

BioEnergy Science Center: An Integrated Strategy to Understand Biomass Recalcitrance

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Coordinator**

Paul Gilna, Director
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Presented to CBES



The BioEnergy Science Center

BESC: A multi-institutional DOE-funded center dedicated to understanding and modifying plant biomass recalcitrance



**322 People
in 19 Institutions**

The BioEnergy Science Center

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

Oak Ridge National Laboratory

National Renewable Energy
Laboratory

Samuel Roberts Noble Foundation

ArborGen, LLD

Ceres, Incorporated

Mascoma Corporation

DuPont

GreenWood Resources



University of Georgia

University of Tennessee

Cornell University

Dartmouth College

West Virginia University

Georgia Institute of Technology

University of California--Riverside

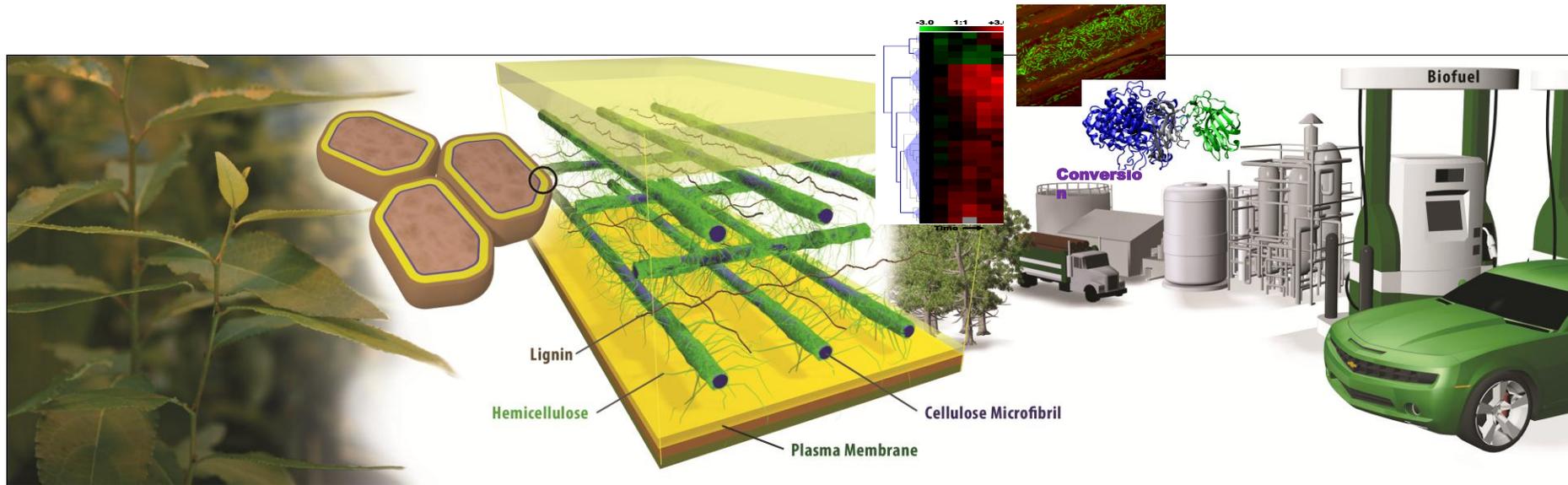
North Carolina State University

University of California—Los Angeles

300+ People in 17 Institutions

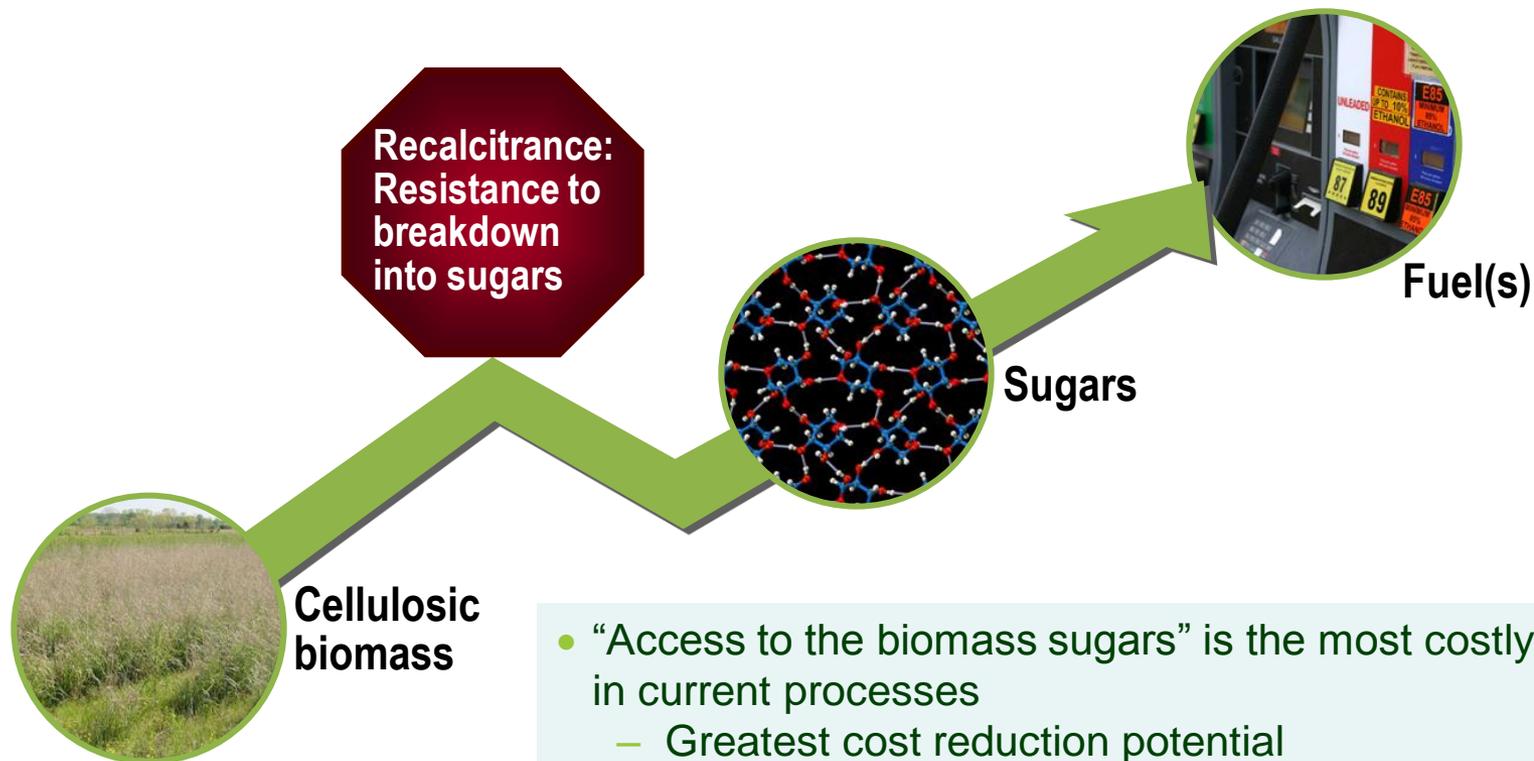


BESC mission statement



To enable the emergence of a sustainable cellulosic biofuels industry by leading advances in science and science-based innovation resulting in removal of recalcitrance as an economic barrier to cost-effective production of biofuels

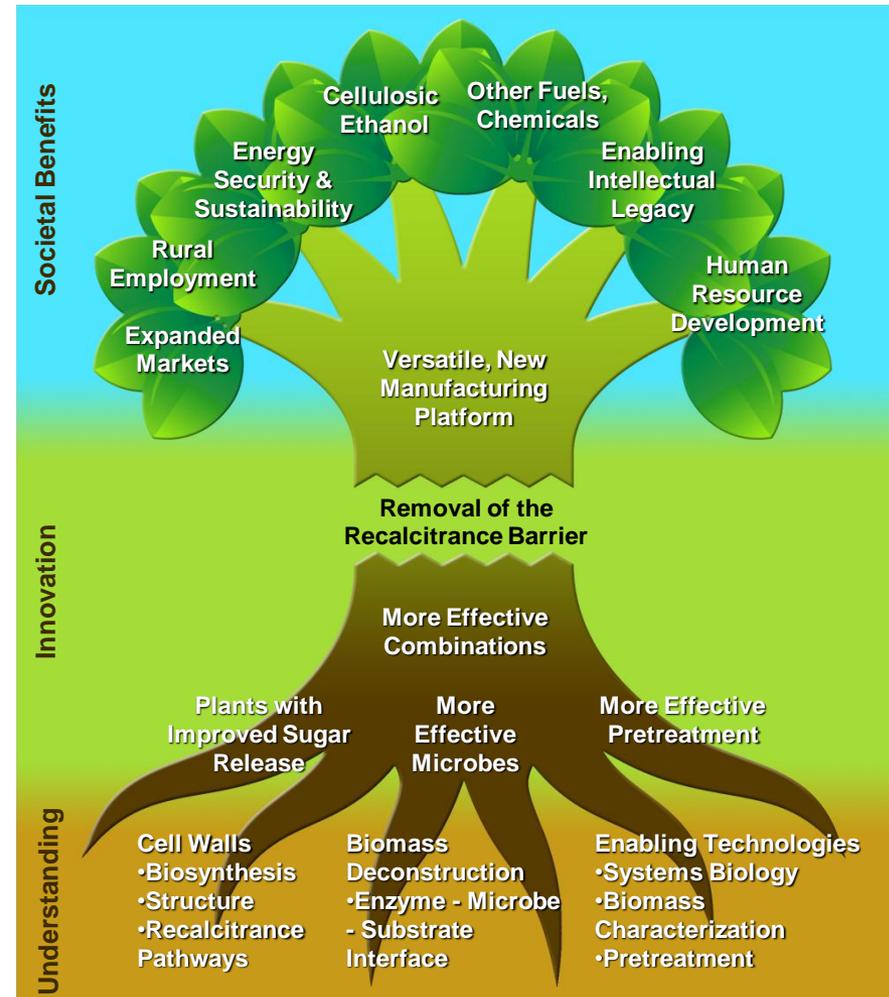
Overcoming biomass recalcitrance remains the single coherent theme for BESC



- “Access to the biomass sugars” is the most costly aspect in current processes
 - Greatest cost reduction potential
 - Advances necessary and sufficient to enable cellulosic biofuels industry
 - Enabling for all products, fuels, co-products
 - ethanol likely first, advanced fuels next

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

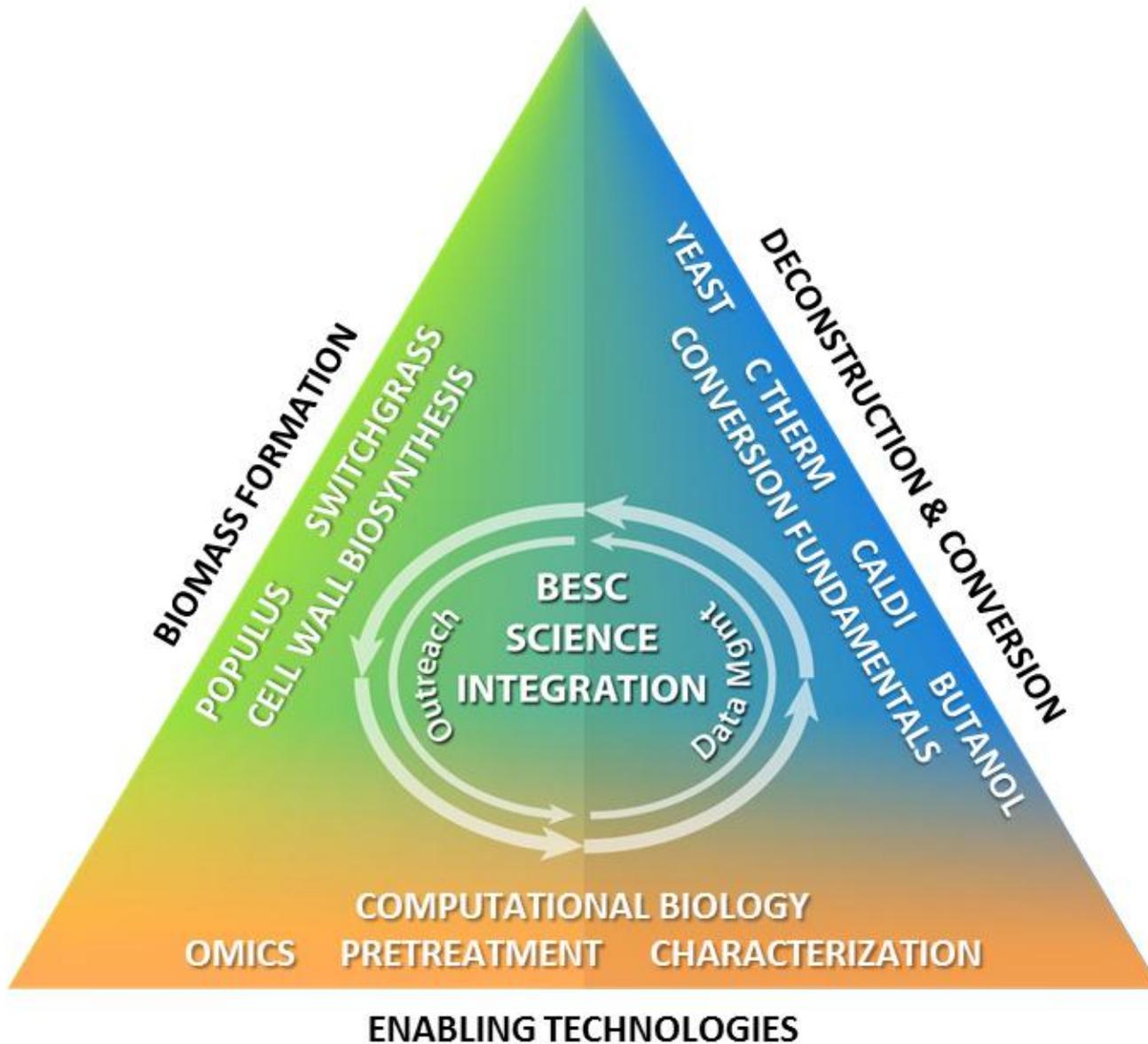
- Overcoming this barrier will cut processing costs significantly and be used in most conversion processes
- This requires an integrated, multi-disciplinary approach
- ***BESC believes biotechnology-intensive solutions offer greatest potential***



Top science accomplishments

- Genetic improvements to switchgrass
- Natural and genetically improved variants of *Populus*
- Development of genetic systems for *C. thermocellum* and *Caldicellulosiruptor*
- Tolerance to key inhibitors of microbial fermentation
- Cellulolytic microbes more effective than cell-free systems
- Modified yeast the basis of commercial development
- Production of isobutanol directly from cellulose
- High-throughput platform handles tens of thousands of feedstock samples

