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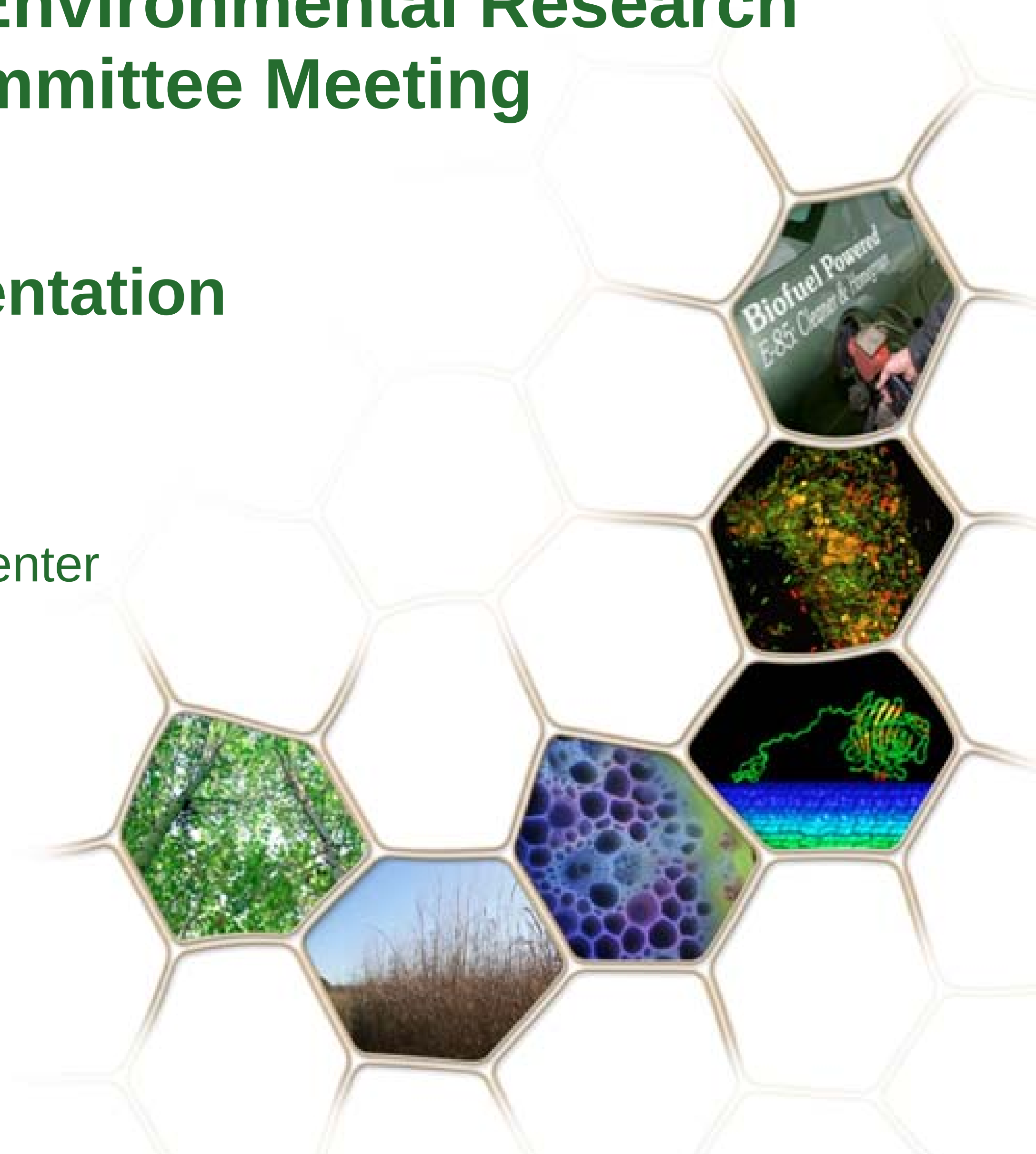
DOE Biological and Environmental Research Advisory Committee Meeting

BESC Director's Presentation



Paul Gilna

Director, BioEnergy Science Center

October 15, 2012

Gaithersburg, Maryland

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Bioconversion of cellulosic biomass: state of technology 2006/7

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- Then Current Generation Technology
  - Concentrated acid or dilute acid pretreatment
  - Engineered microbes that ferment multiple sugars
  - Simultaneous Saccharification and Fermentation (SSF)
    - Added cellulase enzymes with sugar fermenting microbes
  - Utilize existing localized supplies of wastes or ag. residues
- Anticipated Next Generation
  - Improved pretreatments
  - Consolidated Bioprocessing
    - Cellulase production and ethanol fermentation combined
  - Utilize dedicated energy crops
- Envisioned Advanced Generation
  - Beyond “ethanol” to advanced biofuels
  - Improved biofeedstocks for biomass conversion

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Scientific bottlenecks 2006

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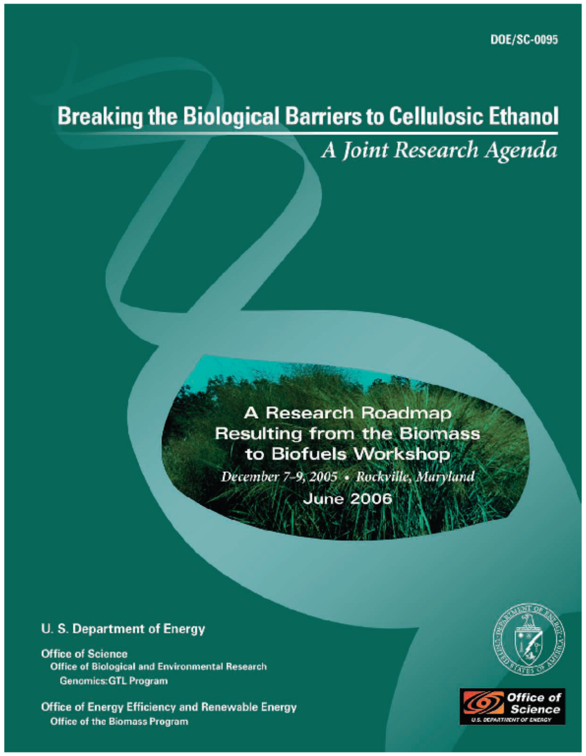
- This Roadmap identified many Barriers and Transformational Challenges
- We need a deeper understanding of:
  - ➢ The resistance of lignocellulosic biomass to deconstruction;
  - ➢ The genetic controls of plant composition and ultrastructure;
  - ➢ Bioenergy crop domestication and sustainability;
  - ➢ The structure and function of cellulases and other plant cell wall depolymerizing enzymes;
  - ➢ The cellular controls for multi-sugar transport, ethanol fermentation, and heterologous expression of enzymes (regulation);
  - ➢ The microbial cell's mechanisms for toxicity response;
  - ➢ Decoupling of cell mass production from glycolysis;
  - ➢ And more…



DOE Biomass to Biofuels Workshop (12/2005)
Roadmap (7/2006)

http://doegenomestolife.org/biofuels/b2bworkshop.shtml

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Scientific bottlenecks 2006 (cont)

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- Enabling technologies needed for success:
  - Gene transfer methods and expression of genes in non-conventional host organisms;
  - Rapid tools for the analysis and modeling of cellular composition and physiological state, (“omics”);
  - High throughput screening methods;
  - Metabolic engineering/synthetic biology;
  - Protein engineering/directed evolution;
  - Evolutionary engineering;
  - Process modeling for ethanol from biomass.

