Identifying and Characterizing *Pseudomonas syringae* mutants with decreased virulence

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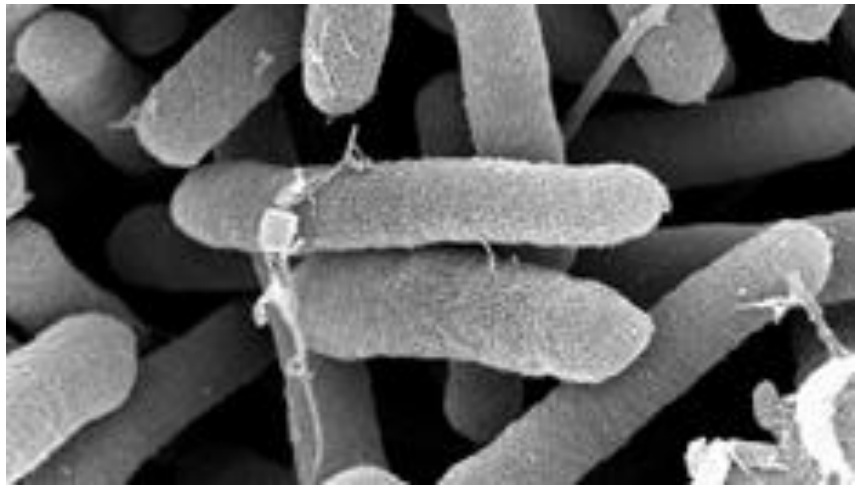
What is *Pseudomonas syringae?*

- Gram negative pathogenic bacteria
- Capable of infecting model organism *Arabidopsis*
- Extracellular foliar pathogen
  - Enters through natural openings/wounds
  - Grows to high levels in extracellular space (apoplast)
- Many pathovars/isolates
  - Wide range of specific hosts