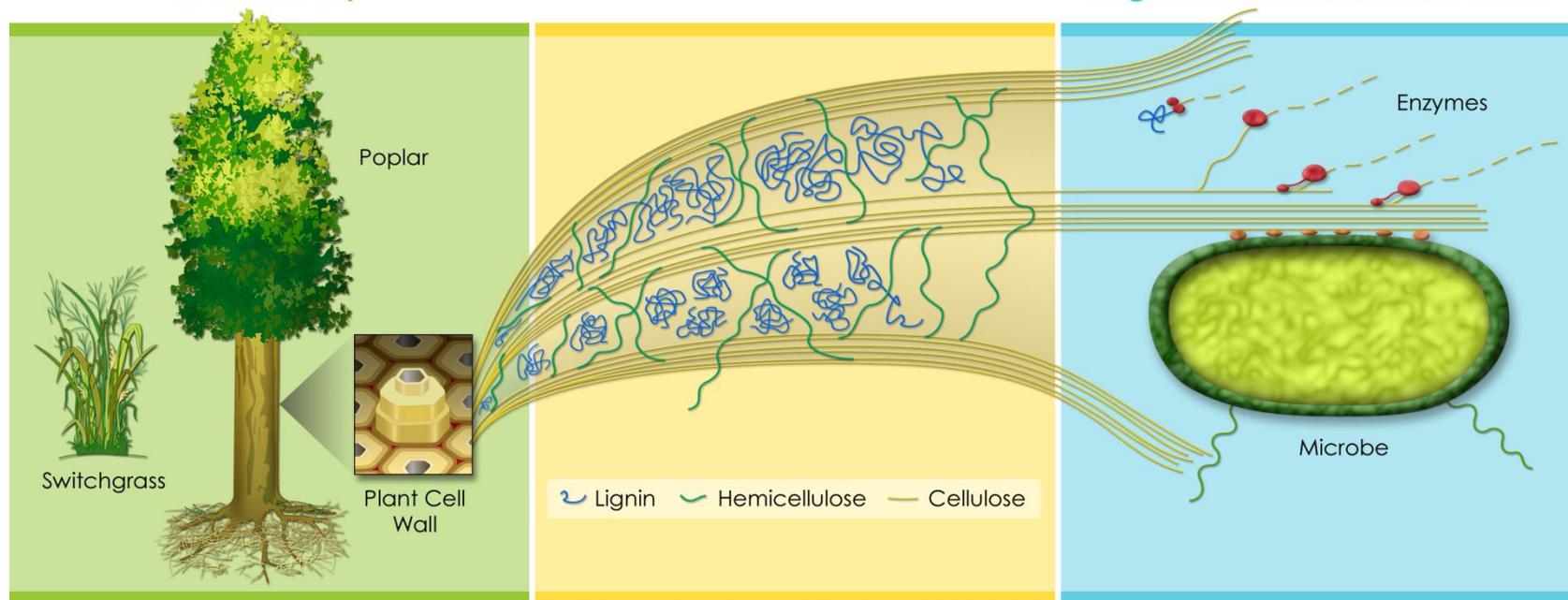
Three main science areas



A two-pronged approach to increase the accessibility of biomass sugars

Modify the plant cell wall structure to increase accessibility

Improve combined microbial approaches that release sugars and ferment into fuels

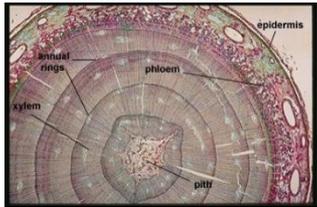


Both utilize rapid screening for relevant traits followed by detailed analysis of selected samples

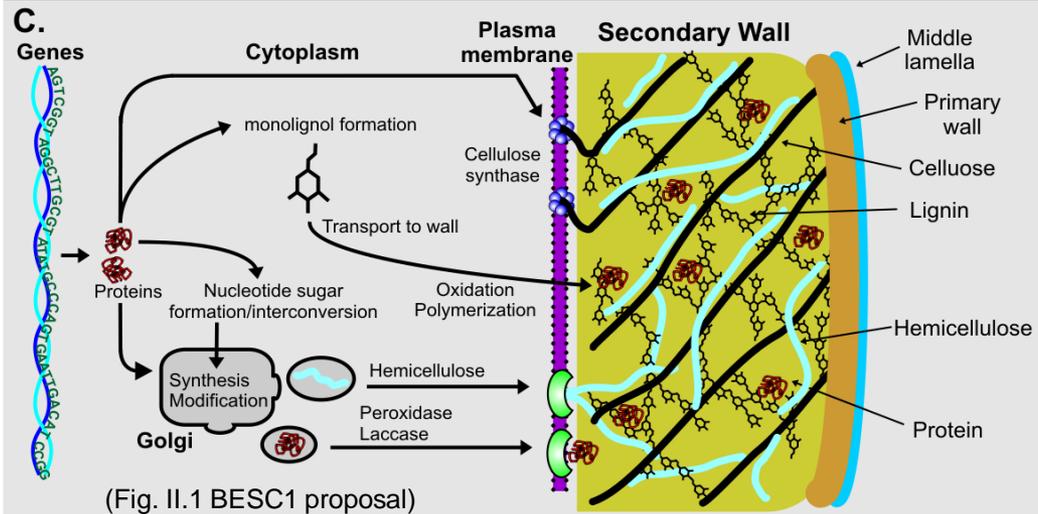
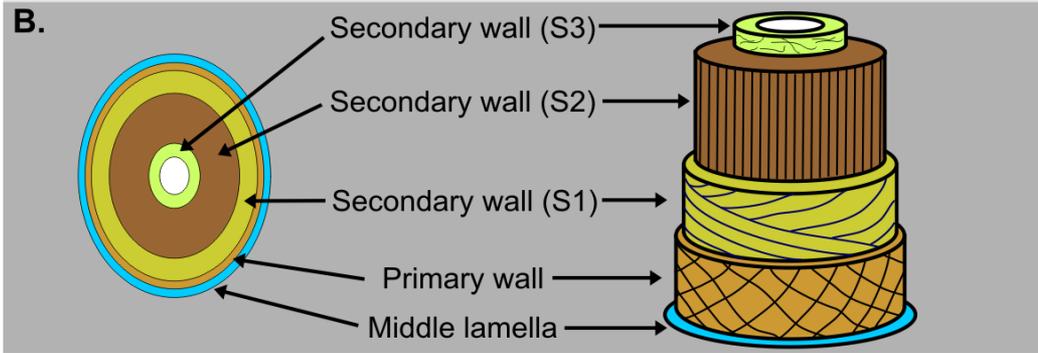
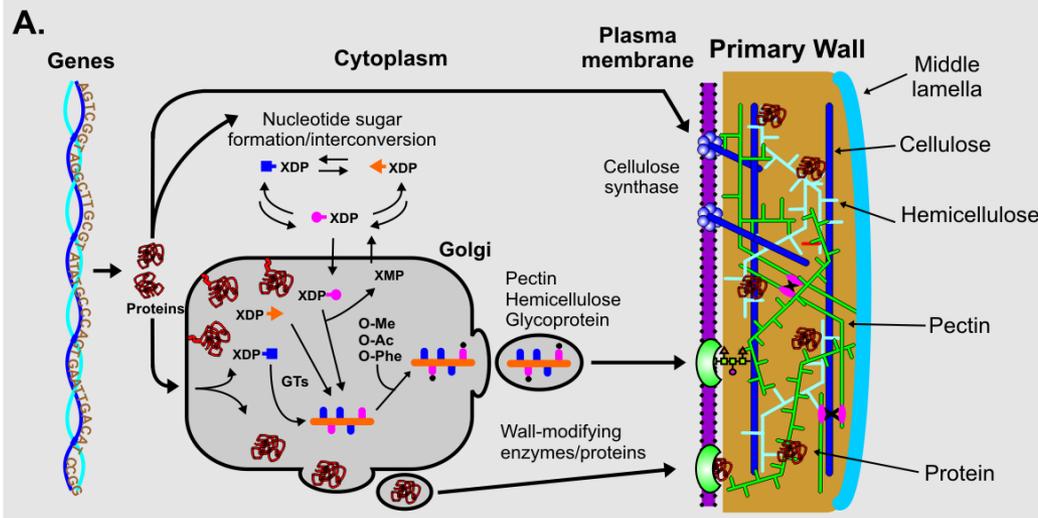
BESC: biofeedstocks



Switchgrass



Populus



(Fig. II.1 BESC1 proposal)

Primary Wall

Pectin
Hemicellulose
Cellulose
(proteins)

Secondary Walls

↓ Pectin
Hemicellulose
Cellulose
Lignin
(proteins)

Lignocellulosic Biomass

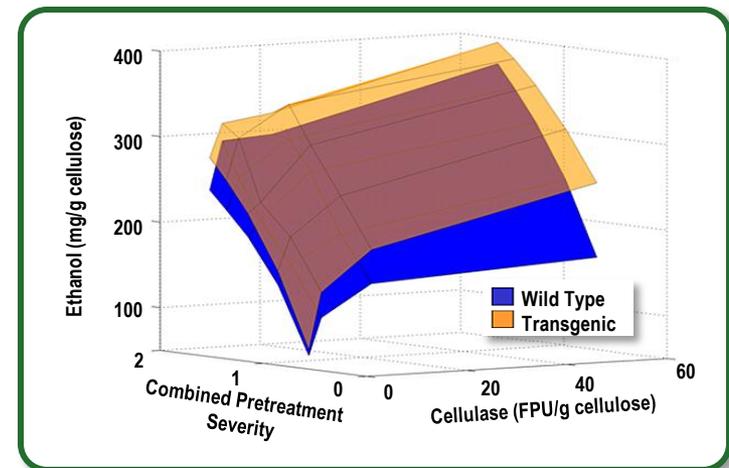
Genetic manipulation of lignin improves biofuel production from switchgrass

Down-regulation of a single gene reduces recalcitrance with no apparent growth defects and leads to:

- Increase in ethanol production by over one third.
- Reduction in needed severity of pre-treatment.
- Evidence that biofuel processing costs can be reduced by at least 20% with 300-400% lower enzyme costs.



Wild-type (L) and 3 transgenic switchgrass plants (R)



Impact of enzyme levels and pretreatment conditions on biofuel production

Modified switchgrass in field trials

Field Trials at UT-Knoxville

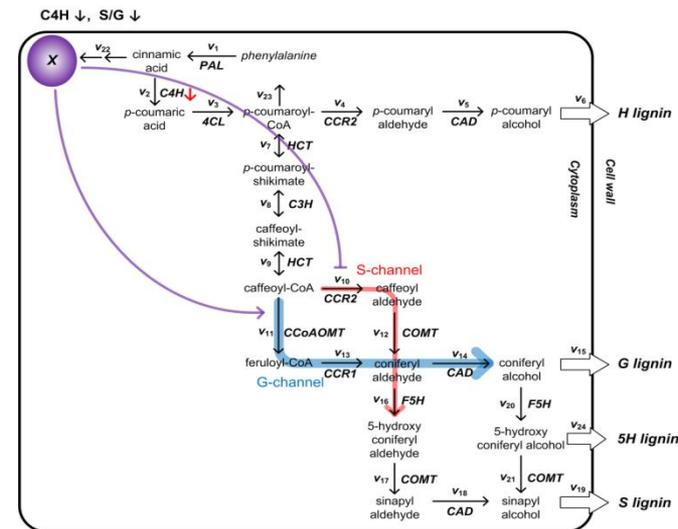


Field Trials at Ceres TAMU facility



Mathematical modeling elucidates control of lignin biosynthesis

- Ratios of lignin monomers can be a significant contributor to recalcitrance of biomass to cellulosic biofuel conversion.
- BESC researchers developed a novel model combining static flux-based analysis with Monte Carlo simulation to identify control points in the lignin biosynthetic pathway.
- The model, built upon experimentally generated S/G monolignol data from stem internodes of transgenic alfalfa (*Medicago sativa* L.) lines, predicted new metabolic channels and novel regulatory control schemes which then were confirmed experimentally (e.g., salicylic acid).
- Such wall biosynthesis pathway models are indispensable tools in the rational design of feedstock crops for efficient biofuels production.



The mathematical model allowed the inference of metabolic channels as well as a significant regulatory mechanism controlling the pathway.



Stem internodes in alfalfa

Lee et al., PLoS Comp. Biol. 2011



Switchgrass highlights

High-throughput *Agrobacterium*-mediated genetic transformation of switchgrass: 90% efficiency!!!



- A transformation efficiency of more than 90% has been achieved.
- It takes only about 4 months to produce *in vitro* rooted plants.
- A large number of independent transgenics (>800) were produced in 6 months.

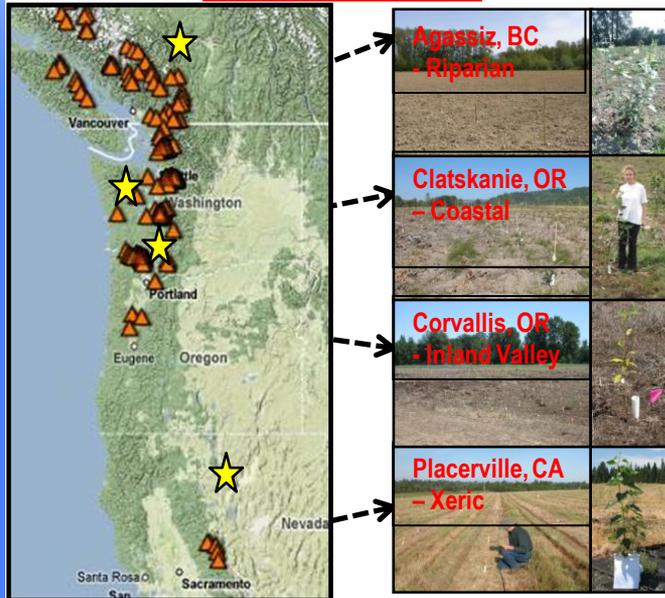
Fu, Wang, Dixon/Noble

Unpublished

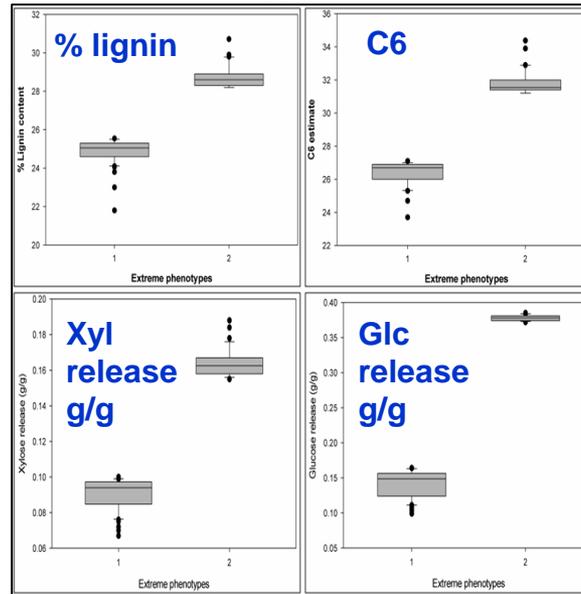
Populus highlights

Populus association genetic mapping yields reduced recalcitrance lines and affected genes in the natural population

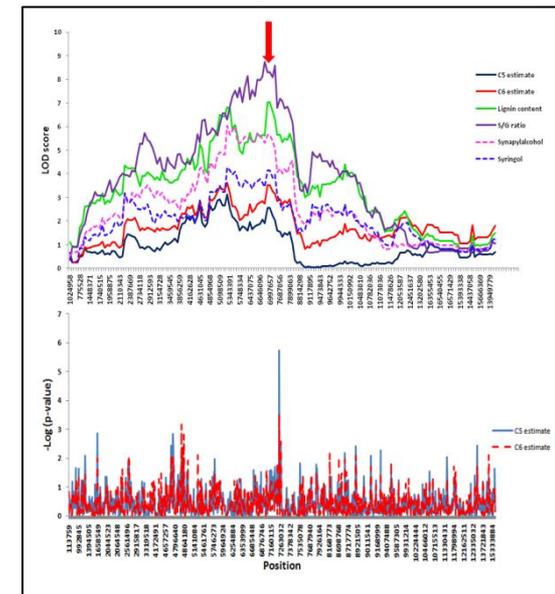
Common Gardens



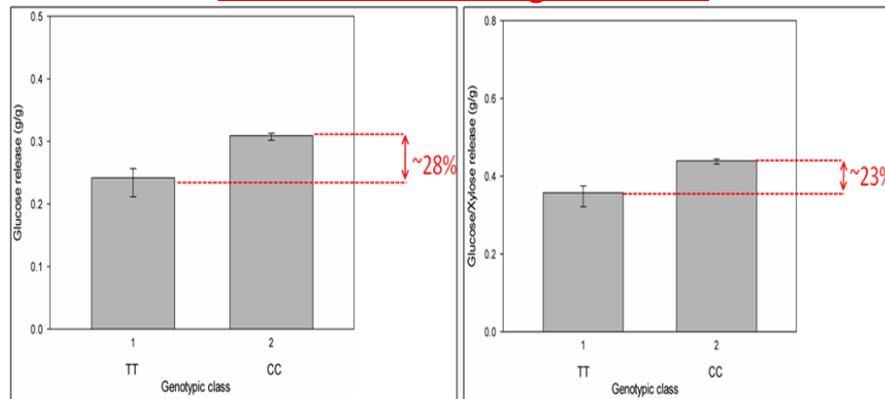
Phenotypic Variation



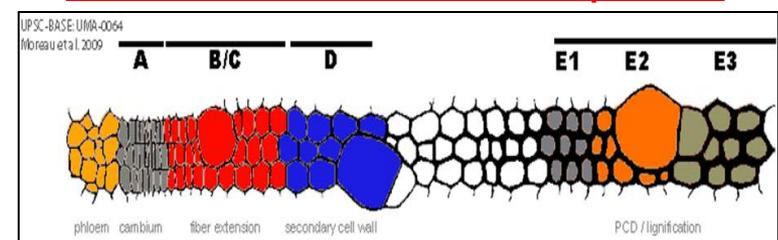
Gene ID using QTL/LD Mapping



Allelic Effects on Sugar Release



Gene Validation & Tissue Level Expression



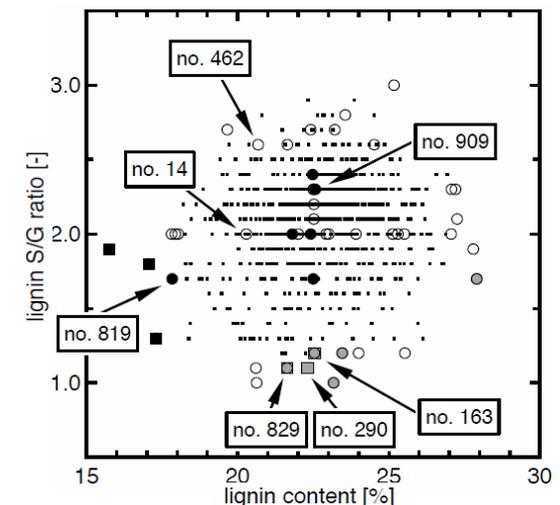
Muchero, Tuskan/ORNL

Lignin content and composition in natural *Populus* variants affects sugar release

- The largest recalcitrance studies of *Populus* natural variants.
- Strong negative correlation between lignin content and glucose release does not apply to biomass samples with an S/G ratio > 2.0.
- Certain natural variants yielded unusually high sugar yields with no pretreatment.
- SNP discovery revealing new recalcitrance genes



Orthogonal relationship plot between lignin content and S/G ratio characterization of 1100 *Populus* natural variants.