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Lignocellulosic biofuels today

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Beta Renewables Crescentino plant

- 20M Gal/yr
- Operational this year
- N. Carolina plant planned
- Will use mixed feedstocks including straw, switchgrass and *Populus*

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The BioEnergy Science Center

A multi-institutional, DOE-funded center performing basic and applied science dedicated to improving yields of biofuels from cellulosic biomass

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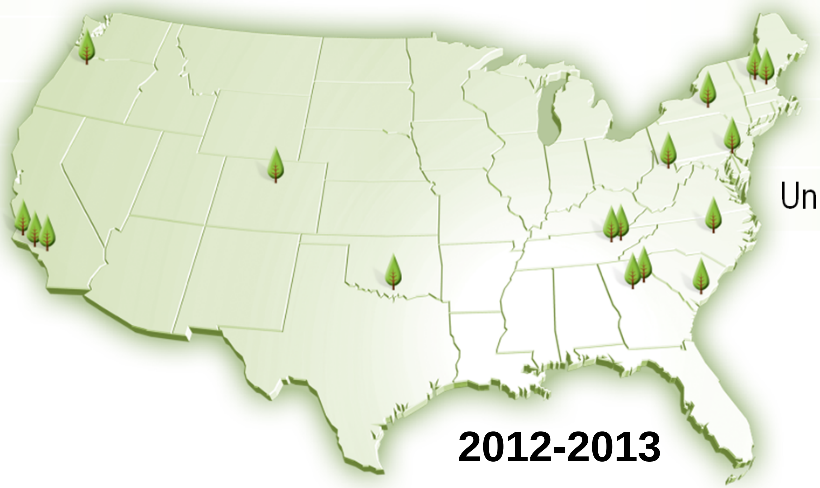
- Oak Ridge National Laboratory
- National Renewable Energy Laboratory
- Samuel Roberts Noble Foundation
- ArborGen, LLD
- Ceres, Incorporated
- Mascoma Corporation
- DuPont
- GreenWood Resources

300+ People in 17 Institutions



2012-2013

- University of Georgia
- University of Tennessee
- Cornell University
- Dartmouth College
- West Virginia University
- Georgia Institute of Technology
- University of California--Riverside
- North Carolina State University
- University of California—Los Angeles

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Access to the sugars in lignocellulosic biomass is the current critical barrier

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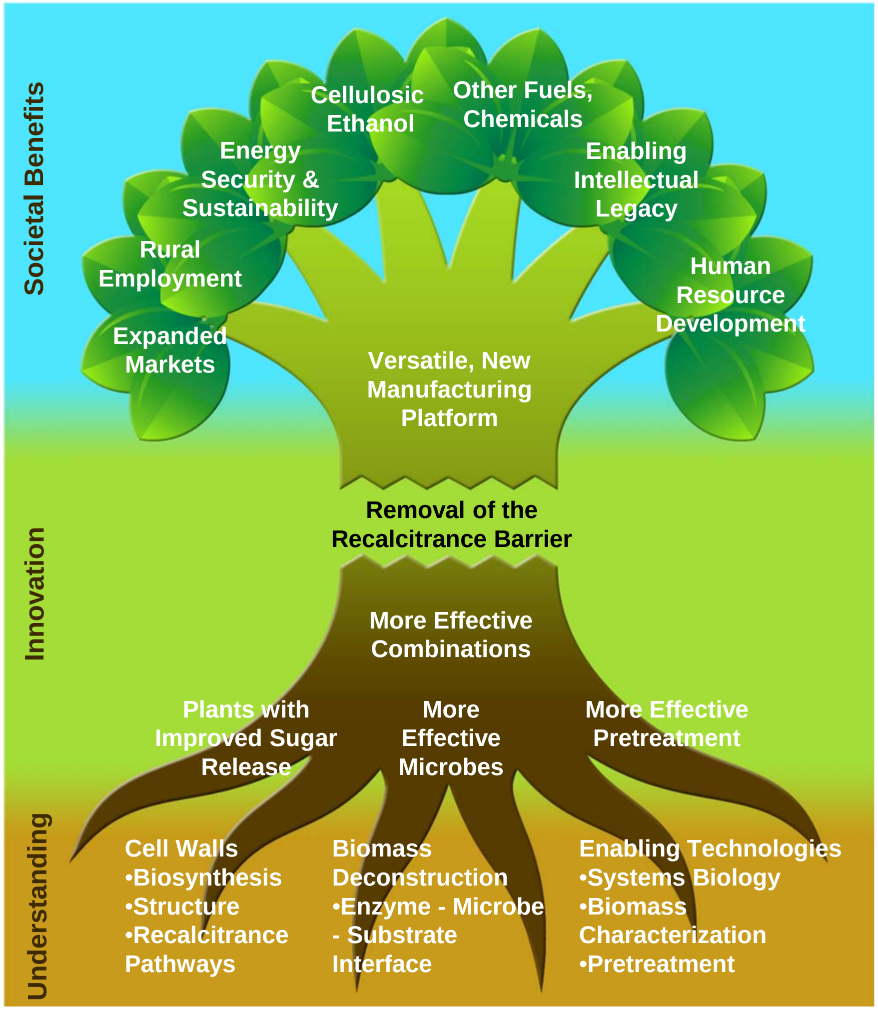
- Overcoming this barrier will cut processing costs significantly and be used in most conversion processes
- This requires an integrated, multi-disciplinary approach
- ***BESC believes biotechnology-intensive solutions offer greatest potential***



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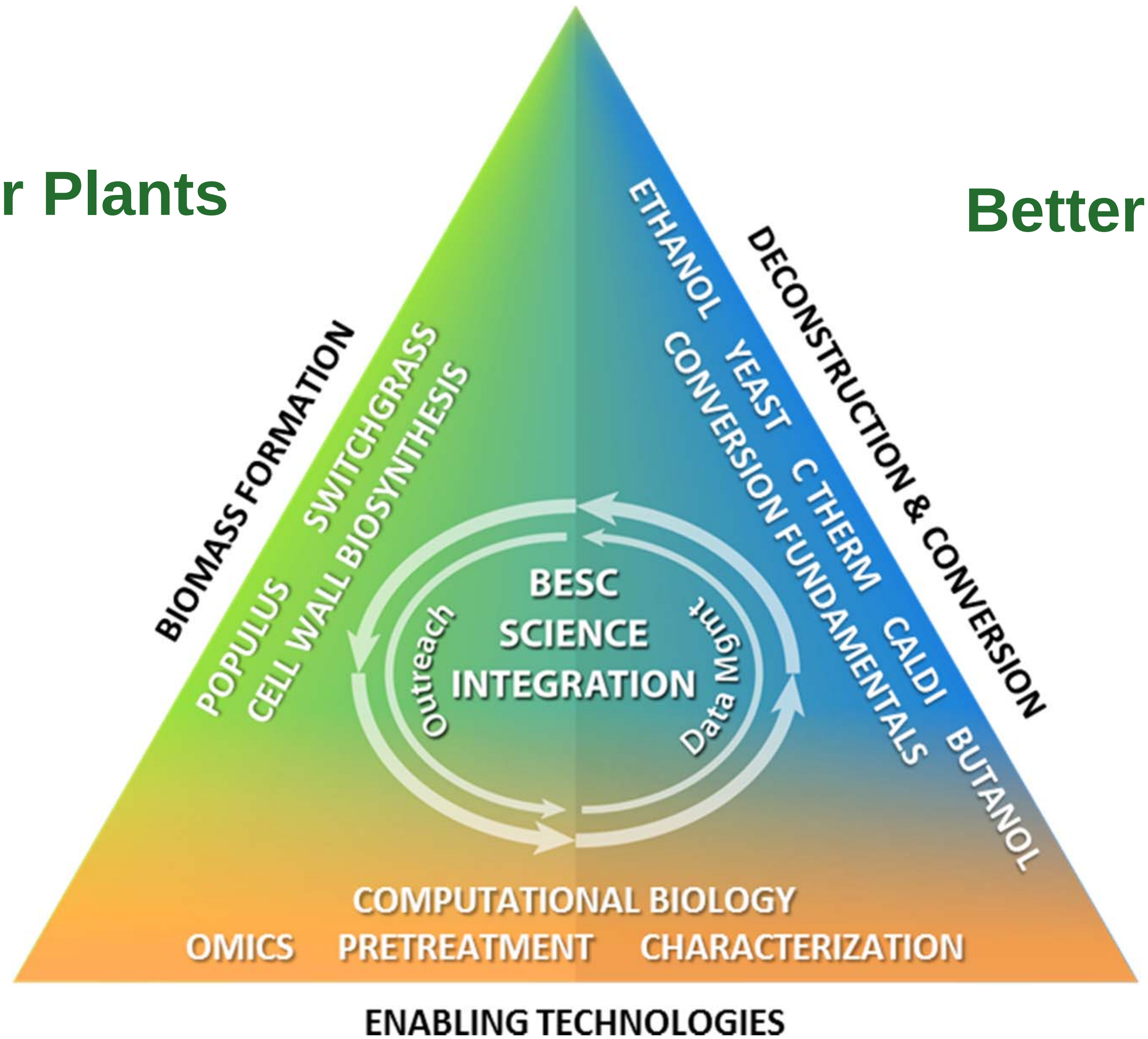
Organized around our strategic goals

