The impacts of microbial genes on plant development

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Outline

- Introduction
- Impact of *iacA* on *Arabidopsis* gene expression over time
- Future directions

Microbial impact on plants

PGPB

- *Pseudomonas*
- *Burkholderia*
- *Bacillus*
- *Azospirillum*

Effects

- Biocontrol
  - Antagonism
  - Predation
  - Signal interference
- Fertilization
  - N_2 fixation
- Phytostimulation
  - Stress control
  - Hormone metabolism

Lugtenberg and Kamilova 2009)

Pseudomonas putida 1290

- Environmental bacterium
- Capable of indole-3 acetic acid (IAA) degradation
- Possesses 10-gene *iac* cluster
- Grows on IAA as a sole carbon and nitrogen source

indole-3-acetic acid   *iacA* →   2-oxoindole-3-acetic acid   Other *iac* genes →→→   catechol
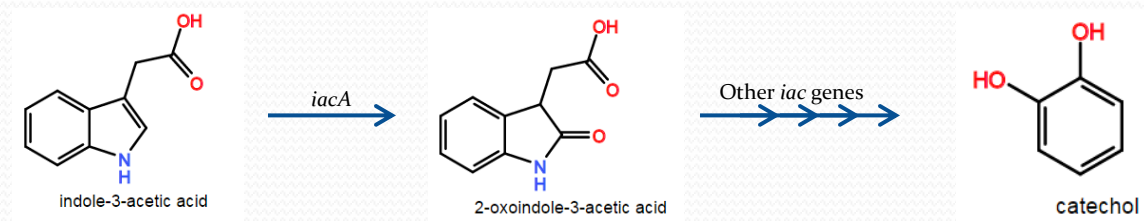
Indole-3 Acetic Acid (IAA)

- What is it?
  - Plant Hormone
  - Produced by plants and some plant pathogens
- Why is it important to plants?
  - Root development
  - Shoot development
  - Embryogenesis
  - Flowering
  - Gravitropism
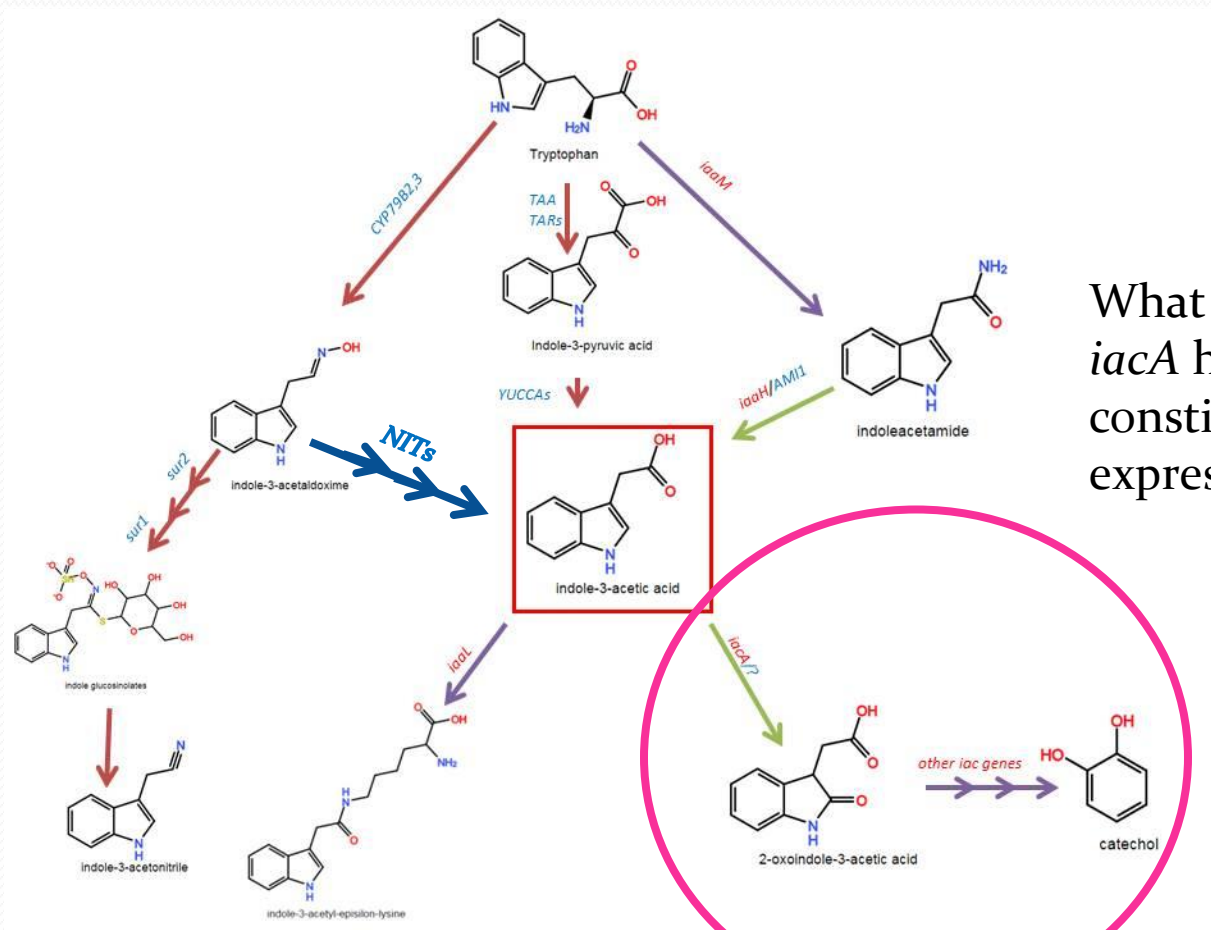
What impact does *iacA* have when constitutively expressed in a plant?

