Microbial Interactions in the Rhizosphere

- Rhizosphere -- biology and chemistry

- Binary interactions – Inter-domain
  *Bacillus cereus* -- oomycete antagonism

- Community interactions –
  *B. cereus* -- rhizosphere community

- Binary interactions – Inter-phylum
  *B. cereus* -- CF co-isolates
Growing root tip--Root hairs look transparent here. As the root pushes into soil, tip cells wear off, providing food for microbes
http://www.morning-earth.org/Graphic-E/BIOSPHERE/Bios-C-PlantsNew.html#soillife
parts of a root tip and areas of the rhizosphere
graphic from Raina Maier et al, 2000, Environmental Microbiology
• Plants secrete 5-21% of their photosynthate from roots
• The rhizosphere is enriched with sugars, amino acids, secondary metabolites, DNA, polysaccharides, and microbes
Culture-Based Study????????

Rare biosphere organisms that appear rarely in deep sequencing of a habitat. Said to be largely unknown and uncharacterized.

In soil, we have >100 years of study of the rare biosphere.
**Figure A**

Venn diagram showing the overlap of culture-independent and culture-based OTUs.

- **3903 OTUs** are culture-independent OTUs.
- **453 OTUs** are shared OTUs.
- **601 OTUs** are culture-based OTUs.

**Figure B**

Heatmap showing the relative abundance of OTUs.

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<thead>
<tr>
<th>OTU</th>
<th>Culture-independent</th>
<th>Culture-based</th>
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26 most abundant OTUs, identified to their closest RDP match:

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Search for biological agents to control oomycete-induced plant disease
*Bacillus cereus* strain UW85
Isolated from an alfalfa root growing in Arlington, WI
Inhibition of *Phytophthora* by *Bacillus cereus* wild type and non-disease suppressive mutants
Bacillus cereus antibiotic

Zwittermicin A
He et al., 1994
Search for Zwittermicin Biosynthetic Cluster

- How is this new antibiotic synthesized?
- Clone gene for ZmA resistance in *E. coli*
- Seek biosynthetic genes flanking resistance gene
Zwittermicin Resistance

BAC library of *B. cereus* genome in *E. coli*

Select for resistance to zwittermicin

ZmaR = deduced sequence has similarity to aminoglycoside acetyltransferases

Mode of action in zwittermicin resistance?
Mechanism of zwittermicin resistance by ZmaR
Zwittermicin Biosynthesis

Emmert et al., 2004
Zwittermicin Biosynthesis

Emmert et al., 2004
Zwittermicin A Biosynthetic Gene Cluster

NRPS/PKS Components:
- ORF1: PKS/NRPS
- ORF2: NRPS
- ORF3: NRPS
- ORF7: PKS
- ORF12: NRPS/PKS
- ORF16: NRPS
- ORF17: Type II Te
- ORF18: NRPS
- ORF19: NRPS
- ORF20: Ppant Transferase
- Hydroxymalonyl-ACP
- ORF4: ACP
- ORF5: Acyl-CoA DH
- ORF8: 3-OH butyryl-CoA DH
- ORF15: FkbH

Aminomalonyl-ACP:
- ORF8: 3-OH butyryl-CoA DH
- ORF9: ACP
- ORF10: Acyl-CoA DH
- ORF11: Adenylation Domain (Ser)

2,3-diaminopropionate (DAP):
- ORF26: Carbamoyltransferase (VioL)
- ORF27: VioB (DAP synthase)
- ORF28: VioK (Amidotransferase)

Modifying Enzymes:
- ORF13: Monooxygenase
- ORF23: Hydrolase
- ORF24: Oxidoreductase

Resistance:
- ORF6: ZmaR (acetyltransferase)

Regulation:
- ORF21: LacI family
- ORF22: Sensory regulator

Transport:
- ORF14: ABC transporter
- ORF25: Membrane Protein

Sequencing: TIGR
Bioinformatics: Handelsman lab, M. Thomas, TIGR
Bacillus cereus zwittermicin producers