Contact: Charlie Wyman (charles.wyman@ucr.edu, 951 781-5703)

Funding Source: DOE Office of Science BioEnergy Science Center

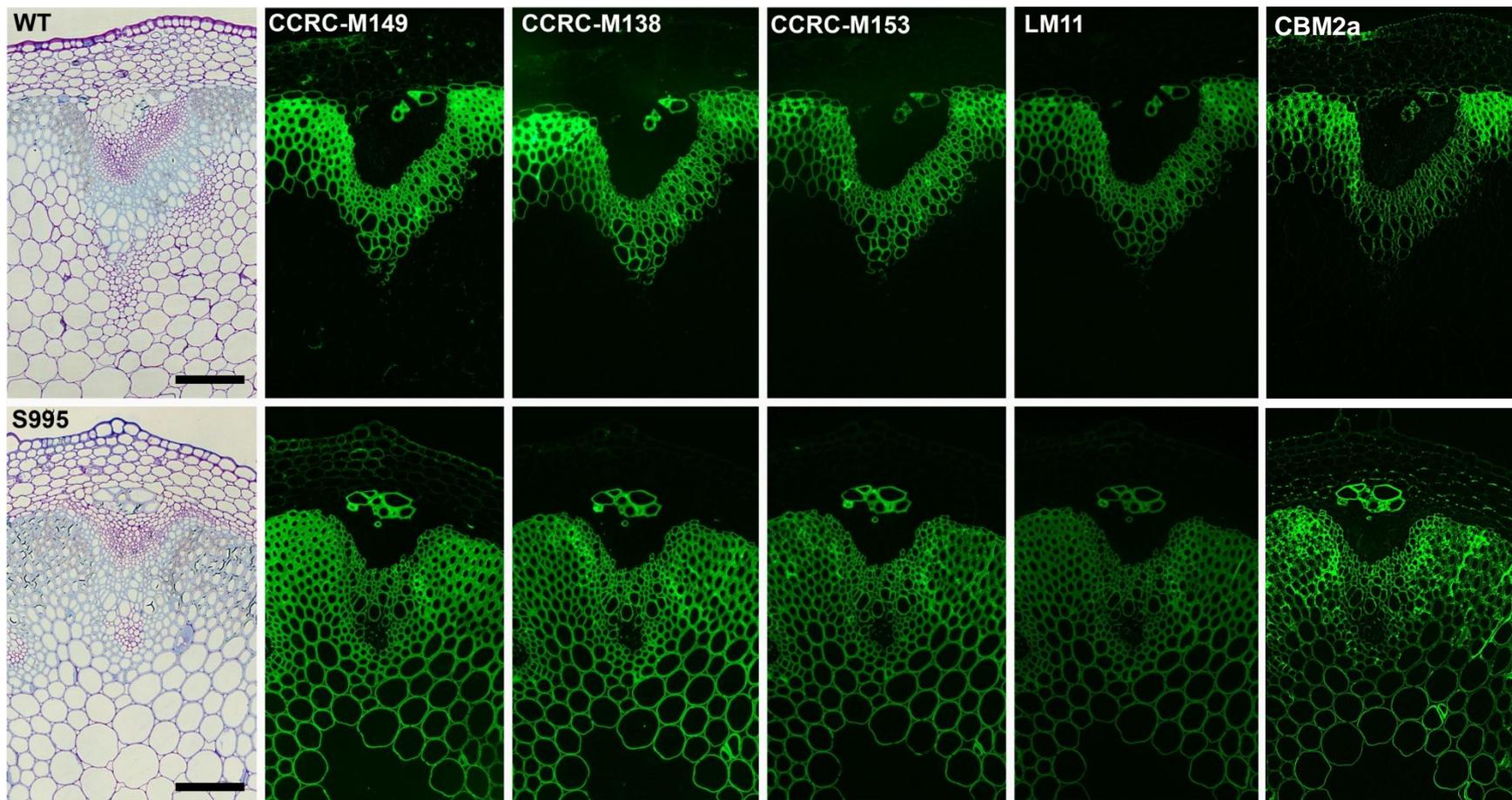
Citation: Studer et al. Lignin content in natural *Populus* variants affects sugar release. PNAS 108:6300-5, 2011

Order-of-magnitude difference in sugar release from natural variants in *Populus*

- Identified **19** genes that control reduced recalcitrance in *Populus*
- Identified **6** individual genotypes that yield significant glucan sugars



Mutation in a WRKY transcription factor leads to ectopic secondary wall deposition in *Arabidopsis*: breakthrough in biomass densification and tool to study lignin:polysaccharide connection



Xylan

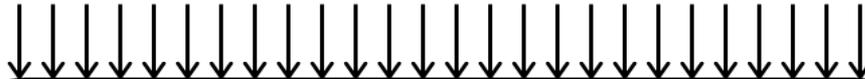
Systems analysis and elite line selection activity will drive BESC in the next years

Overview of TOP40 and ELITE8 line selection process

Selection process for TOP and ELITE *Populus* and switchgrass reduced-recalcitrance lines

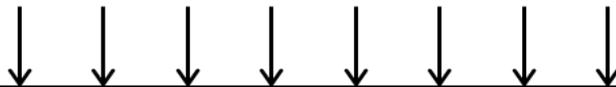
Candidate Reduced Recalcitrant Lines
Bioenergy feedstock lines identified from:

Association genetics
Activation tagged lines
Transgenic lines



TOP40 Lines
TOP20 *Populus* + TOP20 switchgrass

Selected TOP40 lines with greatest reduction in recalcitrance and best agronomic characteristics



ELITE8 Lines
ELITE4 *Populus* + ELITE4 switchgrass

Based on reduced recalcitrance and agronomic characteristics

Year 5-Year 7/8:
continue to identify candidate TOP40 Lines
(based largely on high sugar release)

Year 5:
-begin selection of TOP40 lines
-form TOP40 Systems Analysis teams as each line is selected

Year 8:
begin to select ELITE8
-highest sugar release
-best agronomics
-unique recalcitrance pathways

Year 10:
-develop superior bioenergy feedstocks
-understand mechanism of recalcitrance

High-throughput characterization pipeline for recalcitrance phenotype

Screening thousands of samples

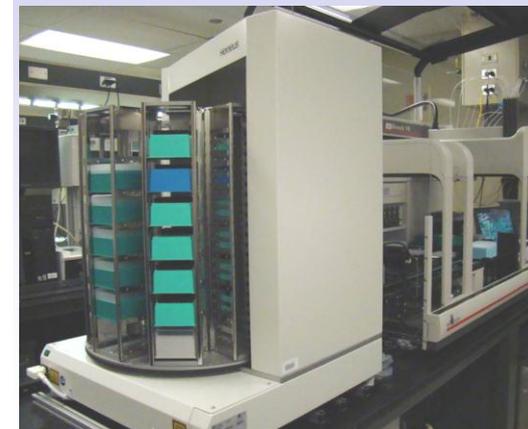
Composition analytical
pyrolysis, IR, confirmed
by wet chemistry



Pre-treatment
new method with dilute
acid and steam



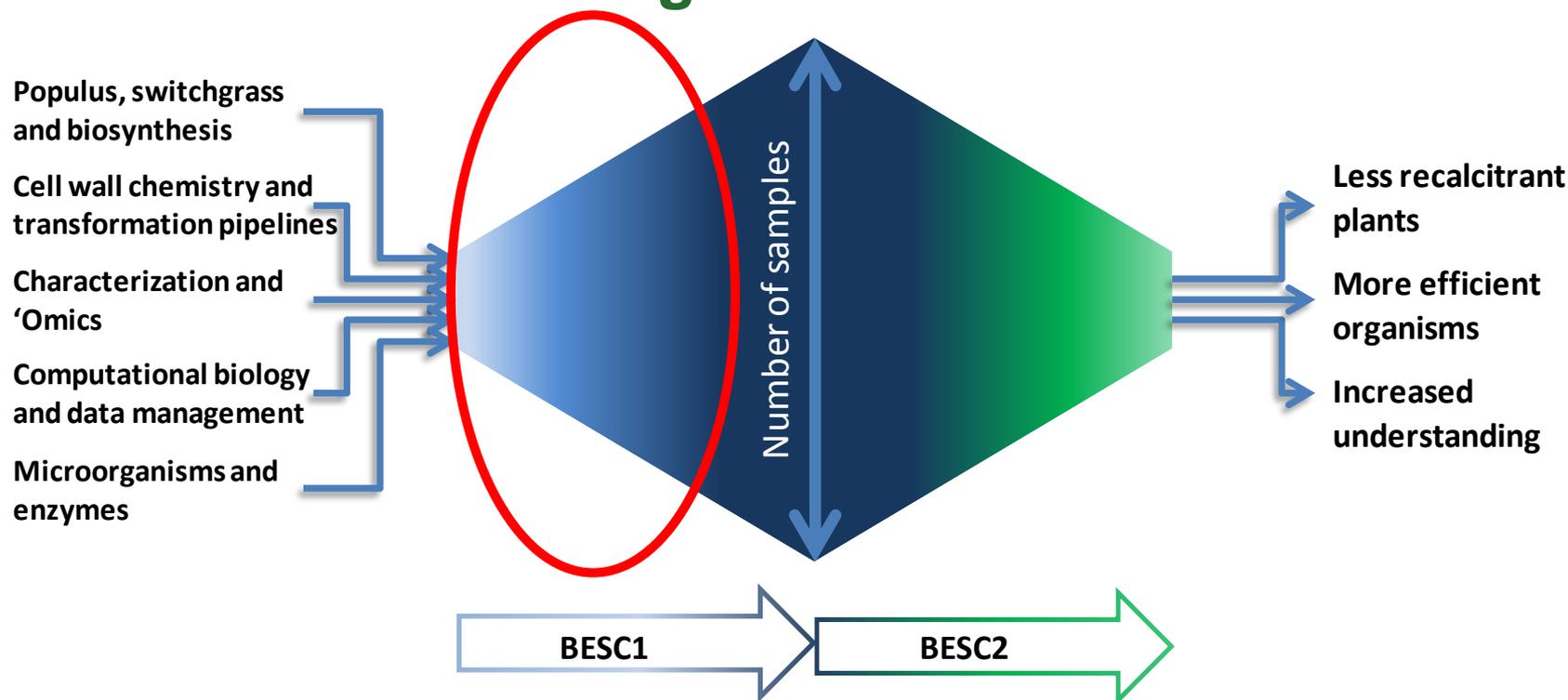
Enzyme digestibility
sugar release
with enzyme cocktail



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)

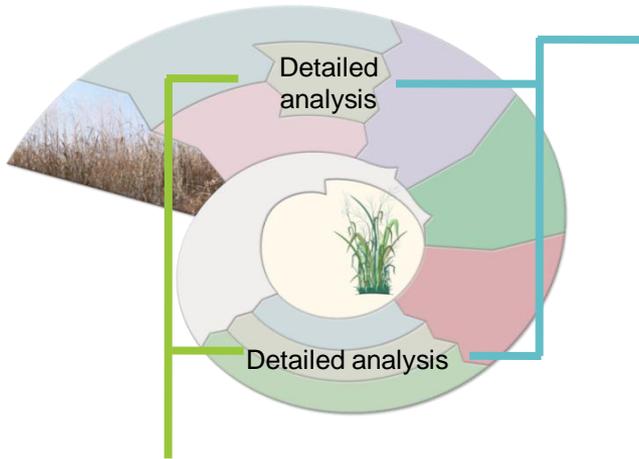
Enabling Technologies provided needed infrastructure for integration in BESC1



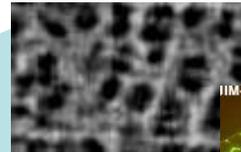
Enabling Technology developments

- High-throughput pipelines for cell wall chemistry and recalcitrance.
- Deployed a unique bioenergy-based knowledgebase (BESC KB).
- Tools predicting biosynthesis and degradation enzymes (CAT).
- A LIMS to gather and report datasets across the BESC.
- Switchgrass Affymetrix gene chips to identify plant cell wall biosynthesis genes.

