

# ***Office of Biological and Environmental Research***

## ***Biological Systems Science Division Update***

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Environmental Research

February 26, 2015



U.S. DEPARTMENT OF  
**ENERGY**

Office  
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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**

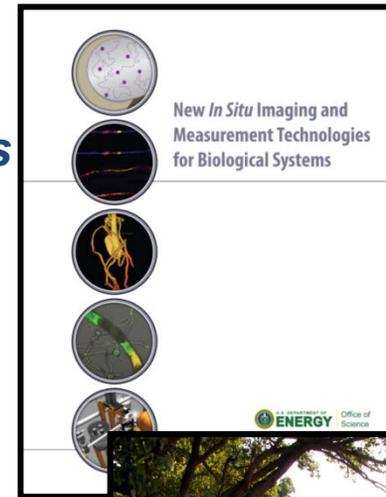
- Panel Reviews for FY 2015 FOAs
- Reviews of Bioimaging Projects at the DOE National Laboratories
- DOE National Laboratory Science Focus Area (SFA) Triennial Reviews
  - ANL, LANL, NREL, ORNL-Biofuels, ORNL-Foundational

# Portfolio Context for the New FOAs

## 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

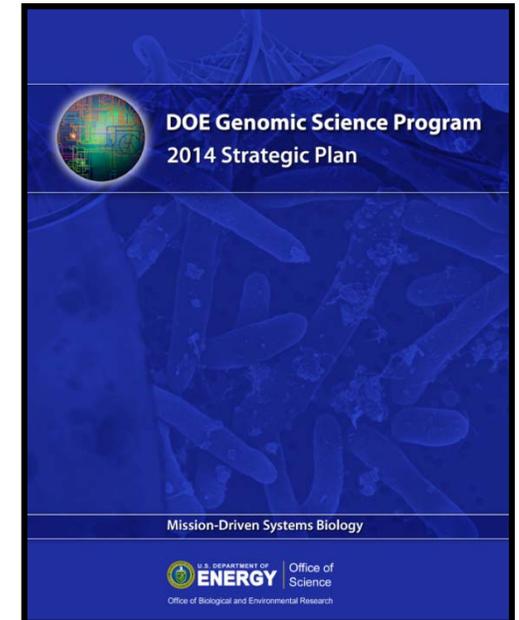


**Panel Reviews Scheduled for Spring**

# *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*



**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*

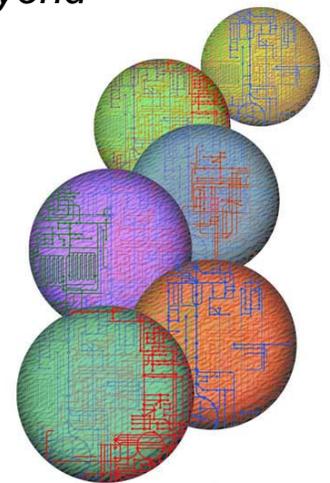
### ***KBase includes (to date):***

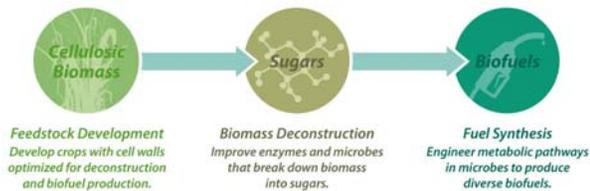
22,253 microbial genomes  
96 eukaryotic genomes (56 plants)  
15,462 metagenomic datasets  
13,111 public reference models  
28 analysis services  
60+ point & click analysis functions

<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)

## 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



# *BRCs actively engage industry in partnerships and license agreements*



# *Field performance of modified switchgrass demonstrates a “Goldilocks Effect”*

## **Objective**

Demonstrate the field performance of transformed, low recalcitrance switchgrass.

