



U.S. DEPARTMENT OF
ENERGY

Office of
Science

Office of Biological and Environmental Research

Biological Systems Science Division Update

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Update on Programmatic Activities

Completed Reviews/Activities

- ✓ Workshop: "Genome engineering for materials synthesis" [Oct 9-11](#)
- ✓ Workshop: "Breaking the bottleneck of genomes: Understanding gene function" across taxa [Nov 1-2](#)
- ✓ Bioenergy Research Center (BRC) Annual Progress Reviews
 - CBI [Nov 14-15](#), JBEI [Dec 4-5](#), GLBRC [Jan 23-24](#), CABBI [Feb 20-21](#)
- ✓ ORNL Center for Structural Molecular Biology (CSMB) reverse site review [Dec 10](#)
- ✓ ORNL Biofuels Science Focus Area (SFA) reverse site review [Dec 11](#)
- ✓ BNL Quantitative Plant Science Initiative (QPSI) reverse site review [Jan 8](#)
- ✓ 2019 Genomic Science Annual PI Meeting [Feb 25-27](#)
- ✓ 2019 Bioimaging Science Annual PI Meeting [Feb 27-28](#)
- ✓ JGI User Meeting [Apr 2-5](#)

Upcoming Reviews/Activities

- ANL Structural Biology Center (SBC) review [May 29- 30](#)
- Panel Review of Early Career FOA Applications [June 7](#)
- Panel Review of Plant Biology FOA Applications [June 25-27](#)
- Panel Review of System Biology FOA Applications [June 25-27](#)
- Panel Review of Bioimaging FOA Applications [June 26-27](#)

New funding Opportunity Announcements for FY 2019

Systems Biology Enabled Research on the Roles of Microbiomes in Nutrient Cycling Processes (DE-FOA-0002059)

Proposals due: 5/17/19 – (Dr. Dawn Adin, Dr. Boris Wawrik)

Genomics-Enabled Plant Biology for Determination of Gene Function (DE-FOA-0002060)

Proposals due: 5/17/19 – (Dr. Cathy Ronning)

New Bioimaging Approaches for Bioenergy

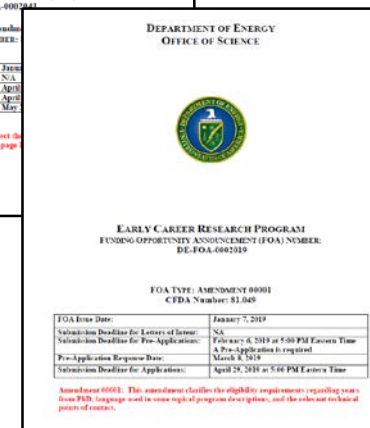
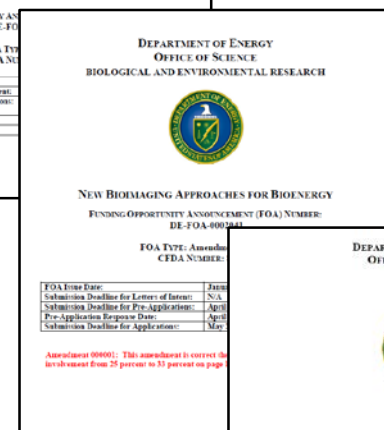
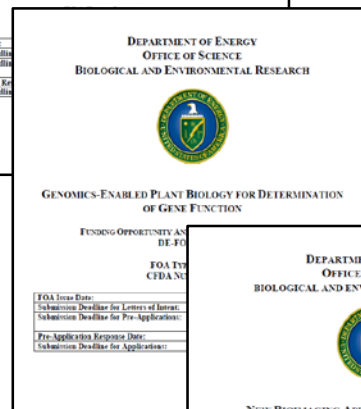
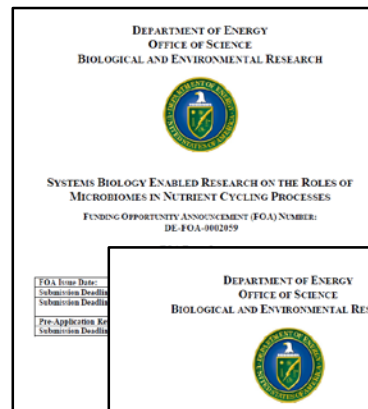
Pre-App due: 4/4/19

