Office of Biological and Environmental Research

Biological Systems Science Division Update

Todd Anderson, Ph.D.
Director, Biological Systems Science Division,
Department of Energy, Office of Biological &
Environmental Research

February 26, 2015

U.S. DEPARTMENT OF
ENERGY

Office
of Science

Office of Biological
and Environmental Research

Programmatic Activities

Completed Reviews/Activities

- Pacific Northwest National Laboratory Foundational Genomic Science SFA review (Nov. 17) - complete
- Bioenergy Research Centers annual reviews (Oct-Nov-Dec) - complete
- Joint Genome Institute (JGI) Triennial Review (Dec 8-10) - complete
- Early Career Panels (Feb 10) – reviews completed
- SBIR review process - in progress
- Genomic Science Annual PI Meeting (Feb 23-25) – earlier this week
- Review of PNNL Pan-Omics Project (Feb 25-26) – today

Upcoming Reviews/Activities

- Panel Reviews for FY 2015 FOAs
- Reviews of Bioimaging Projects at the DOE National Laboratories
- DOE National Laboratory Science Focus Area (SFA) Triennial Reviews
  - ANL, LANL, NREL, ORNL-Biofuels, ORNL-Foundational

Portfolio Context for the New FOAs

New *In Situ* Imaging and Measurement Technologies for Biological Systems

Research for Sustainable Bioenergy: Linking Genomic and Ecosystem Sciences

Workshop Report

Plant Feedstock Genomics for Bioenergy Joint Awards 2006–2014

GenomicScience.energy.gov/research/DOEUSDA/

September 2014

Novel in situ Imaging and Measurement Technologies for Biological Systems Science (DE-FOA-0001192)

- *Academic complement to five projects ongoing at the DOE National Laboratories*

Due Date – December 18, 2014

Systems Biology Research to Advance Sustainable Bioenergy Crop Development (DE-FOA-0001207)

- *Follows a recent workshop on sustainable bioenergy research*
- *Builds on advances in plant and microbial research within the:*
  - *Genomic Science program*
  - *Bioenergy Research Centers (BRCs)*
  - *DOE National Laboratory programs (SFAs)*

Due Date – January 16, 2015

Plant Feedstock Genomics for Bioenergy: A Joint Research Funding Opportunity Announcement USDA, DOE (DE-FOA-0001249)

- *Builds on past FOAs with USDA (ten years!)*
- *Includes a focus on plant-plant pathogen interactions*
- *Complements bioenergy efforts within the BRCs*

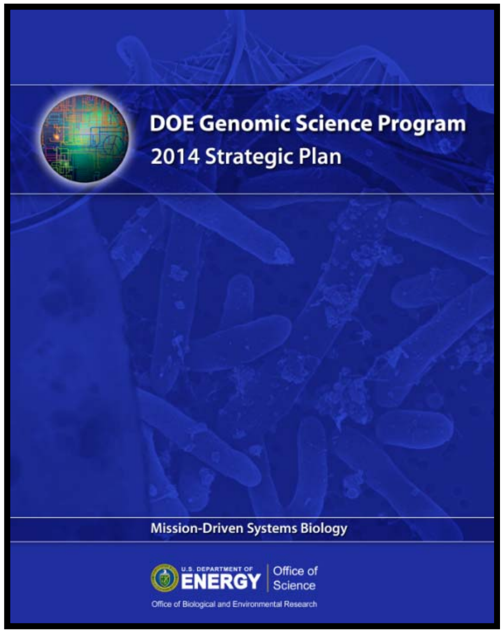
Due Date – March 2, 2015

Panel Reviews Scheduled for Spring

Strategic Planning

Revision of the Genomic Science Program Strategic Plan

- *Systems Biology for:*
  - *Bioenergy Research*
  - *Biosystems Design*
  - *Sustainable Bioenergy*
  - *Carbon Cycling and Biogeochemical Processes*
- *Cross Cutting Computational Biology, Bioinformatics and Predictive Modeling*


DOE Genomic Science Program
2014 Strategic Plan
Mission-Driven Systems Biology
U.S. DEPARTMENT OF ENERGY | Office of Science
Office of Biological and Environmental Research

Available online at: http://genomicscience.energy.gov/index.shtml

Systems Biology Knowledgebase (KBase)

A Community Resource for Predictive Biology

An open-source and open-architecture computational environment for integrating large, diverse datasets, generated by the Genomic Sciences program and other sources, and for using this information to advance predictive understanding, manipulation, and design of biological systems.

Accelerates the use of complex data by biologists:

- *encourages best science practices: access to data, sharing, publishing, reproducibility of analyses*
- *provides access to the best tools for the analysis of large, complex data sets*
- *provides metrics for success and utility of data, tools etc.*
- *lowers the bar for the analysis of complex data sets and modeling with high performance computers*
- *synthesizes biological data from the community to answer questions beyond the ability of the single PI or small teams of researchers*

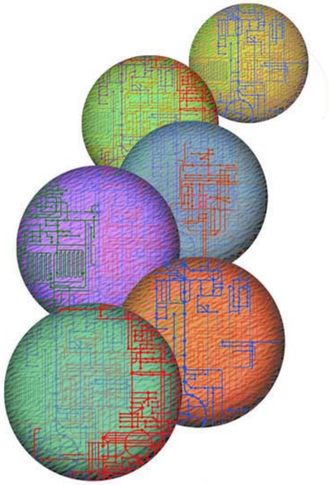
KBase includes (to date):