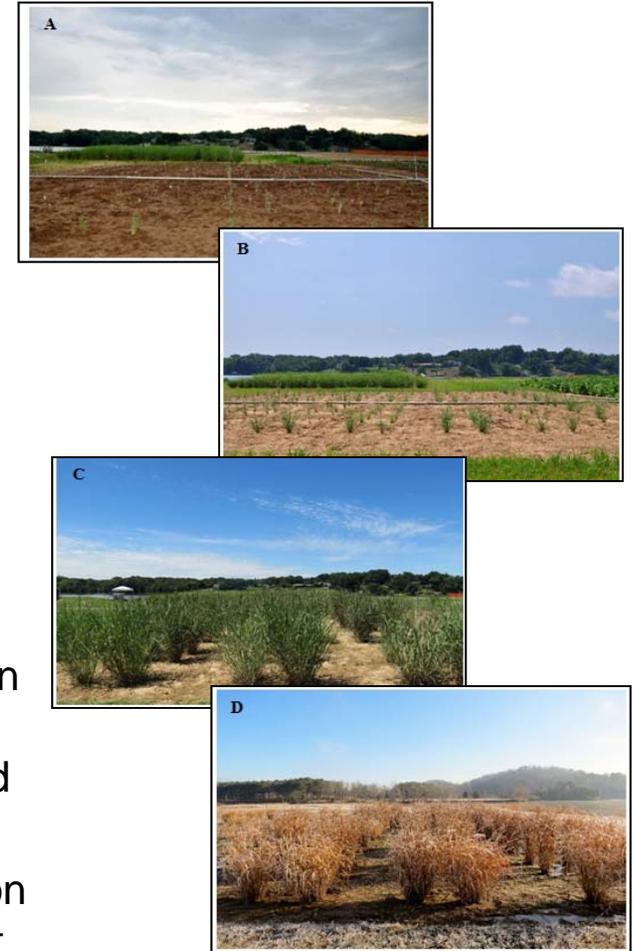
## **Approach**

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.

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



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



## *Lignin Depolymerization for Valorized Aromatics*

### **Objective**

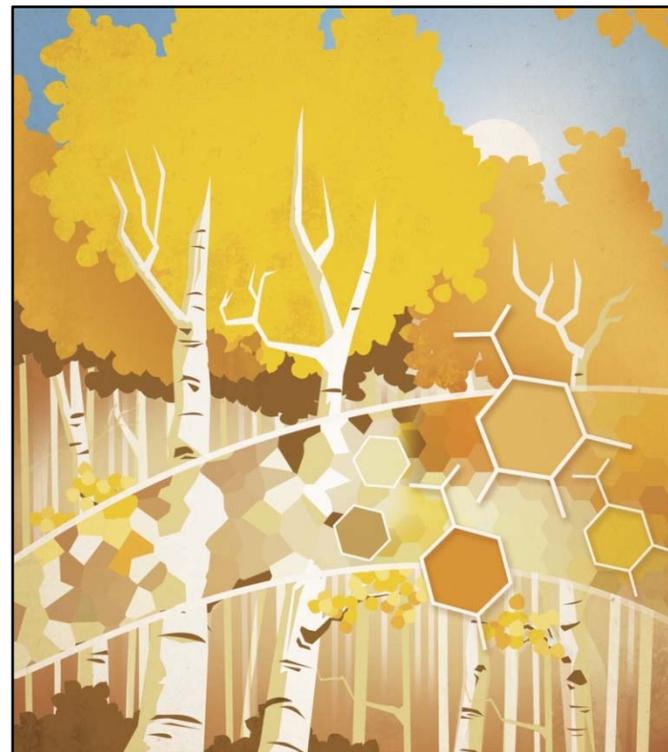
To obtain high-yield, low-molecular weight aromatic compounds that have intrinsic value or are suitable for downstream processing from native sources of lignin.

