Non-coding RNAs in *Agrobacterium tumefaciens*

Presenter: Chris Hernandez
Mentor: Dr. Kan Wang
Acting Mentor: Dr. Keunsub Lee
Outline

1. Agrobacterium
2. Central dogma review
3. The world of ncRNAs
4. The role of ncRNAs In Agrobacterium?
5. Experimental design
6. Results
7. Future directions
8. Acknowledgements
Agrobacterium: plant terrorist or biologist’s tool?

- Microbe responsible for crown gall tumors
- Hijacks plant machinery to make food by inserting new code (DNA) into existing plant program
- Can be used by biologists for genetic engineering
The Central Dogma

- RNA traditionally thought of as only an intermediate
ncRNAs:

- RNA is more than just an intermediate
- Many non-coding RNAs (ncRNAs) have been identified
- ncRNAs are involved in regulatory roles
ncRNAs in *Agrobacterium tumefaciens*

- Initial work has identified regulatory RNAs in *Agrobacterium* that are differentially expressed (Lee *et al*. 2013)
- Question: What are the roles of these ncRNAs in *Agrobacterium*?
7-1: Putative ncRNA regulator

Lee et al. A Genome-wide Survey of Highly Expressed Non-coding RNAs and Biological Validation of Selected Candidates in Agrobacterium tumefaciens. PLOS One 8: e70720. doi:10.1371/journal.pone.0070720 (2013)
7-1 Con’t

Predicted Secondary Structure*

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<tr>
<th>Id - mRNA</th>
<th>mRNA description</th>
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<tr>
<td>phbc</td>
<td>poly-beta-hydroxybutyrate synthase</td>
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<tr>
<td>Atu1751</td>
<td>hypothetical protein</td>
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<tr>
<td>pfs</td>
<td>5’-methylthioadenosine/S-adenosylhomocysteine nucleosidase</td>
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<tr>
<td>xynA</td>
<td>endo-1,4-beta-xylanase</td>
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<td>mgsA</td>
<td>methylglyoxal synthase</td>
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<tr>
<td>Atu2228</td>
<td>diguanilate cyclase</td>
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*predicted by: http://rna.informatik.uni-freiburg.de/IntaRNA/Input.jsp
Experimental Design

+AS
(induced)

C58
Δ7-1

Extract RNA

qPCR

-Analyze
results

C58
Δ7-1

-AS
Results

Gene Expression of Mutant Strain Δ7-1 Relative to C58

-AS

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<thead>
<tr>
<th>Gene</th>
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Summary and Future Directions

- Gene expression of six potential ncRNA target genes were measured in both wild type strain (C58) and ncRNA mutant strain (Δ7-1) by quantitative PCR. No marked differences were observed.

- Future work would include replications, different growth conditions, and will assay more genes.

- cDNA libraries could be constructed for the mutant strains and RNA-seq will be used to identify differentially expressed genes.
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