22,253 microbial genomes
96 eukaryotic genomes (56 plants)
15,462 metagenomic datasets
13,111 public reference models
28 analysis services
60+ point & click analysis functions

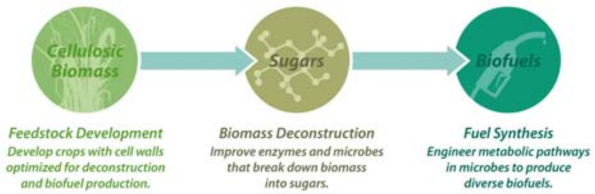
http://www.kbase.us

Beta-Tester comments on new features

"Painless... Finally doable for a non-command line / oldster / PI like myself!"
"The app looks really promising especially for users without computational experience, though it takes a bit to get used to. I plan to recommend it to members of the lab."
"The site looks like you have made some significant improvements. Congrats!"
"The Narrative interface is very nice and easy to use. Your methods and apps are interesting and relevant."
"Interesting site so far! Looking forward to working with it in the future! looks good!!!"



Bioenergy Research Centers (BRCs)



Feedstock Development
Develop crops with cell walls optimized for deconstruction and biofuel production.

Biomass Deconstruction
Improve enzymes and microbes that break down biomass into sugars.

Fuel Synthesis
Engineer metabolic pathways in microbes to produce diverse biofuels.

Scientific goals (summarized) for FY 2015

Great Lake Bioenergy Research Center (GLBRC)

- Develop a functional metagenomics description of the rhizosphere for bioenergy crops
- Modify the cell wall structure in bioenergy crops for reduced recalcitrance and sugar yield
- Engineer efficient biosynthetic pathways for NextGen biofuels

Joint BioEnergy Institute (JBEI)

- Engineer plant cell walls with optimized matrix polysaccharide composition
- Develop and optimize new biomass pretreatment techniques based on ionic liquids
- Continue discovery and engineering of novel hydrocarbon biochemistries and biosynthesis

BioEnergy Science Center (BESC)

- Identify key biomass structural features that increase sugar release
- Generate modified and natural variant bioenergy crops improved for conversion into biofuels
- Establish thermophilic pathways for advanced biofuel production

To date the BRCs have generated :

- **660 invention disclosures and/or patent applications**
- **24 patents awarded**
- **105 licensing agreements**
- **1799 peer-reviewed publications**

BESC
BioEnergy Science Center
jbei
Joint BioEnergy Institute
GREAT LAKES BIOENERGY RESEARCH CENTER

BRCs actively engage industry in partnerships and license agreements

Pacific Ethanol, Inc.
Dow
ceres
Weyerhaeuser
POET biorefining
MONSANTO
SCION forests · products · innovation
novozymes Rethink Tomorrow
Cargill
Borregaard LignoTech
AMYRIS
Lignol
GREENWOOD RESOURCES
evolva
GE
firstgreen PARTNERS
DSM BRIGHT SCIENCE. BRIGHTER LIVING.
IOGEN CORPORATION
BOEING
FuturaGene Yielding the Future™
teselagen BIOTECHNOLOGY
MillerCoors™
allopartis
GINKGOBIOWORKS™
PlantProbes
MASCOMA
ZeaChem
genomatica
LYGOS
Nextval
opxbio good chemistry.
bp
Mendel BIOTECHNOLOGY
ADM
SAFFRON EAGLE
BIOFUELS
ABENGOA
ExxonMobil
LS9, INC.
ARBORGEN
GM
Oakbio Clean Technology, Clean Products
Statoil
Elanco
SuGanit
BASF The Chemical Company
TOTAL
Hyrax ENERGY
Caliper a PerkinElmer company
DUPONT
Show Me Energy Cooperative
empowering the next generation...
...through a flexible model that allows plant matter to be turned into fuel and power

BRC Science Highlight

Field performance of modified switchgrass demonstrates a "Goldilocks Effect"

Objective

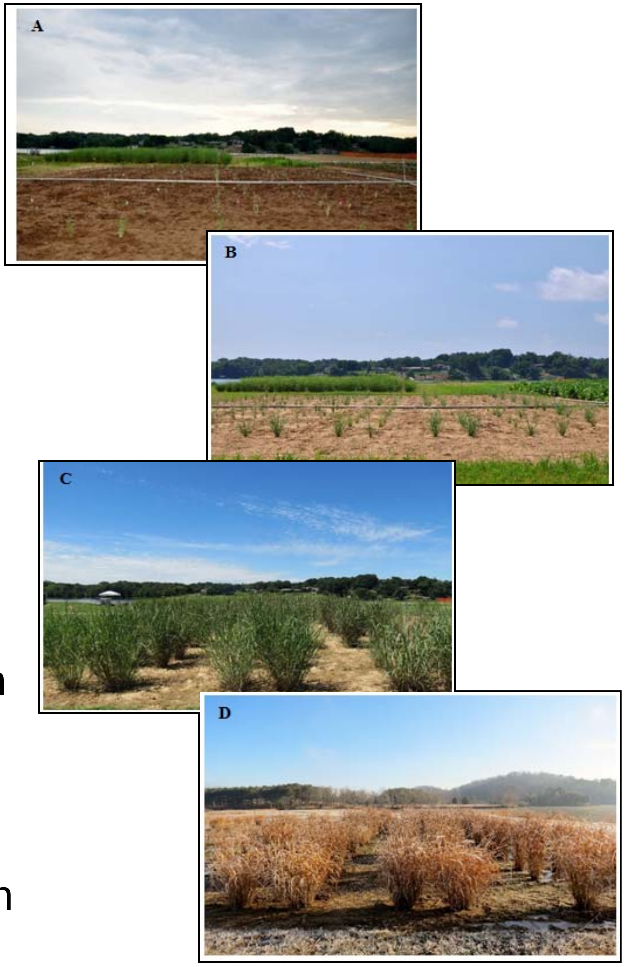
Demonstrate the field performance of transformed, low recalcitrance switchgrass.

