Global Population Genomics of Chickpea-Nodulating Mesorhizobium

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Chickpea Innovation Lab Meeting
ICRISAT, 13 December, 2017
Nitrogen Fixation

\[ \text{N}_2 + 8 \text{H}^+ + 8 \text{e}^- \rightarrow 2 \text{NH}_3 + \text{H}_2 \]

16 ATP \rightarrow 16 \text{ADP} + \text{P}_i
Fixed N is growth-limiting nutrient in most agricultural systems

33-46 Tg N into agricultural systems per year (Herridge et al., Plant and Soil, 2008)

100 Tg N fertilizer consumed per year (FAO Stat, 2013)
Limitations of N Fertilizer

• 80% of African countries are nitrogen deficient
• Limited infrastructure for fertilizer production
Rhizobial bacteria

Epidermis

Inner cortex

Outer cortex

Endodermis

Pericycle

Infection thread

Pre-infection thread


Symbiotic Specificity

The diversity of Sinorhizobium species includes: Sinorhizobium -de- Agrobacteriun, Ancylobacteris, Azorhizobium, Mesorhizobium, Bradyrhizobium, Rhizobium, Rhodopseudomonas, Phyllobacterium, Methylobacterium, Nitrobacter, Afipia, Beijerinckia, Thibacillus, Azorhizobium cauliformans, Aquabacterium, Rhodopseudomonas viridis, Rhodopseudomonas palustris, Rhodoplanes, and Rhodospirillum rubrum.
Symbiotic Specificity

[Diagram showing phylogenetic relationships among various plant families and bacteria, with labels such as Caesalpinioideae crown clade, Mimosoids, and bacterial genera like Sinorhizobium, Rhizobium, and Bradyrhizobium.]
Symbiotic Specificity
Can we use our understanding of chickpea’s biogeography to inform the study of its bacterial symbionts?

What factors influence the distribution of these bacteria (e.g. cultivation history, soil properties, host genotype)?
Isolate 1

Isolate 2

Pacbio sequencing

Symbiosis island(s)
Mesorhizobium species group:

- M. australicum
- M. ciceri
- M. mediterraneum
- M. plurifarium
- M. temperatum

Reference Chickpea Isolate

Reference Genome Source:

- Ethiopia
- India
- Morocco
- Turkey
- North America
Mesorhizobium species group:

- M. australicum
- M. ciceri
- M. mediterraneum
- M. plurifarium
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- Reference Chickpea Isolate
- Reference Genome

Sampling region:
- Ethiopia
- India
- Morocco
- Turkey
- North America
Mesorhizobium ciceri group

Mesorhizobium species group:
- M. australicum
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Sampling regions:
- Ethiopia
- India
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- Turkey
- North America
Mesorhizobium ciceri group

Inoculant in North America and Australia

Mesorhizobium species group:
- M. australicum
- M. ciceri
- M. mediterraneum
- M. plurifarium
- M. temperatum

Reference Chickpea Isolate
Reference Genome Source:
Ethiopia
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Reference Chickpea Isolate
Reference Genome

Sampling region:
- Ethiopia
- India
- Morocco
- Turkey
- North America
<table>
<thead>
<tr>
<th>Plant</th>
<th>Species</th>
<th>Sites</th>
<th>Plant Genotypes</th>
<th>Soil</th>
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<td>C. reticulatum</td>
<td>Oyali</td>
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<td>C. echinospermum</td>
<td>Siv-Diy Roadside</td>
<td>Accession 1</td>
<td>C. echinospermum</td>
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<td>C. echinospermum</td>
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<td>Accession 3</td>
<td>C. echinospermum</td>
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<td>C. arietinum (cultivated)</td>
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<td>Cultivar 1</td>
<td>C. arietinum (cultivated)</td>
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<td>Cultivar 2</td>
<td>C. arietinum (cultivated)</td>
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<td></td>
<td>Cultivar 3</td>
<td>C. arietinum (cultivated)</td>
</tr>
</tbody>
</table>

Measure whole-plant data:
- Nodule number
  - Check subset of nodules for *Mesorhizobium* id
- All-nodule weight
- Leghaemoglobin content
- Shoot weight
- Root weight
- Sequence 16S rRNA genes from rhizosphere samples
- Test selection of *Mesorhizobium* strains with PCR assay of crude nodule-DNA extracts
C. reticulatum
C. echinospermum

M. med / M. temp
M. ciceri

Soil
Plant
16 Symbiosis genes in all Mesorhizobium strains from all hosts.