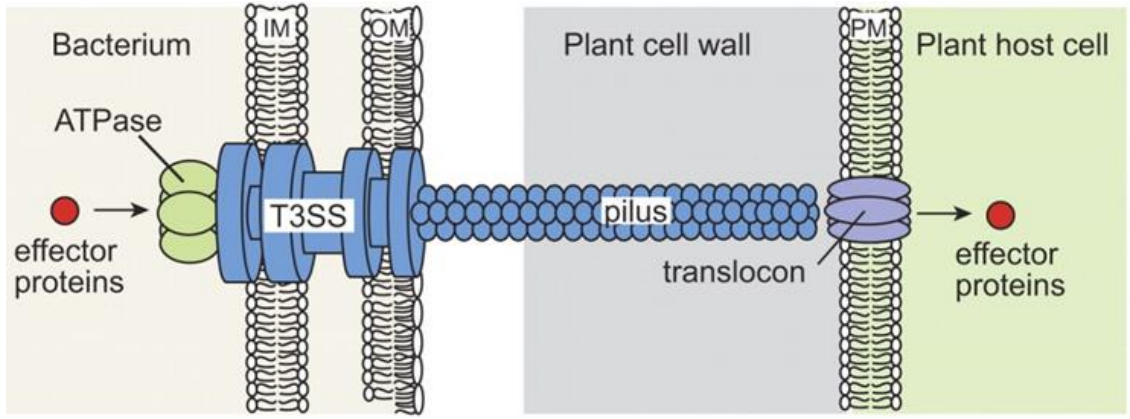


Economically Relevant Agricultural Pathogen

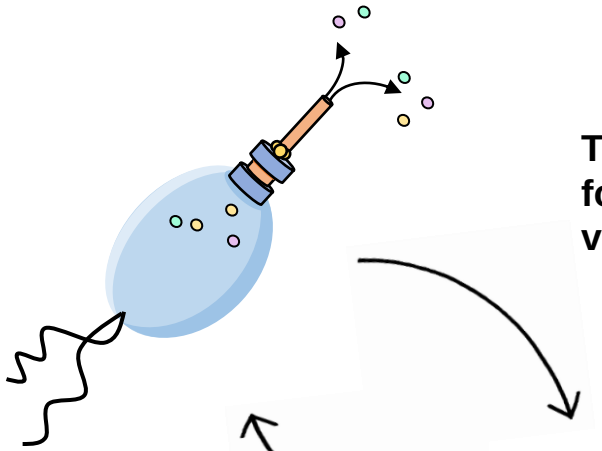
- Susceptible agricultural products include:
  - Grass species
  - Fruit trees
  - Root vegetables
- Specific to Oregon:
  - Sweet Cherry Canker
  - Sugar Beet Blight
  - Bacterial Brown Spot in Beans

Type III Secretion System (T3SS)

- Needle-like structure that secretes effector proteins into the plant cells.
  - **Effector Proteins**: suppress immune response/encourage infection.
  - Enter extracellular space, disrupt innate immune system signals
- Major genes involved in the production of the T3SS is the hrp gene cluster



Büttner D , He S Y Plant Physiol. 2009;150:1656-1664

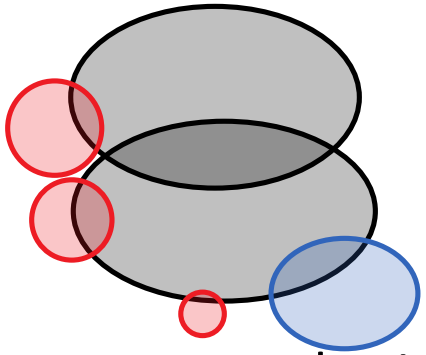


T3SS critical for DC3000 virulence

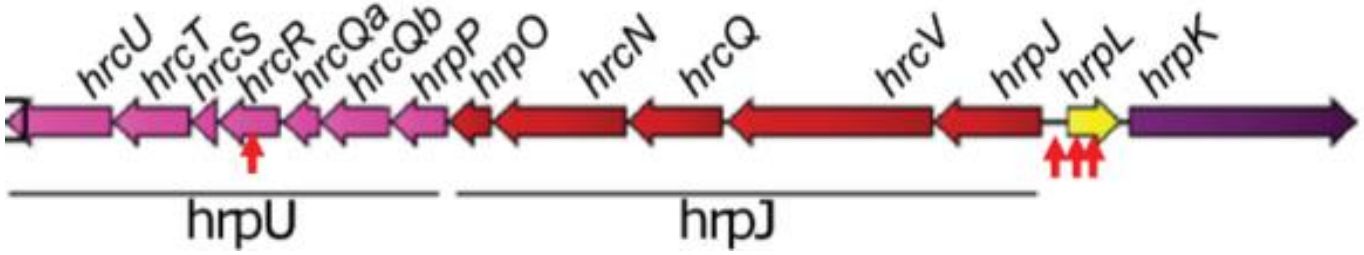
T3SS induced ~1-3 hpi by host signals

Regulation of T3SS

- HrpL is the master regulator of T3SS associated genes (alternative sigma factor)
  - Mutant lacks this regulator = non-virulent
- Hypothesis: hrpL will not grow abundantly in the presence of plant cells