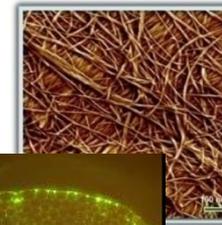
Characterization tools for feedstock and microbial samples



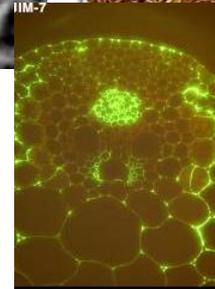
Imaging



Bio-ultraCAT for 3-D density of *Populus* cell walls



AFM of switchgrass showing cellulose microfibrils

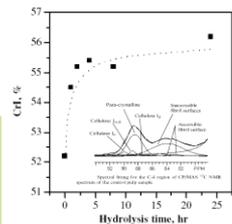


Immunolocalization using wall antibodies on switchgrass

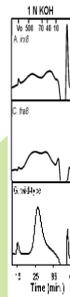


The University of Georgia

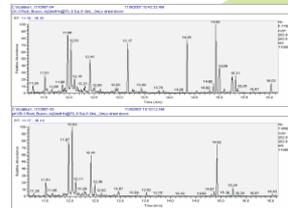
Chemistry



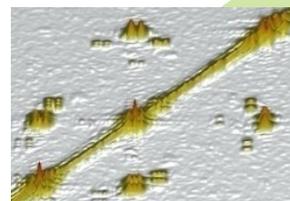
NMR for cellulose crystallinity



Fractionation and chromatography



Mass Spectrometry for key metabolites



2D ¹H-NMR sees altered bonds in polysaccharides and lignin in biomass

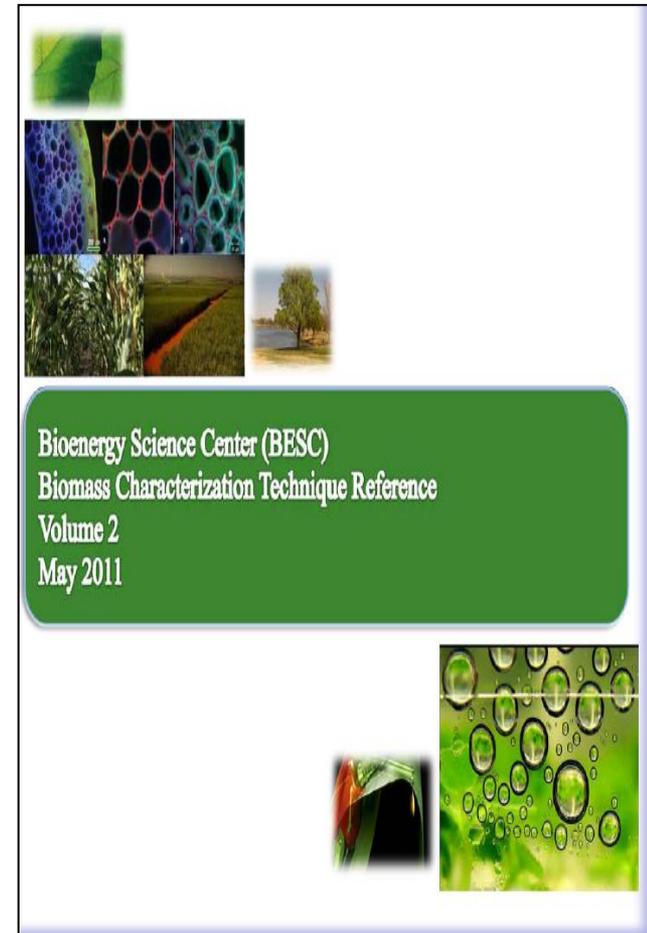


The University of Georgia



BESC compiles Biomass Characterization Technique Reference

- This is a compilation of various established Biomass Characterization Techniques that are available to BESC Researchers. This handbook is being shared with GLBRC and JBEI to help identify unique analytic capabilities.
- Techniques are grouped by capacity:
 - First Tier (capable of >thousand analyses per month)
 - HTP screens for composition by analytical pyrolysis and digestibility by pretreatment and enzyme digestion
 - Second Tier (capable of >hundred analyses per month)
 - Includes glycome profiling, enhanced pretreatment and digestion assays, and carbohydrate measurements
 - Third Tier (lower throughput techniques for specific samples and measurements).
- This includes brief descriptions of the methods, sample preparation, and the contact person.



Impact of lignin down-regulation on cell wall composition and sugar release

Achievement: Sugar release improvement of 150-200% (Alfalfa/Euc) or ethanol yields increases of 30% (Switchgrass)

Alfalfa - p-coumarate 3-hydroxylase (C3H) or hydroxy cinnamoyl-CoA:shikimate/quininate hydroxy cinnamoyl transferase down regulation^{1,2}

- decreases molecular weight of extractable lignin
- changes S/G ratio
- changes inter-unit linkage distribution

Eucalyptus – p-coumarate 3-hydroxylase or cinnamate 4-hydroxylase (C4H) down regulation show similar trends to Alfalfa³

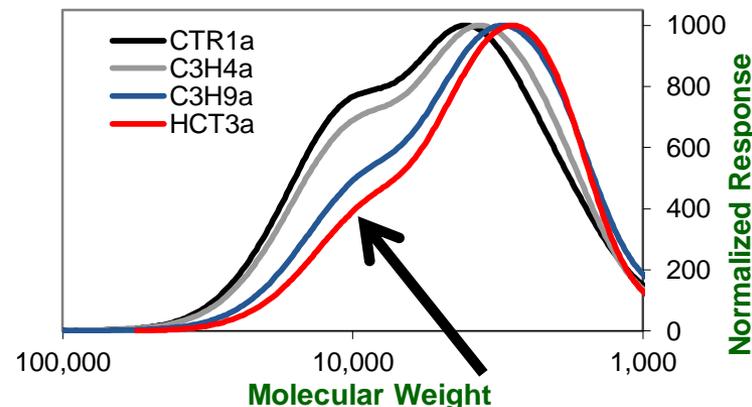
Switchgrass –

Caffeic acid O-methyl transferase (COMT) down regulation

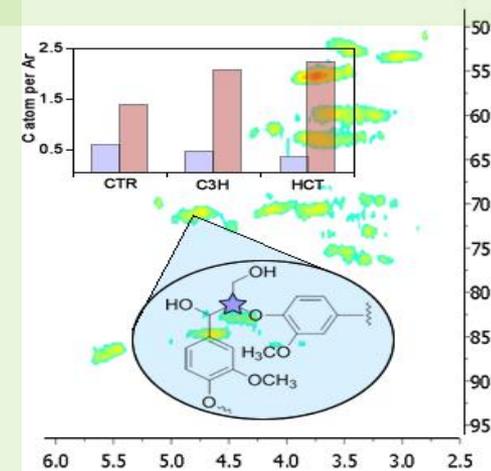
- More G lignin
- New lignin sub-unit characterized⁴
- Increase in ethanol yields⁵

1 – Ziebell *et al.* 2010 *JBC*;
2 – Pu *et al.* 2009 *Bioenergy Res*;
3 – Two manuscripts in preparation;
4 – Samuel *et al.*, under review;
5 – Fu *et al.* 2011 *PNAS*

Alfalfa: Molecular weight distribution of lignin



Understanding the reason for decrease in molecular weight has led to a NREL/ORNL collaborative computational modeling project.



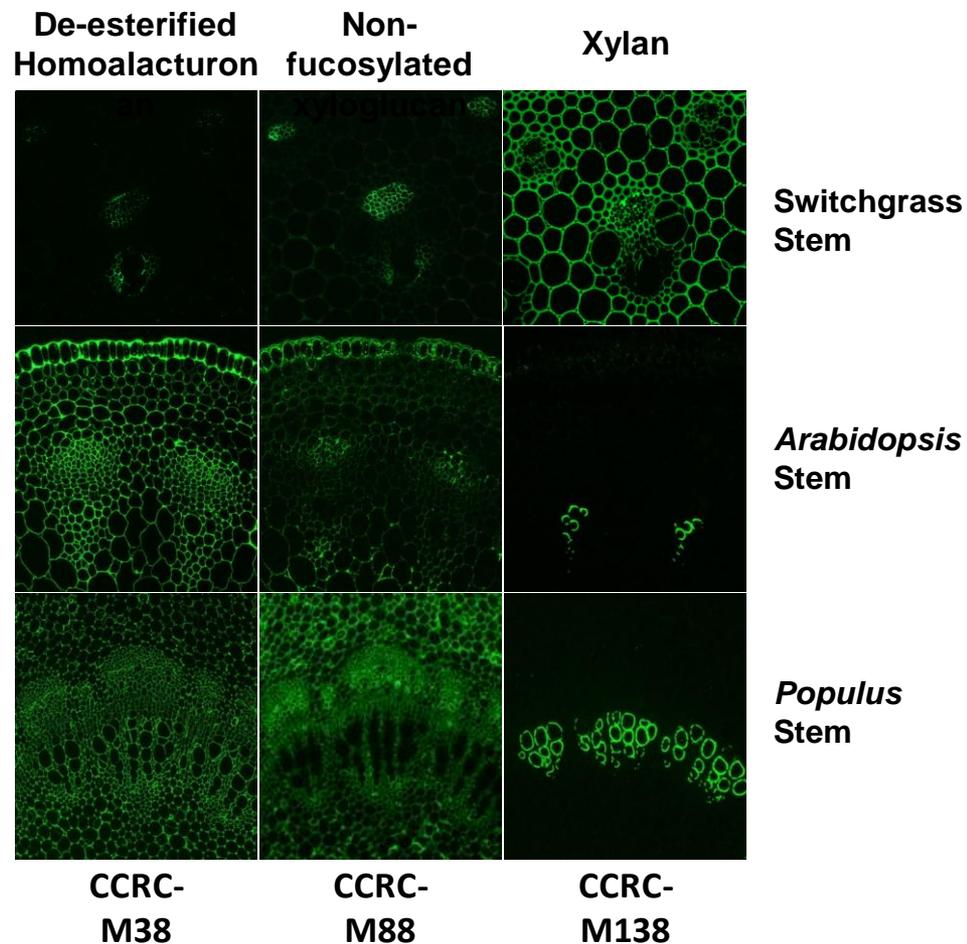
Alfalfa: 2D ¹H-¹³C HSQC NMR identifies changes in lignin

Carbohydrate antibodies enable imaging and profiling of mutants

Panel of >180 antibodies against plant cell wall carbohydrate epitopes: extraordinary tools for understanding wall structure and recalcitrance, e.g. glycome profiling and immunolabeling.

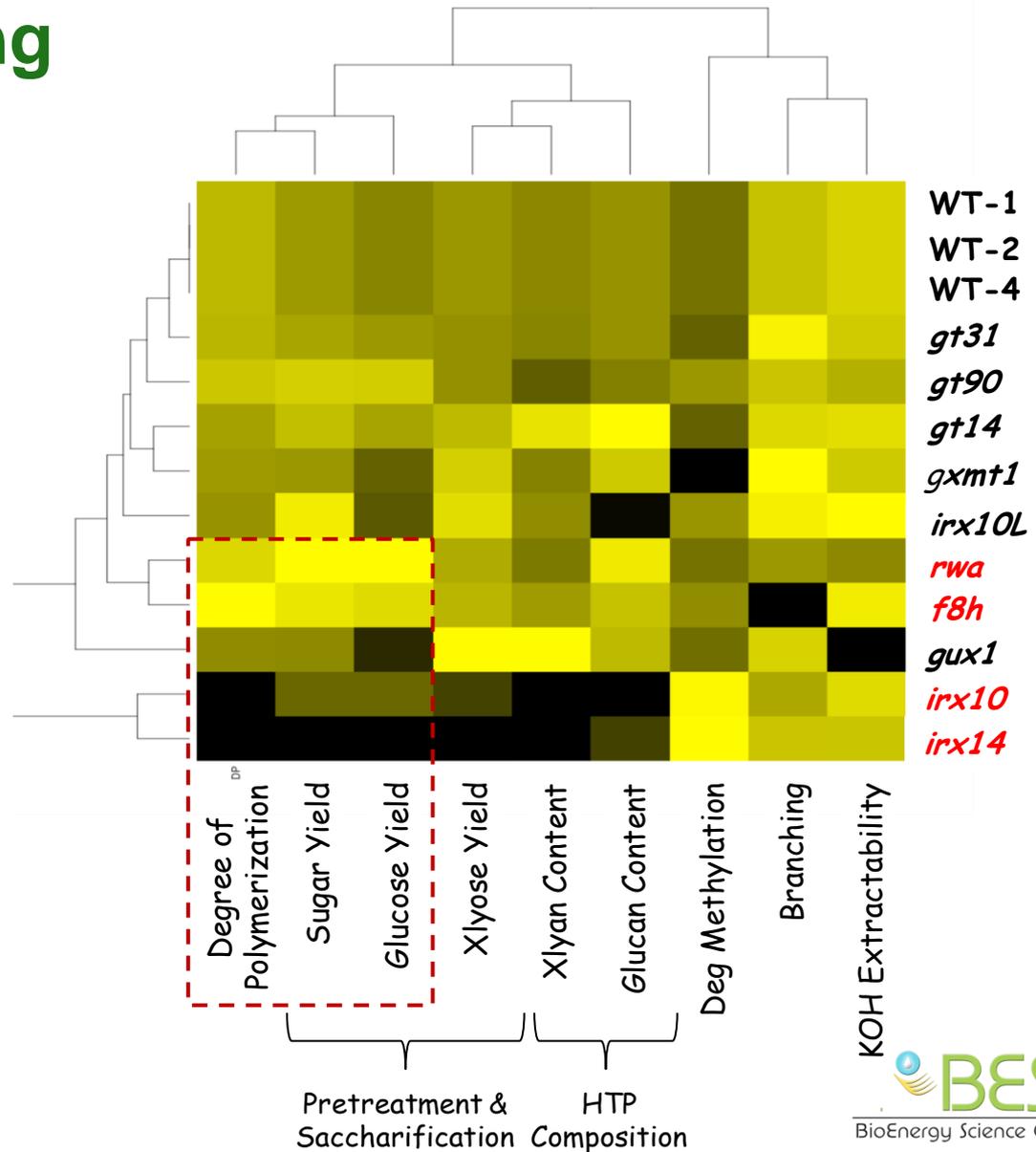


Hahn *et al.*

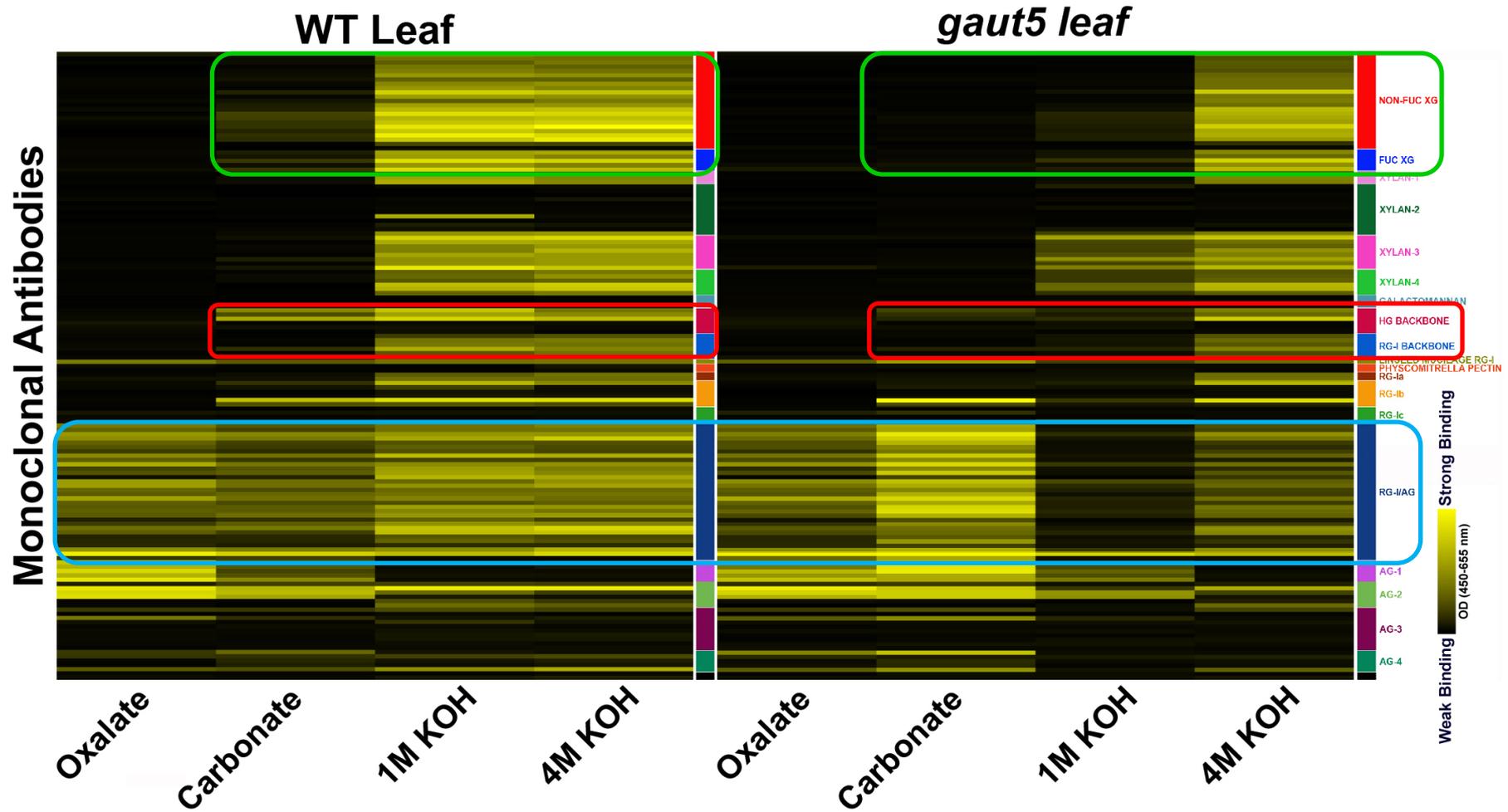


Clustering analysis of cell wall chemotype and recalcitrance properties by glycome profiling

- *Arabidopsis* mutants with altered xylan biosynthesis (CCRC).
- Recalcitrance properties (NREL and UC-Riverside).
- Correlations with the chemical structures of xylan (CCRC).
 - Identified xylan DP is strongly correlated to sugar yield supporting hypothesis that specific xylan structural features affect recalcitrance.
 - Corollary to hypothesis is understanding the relationships between xylan structure and recalcitrance will provide a basis for the development of improved biofuels crops.