### **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.

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

## **Objective**

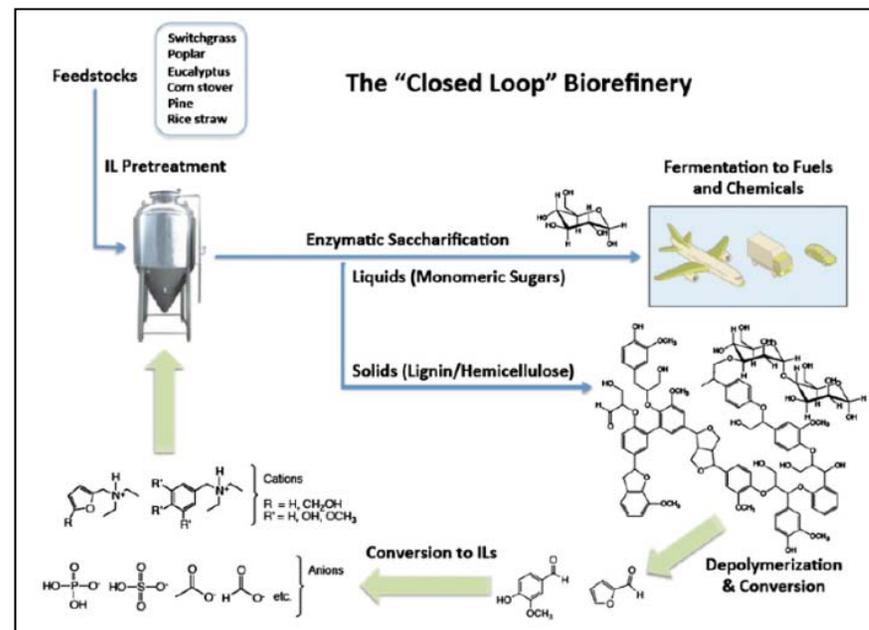
Ionic Liquids (ILs) are an excellent pretreatment solvent for biomass however, the availability and cost of the ILs remains an issue.

## **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

## **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.



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

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.

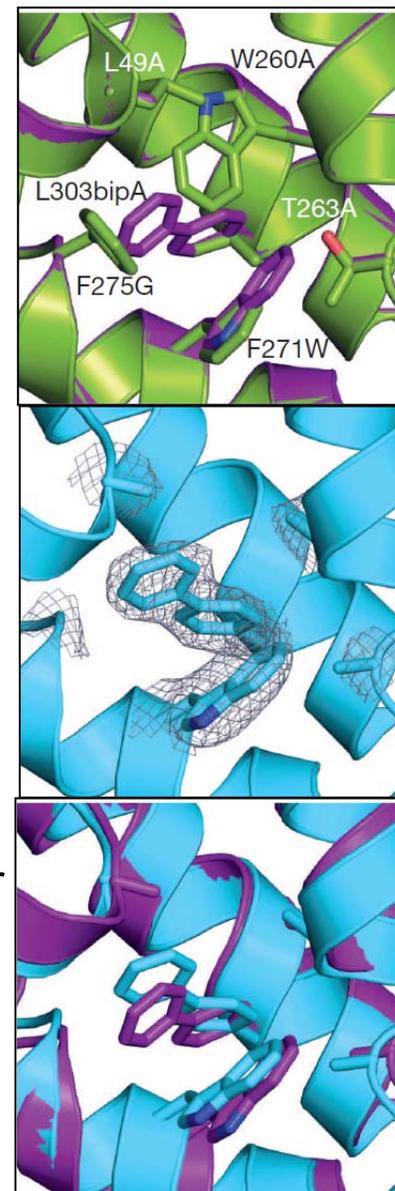
## **Approach**

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<sub>4</sub> production at a permafrost-to-wetland transitional ecosystem in northern Sweden

### ***Approach***

A combination of molecular genetics, isotopic analysis, and CO<sub>2</sub>/CH<sub>4</sub> flux measurement was used to examine relationships between microbial community dynamics and carbon cycle processes



