

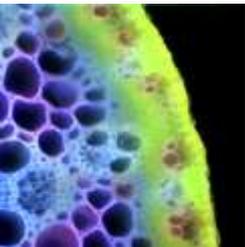
Office of Biological and Environmental Research



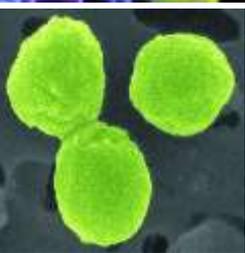
Biological Systems Science Division Update



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October 18, 2018



U.S. DEPARTMENT OF
ENERGY

Office
of Science

Office of Biological
and Environmental Research

Update on Programmatic Activities

Completed Reviews/Activities in FY 2018

- Panel Reviews - Early Career applications (May 7)
 - Microbial Biofuels applications (May 8-9)
 - Bioimaging applications (May 10-11)
 - Plant Feedstocks for Bioenergy applications (Jun 26)
- Panel Review - SFA Projects for KBase applications (Aug 9)
 - Bioimaging Lab Call applications (Aug 10)
- Oak Ridge National Laboratory SFA Review (Aug 28)
- Los Alamos National Laboratory SFA Review (Aug 29)

Upcoming Reviews/Activities in FY 2019

- LBNL ALS BSISB/IDAT Reviews (Nov 5-7)
- Bioenergy Research Centers annual reviews
 - CBI (Nov 14-15) JBEI (Dec 4-5) GLBRC (Jan 23-24) CABBI (Feb 20-21)
- ORNL Center for Structural Molecular Biology Review (CSMB) Review (Dec 10)
- ORNL Biofuels SFA Review (Dec 10)
- BNL Quantitative Plant Science Initiative (QPSI) Review (Jan 8)
- Annual Genomic Sciences Program PI Meeting (Feb 25-27)
- Annual Bioimaging Science Program PI Meeting (Feb 27-28)

Strategic Planning/Activities

BER Workshops

Genome Engineering for Material Synthesis (GEMS) Workshop

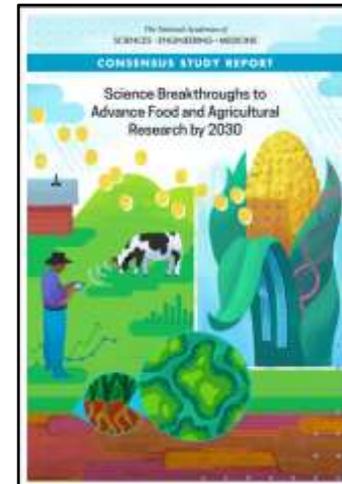
- Exploring possibilities for designed materials using genome engineering techniques
- **Last Week!** - October 9-11 (Brief out by Dr. Seema Singh later)

Breaking the bottleneck of genomes: Understanding gene function across taxa

- Addressing the annotation problem
- November 1-2

National Academy Studies

- **“Developing a Research Agenda for Utilizing Gaseous Carbon Waste Streams.”** Board on Chemical Sciences and Technology and the Board on Energy and Environmental Systems (BER, BES, ARPA-E, FE, EERE)
- **“Breakthroughs 2030: A Process for a 10-year Agenda for Food and Agricultural Research.”**
Board on Agricultural and Natural Resources - Complete (Briefing by Dr. Greg Lowry, Carnegie-Mellon Univ.)
- **“Chemical Engineering: Challenges and Opportunities in the 21st Century”** Board on Chemical Sciences and Technology (initial start)



New Funding Opportunities Announcements for FY 2019

Genomic Science Program

- Plant Genomics Research
- Environmental Microbiome Science
- Computational Biosciences

FOAs will be posted to Grants.gov
“Department of Energy – Office of Science”

Biomolecular Characterization and Imaging Science

- Bioimaging Research

FOAs will be posted to Grants.gov
“Department of Energy – Office of Science”

Facilities & Infrastructure

- Calls for User Proposals at the Joint Genome Institute (JGI)

<https://jgi.doe.gov/user-program-info/community-science-program/how-to-propose-a-csp-project/>

National Microbiome Data Collaborative (NMDC)

- Opportunity at the DOE National Laboratories

New Bioimaging Awards

Bioimaging Research and Approaches for Bioenergy DE-FOA-0001868

13 new projects

Develop new approaches for non-destructive imaging of multiple metabolic processes occurring within the living plant and microbial systems