Approach

Transgenic plants overexpressing the switchgrass MYB4 (PvMYB4) transcription factor gene, which regulates the expression of several lignin biosynthesis genes were tested in the field over two growing seasons.

Results/ Impact

- One transgenic event (out of eight) yielded gains in both biofuel (32% increase) and biomass (63% increase).
- Too high PvMYB4 transgene expression was fatal to plants in the field, whereas too low expression was no different from controls; but when optimal expression is achieved the altered switchgrass produces higher biomass and biofuel yields.
- These gains represent a potential to double biofuel production per hectare over conventional feedstocks. This is the highest potential gain reported to date from any field-grown modified feedstock.



Photos of the field experiments in the first (2012) and second (2013) growing seasons. (a) July 3 2012: day of transplantation; (b) August 1 2012; (c) August 1 2013; (d) December 11 2013

Baxter, *et al*., "Field evaluation of transgenic switchgrass plants overexpressing PvMYB4 for reduced biomass recalcitrance," ***BioEnergy Research**,* 2014, accepted for publication.

Lignin Depolymerization for Valorized Aromatics

Objective

To obtain high-yield, low-molecular weight aromatic compounds that have intrinsic value or are suitable for downstream processing from native sources of lignin.

Approach

➢ Oxidation of a specific C-O bond in aspen lignin followed by depolymerization in a weak acid solution

➢ Determine wt% yield from lignin and identities of the aromatic compounds using LC-MS.

Result/Impacts

- Lignin depolymerization methods typically result in low yields of aromatics; the lignin oxidation step greatly increases the yield (> 60 wt%) and the products are well-defined.
- A more efficient process that potentially may enhance the lignin value chain and improve the commercial and economic viability of lignocellulosic biofuels.

Rahimi, A., Ulbrich, A., Coon, J.J., and Stahl, S.S. 2014. *Formic Acid Induced Depolymerization of Oxidized Lignin to Aromatics*. **Nature**, 515 (7526), 249-252.

jbei
Joint BioEnergy Institute

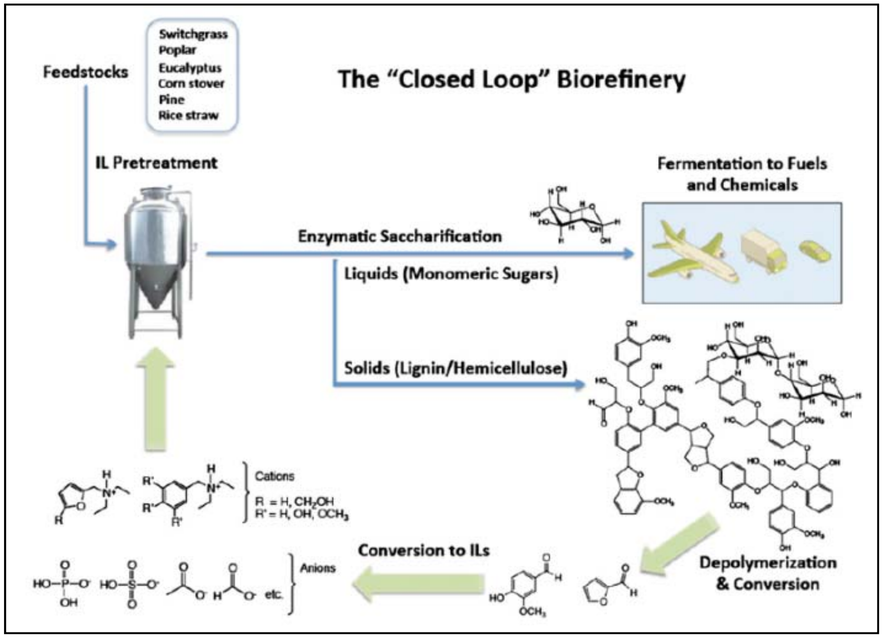
Ionic Liquids (ILs) derived from biomass – provide an effective biomass pretreatment

Objective

Ionic Liquids (ILs) are an excellent pretreatment solvent for biomass however, the availability and cost of the ILs remains an issue.

Approach

- Evaluate the synthesis of ILs from monomers obtained directly from lignin and hemicellulose.
- Use molecular modeling to compare IL solvent parameters with experimentally obtained compositional analysis data.
- Demonstrate effective pretreatment of switchgrass



Process scheme for a closed-loop biorefinery using ILs derived from lignocellulosic biomass

Results/Impact

- Three renewable biomass-derived ILs were produced from lignin and hemicellulose in excellent yields.
- Renewable ILs generated comparable sugar yields relative to current imidazolium-based ILs.
- Cost projections of renewable ILs are $4/kg, much lower than top performing conventional ILs.
- Production of renewable ILs shows significant potential for the realization of a "closed-loop" process for future lignocellulosic biorefineries.

