

Type IV Pili Proteins

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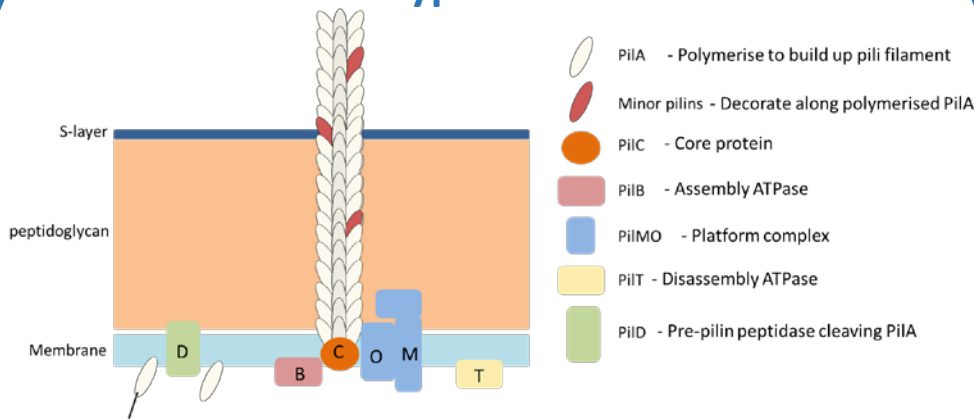
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INTRODUCTION

Clostridium difficile is a multi-drug resistant pathogen causing hospital-acquired infections, which causes diarrhoea, fatal pseudomembranous colitis and toxic megacolon. During the disruption of normal human microbiota, *C. difficile* colonises and forms biofilms along the intestinal epithelium as important processes of its pathogenicity. It has been reported that Type IV Pili (TFP) is involved in these^{1,2}. In order to study the role of minor pilins in TFP pili assembly, we have constructed and characterised a strain containing a fluorescently tagged PilK variant.

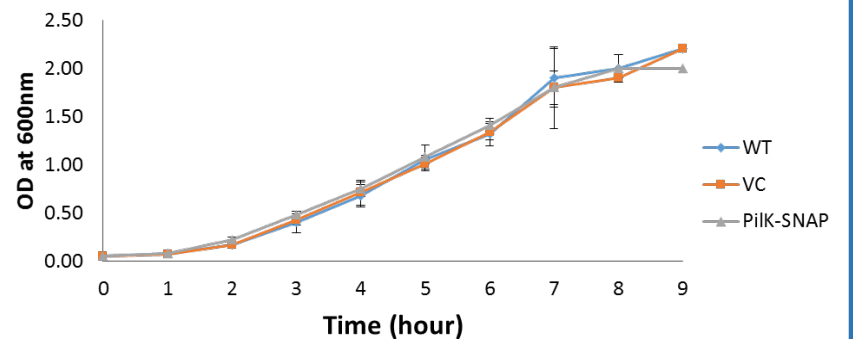
Type IV Pili



- Minor pilins: PilU, PilV, PilK, PilJ, PilW and PilX
- Localisation within the pili and function are still unknown

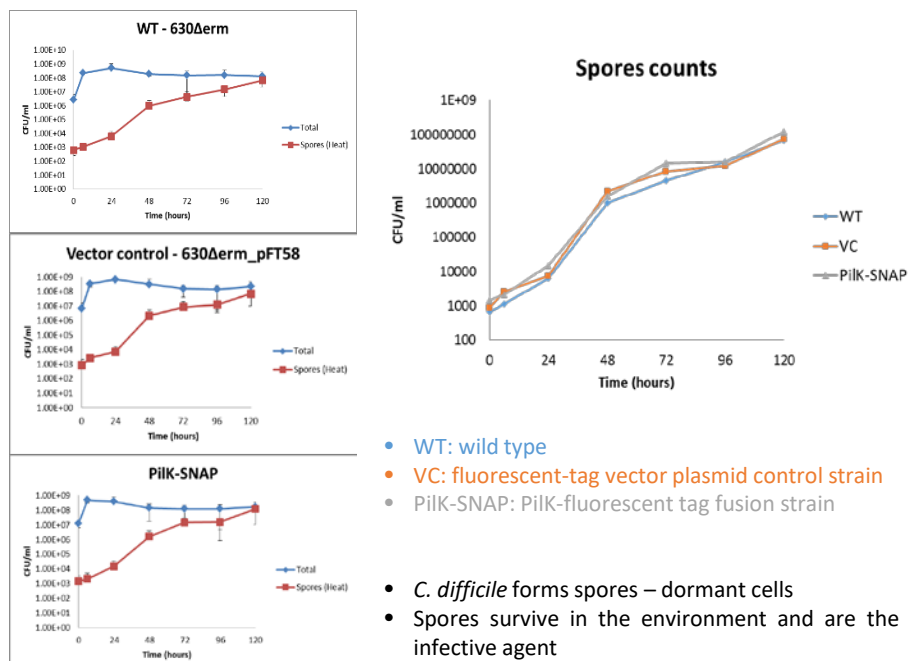
GROWTH CURVES

C. difficile strain growth curve in TY

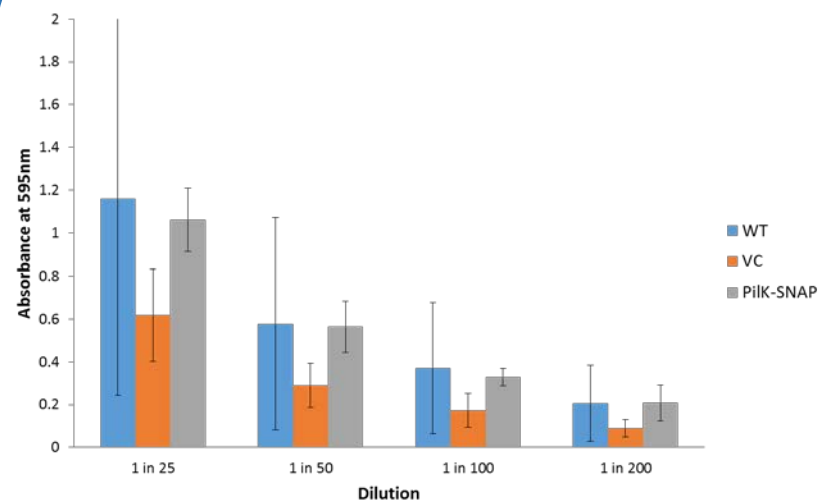


- WT: wild type
- VC: fluorescent-tag vector plasmid control strain
- PilK-SNAP: PilK-fluorescent tag fusion strain

SPORULATION EFFICIENCY



BIOFILM FORMATION



- Biofilms may help *C. difficile* with antibiotic resistance, colonisation of the gut, and may even help competition with the microbiota

CONCLUSIONS

- There is no difference between 3 *C. difficile* strains in growth curve and sporulation assay
- Biofilm assays are inconclusive due to large variations amongst replicates. Improved protocols are being tested
- These biological tests will be used in further studies to investigate the phenotypes of TFP protein deletion strains

FUTURE WORK

- Microscopy to detect localisation of PilK on TFP
- Construct other TFP protein fluorescent tagged strains
- Construct protein deletion mutants from *C. difficile*, targeting proteins: PilW, PilJ and PilD2

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2. Romling U, Galperin M, Gomelsky M. Cyclic di-GMP: the First 25 Years of a Universal Bacterial Second Messenger. Microbiology and Molecular Biology Reviews. 2013;77(1):1-52.