

Office of Biological and Environmental Research

Biological Systems Science Division Update

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U.S. DEPARTMENT OF
ENERGY

Office
of Science

Office of Biological
and Environmental Research

Programmatic Activities

Completed Reviews/Activities

- *Joint Genome Institute (JGI) triennial review (Dec 6-8)*
- *Annual Genomic Sciences Program PI Meeting (Feb 26-28)*
- *Annual Bioimaging Science Program PI Meeting (Feb 28-Mar 1)*
- *Annual JGI User Meeting (Mar 12-15)*

Upcoming Reviews/Activities

- *Panel Review of Early Career FOA applications (May 7th microbial panel and May 11 plant panel)*
- *Panel Review of Systems Biology for Bioenergy –relevant Microbes FOA applications (May 8-9)*
- *Panel Review of Bioimaging FOA applications (May 10-11)*
- *Panel Review Plant Feedstocks USDA, DOE (Jun 26)*
- *Oak Ridge National Laboratory PMI SFA Review (Aug 28)*
- *Los Alamos National Laboratory SFA Review (Aug 29)*
- *Bioenergy Research Centers reviews (Nov., Dec., Jan., Feb).*
- *ORNL Biofuels and CSMB: Review Date - Dec.*

Funding Opportunities for FY 2018

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

- Plant genes/alleles that influence plant responses to pathogens.
- Plant genes/alleles that influence agronomic, yield, and quality traits of non-food oilseed crops

Systems Biology of Bioenergy-Relevant Microbes to Enable Production of Next-Generation Biofuels and Bioproducts **(DE-FOA-0001865)**

- Emerging model microorganisms and/or microbial communities.
- Novel microbial functional capabilities and biosynthetic pathways.

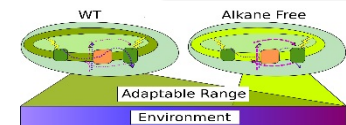
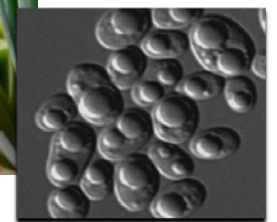
Bioimaging Research and Approaches for Bioenergy **Funding Opportunity Number (DE-FOA-0001868)**

- Development of new, innovative and/or significantly improved instrumentation and imaging approaches for BER research.

And....

Early Career Research Program Funding Opportunity **Number: DE-FOA-0001761**

- Systems-Level Design and Engineering of Microbial or Plant Systems for the Production of Biofuels and Bioproducts

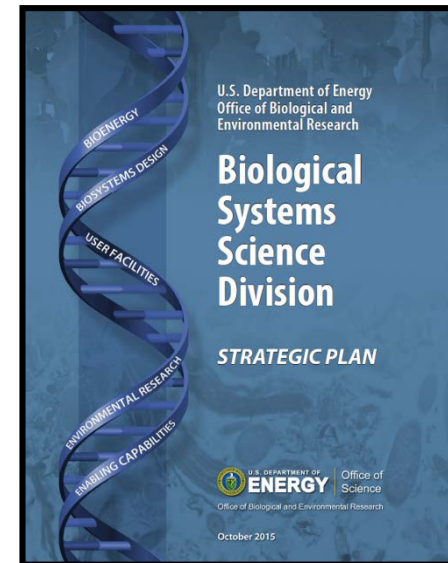


Biological Systems Science Division

Overarching Goal: *Provide the necessary fundamental science to understand, predict, manipulate, and design biological processes that underpin innovations for bioenergy and bioproduct production and to enhance the understanding of natural environmental processes relevant to DOE.*

Objectives

- Provide a basic understanding of plant and microbial biology to underpin the production of biofuels and bioproducts from sustainable plant biomass resources.
- Develop the fundamental understanding of genome biology needed to design, modify, and optimize plants, microbes, and biomes for beneficial purposes.
- Gain a predictive understanding of biological processes controlling the flux of materials (e.g., carbon, nutrients, and contaminants) in the environment and how these processes impact ecosystem function.
- Develop the enabling computational, visualization, and characterization capabilities to integrate genomic data with functional information on biological processes.
- Broaden the integrative capabilities within and among DOE user facilities to foster a more interdisciplinary approach to BER-relevant science and aid interpretation of plant, microbe, and microbial community biology.