Socha, *et al.*, "Efficient biomass pretreatment using ionic liquids derived from lignin and hemicellulose," ***PNAS***, *published online August 18, 2014, http://www.pnas.org/content/early/2014/08/15/1405685111.full.pdf+html*

A new strategy to prevent the proliferation of engineered organisms in the environment

Objective

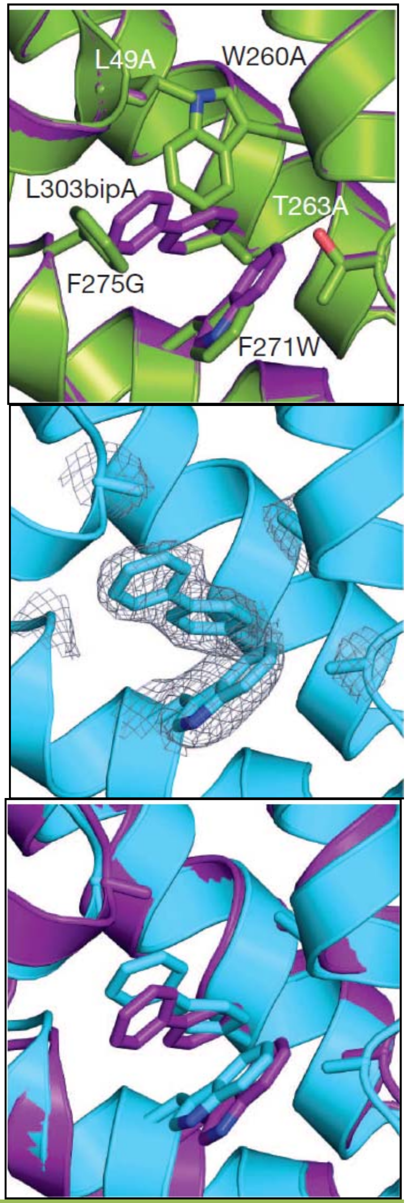
Design a biocontainment mechanism that prevents the growth of engineered microbes outside the laboratory.

Approach

Essential genes in an *E. coli* strain with a “recoded” genome that lacks the UAG codon, were redesigned so that the proteins they code for require the incorporation of a specific non-standard amino acid (NSAA) in newly introduced UAG codons.

Results/Impact

- The redesigned proteins are functional and allow the cell to live and reproduce only if the NSAA is provided in the medium. The lack of that NSAA in natural environments prevents the modified strain from growing outside the laboratory.
- The investigators redesigned three essential genes within the same strain, reducing the number of reversion by mutation or by horizontal gene transfer to undetectable levels.
- The technology will advance toward the safe production of fuels and other chemicals in engineered microbial cells.



Mandell D, Lajoie M, Mee M, Takeuchi R, Kuznetsov G, Norville J, Gregg C, Stoddard B, and Church G. “Biocontainment of genetically modified organisms by synthetic protein design” ***Nature*** (online: January 21, 2015) doi:10.1038/nature14121

Methane Production at a Thawing Permafrost Site Dominated by a Single Microbial Species

Objective

Examine microbial community dynamics and CH_4 production at a permafrost-to-wetland transitional ecosystem in northern Sweden

Approach

A combination of molecular genetics, isotopic analysis, and CO_2/CH_4 flux measurement was used to examine relationships between microbial community dynamics and carbon cycle processes

Results/Impact

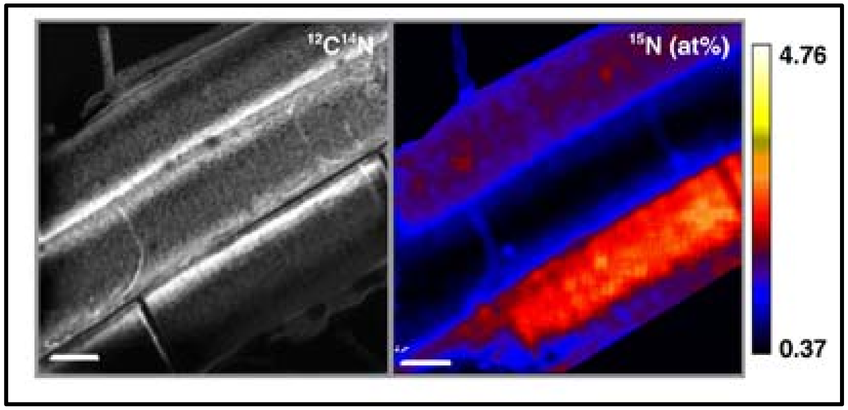
- A distinct successional pattern of CH_4 and CO_2 production was observed over the transitional gradient that strongly correlated with shifts in microbial community structure and associated functional processes.
- CH_4 production in recently thawed sites was dominated by a single microbial species, *Methanoflorens stordalenmirensis,* a potentially "predictive lineage" that has been detected at numerous other thawing permafrost ecosystems.
- Provides a new approach for integrating microbial community structure/function data with isotopic biogeochemistry analyses at the ecosystem scale.

McCalley et al. (2015) ***Nature*** 514: 478-81 doi:10.1038/nature13798

Investigating Nitrogen Fixation in a Photosynthetic Microbial Community

Objective

Examine a cyanobacteria dominated microbial mat community and identify which species provide nitrogen fixing activities



NanoSIMS image of *Lyngbya* spp cyanobacterial filaments from mats incubated in the presence $^{15}N_{2;}$. Color intensity in right panel indicates relative incorporation of ^{15}N into cell biomass. Bar = 5 um

Approach

Used a combination of metatranscriptomics and nanoSIMS analysis to broadly assay functional potential and quantitatively measure N_2 fixation at the single cell scale

Results/Impact

- Metatranscriptomic analysis of gene expression identified several potential candidates as important N_2 fixers, while high resolution functional analysis with nanoSIMS more clearly identified *Lyngbya* spp cyanobacteria as the dominant organisms for this process
- Highlights the importance of pairing genomics and gene expression profiling with complementary approaches that provide quantitative functional measurements.
- Provides a more sophisticated understanding of the mechanisms driving a highly productive photosynthetic ecosystem that serves as an possible model for bioenergy production

Woebken et. al. (2015) *Revisiting N2 fixation in Guerrero Negro intertidal microbial mats with a functional single-cell approach*, ***ISME J.*** 9:485-496.

