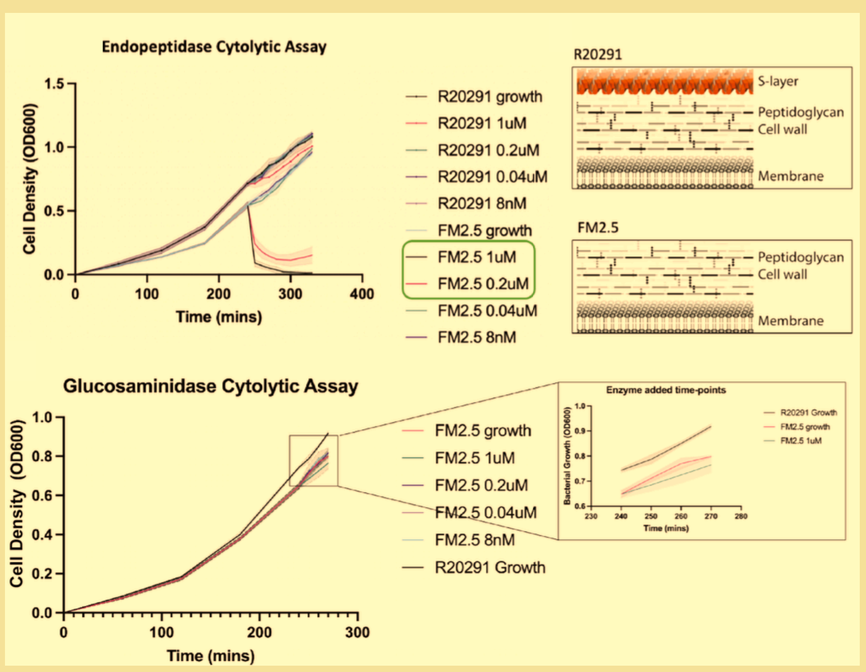
Phage Mutagenesis Pipeline



Difference Between Needle Tips

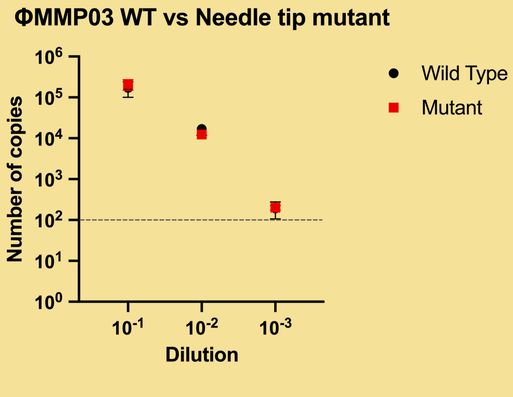


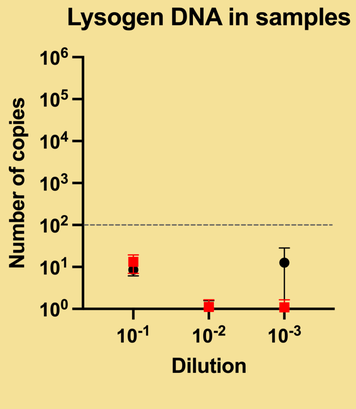
In Vitro Cytolytic Assays



Needle Tip Mutant Assays

qPCR assay





Infection assay

Φ **Wild Type Titre: 2.24 x 10^6**

Φ **Needle Mutant Titre: 2.25 x 10^6**

The results showed no significant difference between the wild type and the mutant, suggesting a potential role of glucosaminidase in infection.

Next Questions

- Does glucosaminidase play a more important role in infection?
- Is there any interaction between endopeptidase and glucosamindase that affects the infection efficiency?
- If not, what is the evolutionary significance of the enzymatic domains in the tip?

Next steps:

- Apply the mutagenesis method to answer if tail length and level of contraction of the phage affect infection efficiency.
- Make phages modular in order to use them as a tool in *C. difficile* research.

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