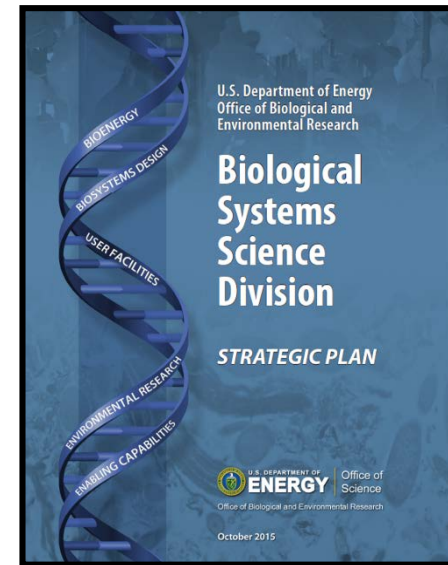


Biological Systems Science Division

Overarching Goal: *Provide the necessary fundamental science to understand, predict, manipulate, and design biological processes that underpin innovations for bioenergy and bioproduct production and to enhance the understanding of natural environmental processes relevant to DOE.*

Objectives

- ***Plant & Microbial Bioenergy Research***
- ***Biosystems Design***
- ***Carbon/Nutrient Cycling and Environmental Microbiology***
- ***Enabling Capabilities***
- ***User Facility Integration***





Chemical genomic-guided engineering of gamma-valerolactone (GVL)-tolerant yeast

GREAT LAKES BIOENERGY
RESEARCH CENTER

Objective

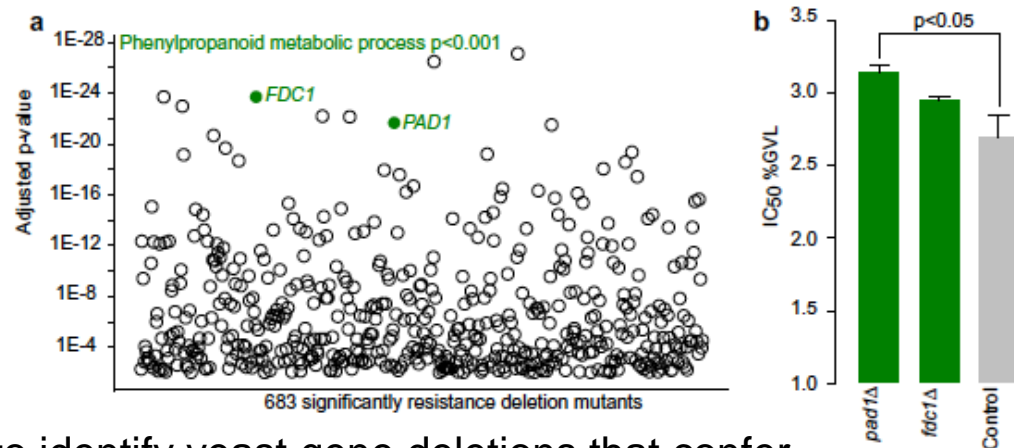
Understand the mechanism of GVL toxicity to enable the engineering of yeast for increased tolerance to GVL.

Approach

- Use a chemical genomic (CG) approach to identify yeast gene deletions that confer sensitivity or tolerance to GVL and predict which cellular processes are affected
- Test CG predictions biochemically and engineer a biofuel-producing yeast strain with increased tolerance to GVL and improved conversion efficiencies

Results/Impact

- CG revealed that deletion of *PAD1* and *FDC1* genes resulted in increased tolerance to GVL; an engineered biofuel microbe showed improved conversion of GVL hydrolysates to biofuel
- CG predicted that GVL affects cellular membranes; ergosterol concentration and biosynthetic enzymes were elevated in the engineered strain, suggesting that one route to GVL-tolerance is via altered membrane fluidity
- CG is an important tool that may be used to rapidly tailor microbes for specific conditions



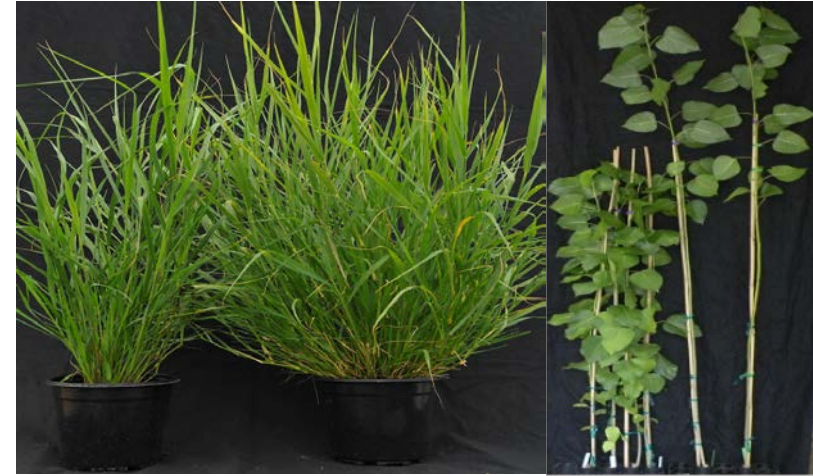
Rewriting Recalcitrance: Genetic Changes Increase Crops' Biomass and Sugar Release