- Track and understand biomass deconstruction processes, the mechanisms of enzymatic deconstruction of lignocellulose and predict lignin's amenability to structural modifications, enzymatic cleavage and degradation
- Visualize and track the spatio-temporal expression patterns of natural or engineered metabolic pathways in microorganisms, and presence of metabolic inhibitors and competitive pathways involved in biomass deconstruction and conversion of lignocellulosic sugars or lignin components to fuels and bioproducts.
- Characterize mechanisms controlling the temporal dynamics of structural changes, and image the spatial and temporal dynamics of the nutrients within the rhizosphere to accelerate the understanding of rhizosphere processes

Lab Awards still pending

New Plant Feedstock Genomics Awards

*Plant Feedstock Genomics for Bioenergy: A Joint Research Funding Opportunity
Announcement USDA, DOE (DE-FOA-0001857)*

6 new projects

H. Cuevas (USDA ARS) *Uncovering new sources of anthracnose resistance in populations of genetically diverse sorghums [*Sorghum bicolor* (L.) Moench]*

T. Jamann (Univ of IL) *Conserved genetic mechanisms for biotic stress in sorghum*

G. Peter (Univ of FL) *Enhanced Resistance Pines for Improved Renewable Biofuel and Chemical Production*

P. Busby (Oregon State Univ) *Identifying plant genes associated with pathogen antagonism in *Populus trichocarpa**

J. Anderson (Univ of MN) *Genetic improvement of seed yield and oil content in field pennycress, a non-food oilseed feedstock*

J. Carlson (Penn State U) *Breeding resilient, disease-resistant switchgrass cultivars for marginal lands*



New SFA Projects for KBase Applications

Improving Draft Metabolic Models in KBase: Tools for Importing, Comparing and Merging Metabolic Annotations

Lawrence Livermore National Laboratory (LLNL) (PI: Rhona Stuart)

Microbes Persist: Building a KBase Foundation for Microbial and Viral Ecogenomics in Soil

Lawrence Livermore National Laboratory (LLNL) (PI: Jennifer Pett-Ridge)

Building a reference-based metagenomics workflow in KBase

Lawrence Berkeley National Laboratory (LBNL) (PI: Paul Adams)

Protein Co-evolutionary Analysis and Structure-based Functional Characterization

Oak Ridge National Laboratory (ORNL) (PI: Julie Mitchel)

Integrating new fungal computational sequence data analysis into KBase's capabilities

Los Alamos National Laboratory (LANL) (PI: Patrick Chain)

New Investments at the Labs for Cryo-EM and Neutron science

Cryo-EM capabilities

Brookhaven National Laboratory (PI: Sean McSweeney)
Operational support for a new cryo-EM facility (in association with New York State) to stand up a functional cryoEM capability at BNL as soon as FY 2020.

Cryo-EM



Stanford Linear Accelerator Center (SLAC) (PI: Wah Chiu)
Operational and instrumental support to complement recent NIH-funded cryo-EM center at SLAC.

Lawrence Berkeley National Laboratory (PI: Karen Davies)
Instrumental support for a screening microscope for cryo-EM prior to accessing the high-end instruments at SLAC.

Neutron Science Capabilities

Oak Ridge National Laboratory (PI: Hugh O'Neill)
Pilot funding to build to demonstrate biological applications in neutron science.

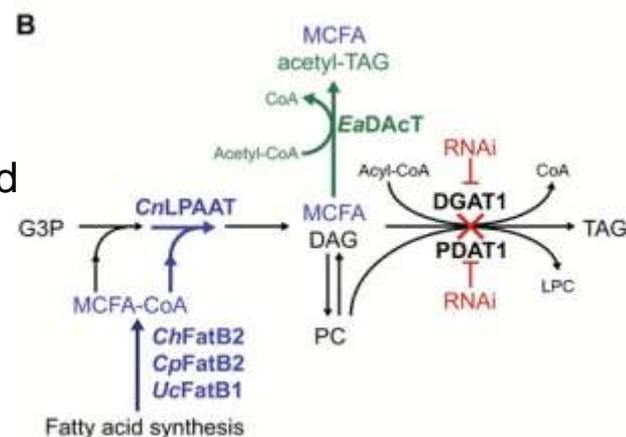
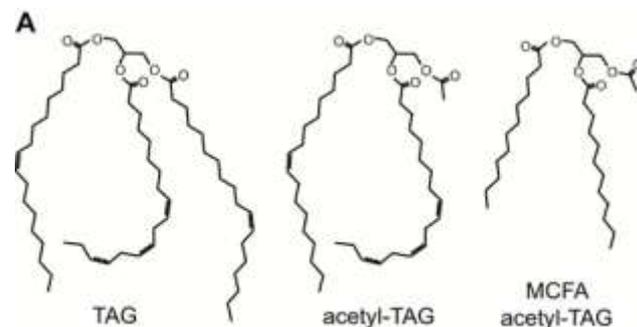
Towards the synthetic design of camelina oil enriched in tailored acetyl-triacylglycerols with medium chain fatty acids

Objective

- To produce 3-acetyl-1,2-diacyl-*sn*-glycerols (acetyl-TAGs) with medium-chain fatty acids (MCFAs) in the oilseed crop *Camelina sativa* by metabolic engineering.