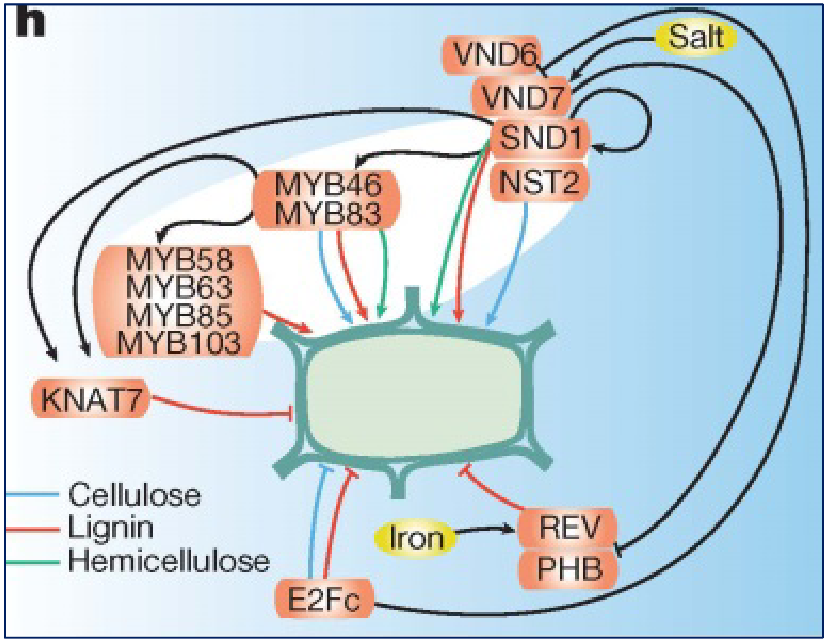
Elucidating control of secondary cell wall synthesis

Objective

Use network-based approach to characterize transcriptional regulation of secondary cell wall biosynthesis

Approach

- Combined high-spatial-resolution gene expression data and literature search to identify genes implicated in xylem cell specification
- Tested ability of model to predict response under abiotic stress (iron deprivation, salinity)



Results/Impact

- Comprehensive, large-scale analysis revealed highly integrated network involving hundreds of genes and protein-DNA interactions
- Combinatorial possibilities allows for subtle adaptation to specific abiotic stresses
- Provides framework to further dissect and refine specific gene functions, and allow targeted manipulation to produce high-yielding plant feedstocks for bioenergy production

Taylor-Teeples et al. 2014. ***Nature*** 517:571-578 doi:10.1038/nature14099

Early Career Highlight

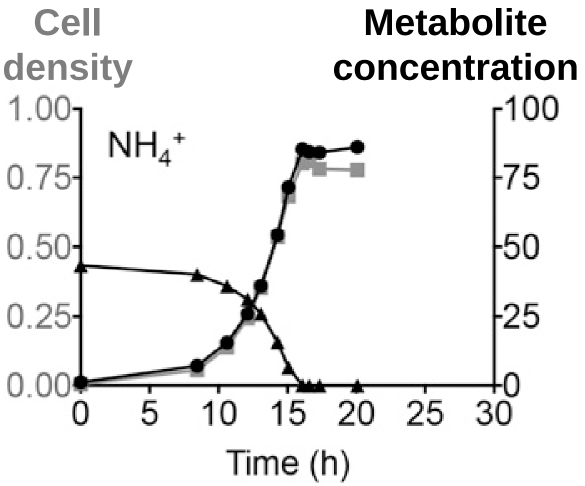
Nitrogen gas is a cost-effective supplement for bacterial production of cellulosic ethanol

Objective

Increase the efficiency of biofuels production from biomass using the bacterium *Zymomonas mobilis*.

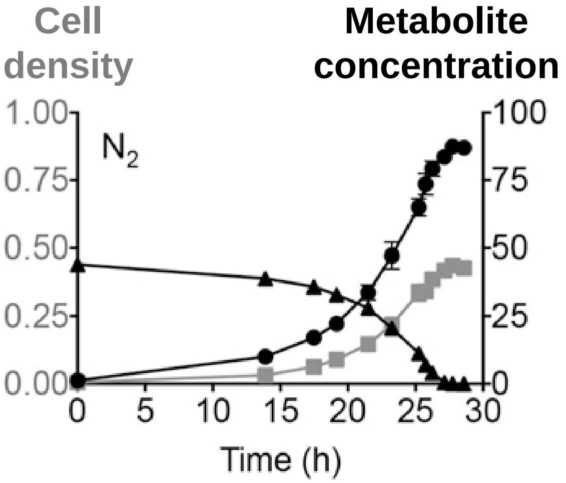
Approach

Compare production of ethanol by *Z. mobilis* growing in bioreactors containing hydrolyzed *Miscanthus* biomass as a feedstock, supplemented with either N_2 gas or chemical nitrogen sources.