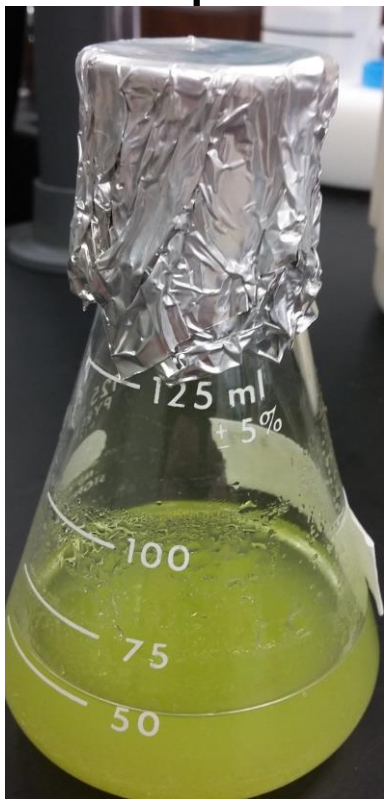


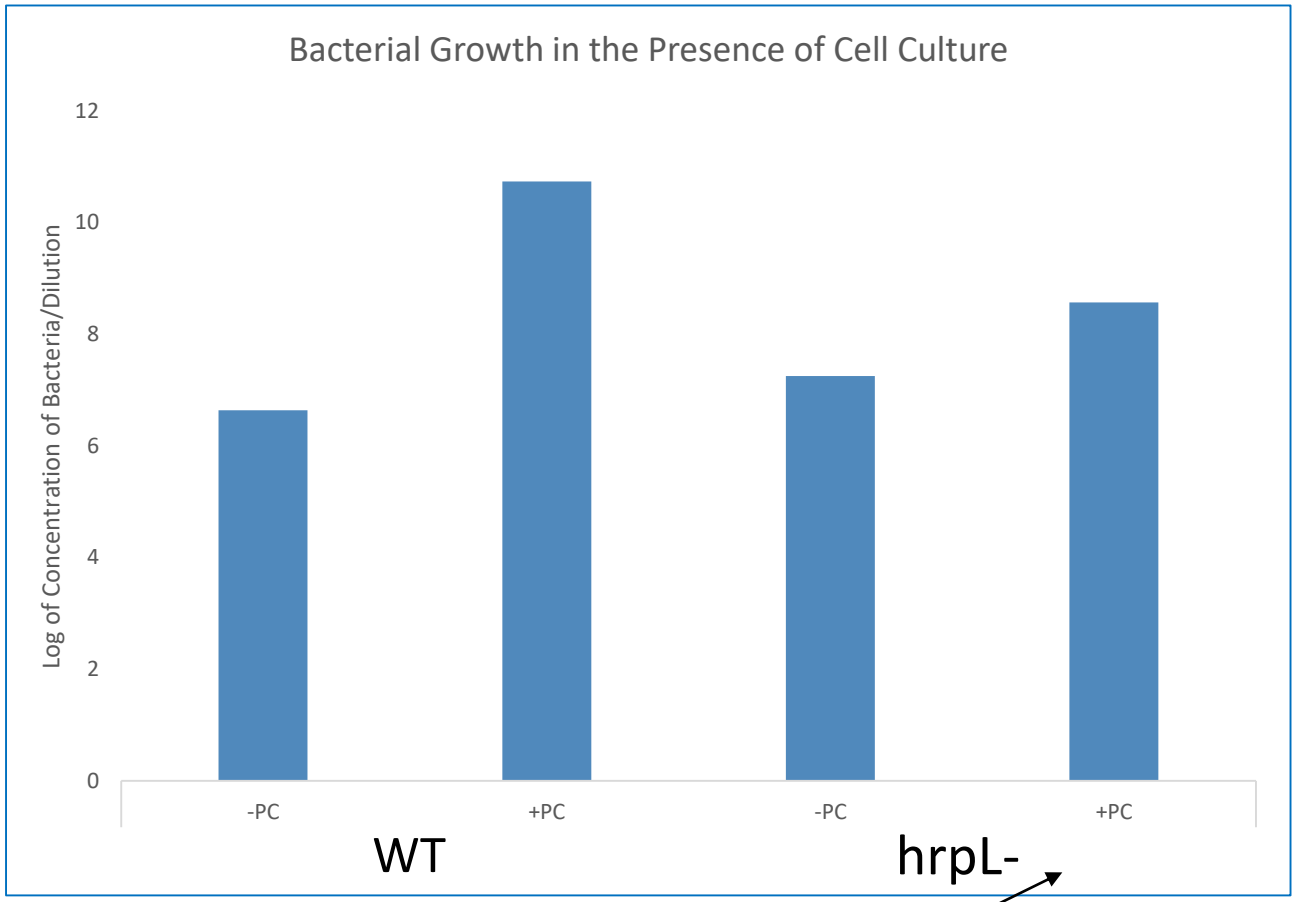
hrpL sigma factor