Approach

- Different transgenic camelina lines that had been genetically modified to produce MCFAs through the expression of MCFA-specific thioesterases and acyltransferases were retransformed with the *Euonymus alatus* gene for diacylglycerol acetyltransferase (EaDAcT) that synthesizes acetyl-TAGs.
- Concomitant RNAi suppression of acyl-CoA: diacylglycerol acyltransferase was designed to increase the levels of acetyl-TAGs.



Strategy for the production of MCFA acetyl-TAGs in biofuel crops

Result/Impacts

- Successfully generated metabolically engineered camelina that produce acetyl-TAG molecules containing medium-chain fatty acids
- These tailored lipids with a designed structure are potentially useful for production of biofuels and bioproducts in crops such as sorghum.

Objective

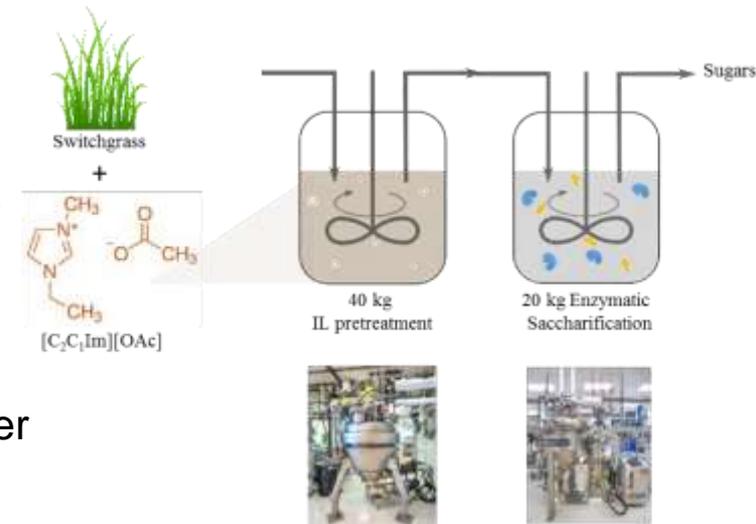
- Demonstrate feasibility of ionic liquid (IL) pretreatment, bridge of gap between lab and bench studies to pilot scale implementation

Approach

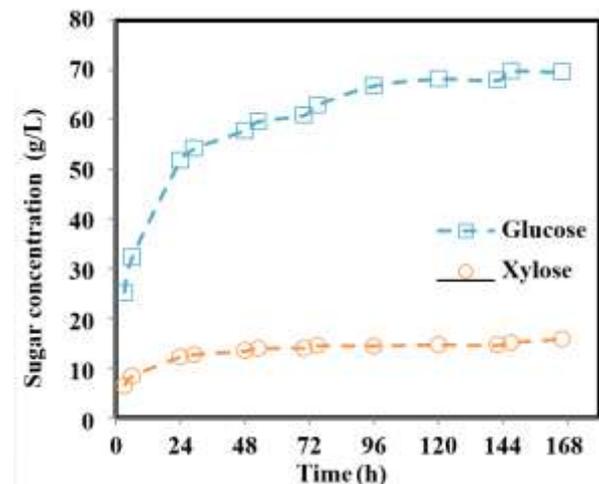
- At ABPDU, scale up 1-ethyl-3-methylimidazolium acetate ($[C_2C_1Im][OAc]$) pretreatment to a 40 kg batch in a 210 liter customized pressure reactor followed by enzymatic saccharification at a 50 L stirred tank

Result/Impacts

- $[C_2C_1Im][OAc]$ pretreatment of switchgrass has been successfully scaled up to 40 kg
- Enzymatic hydrolysis of the pretreated switchgrass resulted in efficient sugar conversions-96% glucan and 98% xylan conversion
- Mass flow of the overall process was established and the major scale-up challenges were identified and discussed
- Identified needs to improve transitional processes such as material transfer, biomass recovery from aqueous IL stream, IL recycling, and improvement of enzyme's tolerance to ILs



Process overview of IL pretreatment and enzymatic saccharification



Sugar release during enzymatic saccharification of $[C_2C_1Im][OAc]$ pretreated switchgrass

One poplar 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase moonlight as transcriptional repressor to regulate lignin biosynthesis