### ***Results/Impact***

- A distinct successional pattern of CH<sub>4</sub> and CO<sub>2</sub> production was observed over the transitional gradient that strongly correlated with shifts in microbial community structure and associated functional processes.
- CH<sub>4</sub> 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

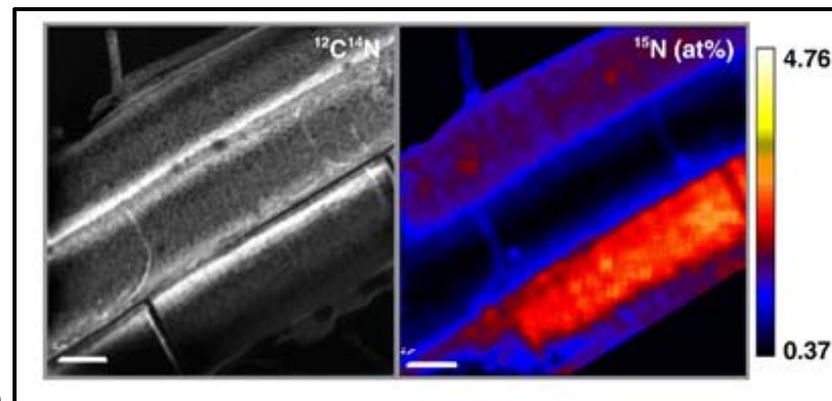
### **Approach**

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  $N_2$  fixation in Guerrero Negro intertidal microbial mats with a functional single-cell approach*, **ISME J.** 9:485-496.



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  $\mu m$

## *Elucidating control of secondary cell wall synthesis*

### **Objective**

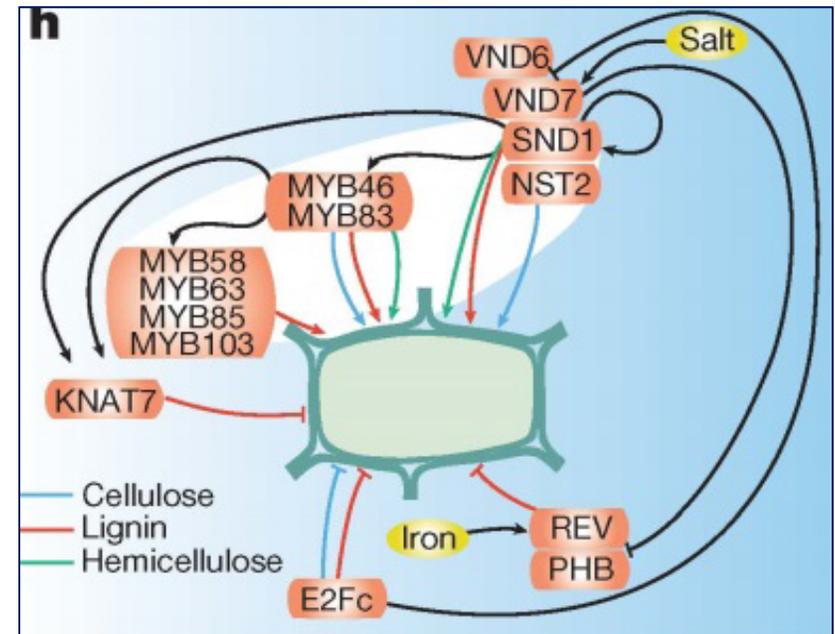
Use network-based approach to characterize transcriptional regulation of secondary cell wall biosynthesis

### **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-Teeple et al. 2014. *Nature* 517:571-578 doi:10.1038/nature14099

