An Essential Endopeptidase is Required for Polar Cell Wall Synthesis in *Agrobacterium tumefaciens*

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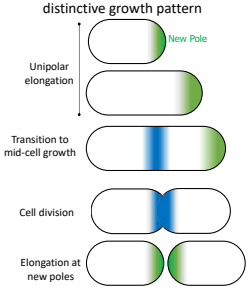
Why study cell wall synthesis in *Agrobacterium tumefaciens*?

A. tumefaciens is causative agent of Crown Gall Disease. Crown Gall limits proliferation of most flowering plants.

A. tumefaciens is capable of inter-kingdom transfer of DNA and has become an important biotechnology tool in the generation of GMO plants.

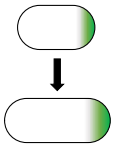
A. tumefaciens has a distinctive growth pattern



Improved understanding of *A. tumefaciens* growth will limit plant disease and promote biotechnology applications

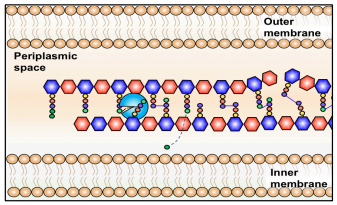
Cell growth requires cell wall hydrolysis and synthesis

How does *A. tumefaciens* grow?

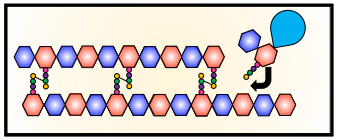


Elongation is the process of cell wall insertion to enable cells to get longer

The bacterial cell wall is comprised of sugars with crosslinked peptide stems that form a net around the cell, called the peptidoglycan

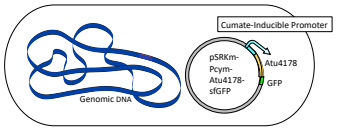


Endopeptidases are space-making enzymes cleave the PG crosslinks

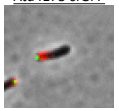


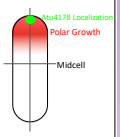
Synthases insert new subunits into the existing peptidoglycan

Endopeptidase Atu4178 localizes to the growth pole



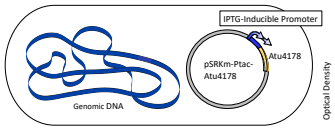
Atu4178-sfGFP

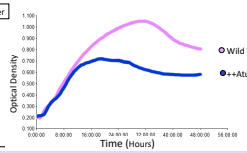




✓ Co-localization of Atu4178-sfGFP and polar growth as determined by staining cells with a red fluorescent-o-amino acid (FDAA)

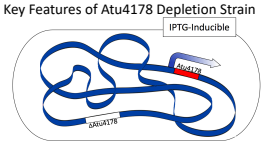
Increased levels of Atu4178 decrease cell growth





What is the impact of depleting Atu4178 on cell growth and division?

Key Features of Atu4178 Depletion Strain



When IPTG is added to the culture, Atu4178 synthesis is "on"
When IPTG is removed, Atu4178 synthesis is "off"

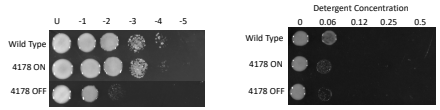
Atu4148 is essential for cell survival

A depletion strain was constructed:

1. Deliver *atu4178* under the control of the *lac* promoter to a neutral site on the chromosome
2. Delete the gene at the native locus

A single inducible copy *atu4178* is present in the depletion strain

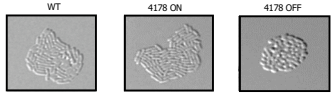
Absence of Atu4178 decreases cell viability



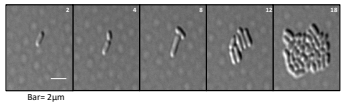
Cells depleted of Atu4178 have an increased membrane permeability and reduced viability

Depletion of Atu4178 leads to compact microcolony formation

Comparison of microcolony formation after 16 hours

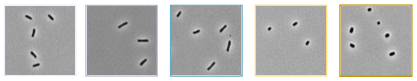


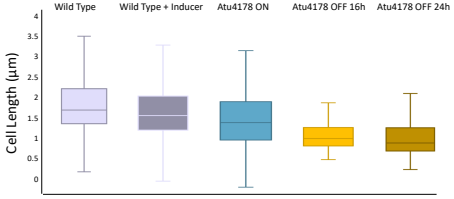
Time-lapse imaging during Atu4178 depletion



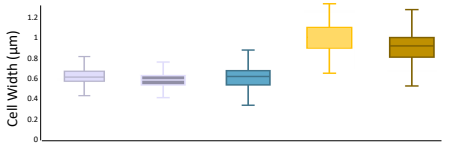
Bar= 2μm

Depletion of Atu4178 causes cells to become shorter and wider than wildtype cells



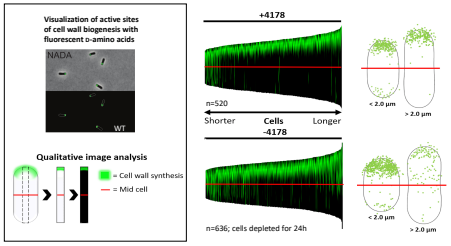


After 24 hours of depletion, most cells have adopted the short, round phenotype

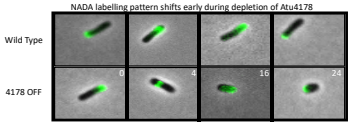


Depleting Atu4178 affects cell viability, colony formation, and produces a rounded phenotype

Polar growth is more dispersed in the absence of Atu4178

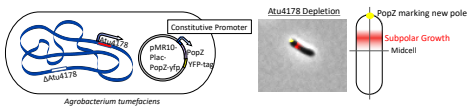


NADA labelling pattern shifts early during depletion of Atu4178



Early during depletion of Atu4178 (4 hr) shifting of active growth to subpolar region is observed

The subpolar region of growth which is visualized during Atu4178 depletion is proximal to the growth pole



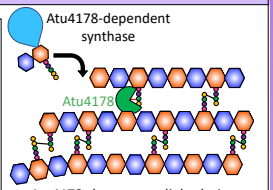
Atu4178 is required for proper polar growth

Summary of Key Results

Absence of Atu4178 causes production of short, round cells

Absence of Atu4178 shifts growth pattern

Atu4178-sfGFP localizes to the growth pole

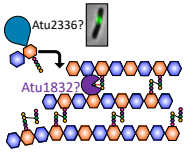


Atu4178 cleaves crosslinks during cell elongation to allow insertion of new cell wall material at the pole

Future Directions: Understanding what happens in absence of Atu4178

Hypothesis

In the absence of Atu4178, another hydrolase may work with alternative synthases to recover the loss of polar elongation



- Does Atu2336 contribute to the subpolar growth?
- Is Atu1832 compensating for the loss of Atu4178?

Acknowledgements

I would like to thank Dr. Brown and the Brown lab team for giving me the opportunity and guidance to conduct this research. I would like to specifically thank my mentor, Dr. Wanda Figueroa-Cuilan, who has put tremendous effort and time towards my training and project. I would also like to thank Peggy and Andrew Cherng, the Panda Charitable Foundation, and the University of Missouri Honors College for their support of my research over the summer.