Pre-App Response: 4/19/19

Proposals due: 5/20/19 – (Dr. Prem Srivastava)

Early Career Research Program (BSSD)

Proposals due: 4/29/19 – (Dr. Pablo Rabinowicz)



Informative Workshops for BSSD Planning

BER Workshops

“Genome Engineering for Material Synthesis (GEMS)”

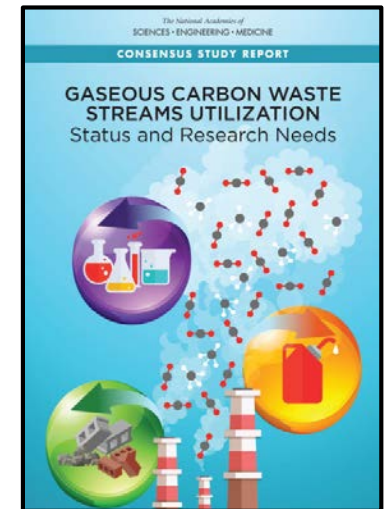
- Exploring possibilities for designed materials using genome engineering techniques
- October 9-11, 2018 (Brief out Friday – by **Dr. Caroline Ajo-Franklin** – LBNL)

“Breaking the bottleneck of genomes: Understanding gene function across taxa”

- Addressing the annotation problem in genomics
- November 1-2, 2018 (Brief out Today – by **Dr. Robin Buell** - MSU)

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) (Brief out Today – by **Dr. Michael Burkart** UCSD remote)



New Bioimaging Projects at the National Labs

Five new projects at the DOE Labs

“Detecting Chemical Signals in the Soil with QDaptamer-optical Fibers”

Ames National Laboratory (AMES) (PI: **Marit Nilsen-Hamilton**)

“Development of a Full-Field X-ray Fluorescence Imaging System for Near Real-Time Trace Element Microanalysis of Complex Biological Systems”

Brookhaven National Laboratory (BNL) (PI: **Ryan Tappero**)

“Illuminating the rhizosphere: Developing an adaptive optics, multiphoton microscope for 3D label-free live imaging of microbes and organic matter in soil and roots”

Lawrence Livermore National Laboratory (LLNL) (PI: **Peter Weber**)

“Intrinsically Co-registered Chemical Imaging of Living Plant and Microbial Systems via 3D Nonlinear Optical Mapping and In Situ-Liquid Extraction-Mass Spectrometry”

Oak Ridge National Laboratory (ORNL) (PI: **John Cahill**)

“Multimodal Chemical Imaging Across Scales to Visualize Metabolic Pathways in Live Plants and Microbial Systems”

Pacific Northwest National Laboratory (PNNL) (PI: **Scott Lea**)

Complements the 13 new University-led projects funded last year

Opportunities and Capabilities at the DOE Labs

Continuing to promote access to cryo-EM capabilities

Initiated procurement of a new NanoSIMS instrument at LLNL

- ✓ *Replaces an existing BER-funded instrument (16 yrs old)*
- ✓ *Co-funded with LLNL*

Secure Biosystems Design Pilot Projects

- ✓ *Requested ideas for small 1-yr projects*
- ✓ *ORNL, PNNL, ANL and SLAC*

Initiated plans for a National Microbiome Data Collaborative

- ✓ *Tasked LBNL with a multi-Lab effort for a computational infrastructure to support microbiome science*
- ✓ *Workplan is under development*

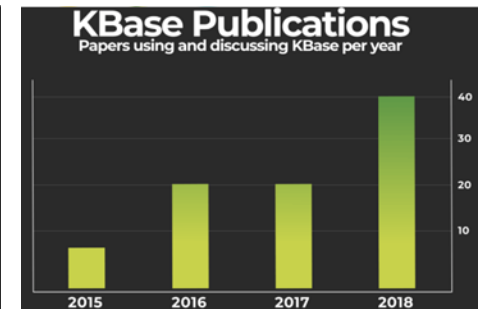
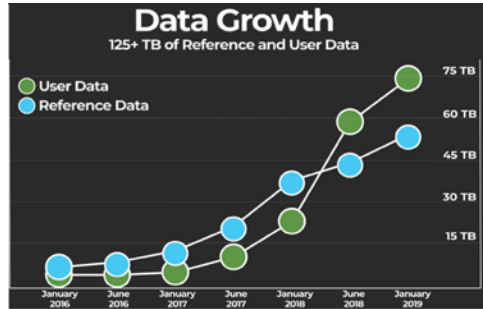
More info later as these efforts solidify