Better Plants

Better Microbes



Better Tools and Combinations

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Genetic block in lignin biosynthesis in switchgrass increases ethanol yields

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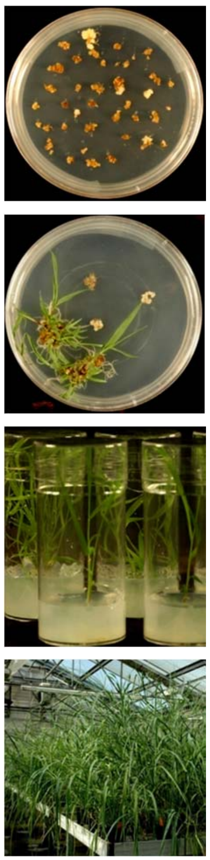
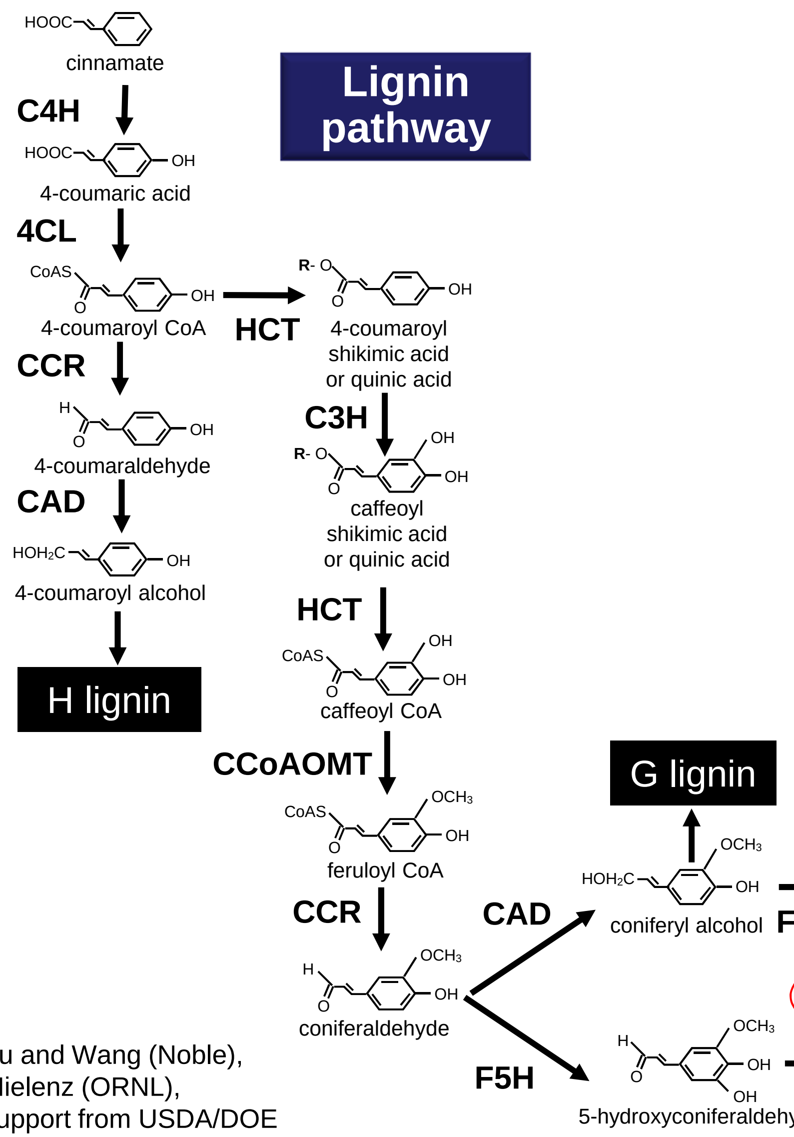
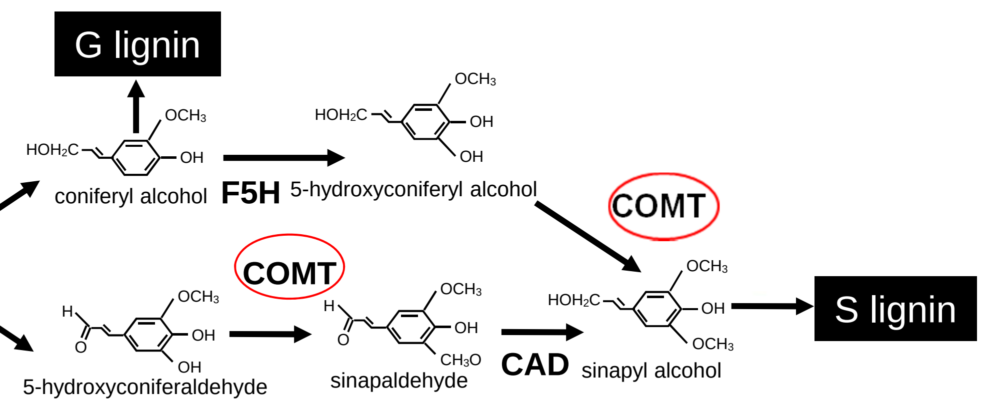
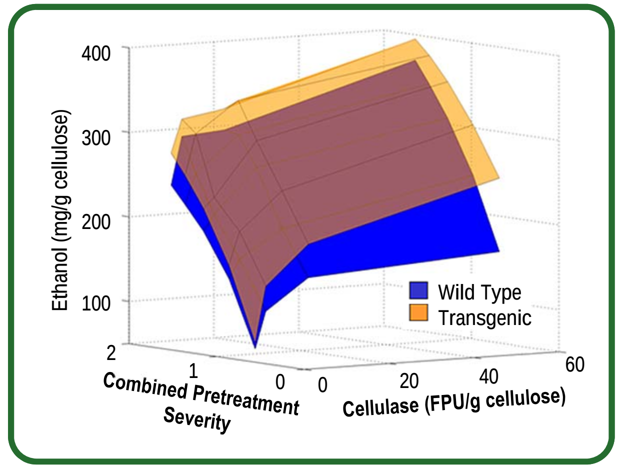
Agrobacterium-mediated transformation of switchgrass