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

### **Objective**

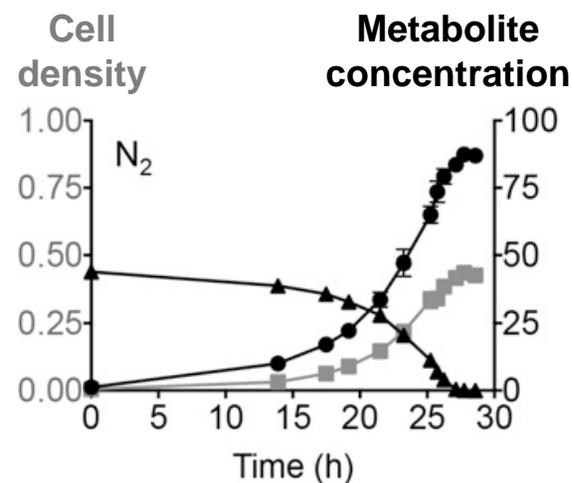
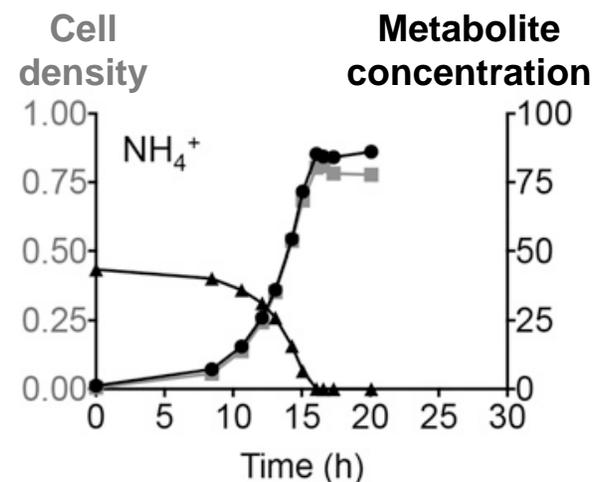
Increase the efficiency of biofuels production from biomass using the bacterium *Zymomonas mobilis*.

### **Approach**

Compare production of ethanol by *Z. mobilis* growing in bioreactors containing hydrolyzed *Miscanthus* biomass as a feedstock, supplemented with either N<sub>2</sub> gas or chemical nitrogen sources.

### **Results/Impact**

- When N<sub>2</sub> 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<sub>2</sub> gas was supplied as the nitrogen source.
- The use of N<sub>2</sub> 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 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

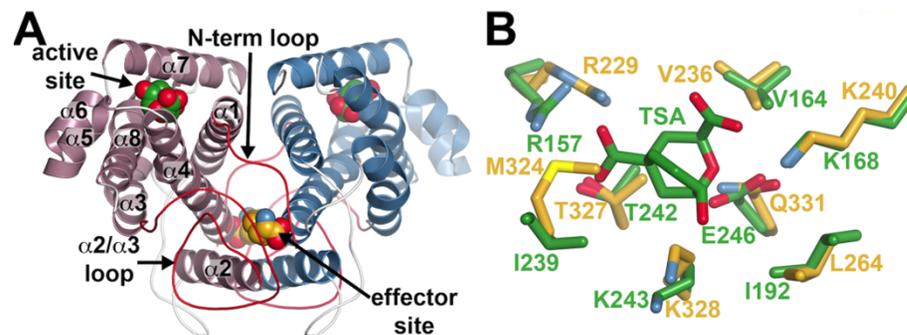
### Approach

- Obtain crystal structures of chorismate mutase isoform 1 from *Arabidopsis 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-phenylethanol 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].