KBase Updates

Increasing User Impact

- Data > 125 TB
- Narratives > 10K
- Publications > 40
- Working with SFAs on data and tool development



Examples of Differentiating Platform Technologies

First Knowledge Engine App

Similar Data

Linked Data

- DifferentialExpressionMatrix - 78 total
- DifferentialExpressionMatrixSet - 3 total
- ExpressionMatrix - 91 total
- ExpressionSet - 6 total
- FBA - 2 total
- FBAModel - 3 total
- FeatureSet - 95 total
- Genome - 14 total
- PairedEndLibrary - 156 total
- RNASeqAlignment - 86 total
- RNASeqExpression - 86 total
- RNASeqSampleSet - 6 total
- ReadsAlignmentSet - 6 total
- ReadsSet - 4 total
- SingleEndLibrary - 8 total

Create Orgs to Share Data and Facilitate Collaborations

KBase KBase: DOE Systems Biology Knowledgebase
Systems Biology, Synthetic Biology, Microbiology, Metagenomics, Plant Biology, Community Ecology, Genomics, Functional Genomics, FAIR Principles, Reproducible Science, Open Science
owner Adam Paul Arkin
created Jan 7, 2019

JGI Joint Genome Institute
Functional genomics of microbes, plants and fungi, and communities of organisms. Synthetic biology. Secondary metabolites. Metabolomics.
owner Nigel Mouncey
created Feb 16, 2019

Arkin Laboratory
Systems Biology, Synthetic Biology, Computational Biology
owner Adam Paul Arkin
created Jan 25, 2019

Wetland Hydrobiogeochemistry SFA
Hydrobiogeochemical controls of water quality within wetlands
owner Ken Kettner
created Feb 8, 2019

Genome Homology Service Co-developed with JGI

Data View for Pseudomonas_fluorescens_SBW25

Similar Data

No linked data results.

Similar Assemblies

Distance	Name	Knowledge Score	Source
+ 0	Pseudomonas fluorescens SBW25	74	NCBI Refseq
+ 0.0107968	Pseudomonas fluorescens	1	NCBI Refseq
+ 0.0108013	Pseudomonas fluorescens	1	NCBI Refseq
+ 0.0103531	Pseudomonas sp. OVS46	1	NCBI Refseq
+ 0.0183979	Pseudomonas marginalis	1	NCBI Refseq
+ 0.0803	Pseudomonas sp. ChM02	1	NCBI Refseq
+ 0.0822272	Pseudomonas azotoformans	1	NCBI Refseq
+ 0.0837424	Pseudomonas fluorescens	1	NCBI Refseq
+ 0.0853563	Pseudomonas fluorescens	13	NCBI Refseq
+ 0.0868982	Pseudomonas fluorescens	1	NCBI Refseq
+ 0.0873037	Pseudomonas sp. NFPPII	1	NCBI Refseq
+ 0.087349	Pseudomonas sp. NFPPO4	1	NCBI Refseq
+ 0.0874398	Pseudomonas azotoformans	1	NCBI Refseq
+ 0.0874398	Pseudomonas azotoformans	1	NCBI Refseq
+ 0.0874852	Pseudomonas azotoformans NBR...	1	NCBI Refseq

Redirecting metabolic flux in *E. coli* via combinatorial CRISPRi-mediated repression for isopentenol production