Naming Clarification

- Col-0: Wild type "Columbia" *Arabidopsis thaliana*
- pMD1iacA: *A. thaliana* constitutively (from a 35S promoter) expressing *P. putida* 1290 gene *iacA* (Columbia background)
- pMD1iacA5 and pMD1iacA8 are independent transformants both constitutively expressing *iacA*.

Does *iacA* expression impact:

- Root Phenotype?
  - Delayed root growth in young plants (9 days post sowing)
- Disease Resistance?
  - No significant difference in response to *Pseudomonas syringae pv. tomato DC3000*
- Root Transcriptome?

Impact of *iacA* on *Arabidopsis* transcriptome?

Differentially Expressed Genes

- Nutrient Metabolism?

- Hormone regulation/transport?

- Disease response?

- Stress response?

Methods

- Trizol RNA extraction from *Arabidopsis* roots
  - Grown on sterile Hoagland media
  - Samples taken at 6 and 9 days post-germination
  - ~40 plants pooled/biological replicate
  - 4 biological reps, 3 cultivars, 2 time points (24 samples total)
- RNA Clean-up
  - Zymo kit (DNAse step very important)
- Bioanalyzer
  - Measure RNA integrity (RIN)
  - Ratio of 28S:18S RNA
- Poly-A Enrichment
  - Select for mRNA
- Library Preparation, sequencing, and analysis

Transcriptional Comparisons

How do the mutants compare to wild type within each time point?
How do the mutants and wildtype compare between time points?
How do the mutants and wildtype compare to each other between time points?
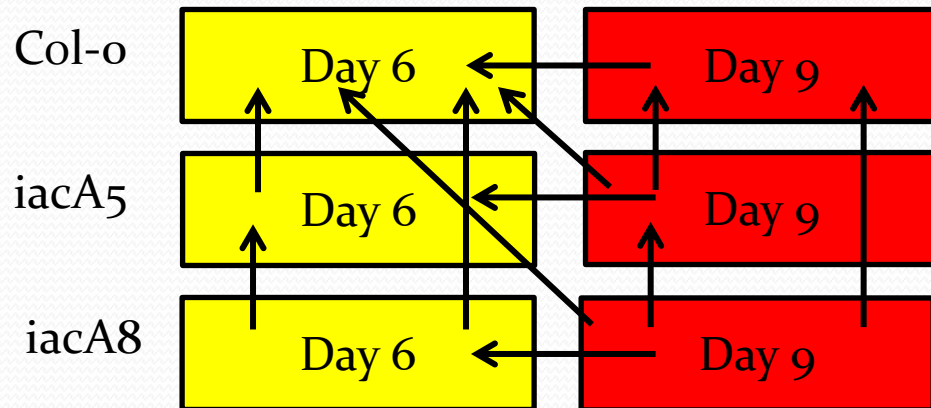
- Look at genes that are differentially expressed by at least a factor of 1.5

- p value < .05

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