Impact of enzyme levels and pretreatment conditions on biofuel production

Noble Foundation transgenic switchgrass

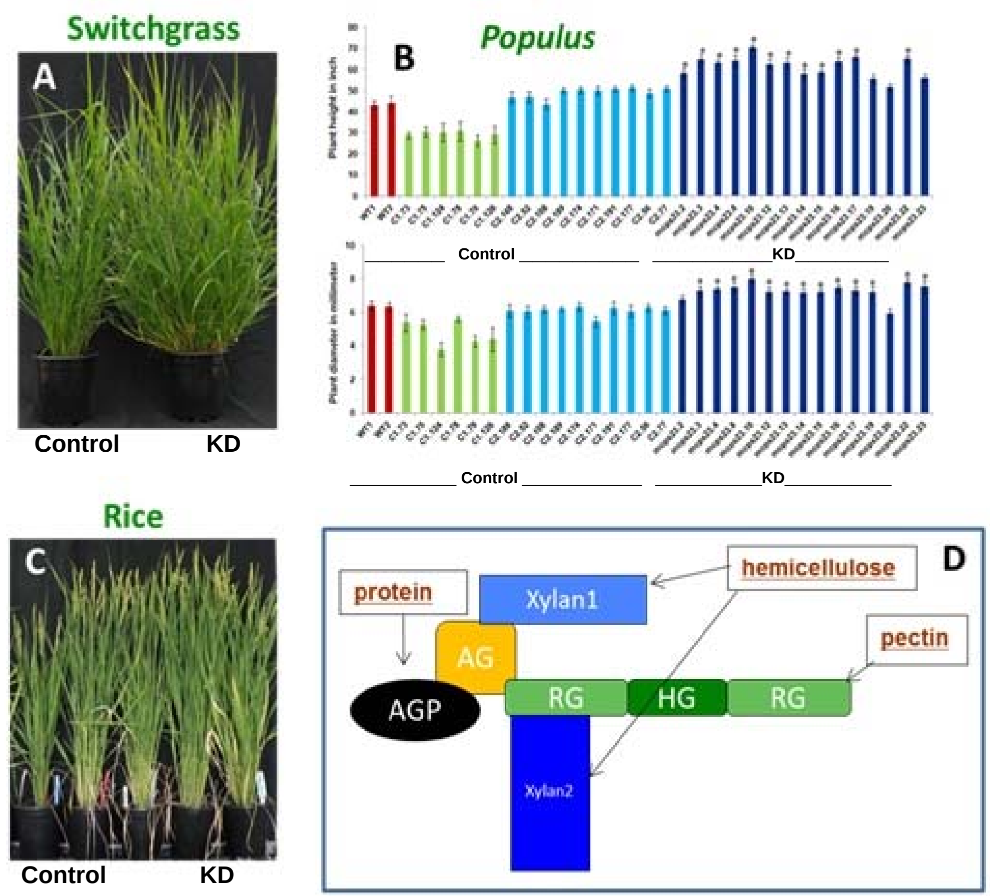
THE SAMUEL ROBERTS NOBLE FOUNDATION

Fu and Wang (Noble), Mielenz (ORNL), support from USDA/DOE
Fu et al. *PNAS* 2011

Beyond lignin: pectin

Pectin synthesis genes have significant effects on recalcitrance and growth in *Populus* and switchgrass:

- Result is surprising since pectin is important in primary cell walls but represents only a small fraction of secondary walls and walls in grasses.



Beyond lignin: MYB4

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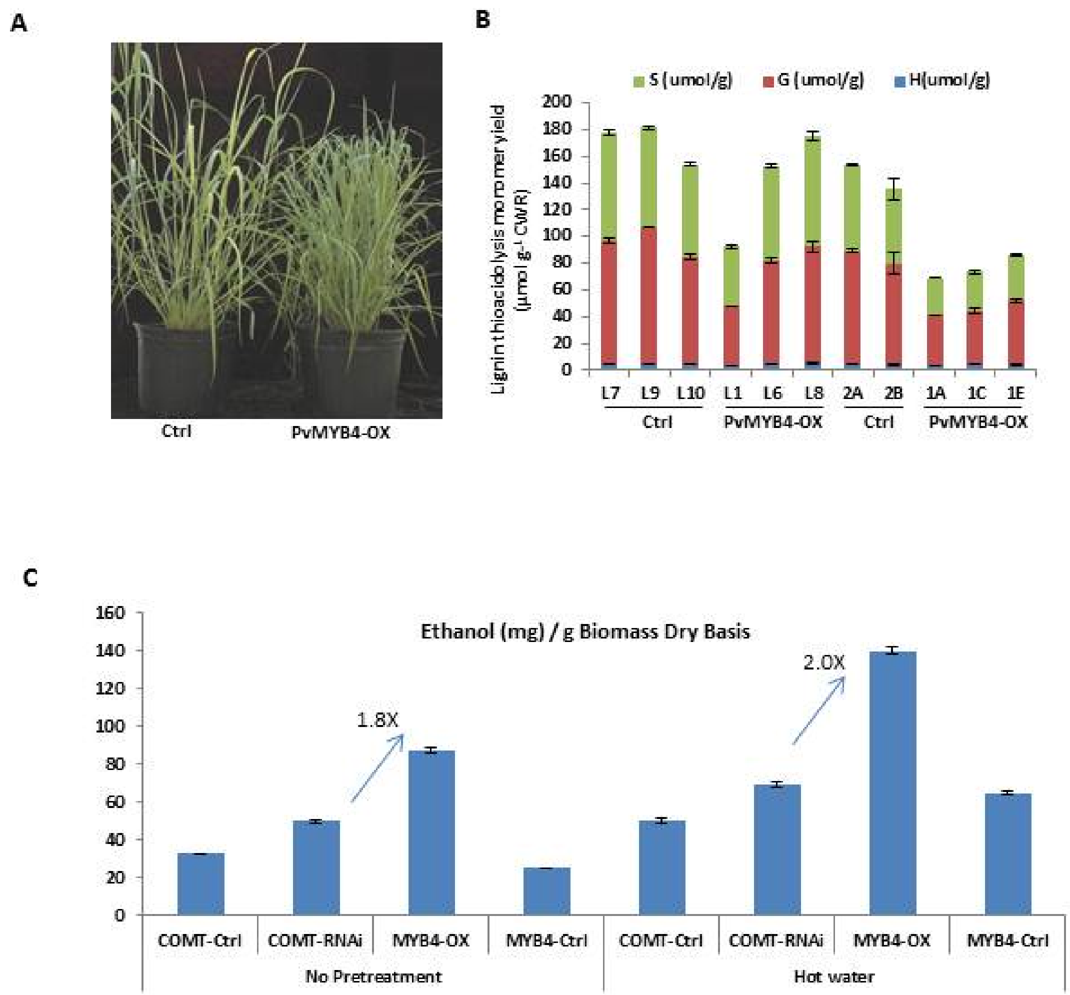
Overexpression of MYB4, a regulatory transcription factor in switchgrass, yields more than a 2-fold improvement in sugar release.