Objective

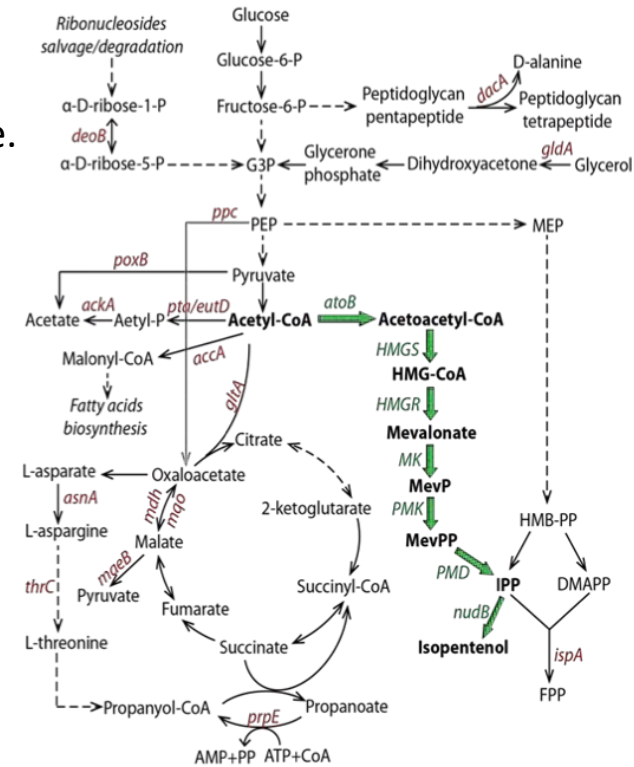
Use CRISPR interference (CRISPRi) to knock down endogenous genes in competing pathways to redirect metabolic flux toward target metabolite.

Approach

CRISPRi-mediated multiplex repression system to silence transcription of several endogenous genes to increase precursor availability in a heterologous isopentenol biosynthesis pathway.

Results/Impacts

- ✓ Designed a single-gRNA library with 15 individual knockdown targets, where 3 gRNA cassettes targeting genes *asnA*, *prpE*, and *gldA* increased isopentenol titer by 18–24%.
- ✓ We then combined the 3 single-gRNA cassettes into a two- or three-gRNA array and observed up to 98% enhancement in production by fine-tuning the repression level through titrating dCas9 expression.
- ✓ Our strategy shows that multiplex combinatorial knockdown of competing genes using CRISPRi can increase production of the target metabolite.



Re-directing metabolic flux toward isopentenol production using CRISPR-dCas9 repression system. (A) Pathway map of gene knockdown target. Red colored genes are the knockdown targets, green colored genes are overexpressed on two plasmids. (B) Isopentenol production with dCas9-gRNA plasmids targeting different genes. (C) Combinatorial multiplex repression using CRISPR-dCas9 system for isopentenol production.

Tian et al. (2019) *ACS Synthetic Biology*, doi: 10.1021/acssynbio.8b00429

Genome-Wide Analysis of Nitrate Transporter (NRT/NPF) Family in Sugarcane *Saccharum spontaneum* L.

Objective

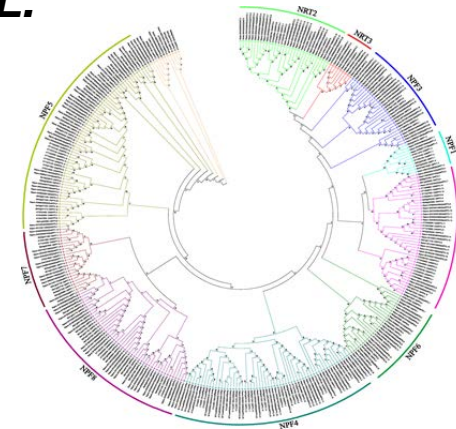
Plants take up nitrate using transmembrane proteins of the Nitrate Transporter (NRT)/Peptide Transporter family (NPF). Understanding nitrogen uptake, translocation, and utilization is key to improve nitrogen-use efficiency (NUE).

Approach

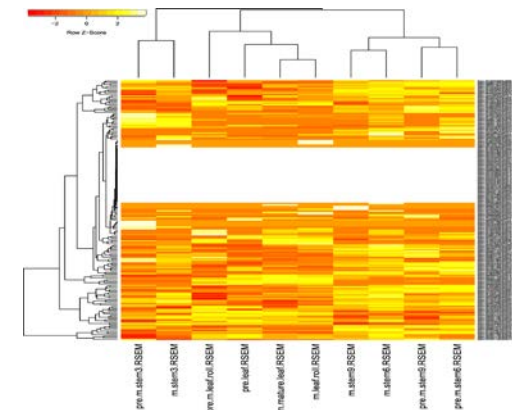
Researchers conducted a large, comprehensive genome-wide analysis of NRT/NPF genes in *S. spontaneum*, which included a BLAST search, phylogenetics, gene structure, protein motif analysis, and RNA-seq data analysis.