Bc  $10^5$ /gm soil

10-20% carry zmaR

Almost all those tested produce zwittermicin

Most common antibiotic producer in soil?
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Hypothesis

*Bacillus cereus* alters microbial community on roots

Altered community protects root from pathogens
untreated soybean seeds → UW85 treated soybean seeds → harvest shortly after emergence (50 per treatment) → suspend in water, sonicate, and plate on 10% TSA → test each isolate for 50 physiological tests by replica plating → multivariate analysis → patch for replica plating → grow aerobically
Rhizosphere Community Ecology

• Are there physiological traits that discriminate soil bacteria from rhizosphere bacteria?

• Are there traits that discriminate bacteria in the untreated rhizosphere from those in the rhizosphere exposed to *B. cereus* UW85.

• Discriminant analysis – identify features of bacteria that predict whether they are members of rhizosphere or soil.
The Camouflage Hypothesis

Plant disease is suppressed by treatments that modify the microbial community of the root to make it more like the community in soil.  

(studies from 1904 to present)

— Root pathogens “see” host by rhizosphere signals
— Rhizosphere created by bacteria that live in it
— Treatments suppress disease and increase “soilness” of rhizosphere
  • Genes for disease resistance
  • Soil amendments that suppress disease
  • Bacterial biocontrol agents that suppress disease
Effects of *Bacillus cereus* on Rhizosphere

- Community undergoes global shift, although *B. cereus* no longer detectable
- Flush of *Flavobacterium-Cytophaga* bacteria (as much as 10-fold)
- Culturing *Bacillus cereus* from roots – found *Cytophaga-Flavobacterium* hitchhikers
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Further evidence of an interaction between *B. cereus* and Cytophaga-like bacteria

Collect field grown soybean plants, isolate bacteria from roots by sonicating, plate

Pick *B. cereus* colonies and purify by restreaking

Patch colonies, incubate at 4ºC

Collect coisolates, purify and identify by 16S gene sequence
Bacteria “co-isolated” with *Bacillus cereus* from soybean or alfalfa roots

- *Flavobacterium* sp.
- *Aureobacterium* sp.
- *Pseudomonas* spp.
- *Pseudomonas aureofaciens* “C16”
- *Cytophaga* spp.
- Unidentified Gram (-) bacteria
Bacteroidetes over-represented among co-isolates compared with rhizosphere
Do *B. cereus* and Cytophaga-Flavobacteria interact in culture?

- *Flavobacterium johnsoniae* does not grow in soybean root exudate alone

- Surprising – all isolates of CF from soybean rhizosphere
Bacillus cereus enables Flavobacterium johnsoniae to grow in root exudate
Effect of *Bacillus cereus* on growth is specific to the Cytophaga-Flavobacterium group
Growth of *Flavobacterium johnsoniae* enhanced in alfalfa root exudate medium by addition of *B. cereus* peptidoglycan
*Flavobacterium johnsoniae* utilizes peptidoglycan as sole carbon source.
Questions about the *B. cereus*- *Flavobacterium* Interaction

• Does peptidoglycan contribute to community shift associated with *B. cereus* addition?
• Is it the basis for hitchhiking FB?
• Are there other hitchhikers and hosts in soil?
Microbial Interactions in the Rhizosphere

Binary interaction – *Bacillus cereus* – oomycete antagonism mediated by zwittermicin common antibiotic is soil exotic structure and mode of synthesis

Community interactions – *B. cereus* -- rhizosphere
Bc transforms community greater resemblance to soil community camouflage root?

Binary interactions – *B. cereus* – CF co-isolates intimate physical association nutritional relationship through peptidoglycan
# Acknowledgments

<table>
<thead>
<tr>
<th>Laura Silo-Suh</th>
<th>Jon Clardy</th>
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<tr>
<td>Greg Gilbert</td>
<td>Haiyin He</td>
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<tr>
<td>Ashley Shade</td>
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**NSF, USDA**

**Howard Hughes Medical Institute**