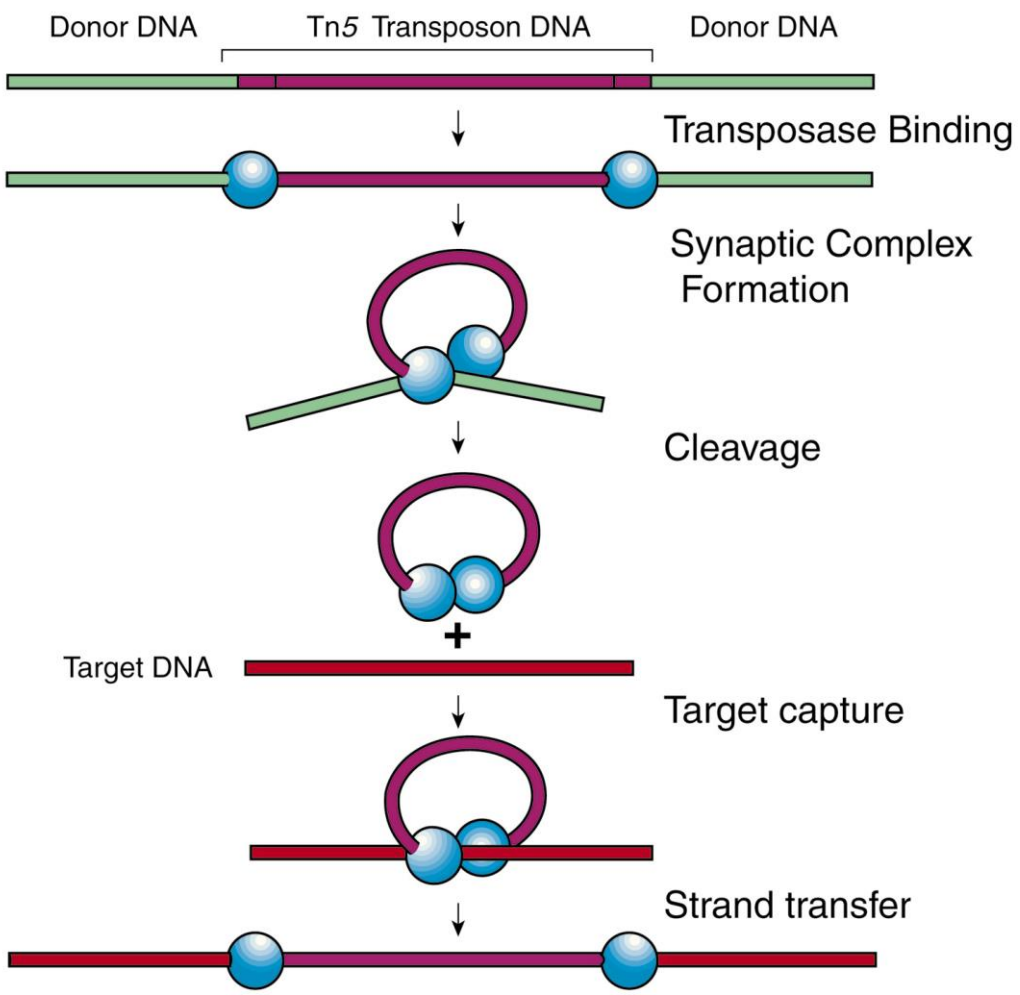
P. syringae hrpL growth in Presence of Arabidopsis