Result/Impacts

- ✓ Identified 178 NPF genes, 20 NRT2 genes and NRT3 genes
- ✓ Both SsNPF and SsNRT3 genes showed diversified gene structures and expression patterns, suggesting the possibility to fine-tune the homeostasis of nitrate to cope with the changing environment.
- ✓ The NRT/NPF genes identified provide the basis for further research of nitrate transporter functions in sugarcane to help optimize nitrogen management in bioenergy crops.



Phylogenetic relationship of NRT/NPF proteins among *Arabidopsis*, *Sorghum*, and *Saccharum*.



Heat map of tissue-specific expressions of NRT/NPF genes from *Saccharum*.

Wang, J., Li, Y., Zhu, F. et al. (2019) "Genome-Wide Analysis of Nitrate Transporter (NRT/NPF) Family in *Sugarcane Saccharum spontaneum* L.", **Tropical Plant Biology**, DOI: 10.1007/s12042-019-09220-8

Knock-down of Galacturonosyltransferase-4 gene leads to reduction in lignin-carbohydrate crosslinking in switchgrass

Objective

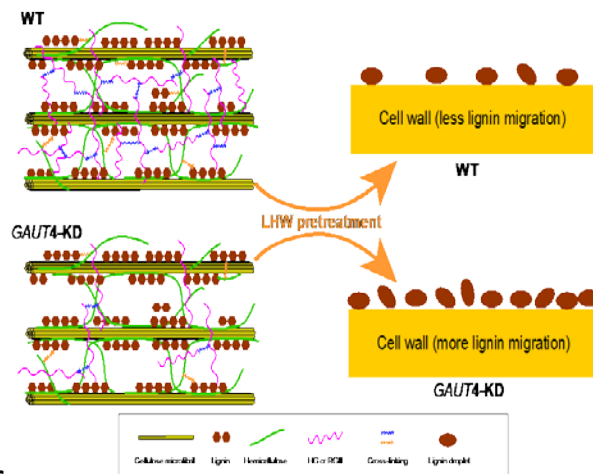
Investigate downregulation of a pectin biosynthetic gene, GAUT4, on the structures of lignin and their importance to the recalcitrance of switchgrass.

Approach

Chromatographic and NMR analyses of the molecular structure of lignin and hemicellulose as well as the level of lignin-carbohydrate crosslinking in GAUT4-downregulated switchgrass.

Results/Impacts

- ✓ GAUT4-KD lines have a lower abundance of ferulate and lignin-carbohydrate complex cross-linkages, reduced hemicellulose molecular weights, and reduced amounts of residual arabinan and xylan in lignin-enriched fractions, in comparison to the wildtype (WT).
- ✓ These structural changes in lignin and hemicellulose lead to a greater coalescence and migration of lignin after hydrothermal pretreatment. The results reveal the roles of both decreased lignin-polymer and pectin cross-links in the reduction of recalcitrance in GAUT4-KD switchgrass.



GAUT4-KD lines have reduced ferulate and lignin-carbohydrate levels, and reduced retention of lignin on liquid hot water (LHW) pretreated biomass, leading to the proposed mechanistic model of reduced recalcitrance in hydrothermally pretreated biomass.