Objective

Target gene associated with cell wall formation to reduce recalcitrance and improve biomass yield

Approach

- Pectin biosynthesis was downregulated by genetic engineering of GAUT4-KD



Greenhouse grown switchgrass (left) and poplar (right) where a key cell wall synthesis gene (GAUT4) was downregulated exhibited increased biomass (on the right of each image) compared to the controls

Results/Impact

- All downregulated GAUT4-KD grasses and trees showed enhanced growth in the greenhouse and improved enzymatic sugar release and fermentation into ethanol.
- GAUT4-KD switchgrass lines grown 3 years in the field provide up to 7-fold increased extractability of cell wall sugars and ethanol production, and 6-fold more biomass yield over field-grown controls.
- GAUT4 is an effective gene target for improved biomass production with improved properties for fuel production

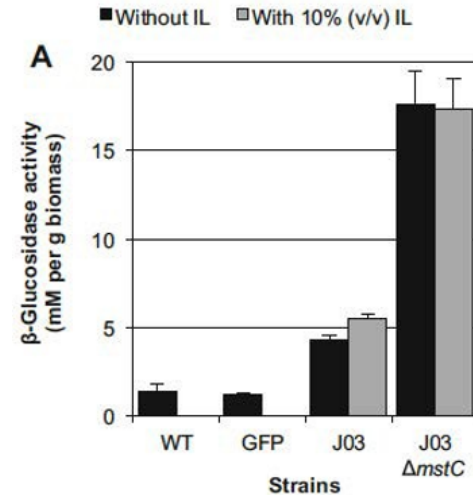
*Novel gene targets discovered for improving heterologous enzyme production in *Aspergillus niger**

Objective

Enhance the production of secreted heterologous proteins in fungal host *A. niger*

Approach

- Developed a forward genetics screen coupled with whole-genome resequencing to identify specific lesions responsible for a protein hyper-production phenotype in *A. niger*
- Use bioinformatics to identify loci associated with heterologous enzyme hyper-production



Loss of the *mstC* locus enhances heterologous enzyme production.

Results/Impact

- Strategy successfully identified novel targets, including a low-affinity glucose transporter, MstC, whose deletion significantly improved secretion of recombinant proteins driven by a glucoamylase promoter. Deletion of *mstC* loci improved by 4-fold heterologous enzyme production driven by a glucoamylase promoter.
- Mutations found in the Ypt/Rab GTPase-activating protein of both tested strains might further increase production of heterologous beta-glucosidase if introduced into other heterologous strains.

Multiplex Genome Engineering of Polyploid Industrial Yeast Strains using an Optimized CRISPR/Cas9 System

Objective

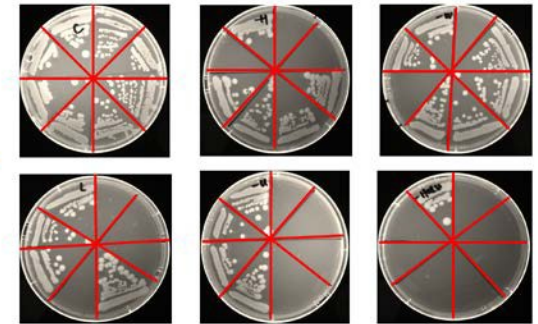
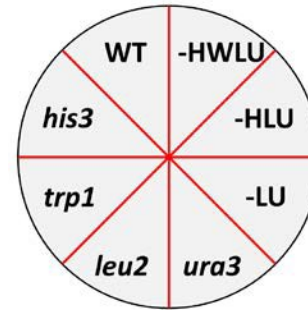
Develop an efficient CRISPR/Cas9 system for industrial yeast strain engineering

Approach

- Increasing gRNA abundance via super-high copy number plasmids.

Results/Impact

- Plasmids with higher copy numbers increased gRNA levels, resulting in higher genome editing efficiencies (up to 100% efficiency).
 - Up to 12 alleles were disrupted in a single step with 100% knock-out efficiency.
 - Facile engineering of industrial yeast strains for practical applications, i.e. biofuel production
- Optimized CRISPR/Cas9 system provides a powerful tool for the development of industrial yeast based microbial cell factories



All the single, double, triple, and quadruple knock-out strains were constructed in a single step with 100% efficiency.