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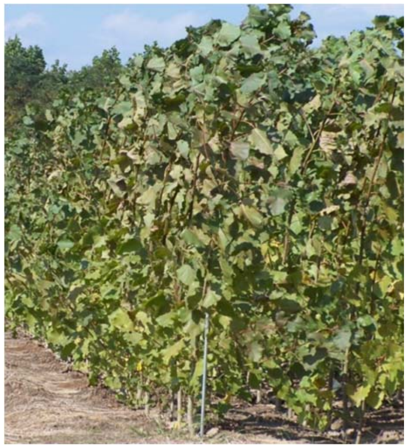
Field testing of improved feedstocks



40+ *Populus* constructs in stool beds (South Carolina)

1000+ *Populus* genotypes in 4 common gardens (Pacific Northwest)

Field assessment of genetically improved switchgrass (Texas)

Field assessment of genetically improved switchgrass (Tennessee)

Sequencing and analysis of a reference genome for the model plant Setaria

New Science:

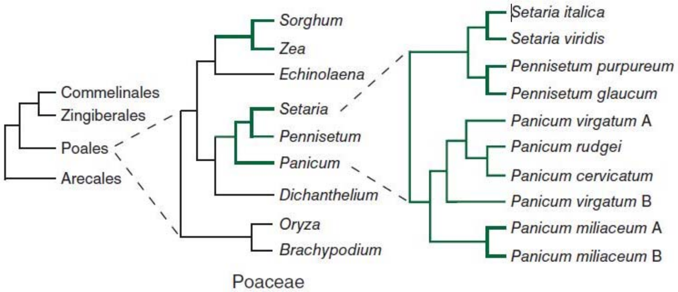
- The high quality whole genome assembly of the diploid *S. italica* is proving valuable as a framework in assisting with the genome assembly of other polyploid sequenced grasses, notably switchgrass.

- Comparison between the genomes of *S. italica, S. viridis*, switchgrass, sorghum and rice have revealed both shared and unique properties of the independent adaptations of several grasses to life on earth.

- Phylogenetic analysis of five nuclear genes shows that millet and switchgrass lineages underwent independent polyploidization events, both of which occurred after the divergence of Panicum from Setaria.

Significance:

- Grasses from the genus Setaria, in particular the food crop *S. italica* (foxtail millet) and the ancestral weed *S. viridis* (green foxtail) have served as informative model species for biofuel crops such as switchgrass (*P. virgatum*).



Bennetzen et al. Sequencing and Analysis of a Reference Genome for the Model Plant Setaria, *Nature Biotechnology*, (2012)

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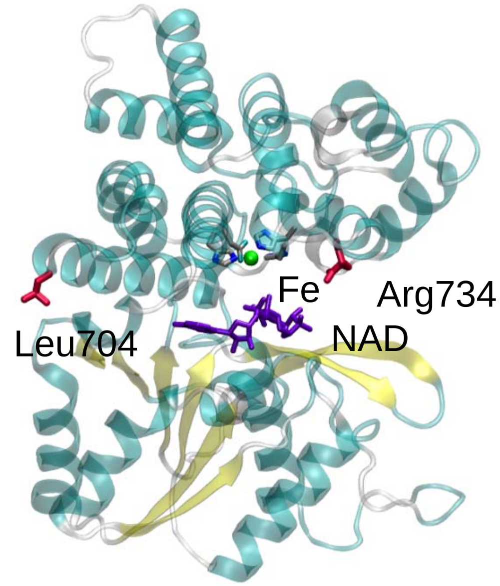
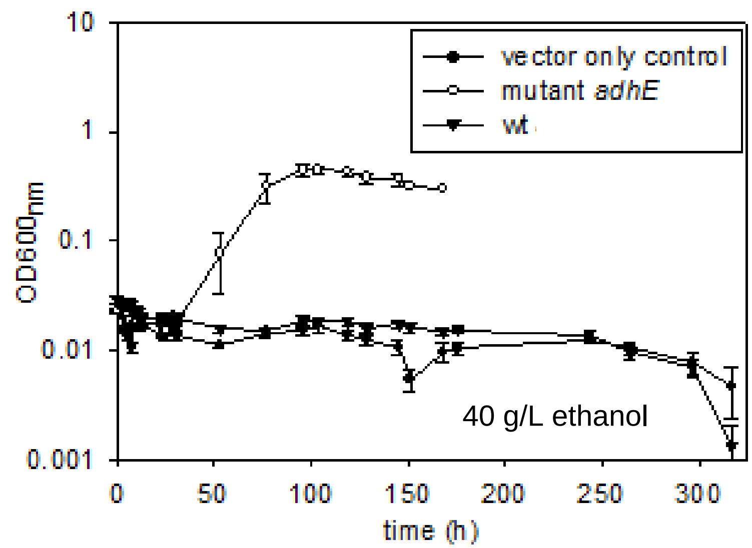
Feedstocks: where we have come

- Proved core concept that multiple genes control plant cell wall recalcitrance
- Used BESC transformation pipeline to identify a panel of 37 candidate genes to date
- Demonstrated reduced recalcitrance in *Populus* and switchgrass
- Dozens of lines now in field trials

Single microbial gene linked to increased ethanol tolerance

- Ethanol intolerance is an important metric in terms of lignocellulosic biofuels process economics
- Tolerance has often been described as a complex and likely multigenic trait for which complex gene interactions come into play
- A mutated alcohol dehydrogenase (AdhE) with altered co-factor specificity was shown to enhance ethanol tolerance in *Clostridium thermocellum*, a candidate consolidated bioprocessing microbe
- The simplicity of the genetic basis for this ethanol-tolerant phenotype informs rational engineering of mutant microbial strains for cellulosic ethanol production





U.S. 61/346,660

Brown et al. 2011. *PNAS* **108:**13752–13757.

Application of genetic tools:
C. thermocellum

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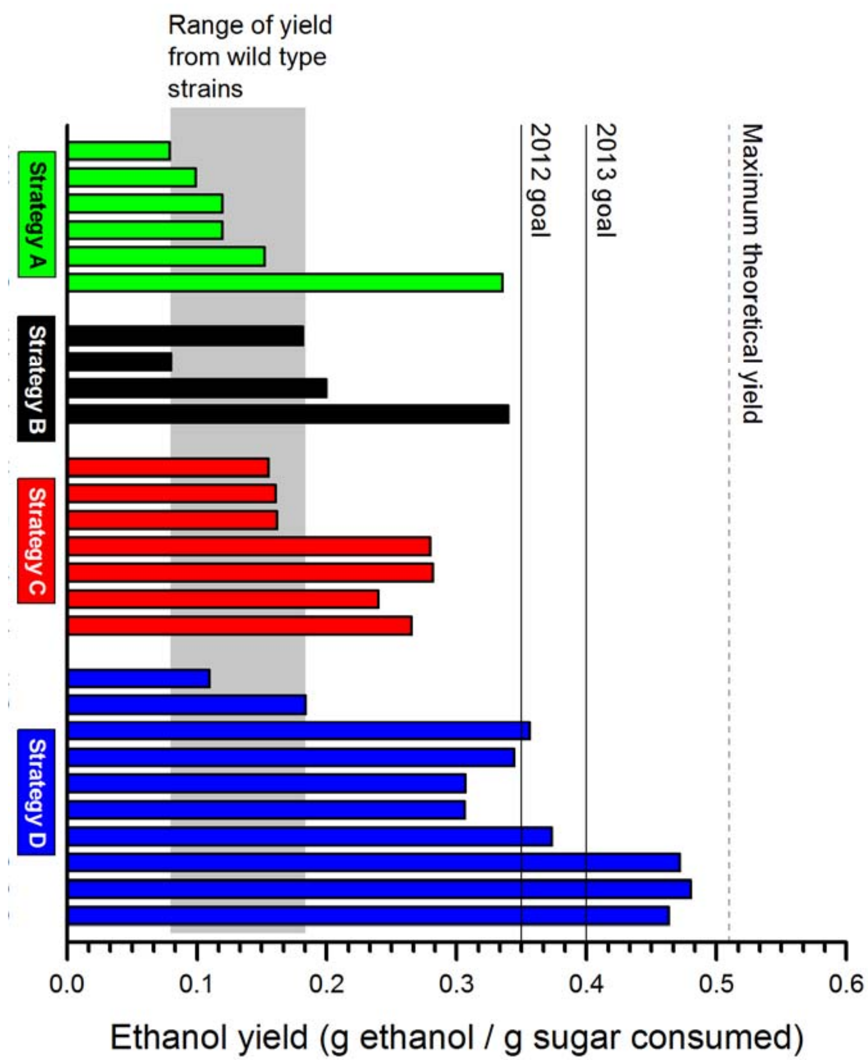
Progress towards ethanol yield goal