Arabidopsis thaliana Cell Culture





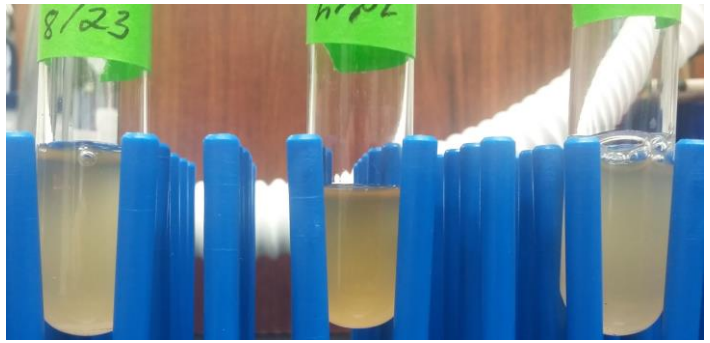
Main Question: What genes are contributing to T3SS^{-} independent growth?



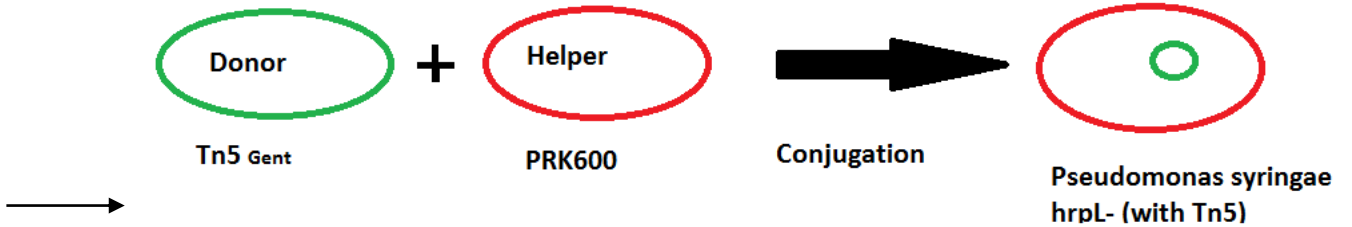
Mutagenesis: How do I generate mutants?

- **Transposon:** A mobile piece of DNA that can randomly insert itself into an organism's genome, disabling or activating genes.
- ~20,000 colony insertions
  - For a 6 million base pair genome