Objective

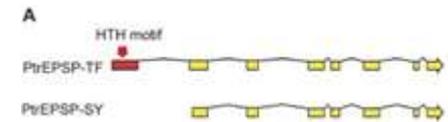
- Understand how lignin biosynthesis is regulated in poplar

Approach

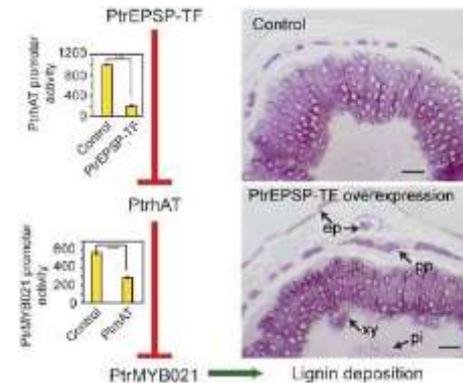
- Used GWAS to define the linkage of *PtrEPSP-TF* and lignin biosynthesis
- Validated linkage by assessing *PtrEPSP-TF* overexpressing poplars for lignin deposition, secondary metabolism, and gene expression levels
- Investigated underlying molecular mechanism by protoplast-based assays

Result/Impacts

- EPSP synthase shown to co-function as a transcriptional repressor
- *PtrEPSP-TF* overexpressing poplars had increased lignin deposition, accumulation of lignin-related metabolites, and expression of lignin-related genes.
- *PtrEPSP-TF* directly represses the expression of *PtrhAT*. *PtrhAT* then directly represses the expression of *PtrMYB021*. Consequently, *PtrEPSP-TF* positively regulates *PtrMYB021* expression and lignin biosynthesis in poplar



The additional HTH motif endows new functions to *PtrEPSP-TF*. A) Protein structure of *PtrEPSP-TF*



PtrEPSP-TF activates *PtrMYB021* expression and lignin deposition. Left panel, the hierarchical regulation of *PtrEPSP-TF*, *PtrhAT*, and *PtrMYB021*. Right panel, ectopic lignin deposition (indicated by black arrows) in stems of poplars overexpressing *PtrEPSP-TF*. Lignin shows red color after staining.



Process and field-scale ethanol yields of five lignocellulosic feedstocks

Objective

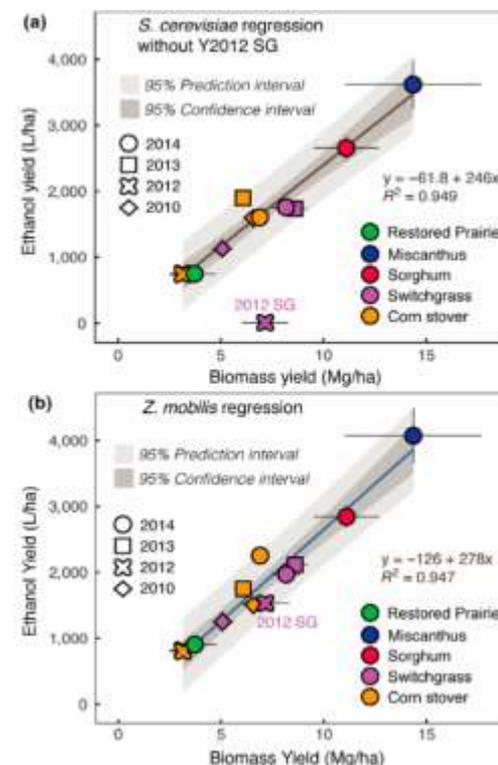
- Determine if lignocellulosic biorefineries can accept and process a variety of feedstocks.

Approach

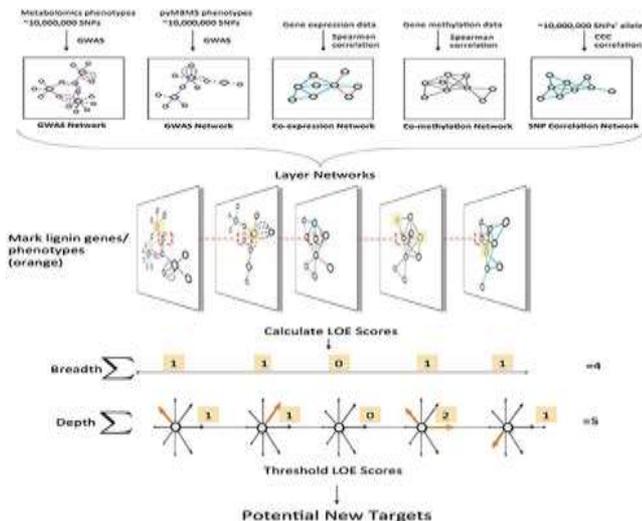
- Test corn stover, energy sorghum, switchgrass, miscanthus, and restored prairie feedstocks
- Pretreat with ammonia fiber expansion (AFEX)
- Ferment separately with yeast (*Saccharomyces cerevisiae* Y128) or bacteria (*Zymomonas mobilis* 8b).

