Methylo trophs for Sustainable Agriculture
PAG XXV – Exploring Phytobiomes Workshop

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Microbials and the Future of Agriculture

- Challenge: adoption of sustainable agricultural technologies to feed 9 BB people

- New GM crop costs 10 years and $150 MM

- New Ag chemical costs 12 years and $250 MM

- Microbial solutions have accelerated path-to-market

- Mainstream Agriculture embraces Microbials as major disruptive technology and growth opportunity
Microbiome – “Second Plant Genome”

PHYLLOSHERE
A plant’s leaves and stems can have up to 10 million microbes per square centimeter

ENDOSPHERE
Endophytic populations can reach up to 10 million microbes per gram of tissue

RHIZOSPHERE
1 gram = up to 10 billion microbes and up to 30,000 different species
Microbial Plant Enhancement “Traits”

- Phytohormones
  - IAA production
  - ACC deaminase
  - Acetoin, 2,3 butanediol etc.
- Biotic stress mitigation
  - Induced Systemic Resistance
  - Biopesticidal compounds etc.
- Abiotic stress mitigation
  - Drought (WUE, increased rooting, ABA), temperature, salinity
  - Reduction of ROS
- Nutrient acquisition
  - Phosphate solubilization
  - Nitrogen fixation
  - Iron scavenging etc.

Bulgarelli et al. (2013)
NewLeaf Symbiotics Strategy

- Methylotroph-based Microbial products as drop-in solutions for sustainable agriculture
- Methylotrophs are ubiquitous colonizers of plants (phylloplane, rhizosphere, endosphere)
- Rich in Plant Enhancement “Traits”
- Discover and Develop the best fit strain(s) to each product concept (crop, BioYield, BioControl, application method)
NewLeaf Is Mining a Rich Vein

- ~1,500 Methylotrophic strains from roots, leaves and endosphere of wild crops (~7,000 strain by end 2017)
- All genomes sequenced, assembled and annotated
- Plant enhancement “trait” discovery rate is very high
- Very “productizable” for non-spore formers
Methylo troph Pan-genome Analysis

- Typical Methylo troph genome
  - ~ 5,200 genes per genome
  - 54% genes with assigned function
  - 46% genes no assigned function (70% of gene clusters no assigned function)

- Align all protein sequences from all genomes against themselves

- Cluster genes around protein homology
  - Core genes: >= in 95% genomes
  - Shell genes: in multiple genomes
  - Cloud genes: in only few or one genome

Graph clustering using Markov chain and flow simulation (MCL).

Genes Over Genomes

M. populi, 30 genomes

Cloud: 12,906 (64%)
Shell: 5,021 (25%)
Core: 2,226 (11%)

M. extorquens, 500 genomes

Cloud: 102,217 (94%)
Shell: 4,667 (4%)
Core: 1,721 (2%)

Methylobacterium, 1000 genomes

Cloud: 170,814 (96%)
Shell: 6,789 (4%)
Core: 1,054 (0%)

Key
- Conserved genes
- Total genes
Gene Presence Phylogeny

- Pan-genome enabled analysis
- Identify uniquely all genes over all genomes
- Use genes presence/absence to infer distance
- Protein sequences cluster agnostically to function
- Sufficient phenotype data allows genes/traits linkage identification
Prescriptive Biologics Knowledge Base™

- Standardize, store all primary and meta-data from all NLS data sources
- Critical mass of experimental results enables predictions, prescriptions
- \textit{In silico} phenomics – hypothesis driven experiments
- Deciphering Phenotype x Genotype x Environment
NewLeaf R&D Workflow

Microbial Genotyping

Strain Phenotyping

In Planta Phenotyping

Bioinformatics Analysis

Field Trials
US Field Trial Locations

- Corn & soya
- W-wheat, corn & soya
- W-wheat
- S-wheat & soya
- Peanut
Colonization of Plant from Seed
Suppression of Fungal Pathogens

Head Blight - Wheat

- Untreated check
- Microbial treatment

Grey Leaf Spot - Corn

- Untreated check
- Microbial treatment
Increase of Yield Over Locations x Years

### 2015/16 Soy Seed Treatment
(14 locations; over locations x years)

- UTC: 61.5
- Strain A: 63.0
- Strain B: 64.5
- Strain C: 65.0

### 2015/16 Corn in-furrow Application
(14 locations; over locations x years)

- UTC: 206
- A: 210
- B: 218
- D: 220
Mitigation of Agricultural Pests

Lead CRW biocontrol strain delivered:
- ~13 bushel per acre advantage across two years
- ~60% reduction in root damage across two years
- Comparable to in-furrow chemical insecticide (pyrethroid)

Lead strain offer an Integrated Pest Management option
Modification of Microbiome

Untreated check

Microbial treatment (significant yield advantage)
Combination of Lead Strains

- Lead strains provide over seven bushel advantage to the check
- Combination of lead strains with strain E provide additional over seven bushel advantage
- Stand retention delivers yield; resistance to lodging delivers “harvestable” yield at high planting rates

![2016 Corn in-furrow Application (across two locations)]
Production and Formulation

- Production patent granted in 2015 provides a unique position (US Patent 9181541)

- Scaling from pilot scale to 100,000L production in 2017

- Formulations are drop-in solutions with current agronomic inputs (traits, chemistries, fertilizers, practices)

- First sales revenue in US markets in 2017
Acknowledgements