Results/Impact

- When N_2 gas was supplied as a nitrogen source it was readily utilized by *Z. mobilis* without decreasing ethanol production, which was close to the theoretical maximum.
- Biomass production in the bacterial culture was reduced by half when N_2 gas was supplied as the nitrogen source.
- The use of N_2 as nitrogen source in bioreactors can decrease the nutrient costs up to ten-fold, making *Z. mobilis* an alternative to yeast for high-yield ethanol production.



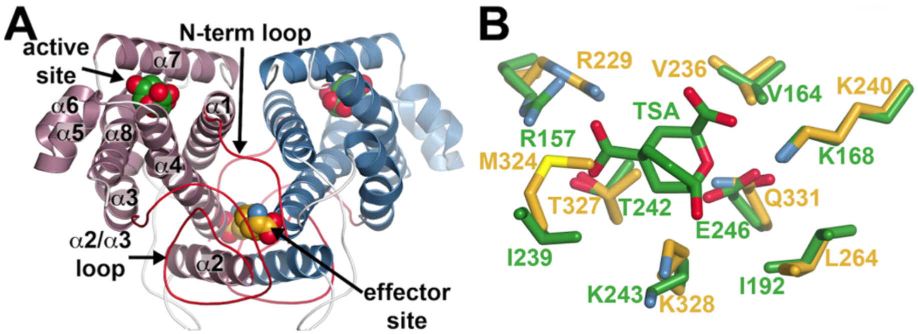
Kremer T, LaSarre B, Posto A, and McKinlay J. ***Proc Natl Acad Sci USA*** (Feb 2, 2015)
http://www.pnas.org/content/early/2015/01/27/1420663112

Structural Biology Facility Access

Structural Evolution of Differential Amino Acid Effector Regulation in Plant Chorismate Mutases

Objective

Understand the structural basis for how a key enzyme regulates production of valuable biofuel and chemical products in plants



A, ribbon diagram showing the chorismate mutase isoform 1/phenylalanine complex dimer. The-helices of monomers A and B are colored rose and blue, respectively.
B, overlay of active site residues from the *A. thaliana* chorismate mutase isoform 1/phenylalanine complex (gold) and the yeast chorismate mutase transition state analog (TSA) complex (green).

Approach

- Obtain crystal structures of chorismate mutase isoform 1 from *Arabadopsis thaliana* in complex with activating and inhibiting small molecules, using beamline 19-ID at the Structural Biology Center (Argonne)
- Compare with structures of the enzyme obtained from other species such as yeast

Result/Impacts

- The structures of the enzyme complexes show how the pathway is controlled by metabolites such as tyrosine and phenylalanine
- The valuable industrial chemical 2-phenylethanl can be produced using prephenate, a product of the chorismate mutase pathway; the new information will help understand how to optimize production of this chemical in plants.

C.S. Westfall, A. Xu & J.M. Jez, “Structural evolution of differential amino acid effector regulation in plant chorismate mutases”. *J Biol. Chem.*, **289**(41), 28619-28 (October 10, 2014) [19ID].

DOE Joint Genome Institute

Next Community Science Program (CSP) Call - OPEN

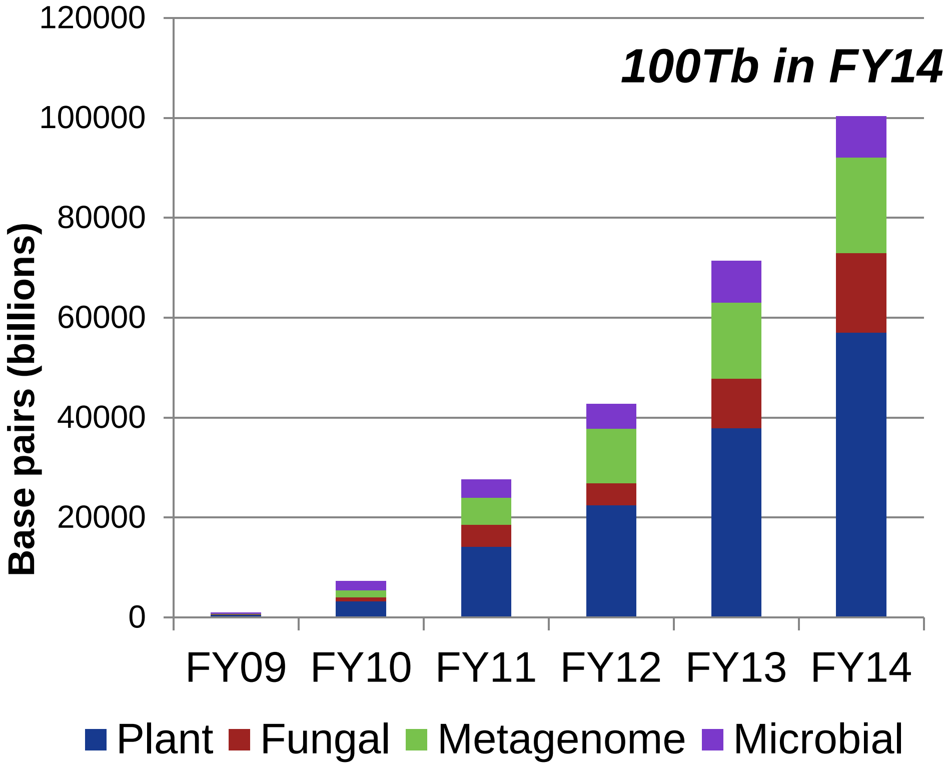
- ➢ *Letters of Intent due: April 16, 2015*
- Functional Genomics and Microbiomes of DOE JGI Flagship Plants
- Function-driven Microbial Genomics
- Extreme Environments including Deep Subsurface
- DNA Synthesis Projects