Result/Impacts

- Most feedstocks showed similar process ethanol yields
- To increase ethanol yield in high-yielding crops, focus should be on improving biomass quality or conversion efficiency, whereas for low-yielding crops, focus should be on increasing crop yield.
- Results suggest that lignocellulosic biorefineries should be able to accept a wide range of feedstock types, which may improve the environmental footprint of the facility; biorefinery economics; and harvest, storage, and transportation logistics.



Systems biology leads the way to exascale computing on Summit supercomputer



Weighill, et al, "[Pleiotropic and epistatic network-based discovery: Integrated networks for target gene discovery](#)..." *Frontiers in Energy Research* 6, 30 (2018). [DOI: 10.3389/fenrg.2018.00030]

Objective

Use new computational approaches to utilize HPC for analysis of large amounts of genetic data with complex interactions to identify genes controlling important traits to target biofuel and bioproduct production

Approach

Data on 28 million genetic variations in *Populus* combined with co-expression, co-methylation and SNP correlation networks in a multi-omic, data layering approach

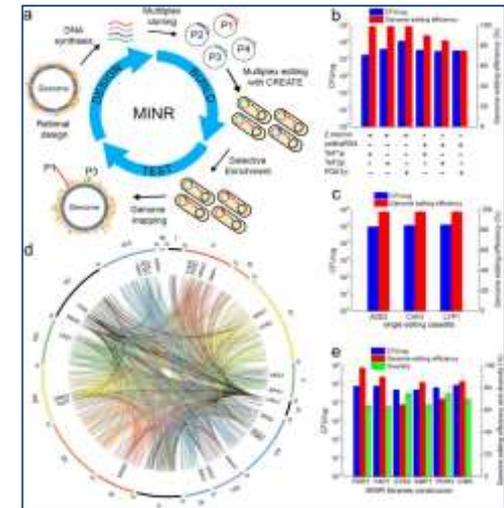
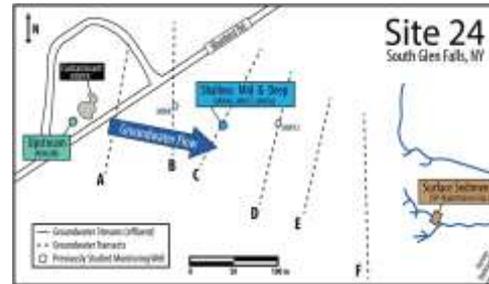
Result/Impacts

- Using the comparative genomics application combinatorial metrics, epistatic interactions were determined across the *Populus* genome
- Resulted in identification of new potential target genes involved in lignin biosynthesis/regulation
- Algorithm used in this work has been used to break the supercomputing exascale barrier for the first time anywhere in the world
- Ability to use exascale computing opens up possibilities to study highly complex and interrelated molecular processes in cells at a level of detail not previously possible
- **Fastest scientific calculation ever done**



What's new in KBase?

- 138+ Apps spanning assembly, annotation, comparative genomics, metabolic modeling, expression analysis, RNA-seq, etc.
- Released
 - Improved general and JGI search
 - JGI metagenome assembly pipeline
 - MetaBAT2
- In community testing
 - Genome homology service
- KBase users
 - > 4700 new users
 - > 2700 return users
- Flagship publication released July 2018



Online Webinars (<https://goo.gl/forms/0u4iWjRxJkWH8Zkm1>)

October 17 - 1PM EST

Analysis of Isolate Prokaryotes in KBase
Ben Allen - Oak Ridge National Laboratory

October 24 - 1PM EST

Metabolic Modeling in KBase
José P. Faria & Janaka Edirisinghe - Argonne National Laboratory

November 7 - 1PM EST

Analysis of Microbial Community Genomes in KBase
Dylan Chivian - Lawrence Berkeley National Laboratory

November 14 - 1PM EST

KBase Software Development Kit & Execution Engine
James Jeffryes & Boris Sadkhin - Argonne National Laboratory

November 28 - 1PM EST

Transcriptomics Workflow in KBase
Vivek Kumar - Cold Spring Harbor National Laboratory