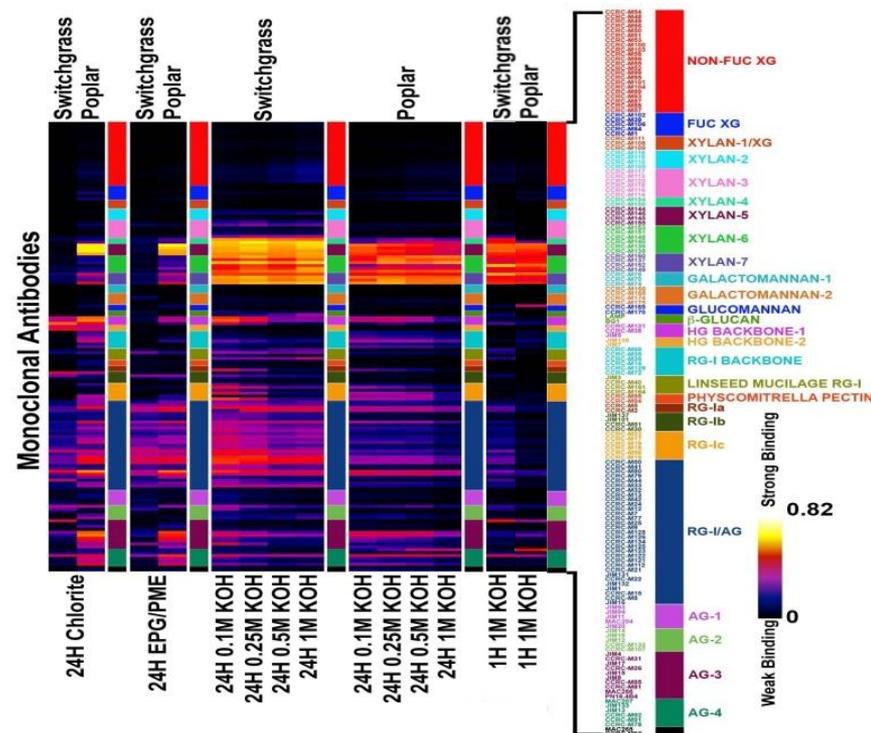


Glycome profiling identifies role of GAUT5 in hemicellulose, pectin and arabinogalactan wall extractability: pushing understanding of wall structure and wall loosening



Investigating how the removal of specific cell wall components affects digestibility

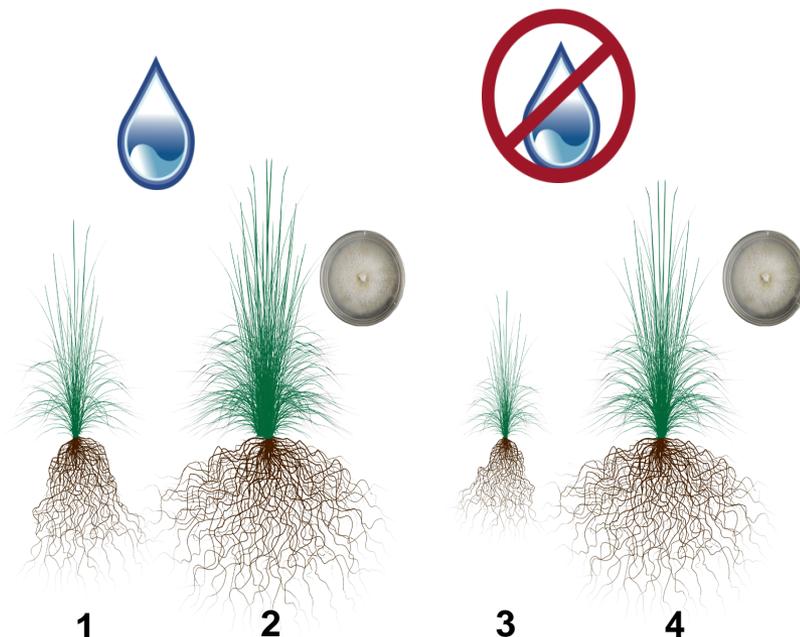
- Chemical and enzymatic extractions produced *Populus* and switchgrass samples varying cell wall composition.
- The samples were characterized using glycome profiling.
- Significant differences were observed between *Populus* and switchgrass, presumably due in large part to cell wall structural differences between dicots and monocots.
- Removal of pectins/arabinogalactans had little effect on digestibility for both *Populus* and switchgrass under pretreatment conditions studied.
- In switchgrass, digestibility was associated with removal of methyl glucuronoxylan, arabinoxylan, and unsubstituted straight chain xylan.
- In *Populus*, digestibility was associated with removal of lignin.



Hahn/UGA, Wyman/UCR

Mycorrhizae inoculation increases switchgrass biomass under drought

- Drought is one of the most important abiotic constraints of plant biomass production worldwide.
- Symbiotic microbes provide several benefits to host plants including drought tolerance.
- BESC researchers conducted greenhouse studies and discovered that colonization of switchgrass roots by the fungus *Sebacina vermifera* increased biomass yield by 258% with normal watering and 132% under severe drought.
- Colonized plants subjected to severe drought (4, at right) produced 173% more biomass than well-watered uninoculated control plants (1, at right).
- Symbiotic microbes hold great potential for bioenergy crop production, or any agronomic crop, grown under a low-input regime.



Normally watered plants without fungus (1) and with fungus (2); drought exposed plant without fungus (3) and with fungus (4).

Contacts: Kelly Craven (kdcraven@noble.org, 580 224 6960)

Funding Source: DOE Office of Science BioEnergy Science Center

Citation: Ghimire SR and Craven KD (2011). The ectomycorrhizal fungus *Sebacina vermifera*, enhances biomass production of switchgrass (*Panicum virgatum* L.) under drought conditions. Applied and Environmental Microbiology (doi:10.1128/AEM.05225-11)

Unusual “C-lignin” polymer discovered in certain plant seeds

- Seeds of both monocot and dicot species contain previously unsuspected lignin polymers constructed almost entirely from catechyl (C) units. This C-polymer is a major component of the seed coat of *V. planifolia*, whereas the stem, leaf, and aerial root have only typical angiosperm G/S lignins.
- Impact:
 - This discovery shows the natural capability that native plants have in producing specific lignin polymers.
 - It supports the radical coupling polymerization hypothesis.
 - This lignin may be new source for carbon fiber manufacturing
 - Selected as the cover story for an upcoming issue in the Proceedings of the National Academy of Sciences and as an “editor’s choice” story in an upcoming volume of Science magazine
- C-lignin polymer is produced in vivo via combinatorial oxidative radical coupling that is under simple chemical control, a mechanism analogous to that theorized for classical lignin biosynthesis.
- A lignin in the monocotyledonous angiosperm Vanilla orchid (*Vanilla planifolia*) that is naturally biosynthesized from the unusual C monolignol, caffeyl alcohol. Similar polymers are found in the seeds of other vanilla species and several species of cacti (which are dicots).
- The absolute levels of the C-lignin in the vanilla seed are substantially higher than the values estimated from the released thioacidolysis monomers because of the unusual structure of the polymer

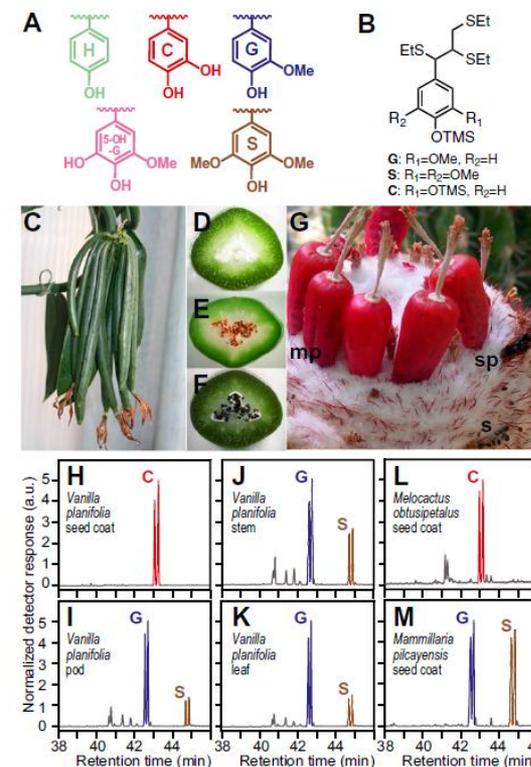


Fig. 1. A C-lignin polymer in plant seeds. (A) Aromatic structures of conventional and novel lignin units (see text and Fig. S1). (B) Diagnostic monomeric compounds released via thioacidolysis of guaiacyl (G), syringyl (S), and catechyl (C) lignin polymers. (C) Mature *V. planifolia* beans. (D–F) Cross-sections of *V. planifolia* beans at 6 (D), 8 (E), and 10 (F) wk postpollination. (G) Terminal cephalium of *Melocactus obtusipetalus*, showing mature seed pods (mp), a senesced seed pod (sp) revealing contents, and individual released seeds (s). (H–M) Partial total-ion chromatograms of thioacidolysis products from *V. planifolia* (H) seed coat, (I) pod (without seed), (J) stem, and (K) leaf, and (L) *Melocactus obtusipetalus*, and (M) *Mammillaria pilcayensis* seed coat.

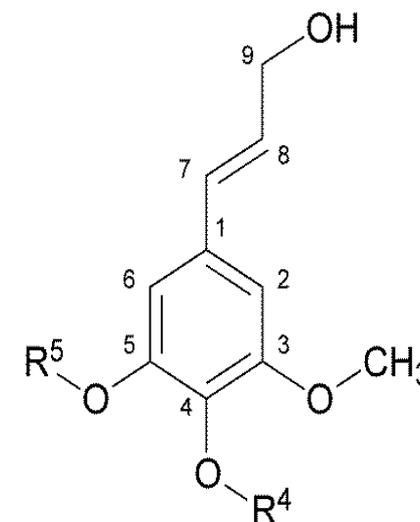
Contacts: (Samuel Roberts Noble Foundation) Richard Dixon (radixon@noble.org, xxx-xxx-xxxx)

Funding Source: DOE Office of Science BioEnergy Science Center, Great Lakes Bioenergy Center

Citation: Chen, F., Tobimatsu, Y., Havkin-Frenkel, D., Dixon, R.A., Ralph, J., A polymer of caffeyl alcohol in plant seeds PNAS 2012 ; published ahead of print January 17, 2012, doi:10.1073/pnas.1120992109

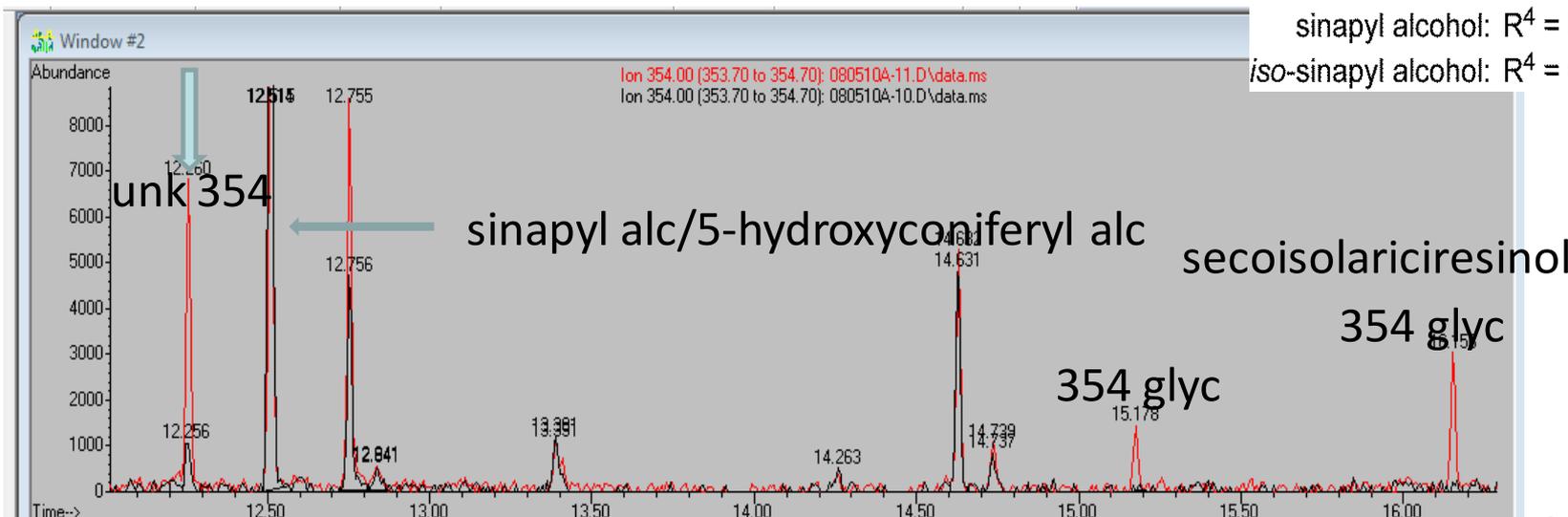
BESC identifies a new monolignol and its synthesis pathway in COMT-deficient switchgrass

- GC-MS metabolomics and NMR confirmed by synthesis for iso-sinapyl and derivatives.
- Reactivity estimated by computation modeling of lignin monomers.