JGI-EMSL Collaborative Science Initiative - OPEN

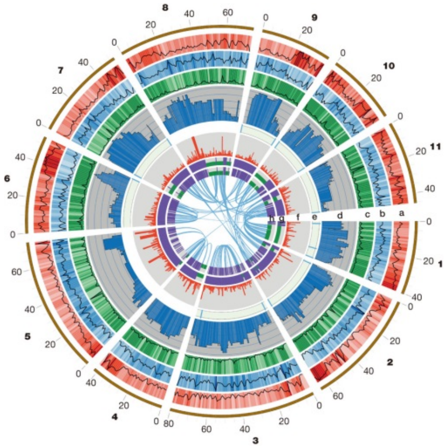
- ➢ *Letters of Intent due: April 6, 2015*
- Biofuels and Bioproducts
- Plant-Microbe Interactions that impact climate

JGI Main Webpage with Links to both Calls

http://jgi.doe.gov/



Eucalyptus Genome Completed



640Mb, 11 chromosomes

Objective

Sequence *Eucalyptus,* the most widely planted hardwood forest trees in the world

Approach

- Eucalypts are a globally important (>20M ha) resource for pulp, paper, biomaterials and bioenergy
- *E. grandis* was sequenced as representative example of this hardwood lineage.

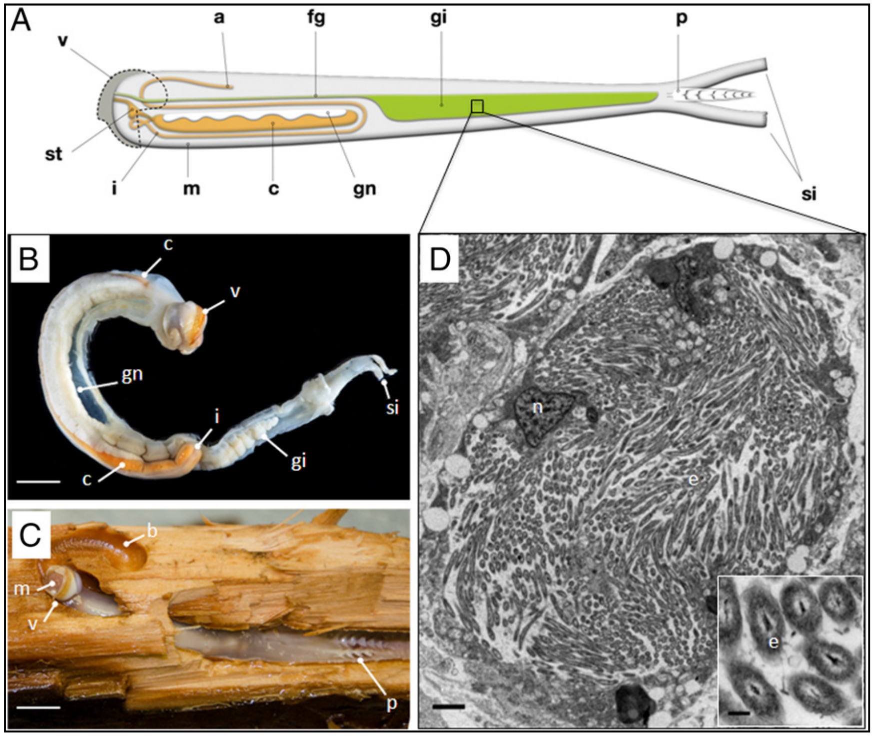
Result/Impacts

- The *E. grandis* genome in combination with other hardwood genomes (*Populus, Citrus, Prunus, Vitis*) provides a comparative basis on which to understand the evolution of hardwoods
- *Eucalyptus* displays a wide diversity of specialized metabolites including terpenes among several other compounds useful for biofuels and biochemicals development

Myburg, AA, et. al.. (2014) The genome of Eucalyptus grandis **Nature** *510:356-362* doi:10.1038/nature13308

JGI
JOINT GENOME INSTITUTE
DEPARTMENT OF ENERGY

Sequencing the microbial community in the shipworm, the "termite of the seas"



Objective

Shipworms are historically famous for their abilities to digest the wood in the hulls of ships.

Approach

Metagenomic sequencing of the microbes in the gills (where the cellulose-digesting microbes are found.)

Results/Impact

- Enzymes from microbes in the gills, not the microbes directly acting in the gut (which is free of bacteria), carry out wood digestion in the shipworm.
- This work expands the known biological repertoire of bacterial endosymbionts to include food digestion and identifies previously undescribed enzymes and enzyme combinations of potential value to biomass-based Industries such as cellulosic biofuel production.

O'Connor RM et al. Gill bacteria enable a novel digestive strategy in a wood-feeding mollusk.
Proc Natl Acad Sci *U S A*. 2014 Nov 25;111(47):E5096-104.

Joint Genome Institute

Selected Publications

Aylward FO et al. Convergent bacterial microbiotas in the fungal agricultural systems of insects. *MBio*. 2014 Nov 18;5(6). pii: e02077-14..

Bartholome J et al. High-resolution genetic maps of Eucalyptus improve *Eucalyptus grandis* genome assembly. *New Phytol*. 2014 Nov 10.

Kirby J et al. Enhancing Terpene Yield from Sugars via Novel Routes to 1-Deoxy-d-Xylulose 5-Phosphate. *Appl Environ Microbiol*. 2014 Oct 17;81(1):130-8.