Li M. et al. "Downregulation of pectin biosynthesis gene *GAUT4* leads to reduced ferulate and lignin-carbohydrate cross-linking in switchgrass", *Communication Biology*. Accepted

Biological funneling of aromatics from chemically depolymerized lignin produces a desirable chemical product

Objective

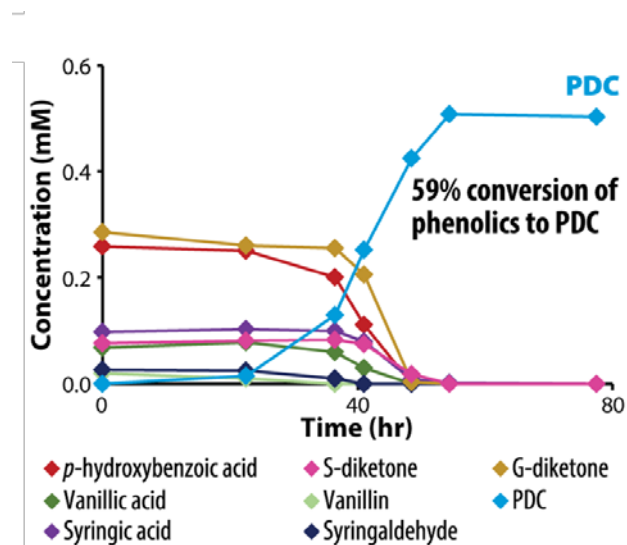
Engineer *Novosphingobium aromaticivorans* to funnel heterogeneous mixtures of lignin-derived aromatic compounds to 2-pyrone-4,6-dicarboxylic acid (PDC), a bioplastic precursor.

Approach

- Develop a model for the degradation of plant-derived aromatic compounds in *N. aromaticivorans*.
- Create a defined set of mutations predicted to result in simultaneous production of PDC from all three major plant-derived aromatic compounds.
- Determine yield of PDC for the mutant strain when grown on a variety of aromatic compounds, including depolymerized poplar lignin.

Results/Impacts

- ✓ The engineered strain converts aromatic compounds into PDC with yields ranging from 22% to 100%; heterogeneous depolymerized lignin is funneled to PDC with a 59% yield.
- ✓ Valuable advance in funneling mixtures of aromatic compounds into defined single commodities.



Perez, J. M. *et al.* "Funneling aromatic products of chemically depolymerized lignin into 2-pyrone-4-6-dicarboxylic acid with *Novosphingobium aromaticivorans*." *Green Chemistry* (2019) [DOI: 10.1039/C8GC03504K].



ENIGMA SFA Highlight

Plasmidome sequencing from ground water microbial communities reveals new genes and an enrichment in metal resistance

Objective

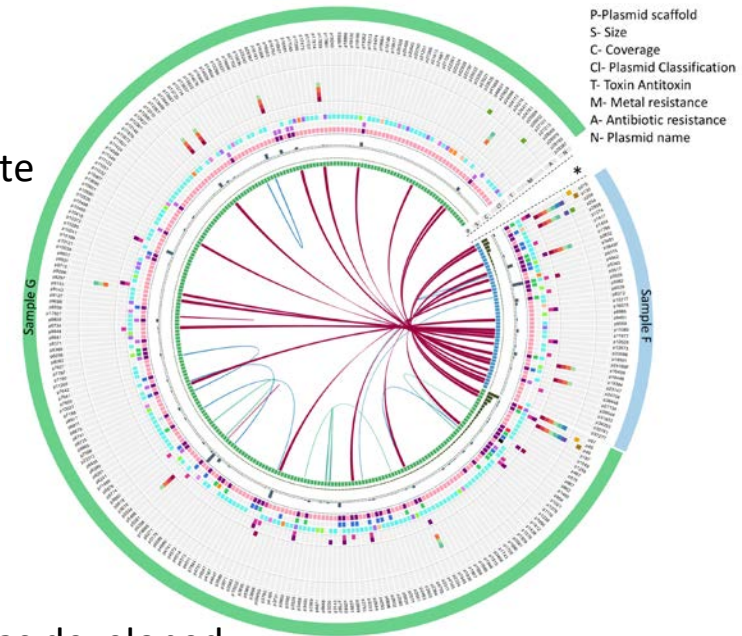
Characterize novel mobile genetic elements in ground water environments to discover new genes and functionality of microbial communities in the Oak Ridge Field Research Center site

Approach

A new plasmid purification method was developed to isolate plasmid populations from microbial communities from ground water with low concentration of cells. New plasmid-encoded functionality was discovered by sequencing and assembling the plasmidome.