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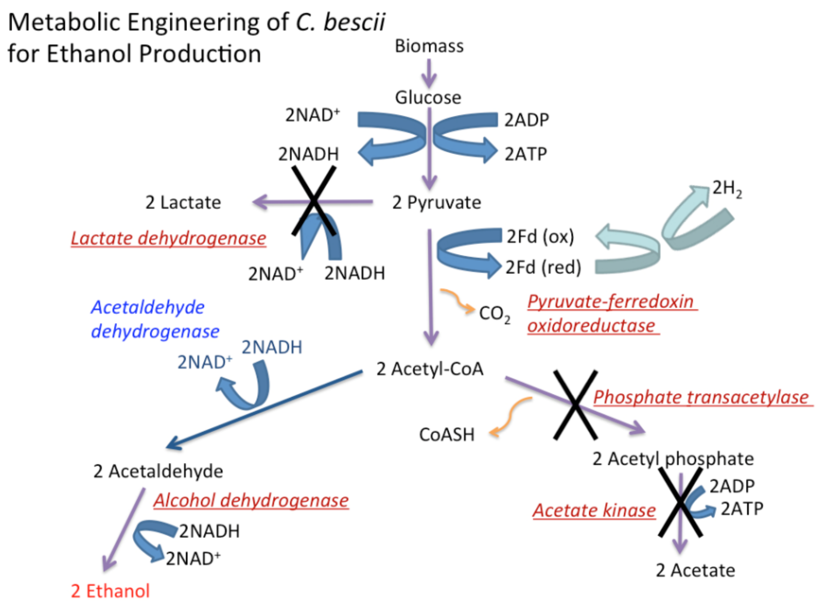
Developed genetic tools for *Caldicellulosiruptor*

New Science:

- First successful DNA transformation of this genus, *C. bescii*:
  - Used Methylation with an endogenous unique *α*-class N4-Cytosine methyltransferase to overcome major barrier to DNA transformation.
  - This efficient method for plasmid and chromosomal DNA transformation and recombination has been extended to other species of the genus.



Significance:

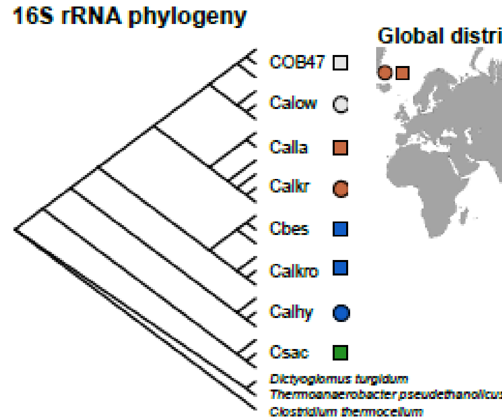
- This technology is being established in BESC partner labs and will allow the metabolic engineering of these bacteria for the direct conversion of lignocellulose to biofuels such as ethanol and butanol.

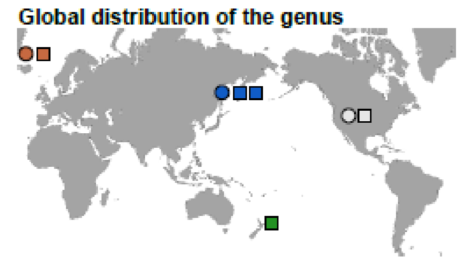
Chung et al. Methylation by a Unique *α*-class N4-Cytosine Methyltransferase is Required for DNA Transformation of *Caldicellulosiruptor bescii* DSM6725. *PLoS ONE*.

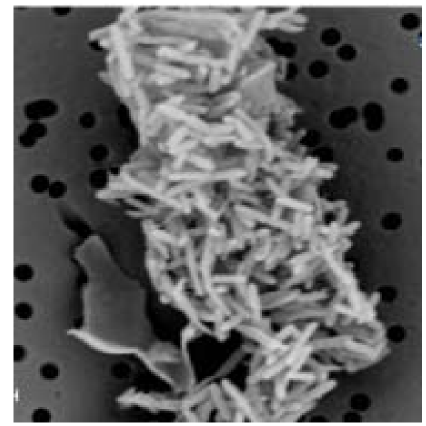
Caldicellulosiruptor pan genome study

New Science:

- Eight closed *Caldicellulosiruptor* genome sequences were examined with respect to their pan- and core-genomes in conjunction with proteomic-based screening, to seek out determinants for the capacity to degrade plant biomass, including crystalline cellulose.
- Cellulolytic ability is linked to modular, multi-domain enzymes (not cellulosomes). Weakly cellulolytic species lost this ability through deletion of a key genomic locus.
- The strongly cellulolytic *Caldicellulosiruptor* species possess novel cellulose-binding adhesins.







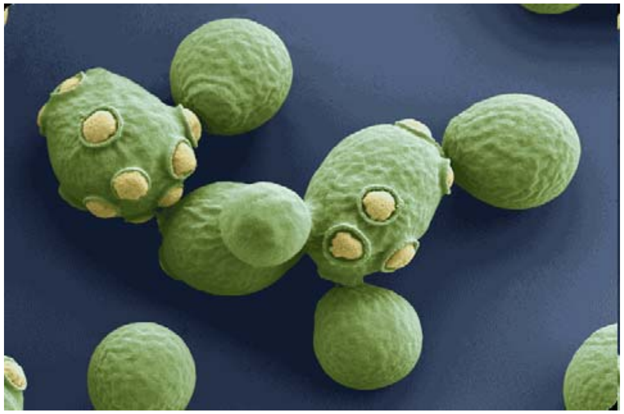
C. bescii **attached to Switchgrass**

Significance:

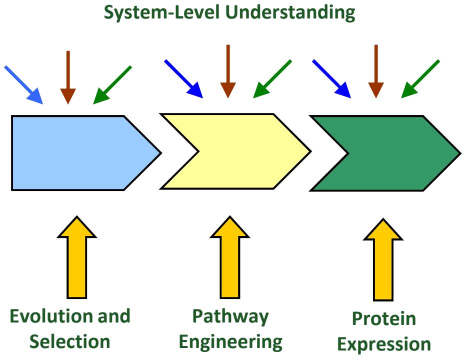
- Biogeography influences phylogeny and synteny among co-located species, but does not predict the ability to hydrolyze crystalline cellulose.

