beneficial plant-microbial interactions in agroecosystems: deciphering the rhizosphere microbial community in two field grown crops

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Many others
Canola Lines Show Variation in Selecting their Rhizosphere Bacterial Members

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Introduction

Plant Microbiome

- Rhizosphere
- Alter soil microbes
- Source of genetic variability
Objectives

- Characterize the core microbiome of *Brassica napus* (Canola).

- Identify bacterial taxa (SNVs) that are differentially abundant among Canola lines.
Methods

How are microbes studied?

- DNA sequencing
- Samples
- Extraction
- “Universal primers”
- Amplify 16S rRNA
- Gene
- NG Sequencing
- Match to a database
Methods

Processing raw sequence reads

Sequencing Data – High-throughput

Trimommatic (Bolger et al., 2014)
Cutadapt (Marcel, 2011)
DADA2 (Callahan et al., 2016)

Sequence (SNV) and Taxonomy table

http://2016.igem.org/Team:Dalhousie_Halifax_NS/Description
Methods

Experimental Design

- Experimental site: Llewelyn Farms near Saskatoon, SK
  - Oilseed ... wheat ... barely ... and fallow
- Sixteen canola (Brassica napus) lines
- Design: RCBD with three blocks + three reps
- Sampling: weekly for ten weeks
Methods

Statistical Analysis

- Core Microbiome:
  - Phyloseq R package (McMurdie and Holmes, 2013)
  - Microbiome R package (Leo et al., 2017)
  - Detection threshold of 0.1 and prevalence 50 to 95 %

- Differential abundance
  - DESEQ2: phyloseq extension (Love, Huber, and Anders 2014)
  - Generalized linear model with negative binomial distribution
  - False discovery rate of 1% (alpha value of 0.01)
  - P value: Benjamini-Hochberg (BH) adjustment (Benjamini and Hochberg 1995)
Results

Major Bacterial Taxa in Canola Rhizosphere

- 49526 taxa in 6 taxonomic ranks

39 DAP Flowering start
60 DAP Flowering end
Results

Major Bacterial Taxa in Canola Rhizosphere

- 49526 taxa in 6 taxonomic ranks
Results

Major Bacterial Taxa in Canola Rhizosphere by Canola Line
Results

Core Microbiome of Canola

- Of observed 49526 taxa

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>50</th>
<th>60</th>
<th>65</th>
<th>75</th>
<th>80</th>
<th>85</th>
<th>90</th>
<th>95</th>
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</thead>
<tbody>
<tr>
<td># Core taxa</td>
<td>39</td>
<td>23</td>
<td>16</td>
<td>8</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

- At 90%:
  - *Pseudarthrobacter* (Actinobacteria)
  - *Bradyrhizobium* (Proteobacteria)
# Results

## Core Microbiome of Canola (75%)

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Genus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteobacteria</td>
<td>Stenotrophomonas</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Bradyrhizobium</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Skermanella</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Skermanella</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>Pseudarthrobacter</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>Arthrobacter</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>unclassified</td>
</tr>
<tr>
<td>Acidobacteria</td>
<td>unclassified</td>
</tr>
</tbody>
</table>
Results

Core Microbiome of Canola

Core Microbiome

Co-occurrence Pattern

Microbial hub
Result

Differential Abundant Bacterial Taxa

- Number of differentially abundant taxa in fifteen Canola lines compared with the reference line
- False discovery rate of 1% (Adj. p value < 0.01)

<table>
<thead>
<tr>
<th></th>
<th>L2</th>
<th>L3</th>
<th>L4</th>
<th>L5</th>
<th>L6</th>
<th>L7</th>
<th>L8</th>
<th>L9</th>
<th>L10</th>
<th>L11</th>
<th>L12</th>
<th>L13</th>
<th>L14</th>
<th>L15</th>
<th>L16</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>273</td>
<td><strong>476</strong></td>
<td>193</td>
<td>375</td>
<td>223</td>
<td>214</td>
<td>274</td>
<td>239</td>
<td>220</td>
<td>274</td>
<td>226</td>
<td>230</td>
<td>244</td>
<td>279</td>
<td>204</td>
</tr>
</tbody>
</table>
Result

Differential Abundant Bacterial Taxa

- Significantly differential abundant bacterial taxa in Line 2 at a false discovery rate of 1%

Up: 156
Down: 117
Result

Differential Abundant Bacterial Taxa

- Significantly differential abundant bacterial taxa in Line 2 at a false discovery rate of 1%
Take Home Message

- Rhizosphere Microbiome can be a potential source of genetic variability needed for breeding the next generation Canola varieties.
THANK YOU

Questions?