Result/Impacts

- ✓ A new method for plasmid purification from water samples was developed
- ✓ Over 600 plasmids from multiple sizes and incompatibility groups were identified
- ✓ An enrichment in metal resistance genes was observed in addition to other traits
- ✓ The plasmid pool was diverse but less than the corresponding microbiome
- ✓ Novel genes discovered in this study will allow to engineer new microbial strains



Kothari et al., 2019. *mBio* 10:e02899-18

Biosystems Design Highlight

Understanding metabolic control in the emerging model green microalga *Chromochloris zofingiensis*

Objective

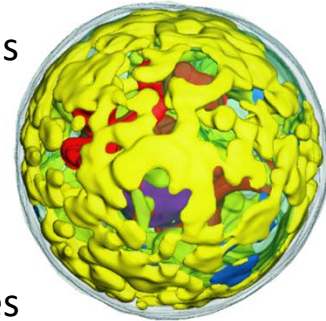
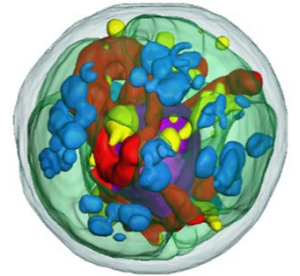
Understand the molecular mechanisms of trophic control of metabolism in microalgae to enable engineering organisms for the production of oils and valuable chemicals

Approach

Using state of the art microscopy, x-ray tomography, mass spectrometry, and transcriptomics analyses, the physiological and genetic responses of the green alga *Chromochloris zofingiensis* to changes in glucose availability was elucidated in detail.

Result/Impacts

- ✓ After a few days of glucose addition to the culture in the presence of light, the algal cells shut off photosynthesis and largely disassemble thylakoids
- ✓ Accumulation of triacylglycerols and other molecules such as carotenoids increase substantially
- ✓ The observed metabolic changes are readily reversible upon removal of glucose
- ✓ The expression of one third of *C. zofingiensis* genes is altered during the trophic changes
- ✓ This work paves the way to identify target genes to develop new engineered strains that accumulate high amounts of biofuels and bioproducts



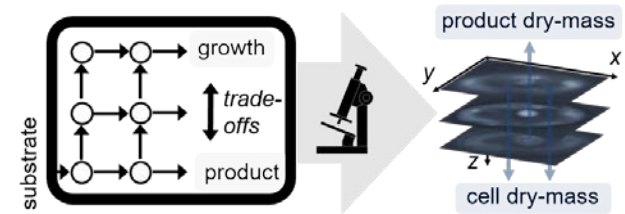
Roth et al., 2019. *The Plant Cell*:tpc.00742.02018.

The cryo-soft X-ray tomography was supported by the Photosynthetic Systems program in the DOE Office of Basic Energy Sciences

Imaging the metabolic trade-off cellular noise

Objective

To develop high-throughput phenotyping of the metabolic trade-offs between growth and the production of biofuel precursors in single, live cells and, as such, to quantify how systems-level noise impacts cellular resource allocations.

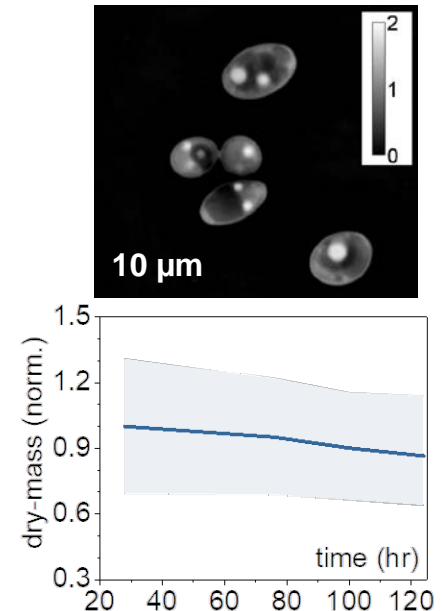


Approach

Interferometric imaging quantifies the phase delay through the cell cytosol and, aided by nanoscale secondary ion mass spectrometry, converts it to the dry-mass of cytosolic compartments via dedicated image processing.