**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).



# DOE Joint Genome Institute

## Next Community Science Program (CSP) Call - OPEN

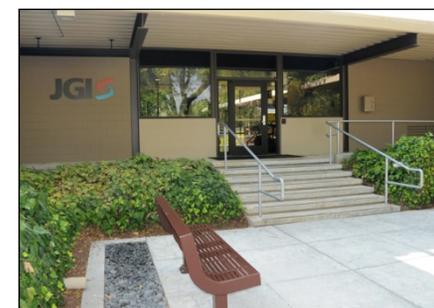
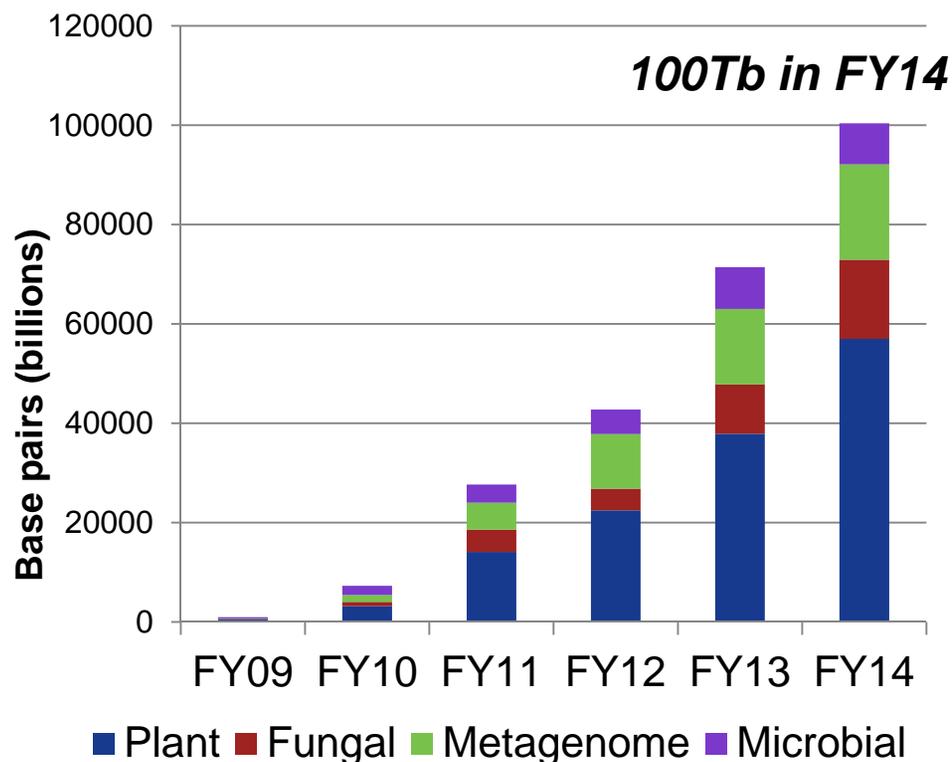
- Letters of Intent due: April 16, 2015
- Functional Genomics and Microbiomes of DOE JGI Flagship Plants
- Function-driven Microbial Genomics
- Extreme Environments including Deep Subsurface
- DNA Synthesis Projects

## 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

## Objective

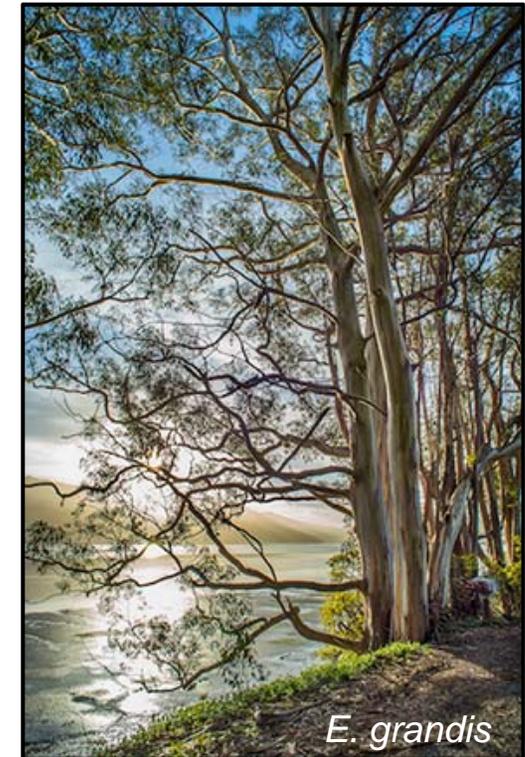
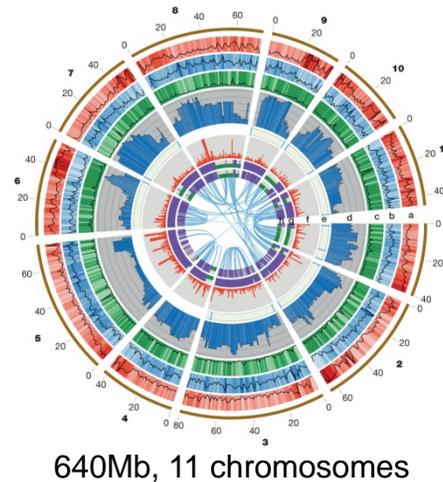
Sequence *Eucalyptus*, the most widely planted hardwood forest trees in the world