Manuscripts citing KBase

Community dynamics and functional characteristics of naphthalene-degrading populations in contaminated surface sediments and hypoxic/anoxic groundwater. (Wilhelm, et. al., 2018, **Environmental Microbiology** doi:10.1111/1462-2920.14309)

Multiplex Navigation of Global Regulatory Networks (MINR) in yeast for Improved Ethanol Tolerance and Production (Liu, et.al., Accepted in **Metabolic Engineering**)

Towards predicting the environmental metabolome from metagenomics with a mechanistic model (Garza et. al, 2018, **Nature Microbiology** 3:456-460)

A new method for precise, high-throughput genome editing in eukaryotes

Objective

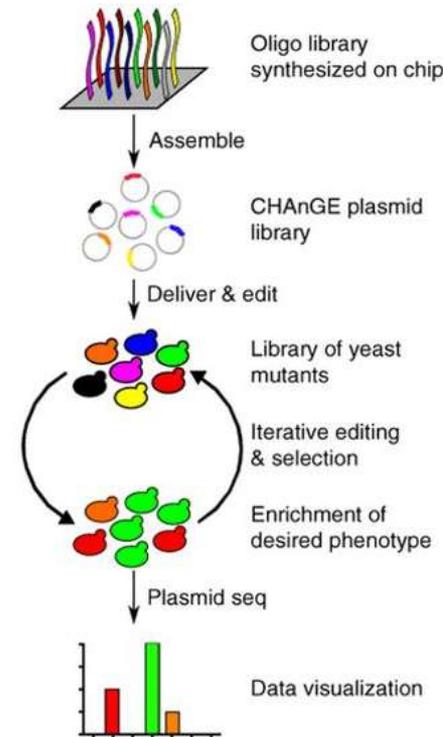
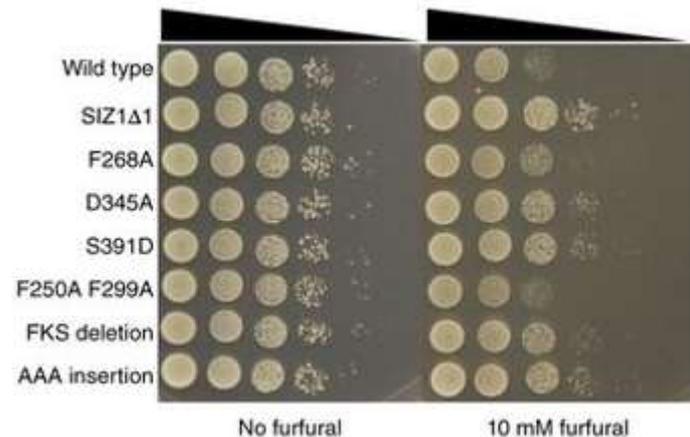
Develop a high-throughput, genome-wide targeted mutagenesis methods to track individual mutations in a eukaryotic organism

Approach

The CHAnGE (CRISPRCas9- and homology-directed-repair-assisted genome-scale engineering) method uses libraries of synthetic oligonucleotides containing a CRISPR guide sequence, gene-specific sequences to target homologous recombination to those selected genes, and unique barcodes to track each mutant strain.

Result/Impacts

- Synthetic CHAnGE cassettes for nearly 25,000 sequences covering virtually all yeast genes resulted in a 98% editing efficiency.
- The technology was effective for the introduction of both small deletion and single-base mutations, as well as for saturation mutagenesis of a single gene.
- This method was applied to engineer furfural tolerance in yeast, showing that it could be used to engineer industrially relevant eukaryotes to advance towards renewable production of biofuels and chemicals.



Z. Bao, M. HamediRad, P. Xue, H. Xiao, I. Tasan, R. Chao, J. Liang, H. Zhao, "Genome-scale engineering of *Saccharomyces cerevisiae* with single-nucleotide precision." *Nature Biotechnology* 36, 505 (2018). [DOI:10.1038/nbt.4132]