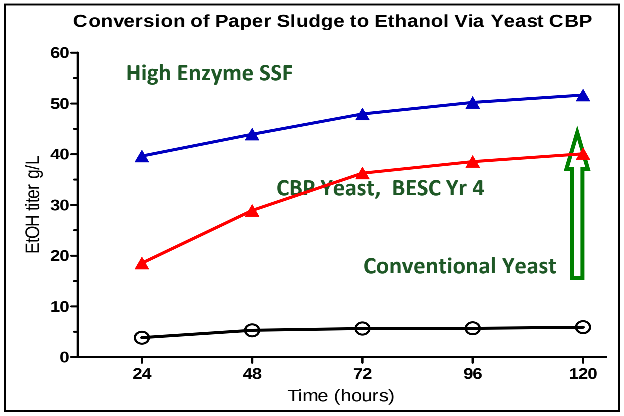
Citation: Blumer-Schuette, et al., Journal of Bacteriology, 2012

Cellulolytic yeast move to pilot tests

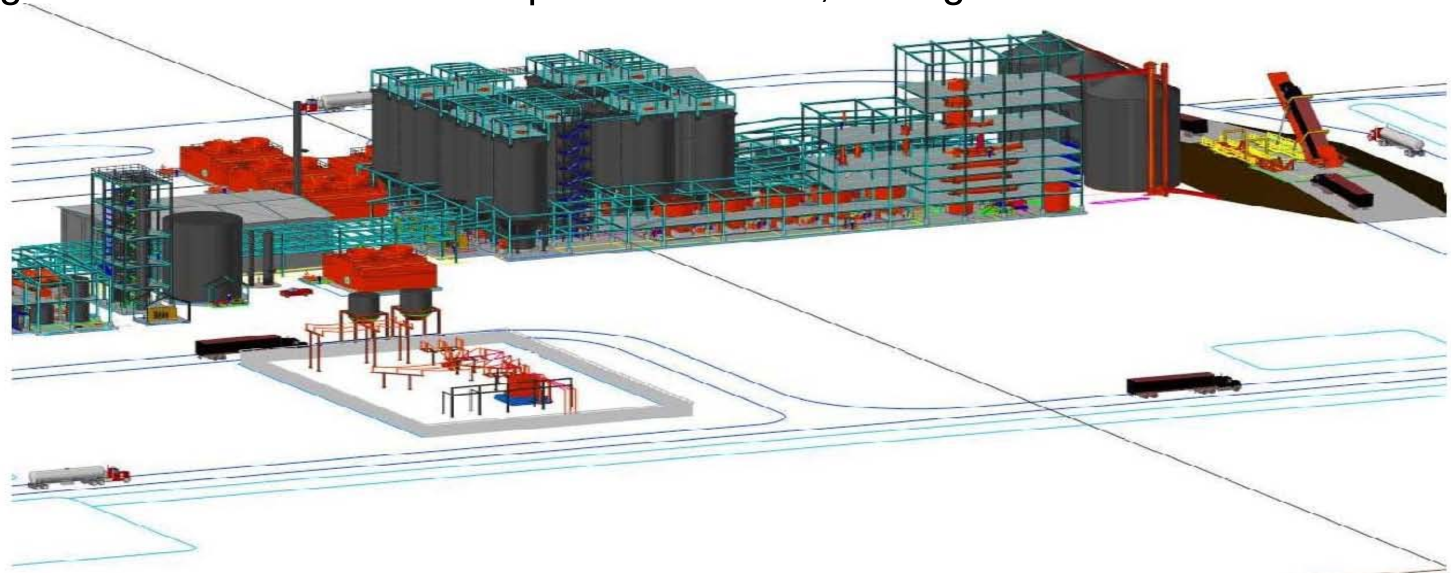


Saccharomyces cerevisiae





Mascoma Corporation has developed yeasts with assistance from BESC that express recombinant cellulases. These reagents will be used in Mascoma's commercial, 20 million gallon hardwood-to-ethanol plant in Kinross, Michigan.



Mascoma's Frontier Renewable Resources, LLC Facility, Kinross, MI

CBP microbes: where we have come

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- New genetic tools for thermophyllic microbes
- Improved ethanol yields in *C. Thermocellum*
- Solubilization of plant cell walls more effective when mediated by cellulolytic microbes

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High-throughput characterization pipeline for recalcitrance phenotype

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Screening thousands of samples

Composition analytical
pyrolysis, IR, confirmed
by wet chemistry

Pre-treatment
new method with dilute
acid and steam

Enzyme digestibility
sugar release
with enzyme cocktail

Detailed chemical and structural analyses of specific samples

Studer, *et al.*, *Biotechnol. Bioeng.*, 2010
Sykes, *et al.*, *Biofuels: Methods*, 2009
Studer, *et al.,* patent pending (US 2010/015570 A1)

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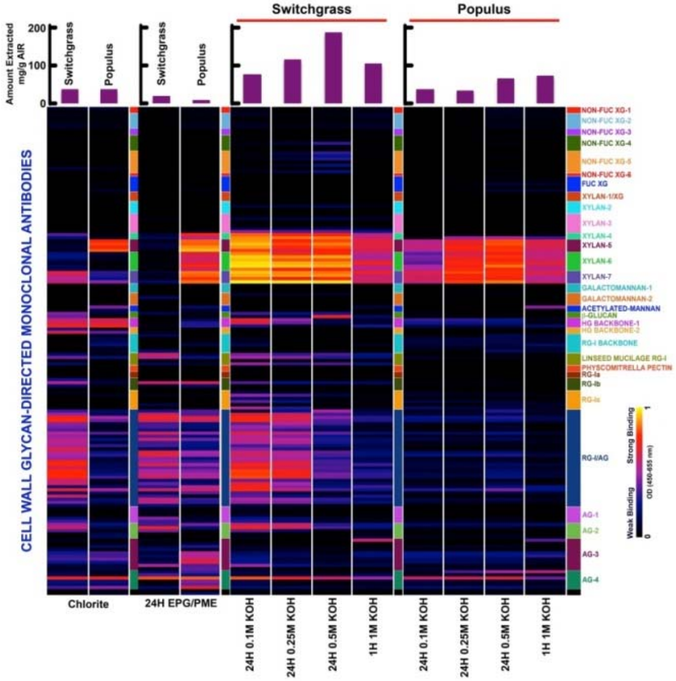
Automated glycome profiling

- Allows for medium-throughput analysis of biomass samples
- Uses 384-well ELISA plates
- Reduces sample size four-fold
- Already used in pretreatment and microbial substrate utilization studies