Kruse T et al. Genomic, proteomic and biochemical analysis of the organohalide respiratory pathway in *Desulfitobacterium dehalogenans*. *J Bacteriol*. 2014 Dec 15. pii: JB.02370-14.

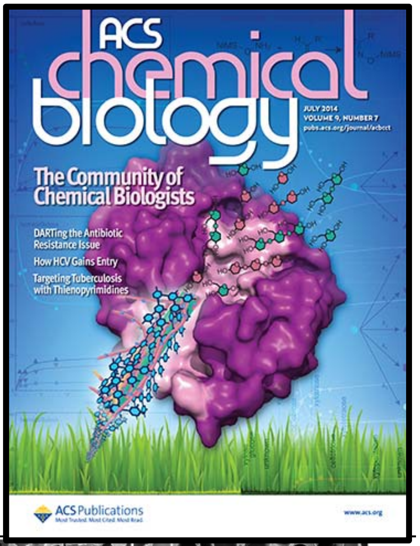
Lim YW et al. Purifying the Impure: Sequencing Metagenomes and Metatranscriptomes from Complex Animal-associated Samples. *J Vis Exp*. 2014 Dec 22;(94).

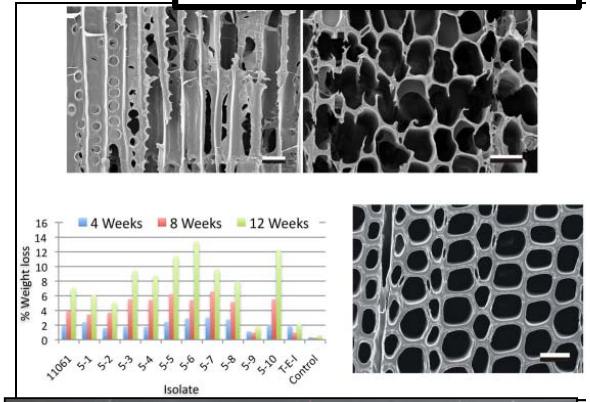
O'Connor RM et al. Gill bacteria enable a novel digestive strategy in a wood-feeding mollusk. *Proc Natl Acad Sci U S A*. 2014 Nov 25;111(47):E5096-104.

Woo HL et al. Complete genome sequence of the lignin-degrading bacterium *Klebsiella sp.* strain BRL6-2. *Stand Genomic Sci*. 2014 Dec 8.

Woyke T et al. Evolution. Searching for new branches on the tree of life. *Science*. 2014 Nov 7;346(6210):698-9.

Woyke T et al. Function-driven single-cell genomics. Microb Biotechnol. 2015 Jan;8(1):38-9.





40 publications since the last BERAC meeting

JGI Publication Highlights: http://www.jgi.doe.gov/News/pubs.html

Systems science to meet DOE mission needs in bioenergy, climate and the environment.

http://science.energy.gov/ber

Thank you!

http://genomicscience.energy.gov

Genomic Science Program
Systems Biology for Energy and Environment

Genomic Science Program

Today, scientists have in hand the complete DNA sequences of genomes for many organisms--from microbes to plants to humans. The U.S. Department of Energy's Genomic Science program (formerly Genomics:GTL) uses microbial and plant genomic data, high-throughput analytical technologies, and modeling and simulation to develop a predictive understanding of biological systems behavior relevant to solving energy and environmental challenges including bioenergy production, environmental remediation,

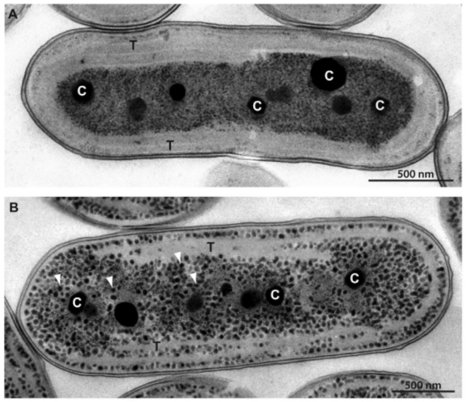
Ideal Photosynthetic Microbe for Bioenergy Applications?

Challenge – Identify a novel cyanobacterial strain that grows rapidly and is amenable to genetic manipulation—ideal traits for a wide range of synthetic biology and metabolic engineering applications.

Approach & Results

- Research team led by Washington University found a new cyanobacterial strain called *Synechococcus elongatus* UTEX 2973 that grows as fast as yeast without requiring special nutrients such as vitamins.
- The team sequenced the genome, characterized the proteome and manipulated the genetic system to turn on and off specific genes, thereby demonstrating the this strain is amenable to easy and rapid genetic modification.

Significance and Impact – The ability to genetically manipulate this strain combined with its rapid growth rates suggests it could have significant potential for industrial biofuel production.



S. elongatus UTEX 2973, a novel cyanobacterial strain that grows rapidly and is amenable to genetic manipulation.

Participants: Washington University in St. Louis, the Department of Energy's EMSL and Pacific Northwest National Laboratory, and the University of Texas at Austin.

Reference: Yu, J, M Liberton, PF Cliften, RD Head, JM Jacobs, RD Smith, DW Koppenaal, JJ Brand, and HB Pakrasi. 2015. "*Synechococcus elongatus* UTEX 2973, a fast growing cyanobacterial chassis for biosynthesis using light and CO_2." Scientific Reports 5:8132. DOI:10.1038/srep08132