## 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

# Sequencing the microbial community in the shipworm, the “termite of the seas”

## Objective

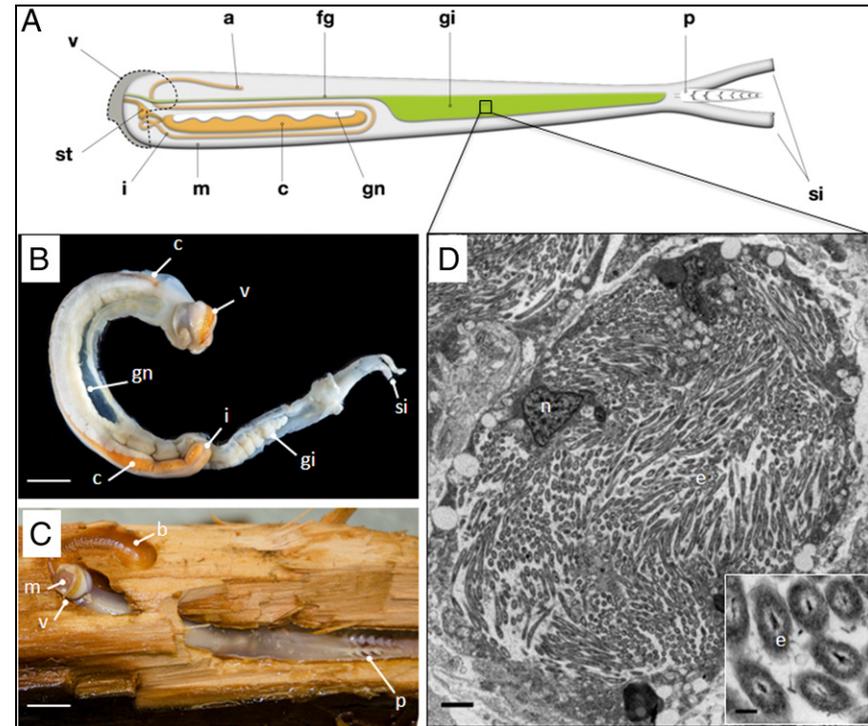
Shipworms are historically famous for their abilities to digest the wood in the hulls of ships.

