Plant-Microbe Interactions in the *Populus* Rhizosphere

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-- Science Lead

Collaborating PIs --

Doktycz, Tschaplinski, Martin, Pelletier, Labbé, Greenberg, Karpinets, Kalluri, Morrell-Falvey, Plett, Kohler, Yang, Hurst, Göran, Muchero & Weston
Early succession tree species -- rapid growth, shade intolerant

Oldest living genet (5000+ yr)

Largest organism on earth (120 ac)

Dioecious (♀ & ♂ genets), undomesticated diploid

Among the most polymorphic organisms (He = 0.35 to 0.48)

Sequenced reference genome
- 485 Mb
- 41,238 genes (V3.0)

Facile Transformation Systems

Tuskan et al. (2006) Science 313:1596-1604
Cell Wall Deconstruction

Dedicated Feedstock
Wood Chips

Cellulose

Lignin

Ethanol, Isobutanol, etc.

Spun Carbon Fibers
Plant-Microbe Interfaces

Understanding the dynamic interface that exists between plants, microbes and their environment

The interface allows for the transfer of energy, information and materials between organisms and their environment.

Can we relate genomic information to ecosystem structure and function?

Dynamic physical, chemical and biological environment

- Multiple length scales

- Numerous components

- Multiple temporal scales
**Diversity Dilemma**

A bazaar may contain a certain number of and types of kiosks, as the market increases in size, there become redundant numbers of similar booths.

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**Does Diversity = Function?**

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**The Functional Bazaar**

- A bazaar may contain a certain number of and types of kiosks, as the market increases in size, there become redundant numbers of similar booths.
Conceptual Approach

How do microbes shape the biology of *Populus*?

**Multi-dimensional Data**
- field collections, metagenomics, plant phenotypes

**Goals and Outcomes**
- Comprehensive knowledge of the *Populus* microbiome
- Rationale design to improve *Populus* fitness

**Targeted biological characterization**
- How do key microbes impact *Populus* growth?

**System reconstitution**

**Field Collections**
- *Populus deltoides* TN & NC
- *Populus trichocarpa* OR

**Genetic selection**

**Functional selection**

**Multiple aspects based assays**

**Model validation**

**Data analysis and integration**

**Aim 2**

**Identify key microbes & functions and how they affect plant**

- Task 2.1
- Task 2.2
- Task 2.3
- Task 2.4

- plant
- fungi
- bacteria
Field Sites – Native River Systems

Caney Fork River, TN

Yadkin River, NC
Common Gardens and Association Genetics

Gradients

Soil pH

Elevation

Moisture

Temperature

Photoperiod
Common Gardens and Association Genetics

- Agassiz, BC – Northern, Riparian
- Clatskanie, OR – Coastal
- Placerville, CA – Xeric
- Corvallis, OR – Inland Valley

- 1100 unrelated genotypes clonally replicated in four contrasting environments
- Randomized complete block design
- 3-4 replicates of each genotype in each trial, 12-14 observations per phenotype
Partitioning the contributions of genotype and environment

- 70% of the OTUs are common between rhizosphere and endosphere, though the relative abundance varies between sphere.
- % variance explained: endosphere vs. rhizosphere > host genotype > location > year/season

~2800 phylogenetically diverse bacterial strains have been isolated

Collection comprised of 7 classes and 89 genera of bacteria:

<table>
<thead>
<tr>
<th>Class</th>
<th># of Isolates</th>
<th># of Genera</th>
<th>Dominant genera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>endo</td>
<td>rhizo</td>
<td></td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>47</td>
<td>104</td>
<td>16</td>
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<tr>
<td>Bacilli</td>
<td>58</td>
<td>130</td>
<td>8</td>
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<tr>
<td>Sphingobacteria</td>
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<td>8</td>
</tr>
<tr>
<td>Flavobacteria</td>
<td>19</td>
<td>51</td>
<td>3</td>
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<tr>
<td>(\alpha)-proteobacteria</td>
<td>178</td>
<td>65</td>
<td>22</td>
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<tr>
<td>(\beta)-proteobacteria</td>
<td>97</td>
<td>65</td>
<td>17</td>
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<tr>
<td>(\gamma)-proteobacteria</td>
<td>116</td>
<td>124</td>
<td>15</td>
</tr>
</tbody>
</table>
Comparative genomics of *Pseudomonad*s

3 distinct
*P. fluorescens* subgroups
+ 1 *P. chlororaphis*
+ 1 *P. putida*

Notable phenotypes within sequenced isolates: Denitrification (5), Phenazine production (1), promotes growth of *Laccaria*, promotes growth of plant roots
Identification and characterization of plant-bacterial inter- and intra-species signaling

AHL-type QS is prevalent in *Populus* microbiome

<table>
<thead>
<tr>
<th></th>
<th>α</th>
<th>β</th>
<th>γ</th>
<th>δ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Populus isolates:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% positive for AHL activity</td>
<td>84% (n38)</td>
<td>20% (n5)</td>
<td>24% (n79)</td>
<td>---</td>
</tr>
<tr>
<td>% positive for luxI homolog</td>
<td>100% (n10)</td>
<td>20% (n5)</td>
<td>17% (n24)</td>
<td>---</td>
</tr>
<tr>
<td>% positive for luxR homolog</td>
<td>100% (n10)</td>
<td>50% (n5)</td>
<td>95% (n24)</td>
<td>---</td>
</tr>
<tr>
<td><strong>IMG database:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% positive for luxI homolog</td>
<td>41% (n278)</td>
<td>43% (n206)</td>
<td>21% (n683)</td>
<td>11% (n53)</td>
</tr>
<tr>
<td>% positive for luxR homolog</td>
<td>64% (n278)</td>
<td>47% (n206)</td>
<td>56% (n683)</td>
<td>11% (n53)</td>
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</tbody>
</table>

Exogenous AiiA-lactonase bioassay to define QS regulon

C. Harwood  A. Schaefer  E.P. Greenberg
Do microbial isolates from *Populus* have aryl-HSL activity?

- Production of aromatic aryl-HSL signals require exogenous addition of plant-provided substrate (e.g. *p*-coumarate)

- Screened cultures from 130 isolates grown presence of 17 aromatic acids (*p*-coumarate, cinnamate, salicylate**, etc.) extract culture supernatant and assay for aryl-HSL activity.