A Plant-Responsive Bacterial Signaling System Senses an Ethanolamine Derivative

Objective

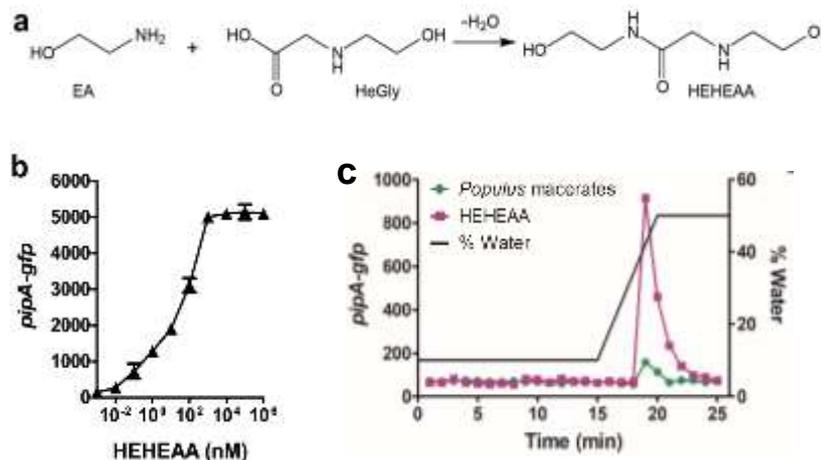
Advance the understanding of non-acyl-HSL-responsive LuxR homologs and how these transcription factors might be responding to their plant host environments

Approach

- To identify a more active PipR coinducer of pipA expression using *Pseudomonas* GM79 with $\Delta pipA \Delta aapA$ mutations containing pPipA-gfp

Result/Impacts

- Identified *N*-(2-hydroxyethyl)-2-(2-hydroxyethylamino) acetamide (HEHEAA) as the compound that binds to the ABC transporter and serves as a potent (pM) PipR-dependent inducer.
- Bioassay-active material in *Populus* leaf macerates co-elutes with HEHEAA by HPLC fractionation, suggesting HEHEAA is present in plant macerates.
- HEHEAA forms spontaneously from ethanolamine (EA), a component of plant phospholipids
- Identification of a GM79 PipR activator sets the stage for understanding interkingdom signaling and for identifying plant-derived signals for other PipR receptors, which are present in dozens of bacterial species associated with economically important plants



Linking production and consumption of soil metabolites to microbial species in a natural environment

EC Awardee
Trent Northen



Objective

Understand the linkage between microbial community structure and *in situ* metabolic activities.

Approach

Applying metabolomics and metagenomics to native biological arid soil crusts (biocrusts) upon changes in water availability, microbe-metabolite relationships were examined in comparison with previous data obtained using bacterial isolates from the same ecosystems.



Results/Impact

- Associations between biocrust exometabolites and microbial community members analyzed *in situ* correlated with those observed in isolated microbes from the same environments.
- Consumed and produced soil metabolites showed negative or positive correlation, respectively, with the presence of certain dominant bacterial taxa.
- The results show that integrating shotgun sequencing and exometabolomics can functionally link microbial community structure with environmental chemistry and carbon cycling.



Molecular Tomography via Three-Dimensional Mass Spectrometry Imaging

Mizzou

University of Missouri



Objective

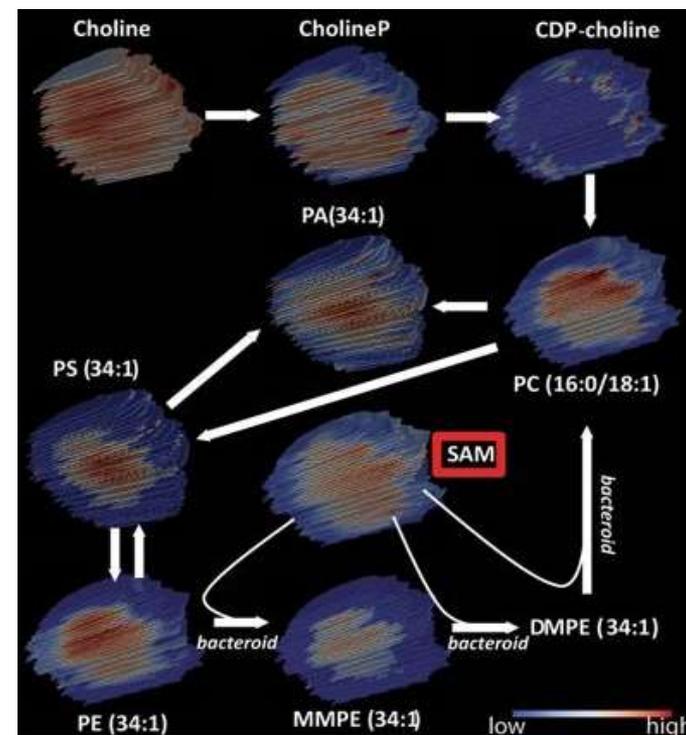
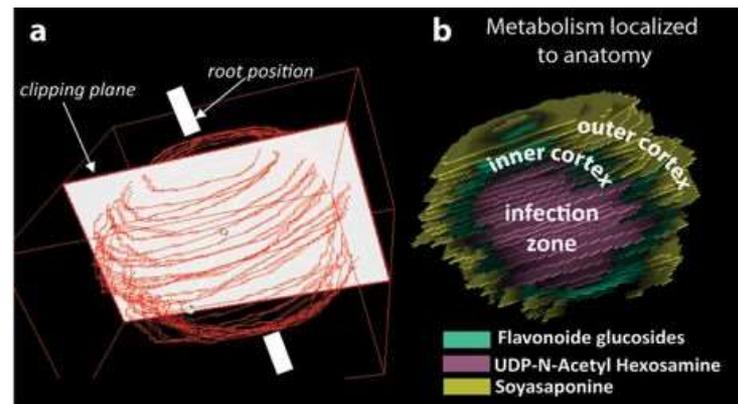
To enable the three-dimensional visualization of metabolite distributions within biological tissues and samples, which will permit researchers to reveal where active metabolic pathways occur.

