Wild Blueberry Phytobiome

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In Progress (NSDA Acceleration 2016-2018)
  - Soil community profiling
  - Wild Blueberry plant growth promotion microorganisms (PGPM)
Why It Is Important to Understand Plant (Wild Blueberry) Microbiome?

Plant perspectives
- Development and nutrient requirement
- Stress levels
- Diseases and how to control it
- Fitness and production

Soil perspectives
- Health (concern about environments)
- Fertility
- Diseases and how to control it

Egan et al., 2013., FEMS Microbiology Reviews
Soil and Plant Community Profiling: General Objectives

Developing general understanding of composition of microbial communities associated with lowbush blueberry plant and soils

Evaluation of effect of management on microbial communities associated with lowbush blueberry

Identifying microbial groups linked to improved plant health, fruit yield and fruit quality

Developing microbiome-based markers for assessment of soil health and potential to support high quality berry production
Data Description

138 bacterial and 130 eukaryotic soil and rhizosphere communities
- 7 blueberry fields, all established at least 30 years ago
- 2 forest areas adjacent to some of the fields

16S rRNA (bacteria)
- 2,184,191 reads
- 8,904 OTUs (individual bacteria)

18S rRNA (eukaryotes, including fungi)
- 439,140 reads
- 8,137 OTUs (individual eukaryotes)
Diversity and Structure: Communities
Identified Microorganisms

15 most abundant eukaryotes

- Annelida
- Basidiomycota
- Nematoda
- Spirotrichea
- Telonemia
- Mucoromycota
- Nematoda
- Filosa-Sarcomonadea
- Acantharea
- Embryophyceae
- Rotifera
- Urochordata
- Prymnesiophyceae
- Arthropoda
- Filosa-Sarcomonadea
- Acantharea
- Embryophyceae
- Ascomycota

15 most abundant bacteria

- Thermoleophilia
- Pedosphaerae
- WPS-2
- Actinobacteria
- Betaproteobacteria
- Saprospirae
- ABS-6
- Gammaproteobacteria
- Ktedonobacteria
- Deltaproteobacteria
- Spartobacteria
- Solibacteres
- Alphaproteobacteria
- Acidobacteriia
- DA052
## Identified Microorganisms

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>18S Eukaryotes</strong></td>
<td></td>
</tr>
<tr>
<td><em>Ascomycota</em></td>
<td>21%</td>
</tr>
<tr>
<td><em>Mucoromycota</em></td>
<td>2%</td>
</tr>
<tr>
<td><em>Basidiomycota</em></td>
<td>1%</td>
</tr>
<tr>
<td><em>Nematoda</em></td>
<td>2%</td>
</tr>
<tr>
<td><strong>16S Bacteria</strong></td>
<td></td>
</tr>
<tr>
<td><em>Acidobacteria</em></td>
<td>36%</td>
</tr>
<tr>
<td><em>Alphaproteobacteria</em></td>
<td>13%</td>
</tr>
<tr>
<td><em>Verrucomicrobia</em></td>
<td>5%</td>
</tr>
<tr>
<td><em>Deltaproteobacteria</em></td>
<td>5%</td>
</tr>
</tbody>
</table>

23% Fungi

1,648 individual fungi
Conclusions

- Understanding of diversity and structure of microbial communities associated with blueberry habitats
Link Between Eukaryotic Communities Structure and Soil Chemical Characteristics

Two groups of fields
Differ in their fertility
  ◦ Organic matter and N
Strong segregation of eukaryotes/fungi based on soil fertility
Major driving factors
  ◦ S, N, OM, P₂O₅, CEC (cation-exchange capacity)

Canonical correspondence analysis (CCA) used to determine which factors are linked to differences in eukaryotic OTUs across samples from bulk soil
Link Between Bacterial Communities Structure and Soil Chemical Characteristics

Two groups of fields
Differ in their fertility
  ◦ Organic matter and N

Minor segregation of bacterial communities based on soil fertility

Major driving factors
  ◦ pH, Al, Mg

Canonical correspondence analysis (CCA) used to determine which factors are linked to differences in eukaryotic OTUs across samples from bulk soil
Conclusions

- Understanding of diversity and structure of microbial communities associated with blueberry habitats
- Soil fertility significantly affect soil eukaryotes/fungi
- Soil fertility has minor effect on soil bacteria
Link Between Microbial Communities Structure and Management (Forest vs. Field)

Effect of management on bulk soil communities

Bacteria: $R^2 = 0.12377$, $p<0.001$

For eukaryotes/fungi: $R^2 = 0.02622$, $p>0.05$
Conclusions

- Understanding of diversity and structure of microbial communities associated with blueberry habitats
- Soil fertility significantly affect soil eukaryotes/fungi
- Soil fertility has minor effect on soil bacteria
- Management has minor effect on soil eukaryotes/fungi
- Management has stronger effect on soil bacteria
Link Between Microbial Communities Structure and Management (Forest vs. Field)

Effect of management on plant rhizosphere comminutes

Bacteria: $R^2 = 0.2222$, $p<0.001$
Eukaryotes/fungi: $R^2 = 0.0689$, $p<0.05$
Conclusions

- Understanding of diversity and structure of microbial communities associated with blueberry habitats
- Soil fertility significantly affect soil eukaryotes/fungi
- Soil fertility has minor effect on soil bacteria
- Management has minor effect on soil eukaryotes/fungi
- Management has stronger effect on soil bacteria
- Management has minor effect on root eukaryotes/fungi
- Management has stronger effect on root bacteria
Eukaryotes affected by Soil Properties, Management and Plant Selective Pressure

Soil fertility

Management

Mean proportion (%)

Difference in mean proportions (%)

99% confidence intervals

q-value (corrected)

Mean proportion (%)

Difference in mean proportions (%)

99% confidence intervals

q-value (corrected)
Microbial Network

*Leotiomyces* - *Ascomycete* fungi causing serious plant diseases
Plant Growth Promotion Microorganisms (PGPM)

Isolation of wild blueberry endophytes
- Selection for flavin secretion
- >100 strains -> B67

Laboratory test
- in progress

Filed test
- In progress

Genome sequencing
- *Agrobacterium rhizogenes ATCC 11325*
  - 16S rRNA, 99% no gaps,

![Graph showing mass of inoculated plants, mg](image)
PGPM: Future Plans

- Isolation of a collection of flavin secreting wild blueberry endophytes
- Select of subset of PGPM
  - Laboratory tests
  - Field tests
- Characterization
- Potential for field applications
Acknowledgements
Question