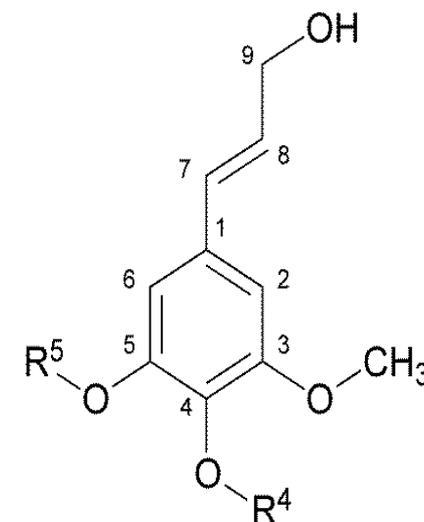
sinapyl alcohol: $R^4 = H$ $R^5 = CH_3$

iso-sinapyl alcohol: $R^4 = CH_3$ $R^5 = H$

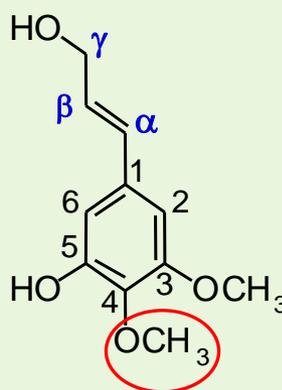
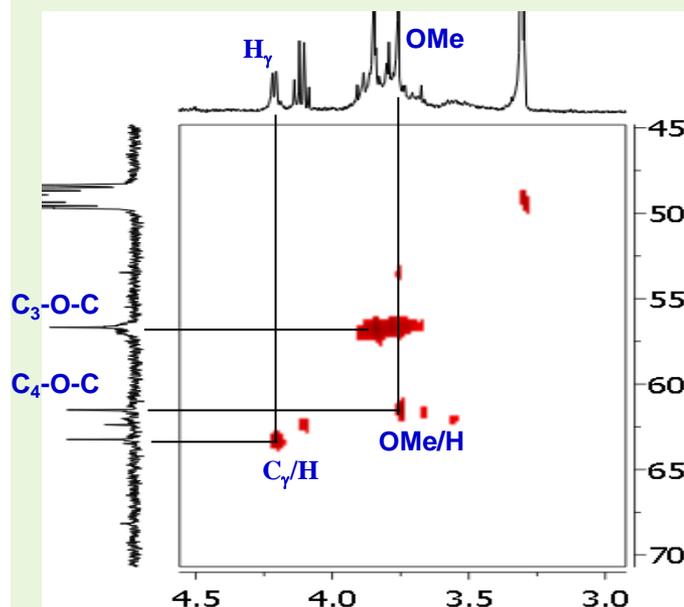


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sinapyl alcohol: $R^4 = H$ $R^5 = CH_3$
iso-sinapyl alcohol: $R^4 = CH_3$ $R^5 = H$



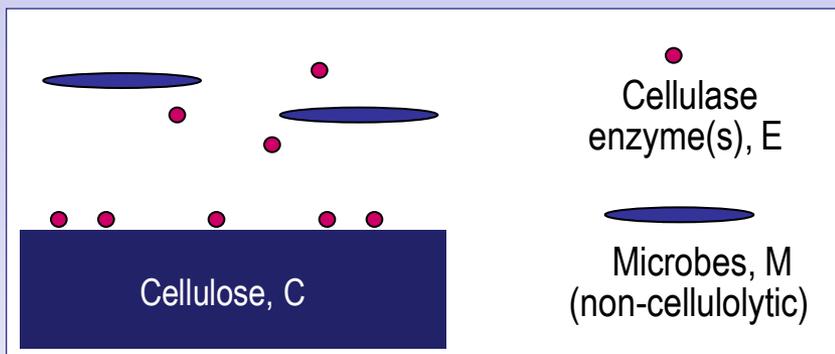
Switchgrass: 2D 1H - ^{13}C HSQC NMR identifies new sub-unit in COMT down-regulated switchgrass lignin

Tschaplinski *et al.*, *New Phytologist*.
in revision 2012

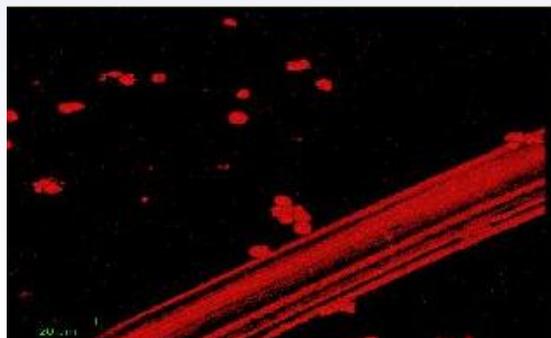
Enzymatic and microbial hydrolysis

A fundamentally different relationship between microbes and cellulose

Enzymatic hydrolysis (classical approach)

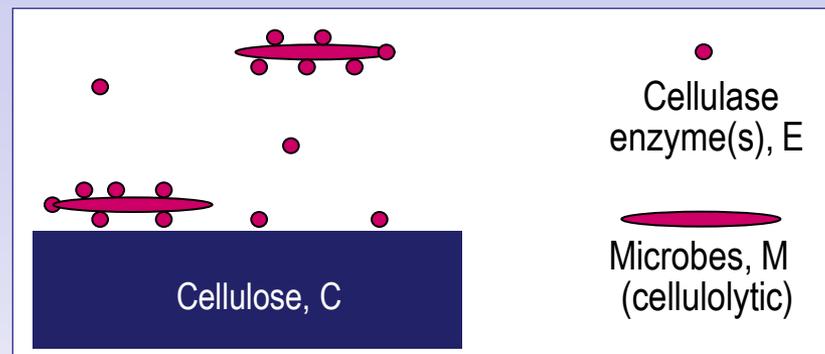


- Hydrolysis mediated by CE complexes
- Enzymes (several) both bound and free
- Cells may or may not be present

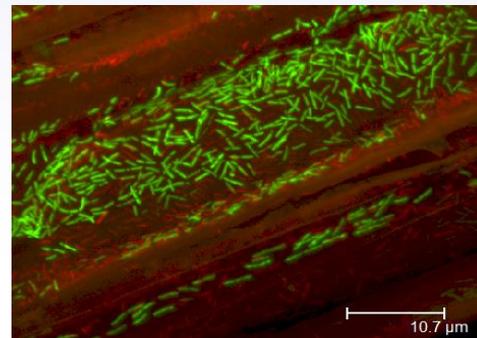


Yeast, enzymes with biomass (Dumitrache and Wolfaardt)

Microbial hydrolysis (CBP)



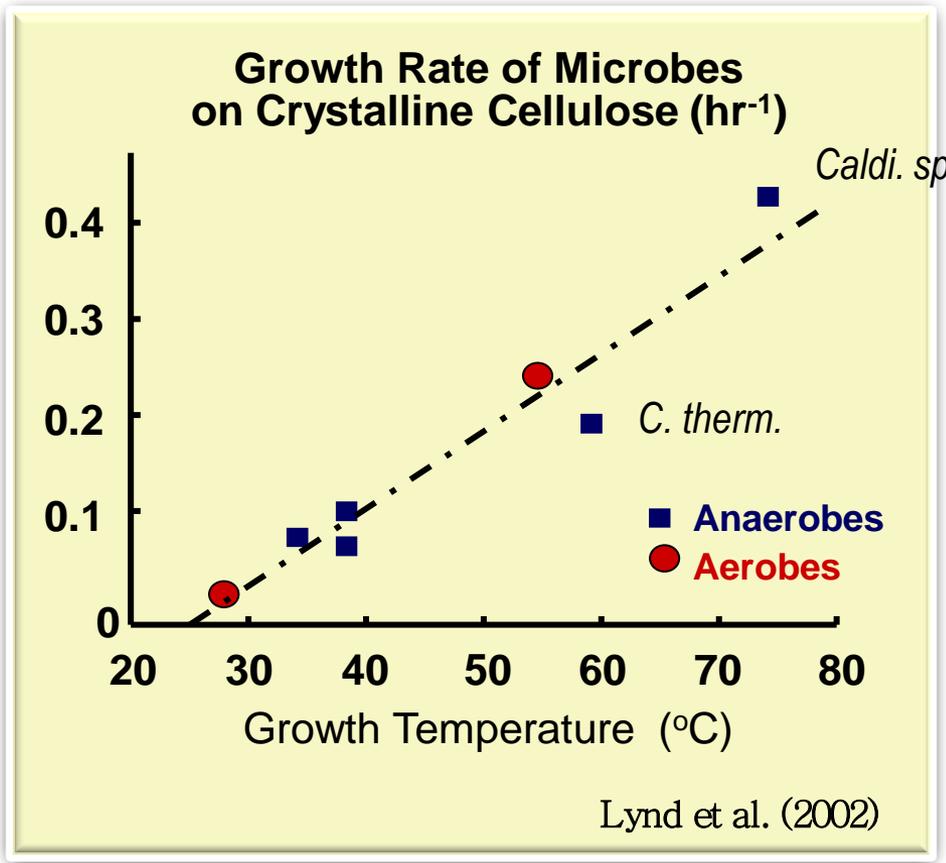
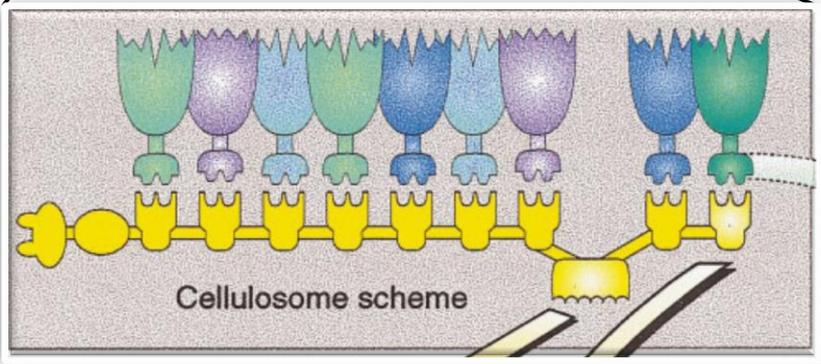
- Hydrolysis mediated mainly by CEM complexes
- Enzymes both bound and free
- Cells both bound and free



C. thermocellum on poplar (Morrell-Falvey and Raman, ORNL)

C. thermocellum as a model system

Cellulose hydrolysis mediated by a “cellulosome” complex with over 70 distinct proteins.

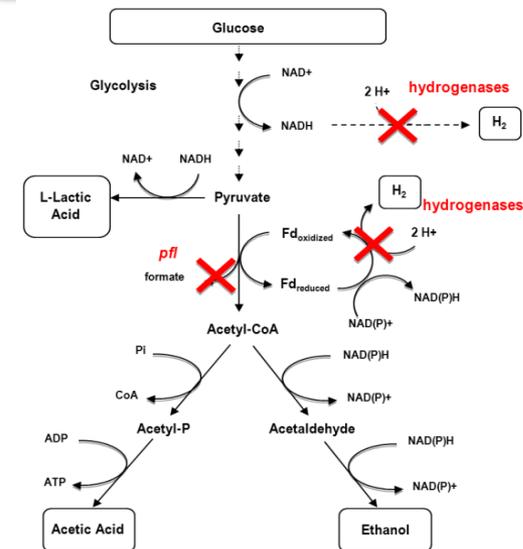


One of the highest growth rates on cellulose among described microbes, but does not ferment pentoses, grows poorly on glucose, makes unwanted fermentation products → requires genetic modification.

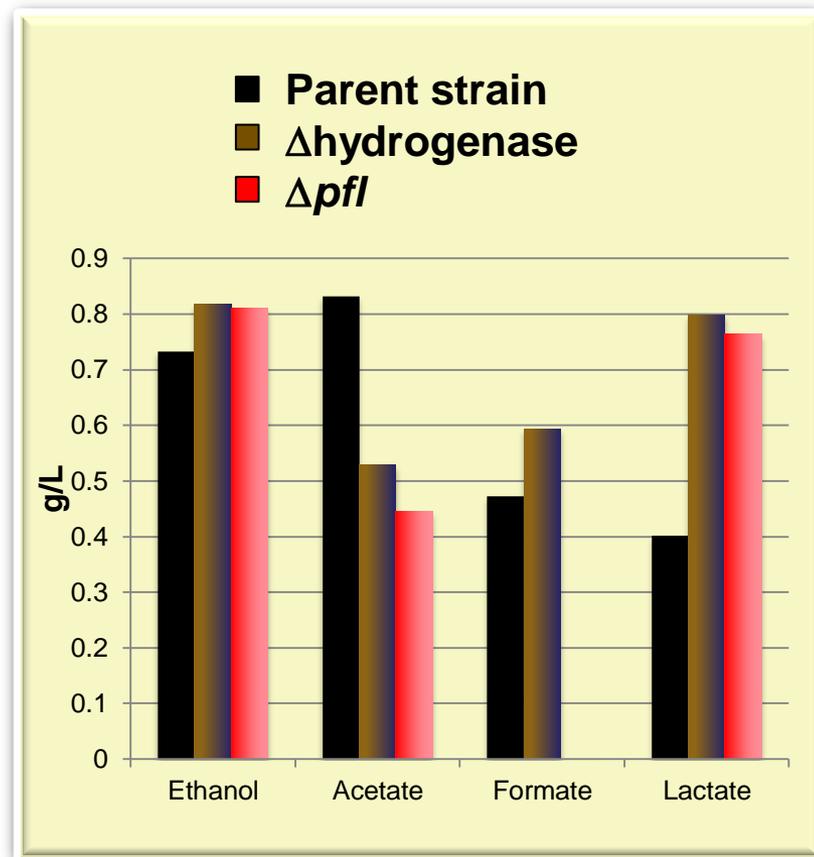
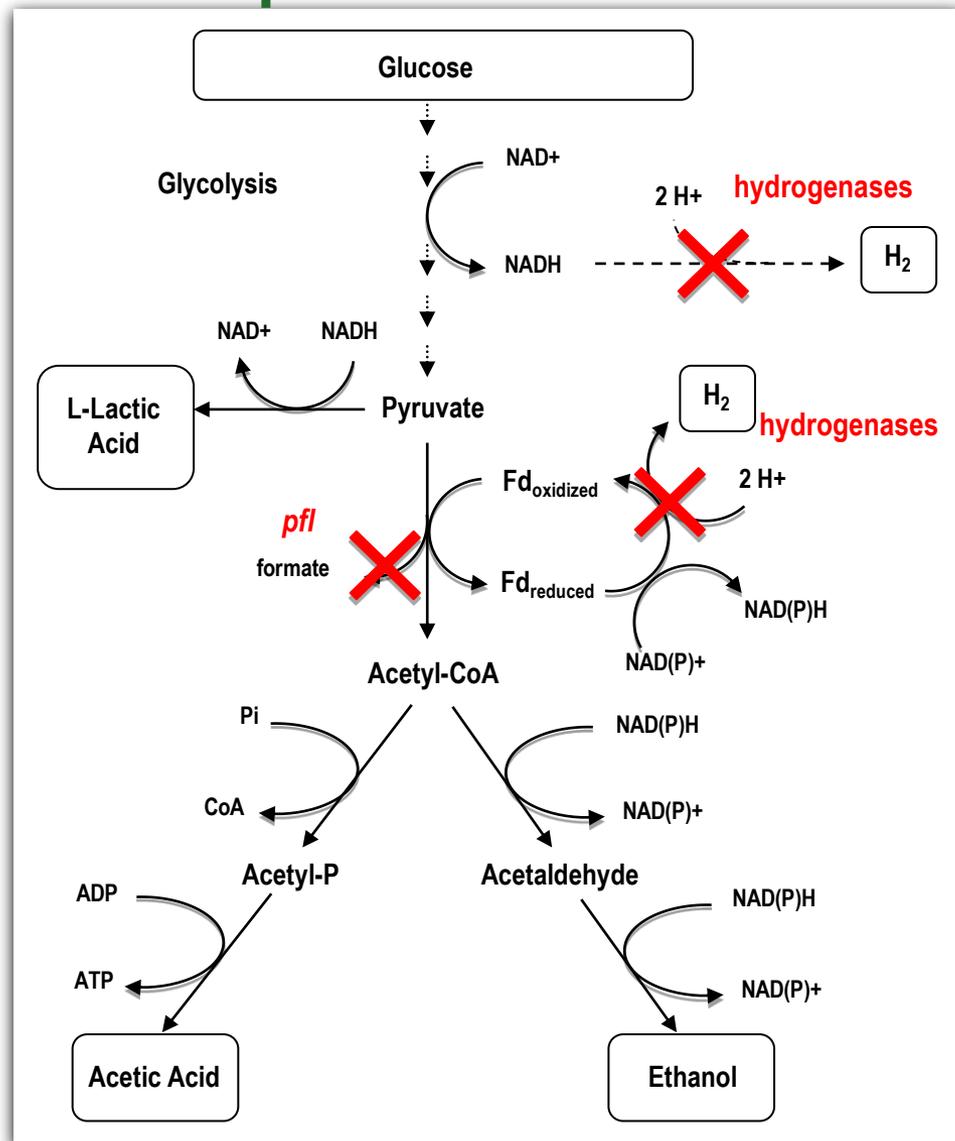
Developed genetic tools for thermophilic CBP microbes

- BESC has developed and applied genetic tools for *Clostridium thermocellum* and *Caldicellulosiruptor* for economical production of biofuels from cellulosic feedstocks.
- BESC has discovered ways to overcome key inhibitors of microbial fermentation efficiency, e.g., microbial strain improvements that lead to enhanced ethanol or acetate tolerance.
- These discoveries are significant since end-product titer, yield and inhibitory byproducts are important contributors to capital and downstream processing costs.