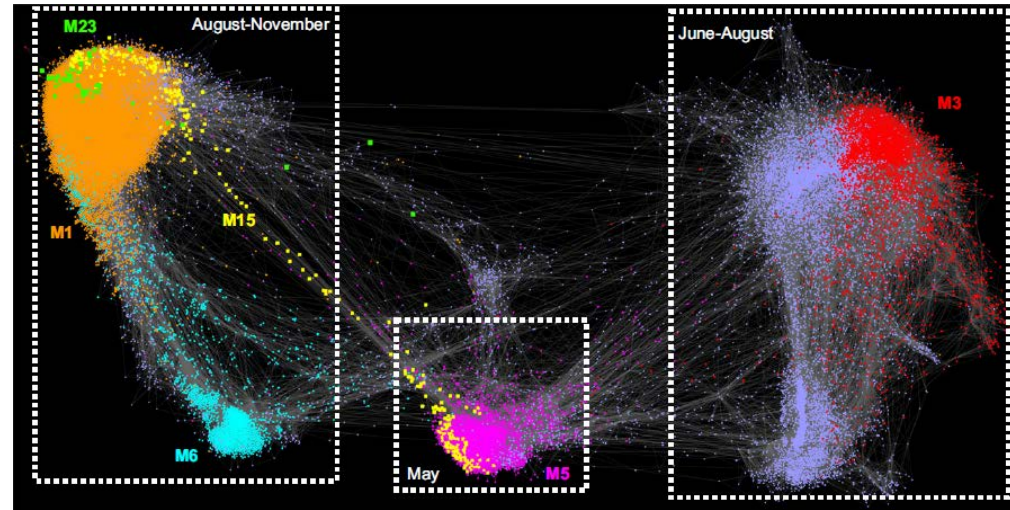
Seasonal below-ground metabolism in switchgrass

Objective

- (a) Synthesize information on cellular processes leading to dormancy
- (b) Provide models that could account for major metabolic pathways present in dormant switchgrass

Approach

- Gene expression data was collected from rhizomes harvested from field-grown switchgrass plants over two growing seasons and analyzed together with metabolite data.



Results/Impact

- Metabolism during switchgrass rhizome dormancy appeared to involve discrete but interrelated events
 - Including cold-related signaling, that may be associated with the translocation of C, N, and other nutrients and regulate resource partitioning between above- and below-ground plant tissues throughout the year
- Dormant switchgrass rhizomes are metabolically active, and pave the way for future studies to extend the range of switchgrass production into more northern climates.

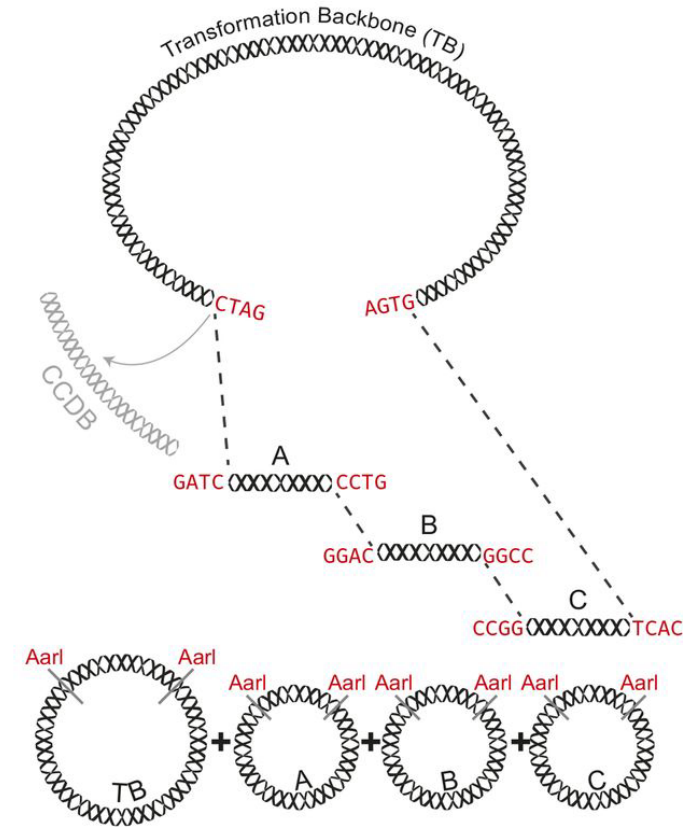
New toolkit for plant genome engineering

Objective

Develop plant genome engineering techniques to allow efficient gene knockouts, replacements, altered transcriptional regulation, and multiplexed modifications.

