Harnessing the Microbiome for Agricultural Sustainability In Bioenergy-based systems

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"We know more about the movement of celestial bodies than about the soil underfoot." ~ Leonardo da Vinci

"We might say that the earth has the spirit of growth; that its flesh is the soil." ~ Leonardo da Vinci

... the Latin name for man, homo, derived from humus, the stuff of life in the soil." ~ Dr. Daniel Hillel

Your Microbes, Your Health
Plant Microbes, Plant Health

Some plant-microbe symbioses are very conspicuous!

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Some plant-microbe symbioses are very conspicuous!
**Symbiosis for Sustainability Group**

**Rationale:** Plants depend on different types of symbionts in all natural environments, especially challenging ones (i.e. nutritionally deficient soils, extreme temps, etc).

**Overarching goal:** Find new symbiotic microbes to promote plant growth under a low-input strategy or other challenging environment(s)

"**Optimize the Plant Phytobiome**"

**Basic Science**

- Omics approaches
- Microscopy and growth tests
- Simplified community analysis
- Modeling

**Application**

- Trait identification
- Compatibility assessment
- Greenhouse/field studies
- Delivery methodology

**"A matter of scale"**

*In vitro* → greenhouse → field

- *In vitro* microbial assays necessary for high throughput screening of potentially beneficial microbes
- *In vitro* association studies (plant + microbe) necessary to study nature of infection via microscopy
- *Greenhouse* studies necessary to control number of variables
- *Field* studies to transition from one-on-one relationships to multi-trophic, ecosystem-like scenarios

**"Improve greenhouse to field congruency"**

**Switchgrass (Panicum virgatum): A Promising Bioenergy Crop**

**Cellulosic Bioenergy Crops**

**Publication Diagram**

**Figure Description**

Development of switchgrass (Panicum virgatum) as a bioenergy feedstock in the United States. (Reference: [source](http://example.com))
Switchgrass (*Panicum virgatum* L.)

- Native C₄ perennial
- High nutrient use efficiency
- Shoot biomass can reach 40 dry Mg/Ha
- Root biomass can reach 8 dry Mg/Ha; excellent carbon sink & erosion control
- Can be grown on marginal, nutritionally depleted lands under a low-input strategy

Switchgrass as a model for low-input agriculture

**Switchgrass endophytic microbes**

- 3 yr survey from native tallgrass prairie and other varied habitats
- Collections in early late April (establishment), July (peak) and early November (senescence)
- Approx. 700 fungal and 800 bacterial endosphere isolates obtained in pure culture from sterilized root and shoot tissues
- Identified by 16S (bacteria) or 18S+ITS (fungi)

Switchgrass endophytic microbes

**Sampling Site: Tall Grass Prairie in Northern Oklahoma**

~450 plants were collected

**Soil analysis results**

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**Experimental Procedure**

- **Switchgrass**
- Root / Shoot
- Pots/Greenhouse
- NF Field
- Metagenomics
- Fungi
- Bacteria
- 16S sequencing
- Screening for beneficial characteristics
- Phosphate solubilizing
- ACC deaminase
- NO Field
- Evaluation of Plant growth promotion

**Fungi**: excised root/shoot bits plated directly

**Bacteria**: root tissue ground in H_2O and plated in serial dilution

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**Regional Distribution of Bacterial Isolates**

- **Ave. 4.9 isolates**
- **Ave. 3.2 genus**

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**Phosphate Solubilizing Bacteria**

- **Bacterial single colonies**
- **Rich medium (step)**
- **Stress by centrifugation**
- **1:10 dilution with LB medium**
- **Dip 3 µl of cell suspension to PVK media**
- **Incubated for 96 hrs, 25°C, dark**
- **Measuring phosphate solubilizing activity**

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**Taxonomic configuration of cultivable bacteria**

- Pseudomonas 55%
- Sphingomonas 3%
- Enterobacter 3%
- Burkholderia 4%
- Xanthomonas 4%
- Stenotrophomonas 4%
- Klebsiella 5%
- Bacillus 6%
- Rhizobium 6%

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**High-throughput ACC Deaminase Assay**

- **Standard reaction (ACC 0 – 500 uM)**
- **Methionine**
- **SAM synthase**
- **ACC synthase**
- **ACC oxidase**
- **ACC deaminase**

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*56 positive out of 551 samples*
**NifH detection**

Total nifH positives: 77

- **Rhizobia**, positive control (+)
- **E. coli**, negative control (-)

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6 strains ACC/phosphate sol. + *Burkholderia* strain is positive for all three traits.

**Acetylene reduction assay with plants grown in Turface-vermiculite mixture**

- **Turface**: vermiculite mixture
- **Ethylene production**
- **Soil drench with 5 ml bacteria dissolved in fertilizer (OD 0.05)**

**Taxonomic Configuration of Beneficial Bacteria**

- **Total 526 ACC**
- **45 NifH**
- **25 Phosphate**

- **Pseudomonas**: 76%
- **Burkholderia**: 24%
- **Rhizobium**: 6%
- **Bacillus**: 6%
- **Klebsiella**: 5%
- **Stenotrophomonas**: 4%
- **Xanthomonas**: 4%
- **Burkholderia**: 4%
- **Enterobacter**: 3%
- **Sphingomonas**: 3%
- **Variovorax**: 1%

**Ongoing research**

**Genomes of approx. 176 endophytic bacteria of switchgrass have been completed (JGI)**

- Comparative genomics (ORNL) to look for commonalities between like phenotypes.

**Comparison** of culturable vs. non-culturable microbe populations

- Targeted isolation

**Fluorescent tagging** of promising strains underway

**Greenhouse trials** initiating “5 best” test: P-solubilizers/N-fixers/ACC deaminase producers

And then there are the fungi…
Symbiotic “consortia” for low-input agriculture

Hormone producing foliar bacteria
Defensive metabolite producing foliar fungus
Nitrogen-fixing root bacterium
Nutrient-solubilizing mycorrhizal fungus

My Lab
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Myoung-Hwan Chi
Kaustav Bandypadhyay
Nikki Charlton
Jeremy Bell
Haiyan Li
JoHanna L’Hereux

Collaborators
Michael Udvardi (Noble)

Joint Genome Institute