Gene	Locus	Description
celS	Cthe2089	Cellulosomal GH48
celY	Cthe0071	Non-Cellulosomal GH48
cipA	Cthe3077	Cellulosomal scaffoldin
cipADdocII	Cthe3077	Domain that attaches CipA to cell surface
ech	Cthe3019-3024	Ech hydrogenase
hfs	Cthe0425-0428	Hfs hydrogenase
ldh	Cthe1053	Lactate dehydrogenase
Gene D01	CtheD01	Central metabolism gene
pta	Cthe1029	Phosphotransacetylase
mf	Cthe2430-2435	Ferredoxin oxidoreductase
spo0A	Cthe0812	Sporulation initiation factor
Gene D02	CtheD02	Central metabolism gene
Gene D03	CtheD03	Central metabolism gene
adhE	Cthe0423	Bi-functional aldehyde/alcohol dehydrogenase
pyrF	Cthe0951	orotidine 5'-phosphate decarboxylase
hpt	Cthe2254	hypoxanthine phosphoribosyltransferase
cat	From pNW33N	Chloramphenicol acetyltransferase
kan	From pIKM1	Kanamycin resistance gene
neo	From pUB110	Kanamycin resistance gene
tdk	From <i>T. saccharolyticum</i>	Thymidine kinase
Gene M01	Thermophilic anaerobe	Central metabolism gene
Gene M02	Thermophilic anaerobe	Central metabolism gene
Gene M03	Thermophilic anaerobe	Central metabolism gene



Deletion of competing electron transport pathways in *C. thermocellum* for enhanced ethanol production

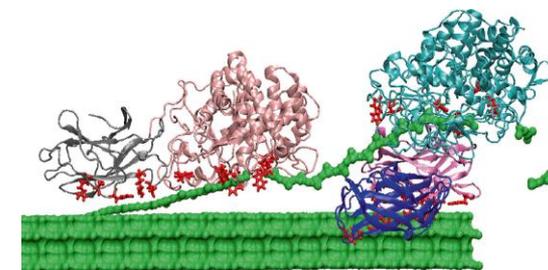
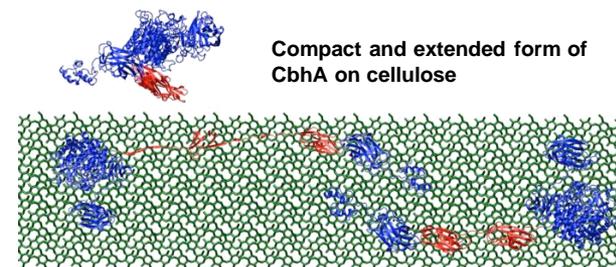


Rationale. Understanding of the mechanisms underlying the action of plant cell wall-solubilizing enzymes is needed to guide the choice of enzymes for the recombinant CBP development strategy and to provide a foundation for improving cellulase enzymes and related cell wall degrading systems.

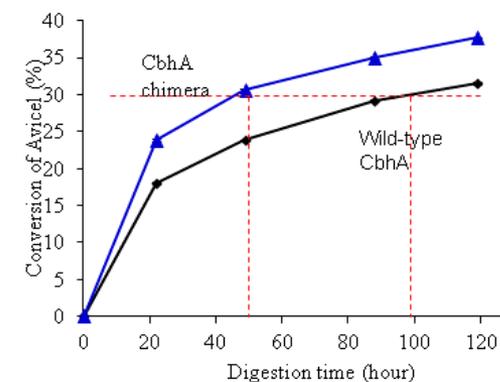
Progress

- New crystal structures from *C. therm.* and *C. bescii* including three family 48 catalytic domains, two family 9 catalytic domains, and one CBM.
- The first complete molecular model of the 7-domain CbhA (*C. therm.*) enzyme → X1 domains possible extensible hidden linkers.
- CelA (*C. bescii*) is as active on BESC pretreated switchgrass as the *C. bescii* broth; confirming that it is the key cellulase in this system.
- CelA (*C. bescii*) is twice as active as a formulation based on native* CBH I at the same protein loading on Avicel.
- Domain-swapping used for *C. therm.* enzymes to improve activity of cellulosomal multi-modular cellulases, demonstrating that the individual enzymes can be improved. Some chimeras twice as active as their wild types at the same protein loading on Avicel.
- Forty minicellulosomes using *C. therm.* Components constructed. 11 of these were the first minicellulosomes able to operate at 60°C and convert substantial fractions (60%) of cellulose to sugars.

[*NREL has developed greatly improved rCBH I variants available to BESC]



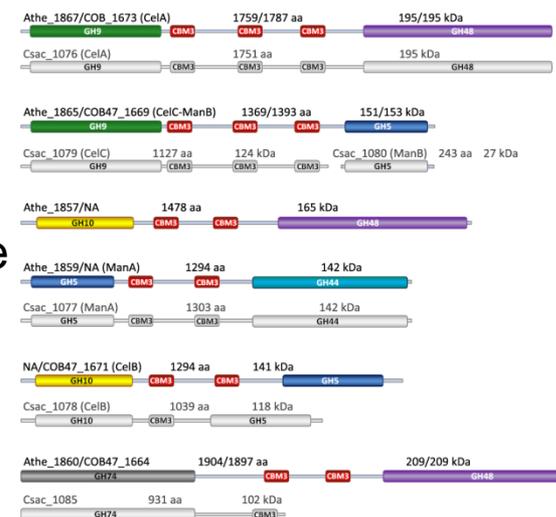
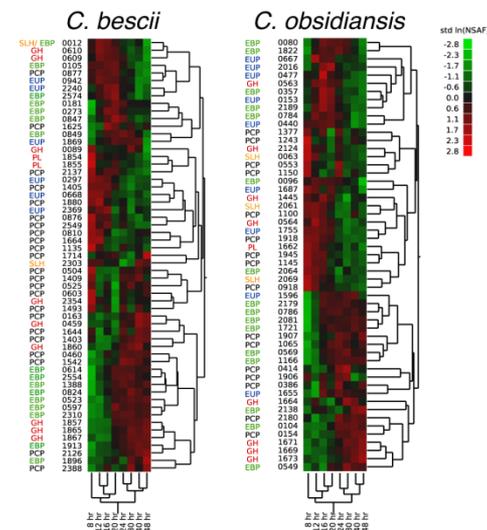
Model of the 5 modules of CelA from *C. bescii*



More efficient CbhA chimera

Quantitative proteomics reveal the secreted, highly thermostable cellulases of two extremely thermophilic bacteria

- 53-57 proteins changed in abundance during the course of cellulose fermentations, including glycosidases, pectate lyases and extracellular binding proteins.
- *Caldicellulosiruptor* spp. secrete a small number of highly abundant multidomain, multifunctional glycosidases with carbohydrate binding domains.
- Glycosidase mixtures efficiently degraded crystalline cellulose at 85°C.
- Glycosidase domains and architecture vary between the two closely related species.

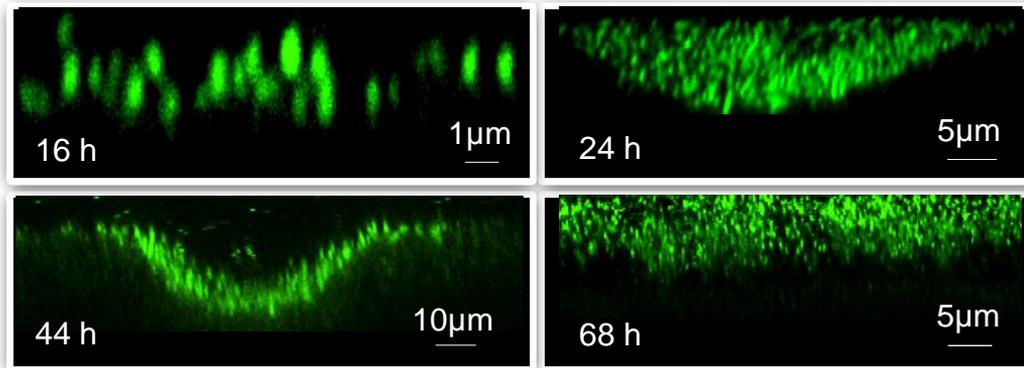


Contacts: Robert L. Hettich, hettichrl@ornl.gov, 865-574-4968, David E. Graham, grahamde@ornl.gov, 865-574-0559

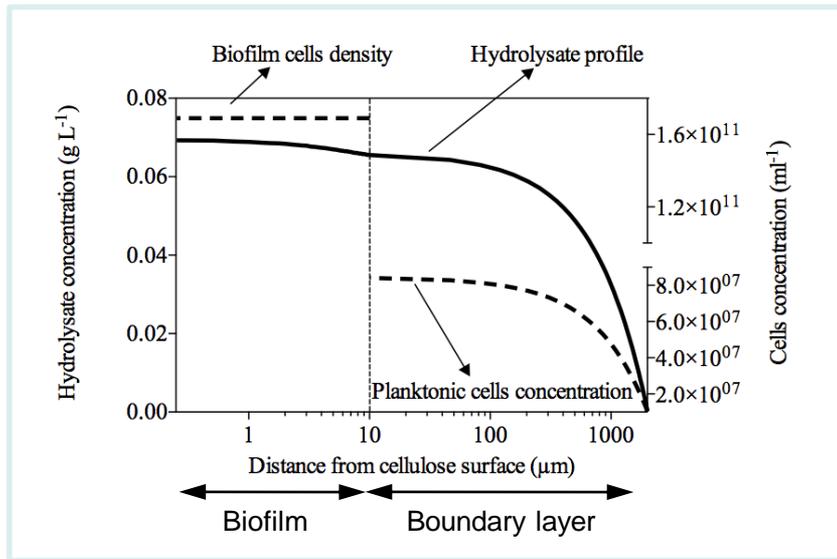
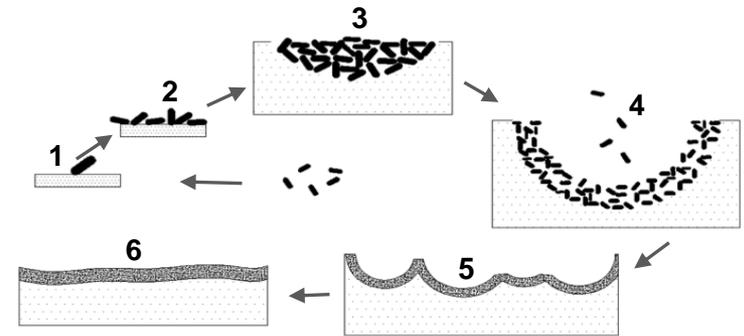
Funding Source: DOE Office of Science BioEnergy Science Center

Citation: A. Lochner, R.J. Giannone, M. Rodriguez Jr., M.B. Shah, J.R. Mielenz, M. Keller, G. Antranikian, D.E. Graham and R.L. Hettich, Label-free quantitative proteomics distinguish the secreted cellulolytic systems of *Caldicellulosiruptor bescii* and *Caldicellulosiruptor obsidiansis*. Applied and Environmental Microbiology. In press. doi:10.1128/AEM.02811-10

Modeling of microbial cellulose utilization



C. obsidiansis cells stained with the DNA dye Syto9 at various timepoints during biofilm formation

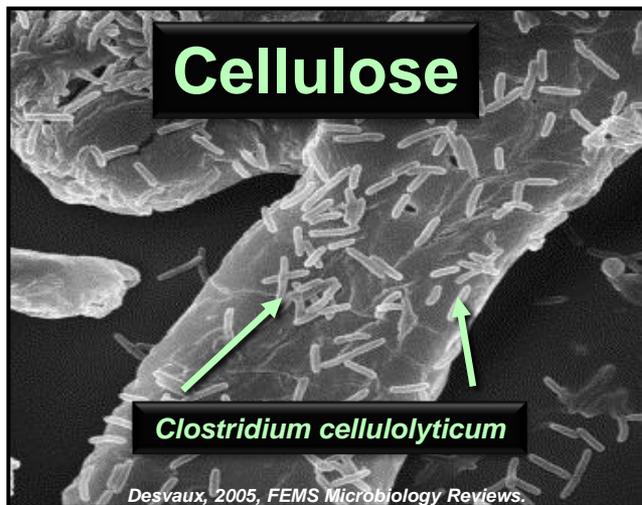


- A small portion of hydrolysate (cellulobiose) is utilized by the cellulolytic biofilm; most of the hydrolysate is released into bulk solution where it supports planktonic cell growth.
- Growth of the cellulolytic biofilm is limited by the rate of soluble substrate utilization rather than the soluble substrate diffusion rate.
- The unexpected results may explain the thin and uniform morphology typically observed for cellulose-degrading microbial biofilms.
- At later stages of the fermentation process, substrate hydrolysis by the biofilm becomes the overall rate-limiting step when considering the biofilm and planktonic cells as a whole system.

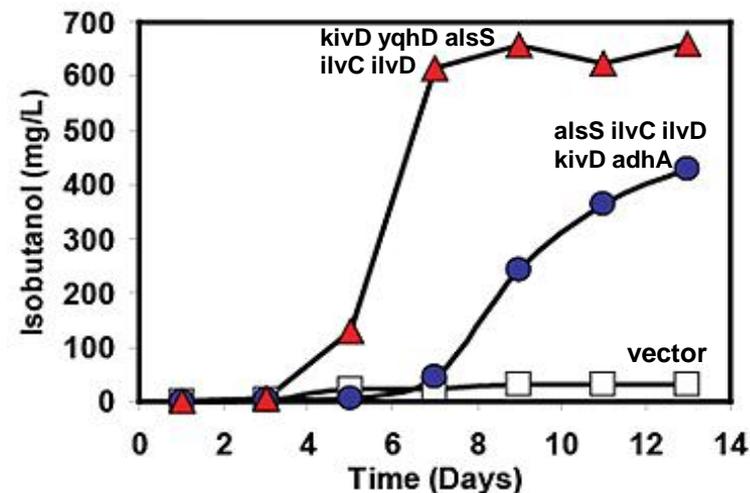
Wang, Z., Hamilton-Brehm, S.D., Lochner, A., Elkins, J.G., and Morrell-Falvey, J.L. (2011) "Mathematical modeling of hydrolysate diffusion and utilization in cellulolytic biofilms of the extreme thermophile *Caldicellulosiruptor obsidiansis*" *Bioresource Tech* 102, 3155-3162.

Microbe engineered to produce isobutanol directly from cellulose

- BESC researchers engineered a native cellulose-degrading microbe, *Clostridium cellulolyticum*, to produce isobutanol.
- Demonstrating the ability to combine CBP (consolidated bioprocessing) with production of next generation biofuels.



C. Cellulolyticum growing on cellulose substrate



Isobutanol production on cellulose:
Isobutanol pathway mutants vs. Vector control

User Name
Password
Keywords
Advanced >

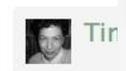
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Mar 8, 14:00

Selected Abstracts
Returned: 1 citations and abstracts
Wendy Higashide, Yongchao Li, Yunfei
METABOLIC ENGINEERING OF CRYSTAL LIGHT
Appl. Envir. Microbiol. 0: AEM.0245
Abstract 1 of 1
Appl. Environ. Microbiol.
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HOME SOLAR ENERGY WIND ENERGY



U.S. Department of Energy Announces New Biofuel to Replace Gasoline

- Speeches
- Congressional Testimony
- Events
- Lab Features
- Email Updates
- DOE Digital Archive
- RSS Feed

March 7, 2011

Energy Department Announces New Advance in Biofuel Technology

Highlights Opportunity to Reduce America's Oil Dependence and Create Jobs in Rural America

U.S. Energy Secretary Steven Chu today congratulated a team of researchers at the Department's BioEnergy Science Center who have achieved yet another advance in the drive toward next generation biofuels: using bacteria to convert plant matter directly into isobutanol, which can be burned in regular car engines with a heat value higher than ethanol and similar to gasoline. This research is part of a broad portfolio of work the Department is doing to reduce America's dependence on foreign oil and create new economic opportunities for rural America.

the WHITE HOUSE PRESIDENT BARACK OBAMA

BLOG PHOTOS & VIDEO BRIEFING ROOM ISSUES

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The White House Blog

Winning the Biofuel Future

Posted by Secretary Steven Chu on March 07, 2011 at 04:59 PM EST

Cross-posted from the Department of Energy blog.

Today, the Department announced that a research team at our BioEnergy Science Center achieved yet another advance in the drive toward next generation biofuels: using a microbe to convert plant matter directly into isobutanol. Isobutanol can be burned in regular car engines with a heat value higher than ethanol and similar to gasoline.