**Aryl-HSL activity**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Plus Aromatic Mixture</th>
<th>No Addition</th>
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<tbody>
<tr>
<td>G1</td>
<td>1500000</td>
<td>0</td>
</tr>
<tr>
<td>G2</td>
<td>500000</td>
<td>0</td>
</tr>
<tr>
<td>G3</td>
<td>0</td>
<td>0</td>
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<tr>
<td>G11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G33</td>
<td>1000000</td>
<td>0</td>
</tr>
<tr>
<td>G41</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

GM01 – *Enterobacter sp.* (Rhizosphere)

GM33 – *Pseudomonas sp.* (Endophyte)
Biofilm Formation Assay

Pseudomonas sp.

Abs (550nm)
Fungal Diversity in the rhizospheric community

- *Populus* has distinct rhizospheric bacterial and fungal communities from *Quercus* and *Pinus*

![Venn diagram showing the overlap of fungal diversity in *Populus deltoides*, *Pinus taeda*, and *Quercus alba*.]

Total richness 304 OTU’s

Based on 10248 16S rDNA sequences

**Trap Plant Experiments**

*Populus deltoide*  
*Pinus taeda*  
*Quercus alba*

**Authors:**  
R. Vilgalys  
G. Bonito
**Fungal Diversity in the rhizospheric community**

- *Populus* has distinct rhizospheric bacterial and fungal communities from *Quercus* and *Pinus*

- *Populus deltoides* (% EcM = <30%)
- *Quercus phellos* (% EcM = >80%)
- *Pinus taeda* (% EcM = >80%)

- ~ 280 new fungal isolates
Mycorrhizal Helper Bacteria

**Bacterial effect on growth and morphology of Laccaria bicolor**

*In vitro bioassay (L. bicolor and P. fluorescens)*

- **BBc6R8**
- **GM17**
- **GM41**

Microscopic observation of *L. bicolor mycelium (10X)*

- **BBc6 effect +**
- **GM17 effect -**
- **GM41 effect ++**

- **Bacteria can facilitate fungal growth and survival**
Mycorrhizal Helper Bacteria

Colony Diameter

Apex number

GM18 with *L. bicolor*
S238N at 12 DPI

GM17 with *L. bicolor*
S238N at 12 DPI

GM41 with *L. bicolor*
S238N at 12 DPI
Mycorrhizal Helper Bacteria

Secondary root length *Populus D124*

- Control
- GM41/S238N
- D124 + GM41
- D124 + S238N
- D124

Secondary root number *Populus D124*

- Control
- GM41/S238N
- D124 + GM18
- D124 + S238N
- D124
QTLs and Genomewide SNP Analysis

QTLs for Populus-Laccaria interaction mapped in regions that showed high genomic divergence between *P. trichocarpa* and *P. deltoides*

- Of the 74 candidate genes, 67 (90%) were serine-threonine kinases of which 49 (66%) were of the D-mannose lectin-type

QTLs and Genome-wide SNP Analysis

• 9 highly significant regions contain genes known to interact with fungi and/or mycorrhizal.

• Within the QTL interval 74 genes with described roles in plant-microbe interactions were identified.
Field Collections
-- *Populus deltoides* TN & NC
-- *Populus trichocarpa* OR

- Direct Pyrosequencing & Metagenomics
- Strain Isolation & Pyrosequencing
- Bait/Trap Experiments & Pyrosequencing

Select a subset of ~200 isolated bacteria based on genetic diversity found in the Bait/Trap and Direct Pyrosequencing efforts.

Genetic Selection

- Quorum Sensing Assay
- Auxin Production Assay
- "Biolog" Assay
- Biofilm Formation Assay
- Mycorrhiza Helper Bacteria Assay
- Reinoculation & Reporter Gene Assays

Functional Selection

- Task 2.1 Plant-Fungal Interactions
- Task 2.2 Plant-Bacteria Interactions
- Task 2.3 Fungal-Bacteria Interactions
- Task 2.4 Constructed Communities

~2800 bacterial isolates
~280 fungal isolates

~2800 bacterial isolates
~280 fungal isolates
30% increase in biomass for inoculated *P. deltoides* when compared to controls

The experiment is being replicated and qPCR is being used to characterize the resulting community

Genetic diversity based assemblages of microbes are being used to inoculate plants
Summary and Conclusions

Characterize and select bacterial and fungi associates for use in pairwise and combinatorial mesocosm experiments.

Future Efforts

- Move beyond measures of OTU’s and 16S indicators of diversity, move to sequence-based genotyping.
- Need better in planta imaging techniques.
- Leverage new long read sequencing technologies.
- Need better single cell capture and culture techniques.
- Apply sophisticated statistical approaches to analyze metagenomics and metatranscriptomics data.

Testing the “Lottery Hypothesis” as a framework for elucidating plant-microbe interactions.
Acknowledgments – Questions/comments?