## 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.



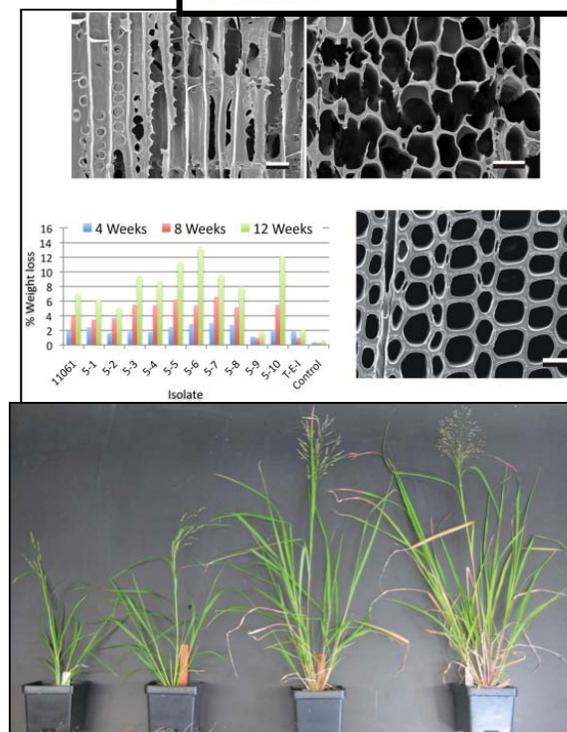
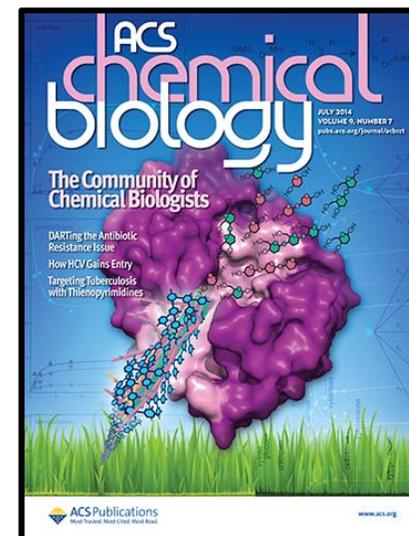
O'Connor RM et al. [Gill bacteria enable a novel digestive strategy in a wood-feeding mollusk.](#) *Proc Natl Acad Sci U S A.* 2014 Nov 25;111(47):E5096-104.

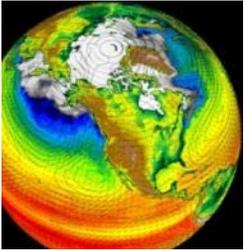
## Selected Publications

- 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**

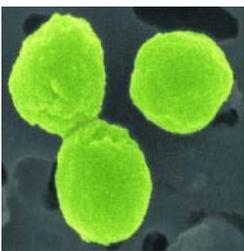
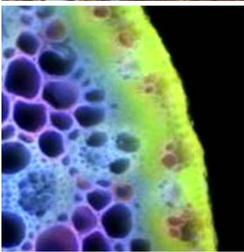
JGI Publication Highlights: <http://www.jgi.doe.gov/News/pubs.html>





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

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



**Thank you!**

<http://genomicscience.energy.gov>



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# Ideal Photosynthetic Microbe for Bioenergy Applications?

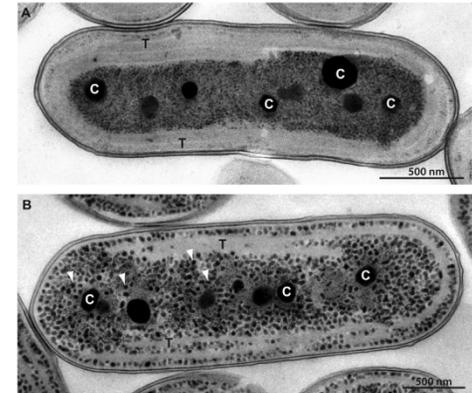
**Challenge** – Identify a novel cyanobacterial strain that grows rapidly and is amenable to genetic manipulation—ideal traits for a wide range of synthetic biology and metabolic engineering applications.

## 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.

**Reference:** Yu, J, M Liberton, PF Cliften, RD Head, JM Jacobs, RD Smith, DW Koppelaar, JJ Brand, and HB Pakrasi. 2015. “*Synechococcus elongatus* UTEX 2973, a fast growing cyanobacterial chassis for biosynthesis using light and CO<sub>2</sub>.” *Scientific Reports* 5:8132. [DOI:10.1038/srep08132](https://doi.org/10.1038/srep08132)



*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.