Approach

- A suit of direct and modular cloning vectors was developed to build TALEN- or CRISPR/Cas9-based editing targeting multiple genes and containing different promoters, reporters, selectable markers, and codon-optimized genes.
- Vector construction is facilitated by an online tool that allows choice of different vector modules for different functions, either in monocot or dicot plants.



Results/Impact

- The toolkit was tested for deletion mutagenesis targeting single or multiple genes, taking advantage of polycistronic transcripts with multiple guide RNAs (gRNA) in a single construct.
- Up to 6 genes were successfully knocked out using multiplexed vectors, and a 58 Kbp genomic region containing 5 genes involved in nitrogen fixation was deleted in a single event.
- The new toolkit will accelerate genome engineering of bioenergy crops providing a flexible and comprehensive set of gene editing options.

Čermák T, Curtin S, Gil-Humanes J, Čegan R, Kono T, Konečná E, Belanto J, Starker C, Mathre J, Greenstein R, and Voytas DA Multipurpose Toolkit to Enable Advanced Genome Engineering in Plants. *The Plant Cell* (2017) 29: 1196–1217 [DOI: 10.1105/tpc.16.00922].

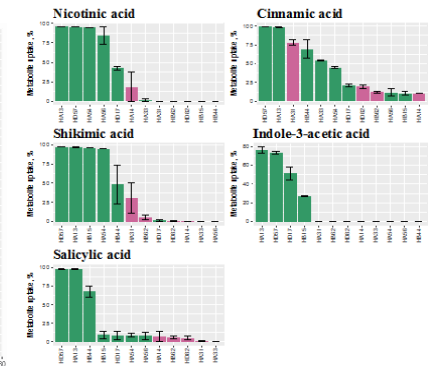
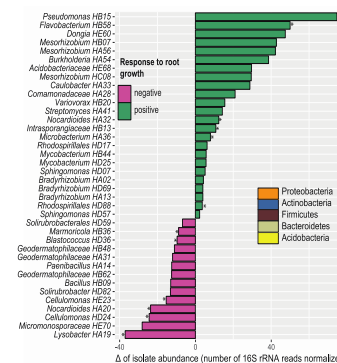
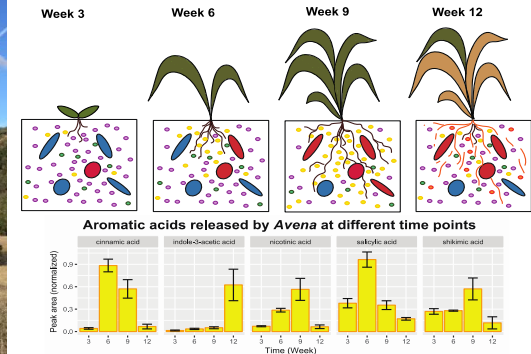
Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly

Objective

Show impact of plant exudates on soil microbiome

Approach

- Large-scale microbial isolation used to create a collection of relevant rhizosphere isolates.
- Microbial community analysis and comparative genomics used to define dynamics of microbial isolates in rhizosphere and identify microbial traits selected in the rhizosphere.
- Exometabolomics used to determine plant exudate composition and substrate uptake preferences of rhizosphere microorganisms.



Results/Impact

- Changes in the root exudate chemistry during plant development and preferential consumption of specific exudate compounds by soil microorganisms is a key mechanism by which plants shape their rhizosphere microbiome.
- Provides a mechanistic underpinning of rhizosphere microbial community assembly and an attractive direction for the beneficial manipulation of the rhizosphere microbiome.

Zhalnina, K. et al. (2018) Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly, **Nature Microbiology** DOI: 10.1038/s41564-018-0129-3

A Functional Genomics Database for Plant Microbiome Studies

Objective: Characterize genes that contribute bacterial adaptation to plants

Approach

- Sequenced, assembled, and compared the 484 genomes of isolated novel bacteria from the root environments of Brassicaceae, poplar trees, and maize
- Compared with thousands of publicly available genomes including bacteria from both plant and non-plant environments