Mesorhizobium species group:
- M. australicum
- M. ciceri
- M. mediterraneum
- M. plurifarium
- M. temperatum

Reference Chickpea Isolate
Reference Genome

Sampling region:
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Reference Genome Source:
100 genes conserved in all chickpea-\textit{Mesorhizobium} symbiosis islands.
Gene presence-absence variation in different components of the symbiosis island.

<table>
<thead>
<tr>
<th></th>
<th>Full symbiosis island</th>
<th>Sym-island start</th>
<th>Right (nodulation) flank</th>
<th>Middle (highly-variable) flank</th>
<th>Right (effector) flank</th>
<th>Sym-island end</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core genes</td>
<td>(99% ≤ strains ≤ 100%)</td>
<td>94</td>
<td>2</td>
<td>34</td>
<td>4</td>
<td>52</td>
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<tr>
<td>Soft core genes</td>
<td>(95% ≤ strains &lt; 99%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>Shell genes</td>
<td>(15% ≤ strains &lt; 95%)</td>
<td>360</td>
<td>80</td>
<td>40</td>
<td>134</td>
<td>88</td>
</tr>
<tr>
<td>Cloud genes</td>
<td>(0% ≤ strains &lt; 15%)</td>
<td>1543</td>
<td>291</td>
<td>47</td>
<td>1101</td>
<td>85</td>
</tr>
<tr>
<td>Total genes</td>
<td>(0% ≤ strains ≤ 100%)</td>
<td>1997</td>
<td>373</td>
<td>121</td>
<td>1239</td>
<td>225</td>
</tr>
</tbody>
</table>

Putative effector genes in highly-conserved “right flank” of symbiosis island are variable in presence.
Normalized Robinson-Foulds distance (measure of phylogenetic-tree similarity): 0.73
Normalized Robinson-Foulds distance for trees composed of 10-gene sliding windows.

Genes 1-44 are from left flank (core signaling and nitrogen-fixation genes).

45-102 are from right flank (type-III and IV secretion systems, potential effectors, &c.).

Average nRF between core housekeeping-gene trees is 0.49
Dear Fulbright committee:

This letter is confirming support by Dicle University for the research of Alex Greenspan, entitled "The effects of domestication on the symbiotic nitrogen fixation in chickpea." We are very eager to support Alex on this research. I worked with Alex and other international collaborators in the spring of 2013 on a project to survey and collect seed from wild chickpea populations in SE Anatolia - Turkey. At the time we discussed Alex's ideas to perform a field experiment on wild chickpea in Turkey. At Dicle University, Alex will have access to the laboratory space and utilities required to perform the chemical and microbiological analysis for his experiment. Diyarbakir is also right in the center of wild chickpea's native range, and within easy driving distance of the field sites Alex prospected last spring. Alex's project will enhance our understanding of the ecology of Turkey. His collaboration will bring expertise and access to genomics technologies that are still underdeveloped in Turkey.

Prof. Dr. Bekir Bukun (Dean)
Dicle University, College of Agriculture, Diyarbakir - Turkey
9/3/2013
Questions?
## Analysis of Molecular Variance (AMOVA)

<table>
<thead>
<tr>
<th></th>
<th>Pangenome gene presence-absence</th>
<th>Core-genome nucleotide variants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Variation Between</td>
<td>% Variation Within</td>
</tr>
<tr>
<td>Mesorhizobium mediterraneum</td>
<td>18.1</td>
<td>81.9</td>
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<tr>
<td>Mesorhizobium temperatum</td>
<td>2.2</td>
<td>97.8</td>
</tr>
<tr>
<td>Mesorhizobium ciceri</td>
<td>16.1</td>
<td>83.9</td>
</tr>
</tbody>
</table>

Test for contribution of site on genetic differentiation

Similar analyses (General AMOVA, BEDASSLE) can use quantitative variables (e.g. soil chemistry, distance, rainfall).