New lignol molecule found in COMT TG SWG extracts

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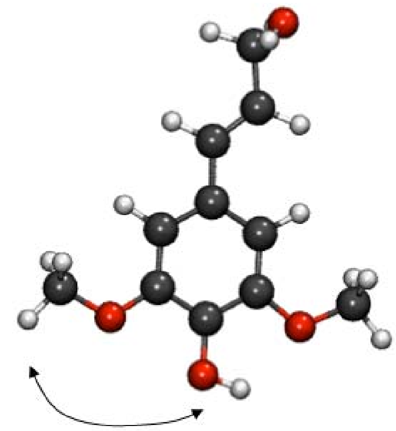
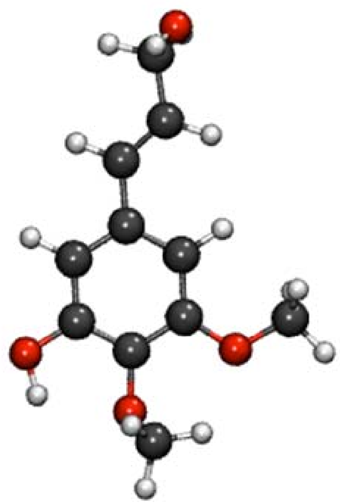
- Down-regulation of the caffeic acid 3-*O*-methyltransferase (COMT) gene in switchgrass resulted in cell walls of transgenic plants releasing more constituent sugars
- Fermentation of both wild-type and transgenic switchgrass after mild hot water pretreatment with no water washing showed that the transgenic switchgrass inhibited *C. thermocellum*
- GC-MS detected numerous compounds including a newly identified isosinapyl alcohol, essentially exclusively in the COMT transgenic lines
- Identity confirmed by chemical synthesis and analysis
- Isosinapyl alcohol was determined to have mild inhibitory properties toward yeast and *E. coli*



Sinapyl alcohol



Isosinapyl alcohol

Citation: Tschaplinski, et al., "Down-Regulation of the Caffeic acid *O*-methyltransferase Gene in Switchgrass Reveals a Novel Monolignol Analog," Biotechnology for Biofuels, (2012), *5:71.*

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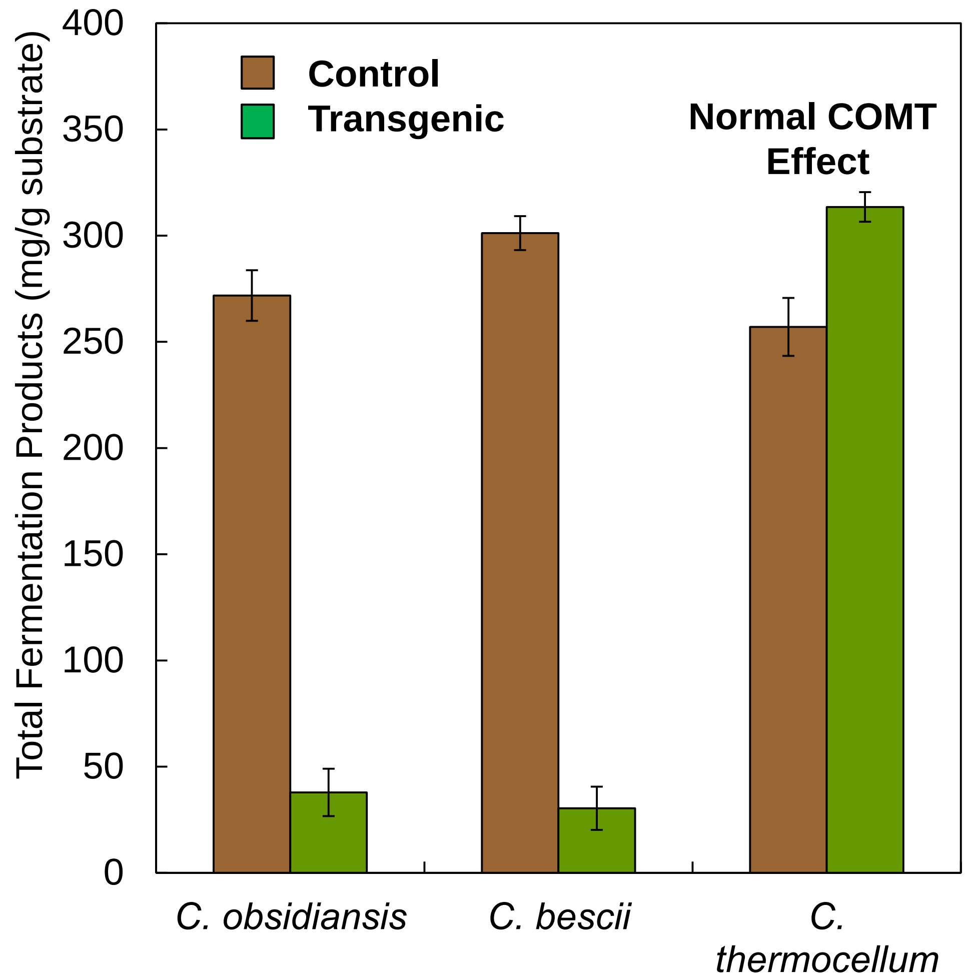
Comparison of fermentation of transgenic and control SWG by three CBP bacteria

- Fermentation conditions:
  - *C. obsidiansis* and *C. bescii*
    - 75° C
  - *C. thermocellum*
    - 58° C
  - Uniform media

- Fermentation of identical washed, pretreated batches of control and transgenic COMT2 switchgrass with *C. obsidiansis*, *C. bescii*, and *C. thermocellum* shows a differential of inhibition between the three CBP microorganisms



Total Fermentation Products (Acetate+Lactate+Ethanol)

Enabling technology: where we have come

- HTP recalcitrance pipeline for composition and sugar release for thousands of samples per year at 4 mg per sample
- Pipeline and material transfer agreement (MTA) data captured in LIMS
- Glycome profiling validated for analyses of cell wall structure and automated
- Tools to infer prediction of carbohydrate active enzymes

BESC reaches thousands of students with 'Farming for Fuels' lessons

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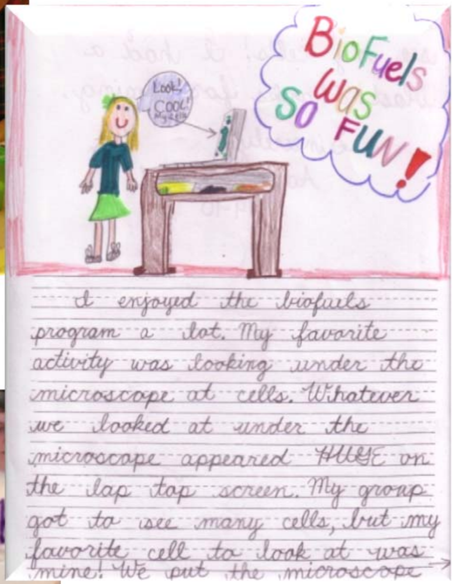
- BESC developed a set of hands-on lesson plans aimed at students in 4th to 6th grades
- Lessons educate students about the use of lignocellulosic biomass as a substrate for the production of biofuels and the technical and economic obstacles to a bio-based fuel economy
- The program has now reached more than 65,000 students, teachers and parents
- Accomplished by partnering with museums and centers in Tennessee, and eight other states.
- Moving towards a self-supporting program



CREATIVE DISCOVERY MUSEUM

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Looking forward: key strategic goals for BESC through Year 10

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- Less recalcitrant plants
  - “TOP40” switchgrass and *Populus*, field trials, pre-commercial testing
- Advanced consolidated bioprocessing (CBP) microbes
  - Realize rate, yield, titer and robustness requirements
- Improved pretreatment, feedstock and organism combinations
  - Optimized combination of BESC plant, microbe and pretreatment

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Elements of BESC's value system

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- Focus on impactful recalcitrance science
- A high-functioning team of world-class scientists
- Emphasis on integration and collaboration
- Close connection to industry increasing potential of impact
- Acceleration of research and technology outcomes
- Growing core of well-trained young research staff

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