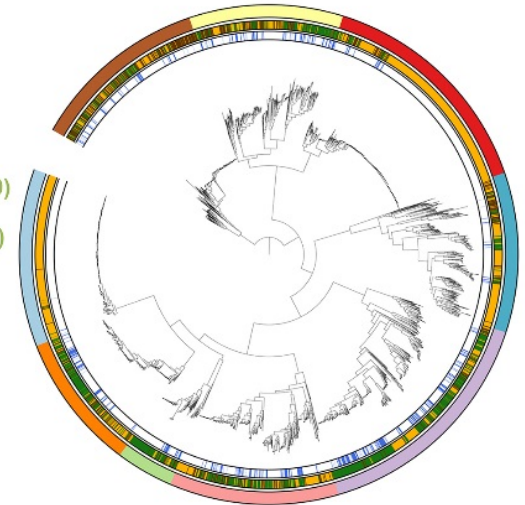
Taxonomic Groups

- Bacillales (n=454)**
- Actinobacteria 1 (n=394)**
- Actinobacteria 2 (n=587)**
- Bacteroidetes (n=409)**
- Alphaproteobacteria (n=610)**
- Burkholderiales (n=433)**
- Xanthomonadaceae (n=147)**
- Pseudomonas (n=349)**
- Acinetobacter (n=454)**

Classification

- NPA (n=2159)**
- PA (n=1160)**
- RA (n=523)**
- soil (n=518)**

Tree scale: 0.1



Results/Impact

- Identified two new, rapidly evolving protein families: one involved in plant colonization, and the other serving in microbe–microbe competition between plant-associated bacteria
- Identified 64 plant-associated protein domains that potentially mimic plant domains; some are shared with plant-associated fungi and oomycetes
- Expands genome-based understanding of plant–microbe interactions & provides leads for efficient and sustainable agriculture through microbiome engineering.



Magic Pools: Parallel Assessment of Transposon Delivery Vectors in Bacteria

Objective

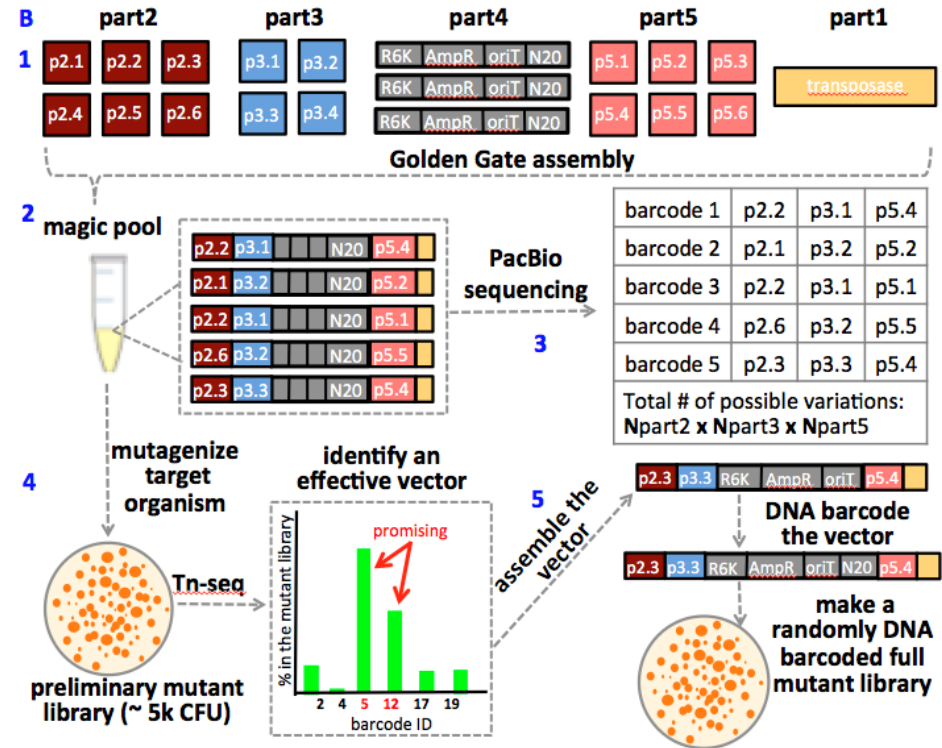
Streamlined approaches for testing hundreds to thousands of genetic systems in parallel against a target bacterium

Approach

- Avoids the labor and cost associated with trial-and-error based approaches.
- Opens door for large-scale genetic studies in diverse bacteria

Results/Impact

- Uses a “parts-based” synthetic biology cloning strategy
- Uses random DNA barcode sequences to track the efficacy of many transposon vectors in parallel
- Successfully adapted the strategy to 5 different genera of bacteria, including 3 from the phylum Bacteroidetes.