Approach

- Advanced cryosectioning techniques, new robotic matrix spraying technology, and leading-edge data processing methods are able to accurately reconstruct highly reproducible two-dimensional matrix-assisted laser desorption/ionization mass spectrometry imaging data of serial sections into molecular tomographs.

Results/Impact

- Illuminated complex and unexpected metabolic behavior within soybean root nodules, a specialized organ formed from the symbiotic interaction between the plant and nitrogen fixing rhizobia. These results revealed that the infection zone of the root nodule is not a homogeneous metabolic compartment.



Veličković et al. (2018) "Observed metabolic asymmetry within soybean root nodules reflects unexpected complexity in rhizobacteria-legume metabolite exchange" *ISME J* (12) 2335–2338 DOI:10.1038/s41396-018-0188-8



Objective

Link changing biogeochemistry to specific microbial types involved in carbon processing in thawing permafrost peatlands.

Approach

Employed a genomic-centric approach to sample and assess the microbial and viral populations present in soils from a site within the Arctic Circle where the permafrost is rapidly changing.

Result/Impacts

- Recovered microbial genomes implicated specific microbial types involved in performing each step of degrading soil organic matter, from complex polysaccharides to CO₂ and methane.
- Doubled the number of known microbe-infecting viral groups worldwide with more than half the viruses being active and a ~1/3 linked to microbial hosts involved in carbon cycling microbes.
- New analysis methods for complex soil communities via a ‘genome-centric’ approach is a powerful new analytic framework to understand the microbial population’s ecology and function.
- Understanding the carbon processing of microbial communities in thawing permafrost is important to accurately predict the magnitude of climate feedbacks from these systems.

Emerson et al. Host-linked soil viral ecology along a permafrost thaw gradient. *Nature Microbiology* 3, 870-880 (2018). DOI: <http://dx.doi.org/10.1038/s41564-018-0190-y>

Woodcroft et al. Genome-centric view of carbon processing in thawing permafrost. *Nature* 560, 49-54 (2018). <https://doi.org/10.1038/s41586-018-0338-1>

76 JGI Publications Since April 25, 2018

Selected Highlights:

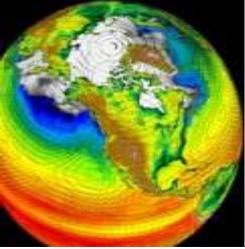
- Genome-centric view of carbon processing in thawing permafrost. *Nature*
- Large-scale replicated field study of maize rhizosphere identifies heritable microbes. *PNAS*
- Single cell genomic and transcriptomic evidence for the use of alternative nitrogen substrates by anammox bacteria. *ISME J*
- Coupled laboratory and field investigations resolve microbial interactions that underpin persistence in hydraulically fractured shales. *PNAS*
- Chemiluminescent Biosensors for Detection of Second Messenger Cyclic di-GMP. *ACS Chemical Biology*
- Evolutionary instability of CUG-Leu in the genetic code of budding yeasts. *Nature Communications*
- The Sphagnome Project: enabling ecological and evolutionary insights through a genus-level sequencing project. *New Phytologist*
- Recovery of genomes from metagenomes via a dereplication, aggregation and scoring strategy. *Nature Microbiology*



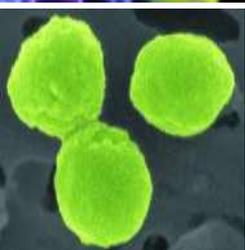
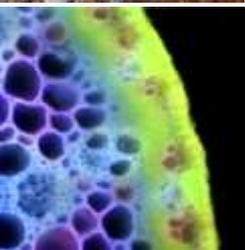
JGI news releases & highlights:

<http://jgi.doe.gov/news-publications/>

<http://jgi.doe.gov/category/science-highlights/>



<http://science.energy.gov/ber>



Thank you!



<http://genomicscience.energy.gov>