Result/Impacts

- ✓ Improved precision of interferometric imaging over conventional, volumetric microscopy.
- ✓ Quantification of cell-to-cell heterogeneity in the density-volume relationship.
- ✓ Systems-level cellular noise impacts growth and production differently.
- ✓ Unlike volumetric imaging, interferometric imaging uniquely unmasks catabolic fluxes under nutrient starvation.



Vasdekis et al. (2019) "Eliciting the impacts of cellular noise on metabolic trade-offs by quantitative mass imaging"

Nature Communications 10, Article number: 848 DOI: <https://doi.org/10.1038/s41467-019-08717-w>

Eucalypt Genome Expands Terpene Synthesis Knowledge

Objective

Characterizing the terpene synthase (TPS) gene family lineage in closely related *Corymbia* and *Eucalyptus* species will enable researchers to develop strategies to increase terpene production for biofuel development.

Approach

- Compared putative genes from *C. citriodora* to known TPS gene sequences from *Eucalyptus* species and other plants.
- The locations of TPS genes and gene clusters were mapped against those of *E. grandis* to find differences in genome organization between the two species.
- Sequenced mRNA from different tissues of *C. citriodora*. From these samples, a total of 127 TPS loci were found, many of which had high sequence similarity to TPS genes from other plants.

Results/Impact

- ✓ Identified 102 total putative functional TPS genes in *C. citriodora*.
- ✓ TPS genes found in *C. citriodora* suggest that these plants synthesize a high level of secondary metabolites which play a part in biotic and abiotic stress responses.
- ✓ Targeting this gene family may enable researchers selection of specific TPS genes to increase terpene production for biofuel production.

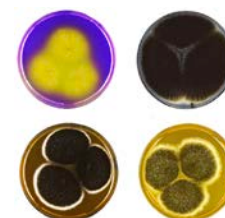
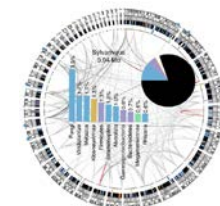
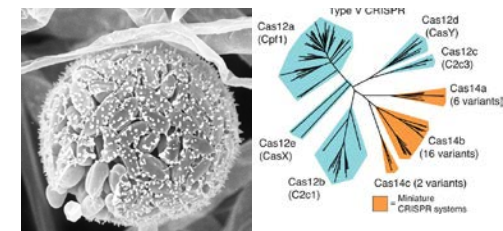


Corymbia citriodora

Butler JB *et al.* Annotation of the *Corymbia* terpene synthase gene family shows broad conservation but dynamic evolution of physical clusters relative to *Eucalyptus*. *Heredity*. 2018 Jul;121(1):87-104. doi: 10.1038/s41437-018-0058-1.

Selected Highlights:

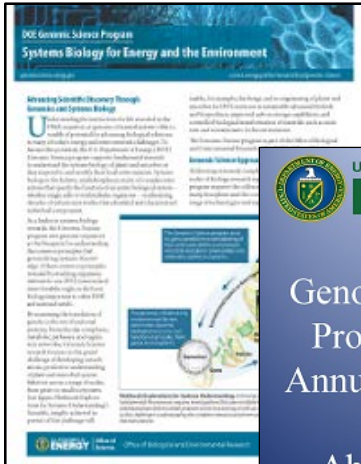
- *Novel insights from uncultivated genomes of the global human gut microbiome.* **Nature**
- *Diversity of cytosine methylation across the fungal tree of life.* **Nature Communications**
- *The genomic landscape of molecular responses to natural drought stress in *Panicum hallii*.* **Nature Communications**
- *Programmed DNA destruction by miniature CRISPR-Cas14 enzyme.* **Science**
- *Minimum Information about an Uncultivated Virus Genome.* **Nature Biotechnology**
- *Hidden diversity of soil giant viruses.* **Nature Communications**
- *Pezizomycetes genomes reveal the molecular basis of ectomycorrhizal truffle lifestyle.* **Nature Ecology & Evolution**
- *Investigation of inter- and intraspecies variation through genome sequencing of *Aspergillus* section *Nigri*.* **Nature Genetics**
- *Leveraging single-cell genomics to expand the fungal tree of life.* **Nature Microbiology**



JGI news releases & highlights:

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

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



Thank you!

<http://genomicscience.energy.gov>

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