First ecosystem-scale demonstration of microbial production of methane in oxygenated soils

Early Career Researcher

Objective

Understand the mechanisms of microbial methanogenesis in oxygen-rich wetlands.

Approach

- Novel methods for measuring methane concentration and flux were used across ecosites with different oxygen concentrations
- Microbial composition and activity in those sites was also studied by metagenomics and metatranscriptomics
- Sequence analysis identified a new methanogenic Archaea (*Candidatus Methanotherix paradoxum*) that not only was the dominant species in the studied wetland but it was also found in many different aerobic environments throughout the world

Results/Impact

- Microbes produce methane in oxygen-rich environments, disproving the accepted theory that oxygen prevents microbial methanogenic activity
- A new Archaea species was found to be a key contributor to methane production in oxygenated environments



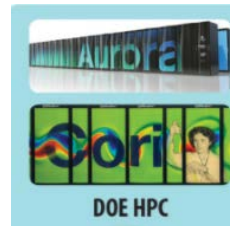
J. Angle *et al.* Methanogenesis in oxygenated soils is a substantial fraction of wetland methane emissions. *Nature Communications* (2017). [DOI: 10.1038/s41467-017-01753-4]

Key accomplishments

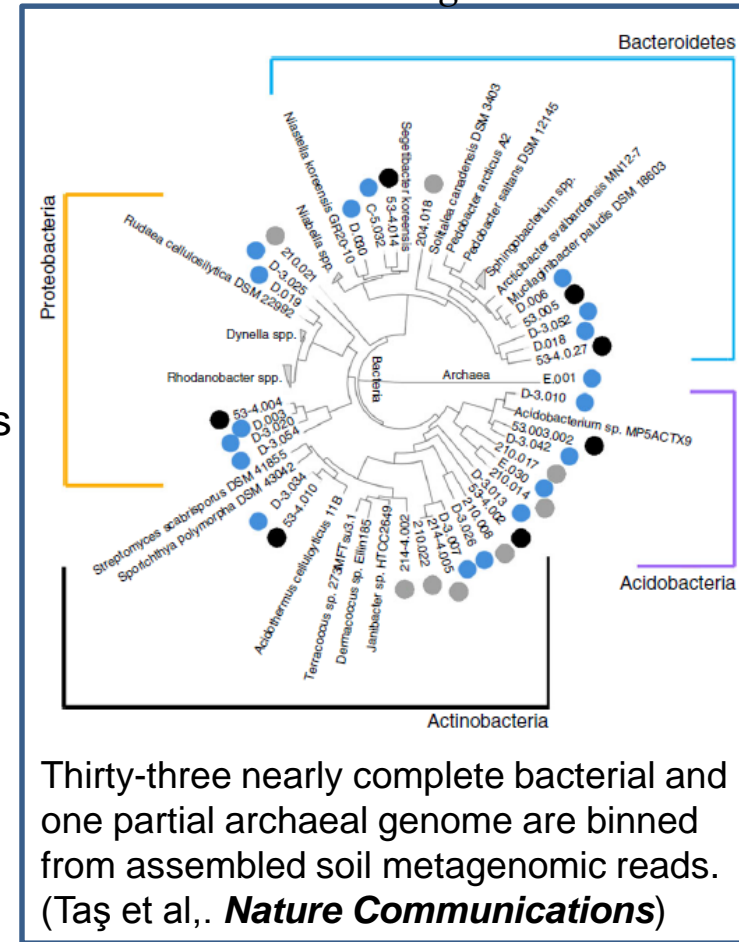
- **Catalyzing user growth**
 - Easier signup, enabled by new authorization system
 - 55% increase in users in 2017
 - 300% increase in data uploaded by users
 - Flagship KBase paper accepted for publication
- **Addressing user needs**
 - 100% increase in apps
 - Many new apps for reads processing, metagenomics and comparative genomics
 - Users can now use JGI search engine, pull JGI data into KBase

Laying foundation for accelerated future growth

- First apps added by external developers using KBase SDK
- Utilizing HPC for compute-intensive apps
- Knowledge Engine proof of concept



Researchers using KBase



<https://kbase.us/publications/>

Towards Custom-made Nanoscale Chemical Factories

BMCs are formed by hundreds of proteins encasing a wide variety of enzymes that carry out highly specialized chemistry