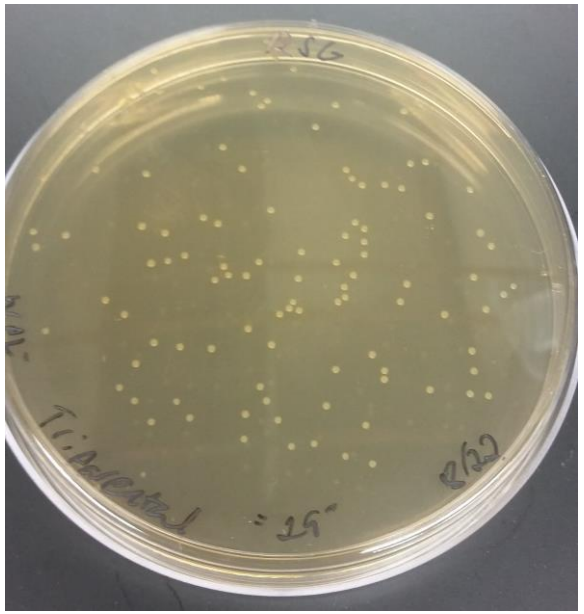
Work-Flow



Overnight Cultures



Triparental Mating

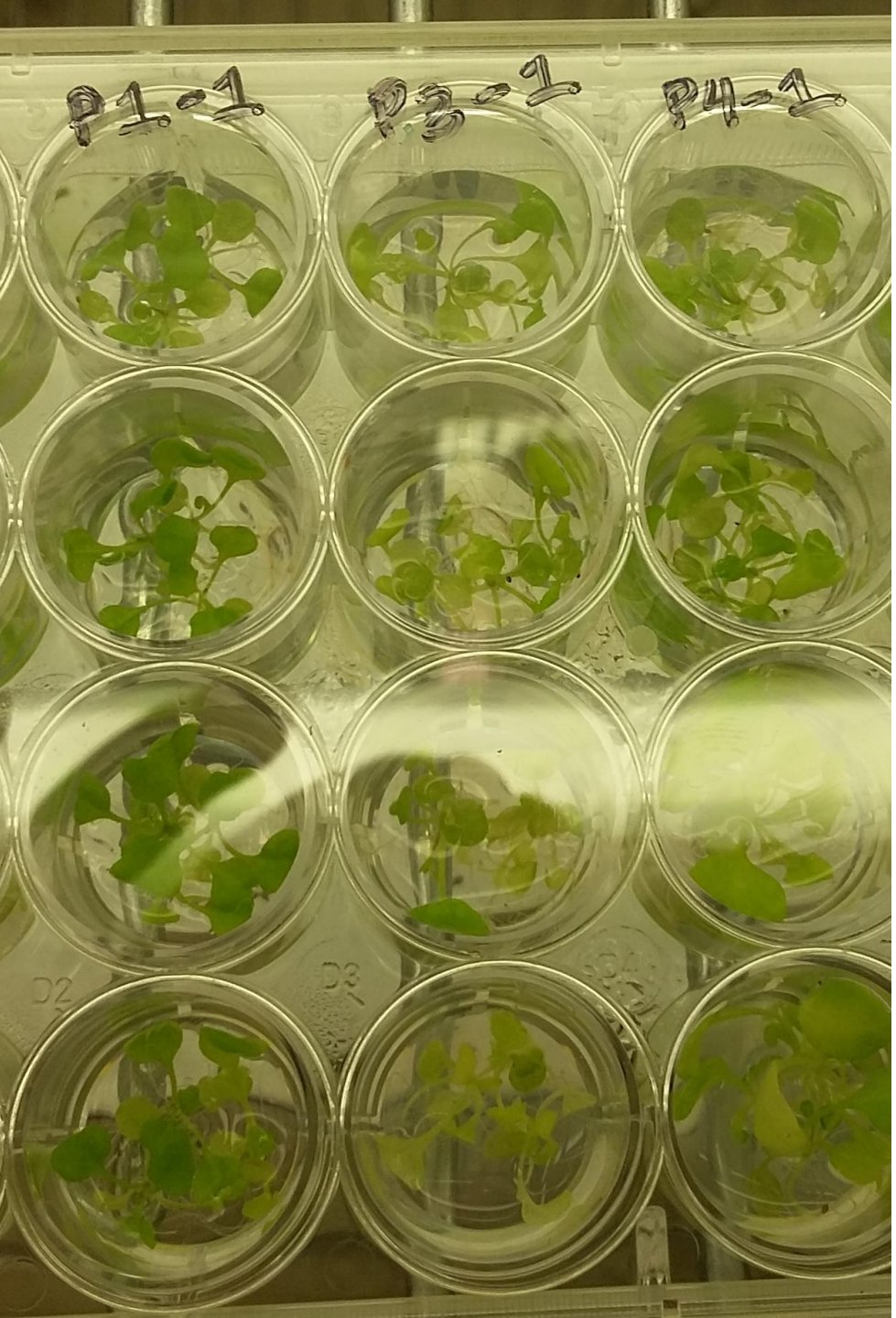


Positive Colonies

Select
Positive
Colonies

Overnight 96 well plate

Frozen Stock



Future Research

- Continue generating library
- Identify bacterial mutants that are defective during infection
  - Test mutants for virulence
  - Screen mutants for growth -/+ plant cells
- Characterize non-T3SS genes involved in *P. syringae* virulence

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Questions?