Objective

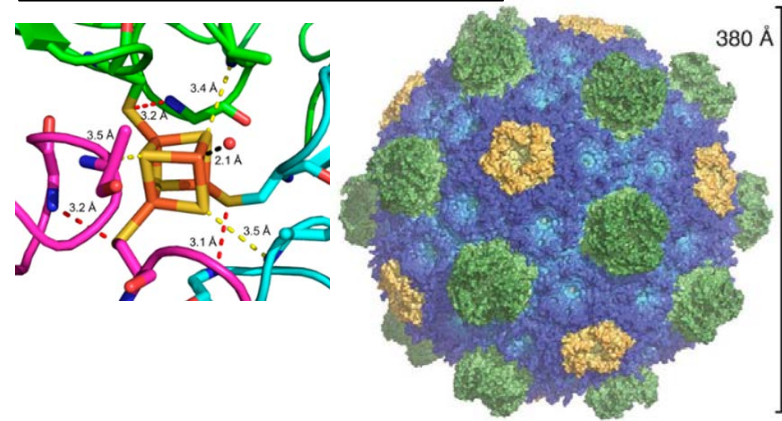
- Understand the architecture of bacterial microcompartment (BMC) mega-complexes
- Design a new functionality to BMCs, expanding the potential to serve as custom-made chemical factories

Approach

- The high resolution crystal structure of an intact BMC shell was determined using SSRL and ALS data with guidance from a low-resolution cryoEM map for initial phasing
- A shell protein was engineered to serve as a scaffold for an iron-sulfur redox cluster [4Fe-4S]



Cheryl Kerfeld and Markus Sutter handling crystallized proteins



Results/Impacts

- Multiple copies of hexameric, 3 types of trimeric and pentameric units compose the shell
- The assembly principles observed in the BMC shells can be used in the rational design of an engineered BMC system
- The complex is remarkably stable during redox cycling which may be attributed to the hydrogen bonding network provided by the main chain of the protein scaffold

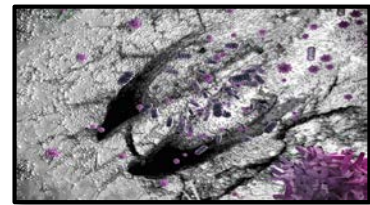
C. Aussignargues, M.-E. Pandelia, M. Sutter, J. S. Plegaria, J. Zarzycki, A. Turmo, J. Huang, D. C. Ducat, E. L. Hegg, B. R. Gibney, C. A. Kerfeld, J. Am. Chem. Soc. 138, 5262 (2016); M. Sutter, B. Greber, C. Aussignargues, C.A. Kerfeld, Science 356, 1293 (2017)

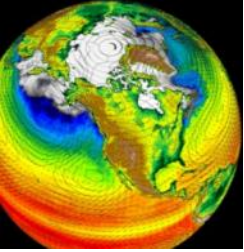
Funded by the Office of Basic Energy Sciences (CSGB) working in part, on BER-supported BL12.2 at SSRL.

95 JGI Publications Since Nov. 17.

Selected Highlights:

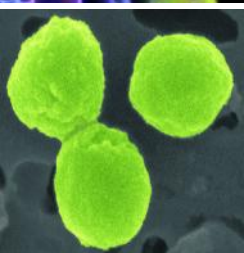
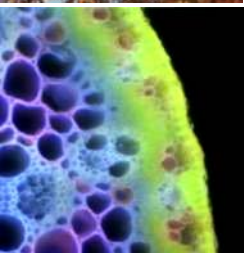
- *Mutant Phenotypes for Thousands of Bacterial Genes of Unknown Function.* accepted **Nature**, 2018.
- *Reference catalog for rumen microbiome – Cultivation, sequencing effort targets economically and environmentally relevant microbes.* **Nature Biotechnology**
- *Metabolic functions of microbial communities vary during a geyser eruption.* **Nature Microbiology**
- *Genomic features of bacterial adaptation to plants.* **Nature Genetics**
- *Insights into carbon fixation in the dark ocean – Nitrite-oxidizing bacteria have bigger role in marine carbon cycle than previously thought.* **Science**
- *Gene content variation in the *Brachypodium* pan-genome correlates with population structure.* **Nature Communications**
- *Succulent genes for water use efficiency – Comparative genomics identifies sequences involved in photosynthesis under reduced water conditions.* **Nature Communications**
- *Genus-wide *Aspergillus* project highlights new functional genome annotation methods.* **PNAS**
- *Bacteria regulate key components of fungal reproductive machinery.* **Nature Communications**
- *How the dry rot *Serpula lacrymans* adapted to a new ecological habitat* **ISME Journal**





Systems science to support DOE's energy, environment and basic research missions.

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



Thank you!



<http://genomicscience.energy.gov>