NEWS

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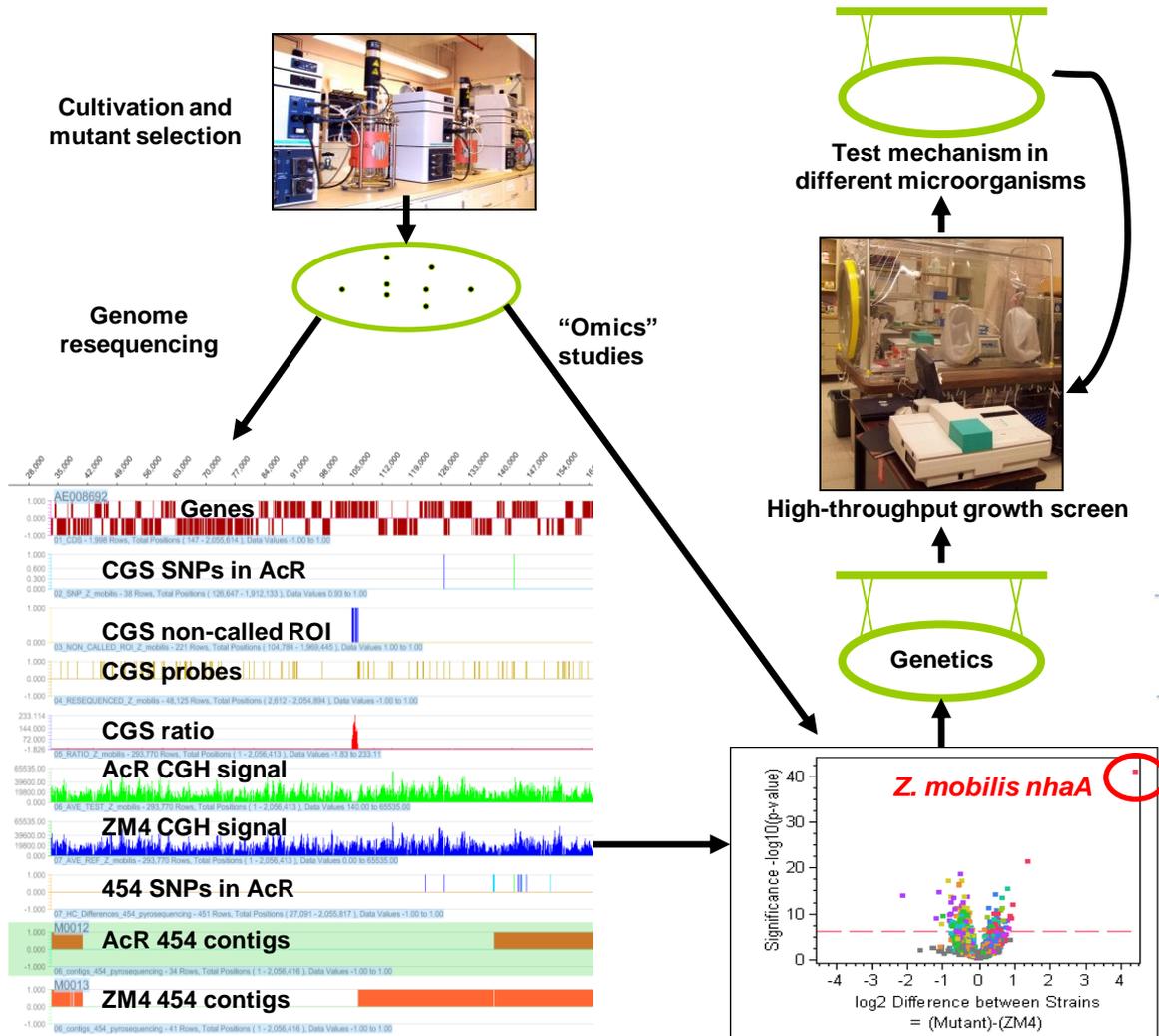
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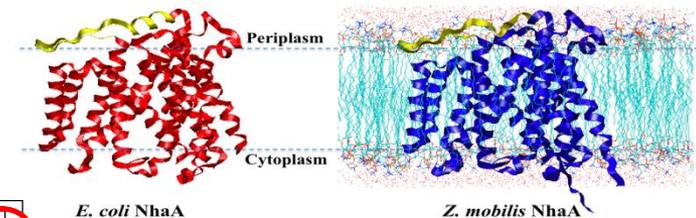
You are here: DOE Home > News > Press Releases > January - March



BESC establishes a paradigm for strain characterization and improvement

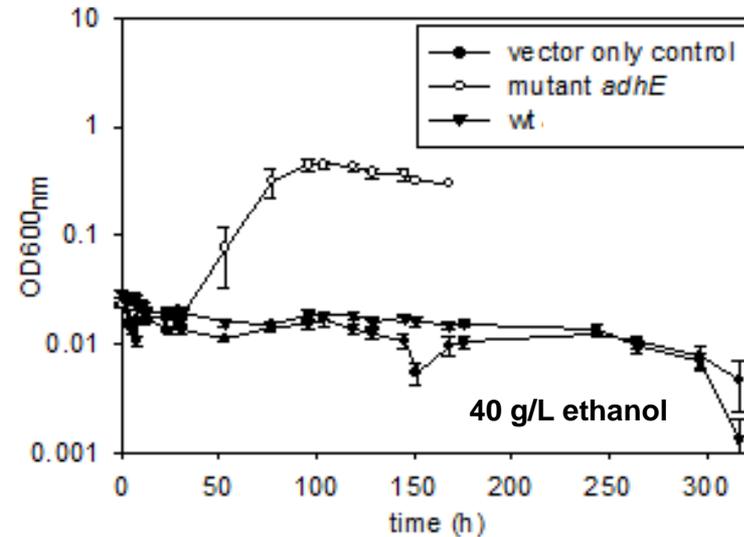
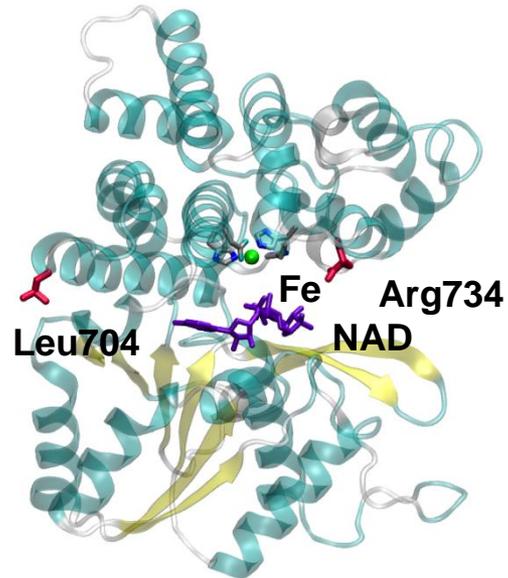


- The application of classical and systems biology tools is a paradigm for industrial strain improvement.
- Identification and over-expression of Na^+/H^+ antiporter genes confers enhanced tolerance to acetate salts.



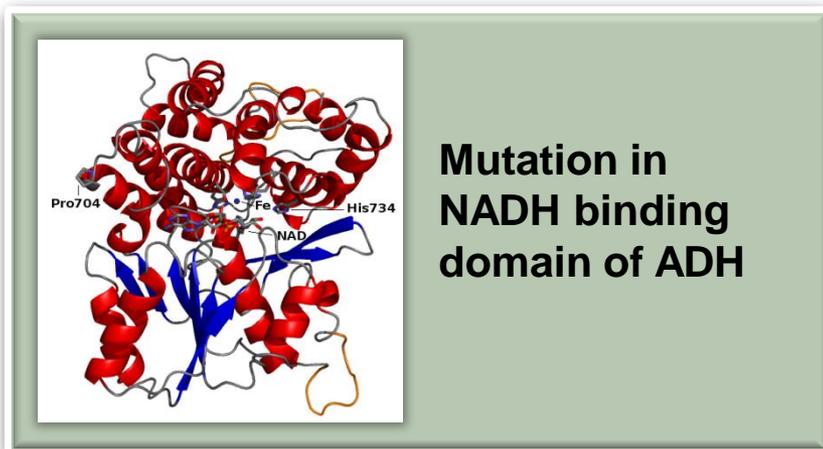
Yang. *et al.* PNAS. 2010

Single microbial gene linked to increased ethanol tolerance



- 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.
- Illustrates systems biology approach including molecular modeling, 'omics, physiological measurements and leadership class computing facilities.

Carbon and electron flow partition differently in AdhE mutant strain

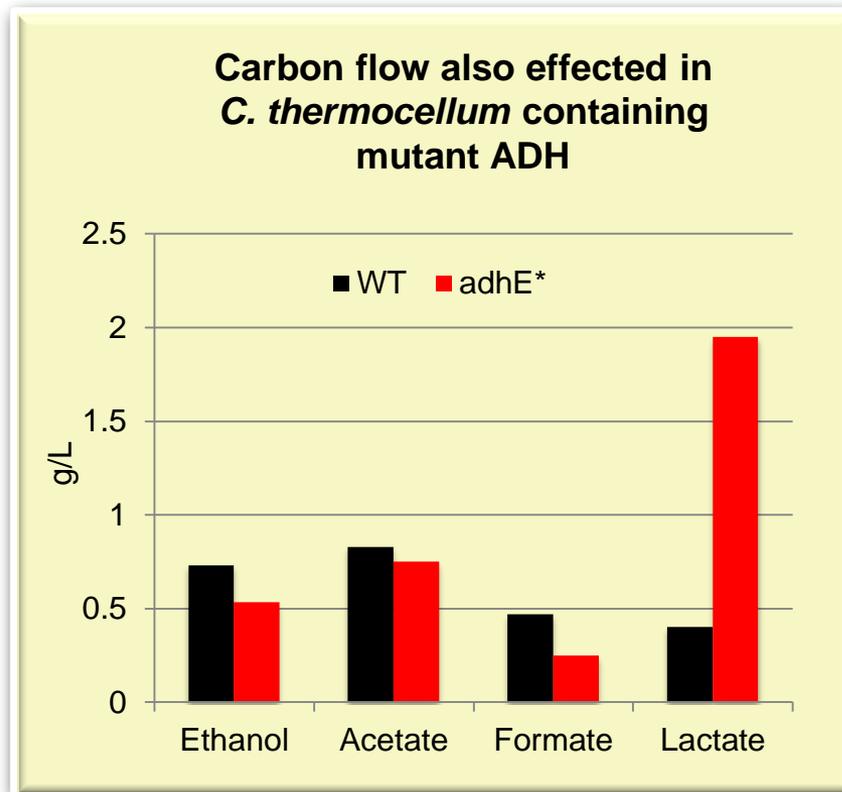


Mutant ADH co-factor specificity changes to NADPH dependence

	Specific Activity ^a (Std dev)	
	NADH	NADPH
WT	2.7 (0.18)	0.025 (0.005)
EA	<0.005 ^b	0.052 (0.007)
adhE*(EA)	<0.005	0.12 (0.03)

^a μg NAD(P)H oxidized.mg crude extract protein-1.min-1

^b Below assay detection limit



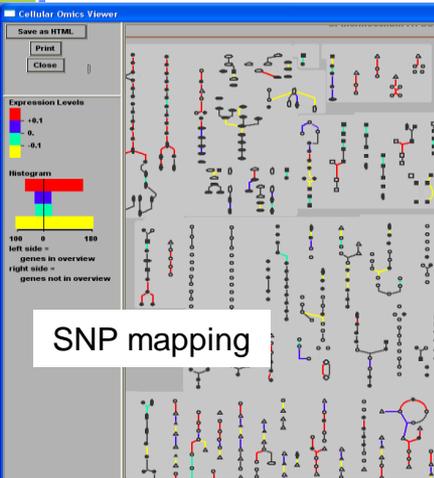
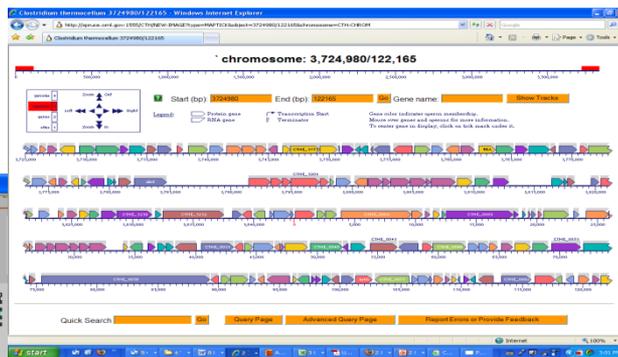
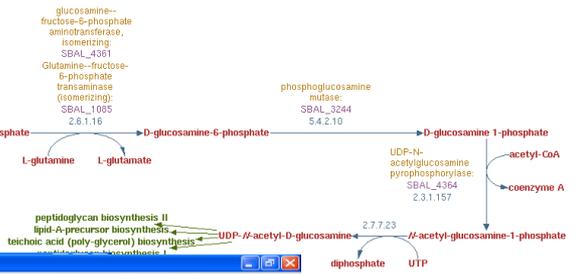
Studies underway to further optimize carbon and electron flow for productivity advances

Integrating Expression, Proteomics, SNPs, Metabolites on Cellular Systems

Clostridium thermocellum
ATCC 27405 proteomics

S. baltica Pathway: UDP-N-acetyl-D-glucosamine biosynthesis

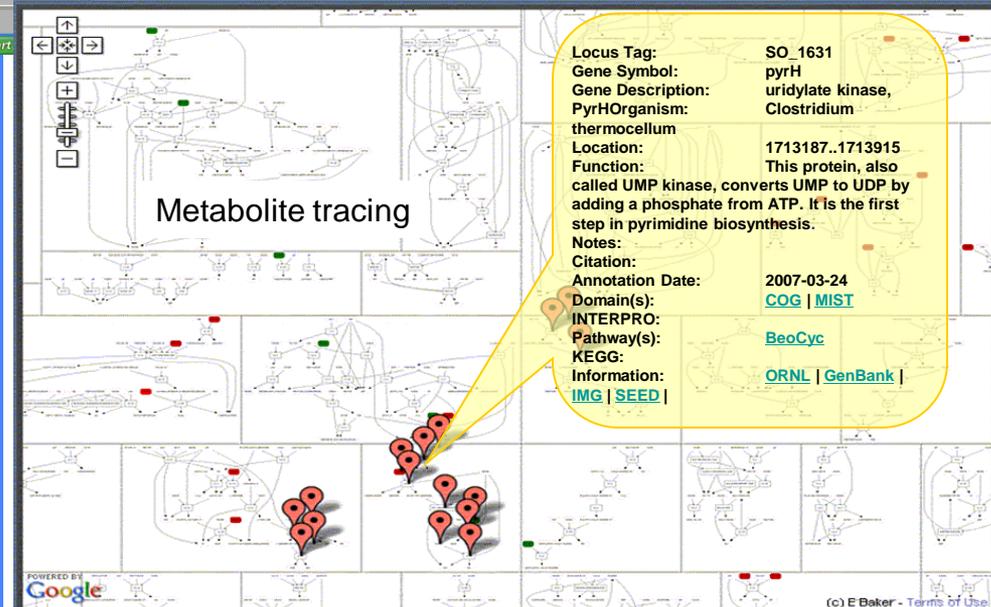
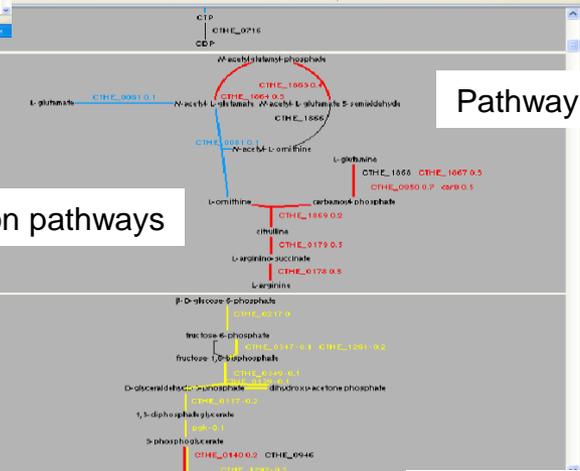
More Detail | Less Detail | Cross-Species Comparison | Download Genes | BioPAX format



SNP mapping

Expression on pathways

Pathway Component Details



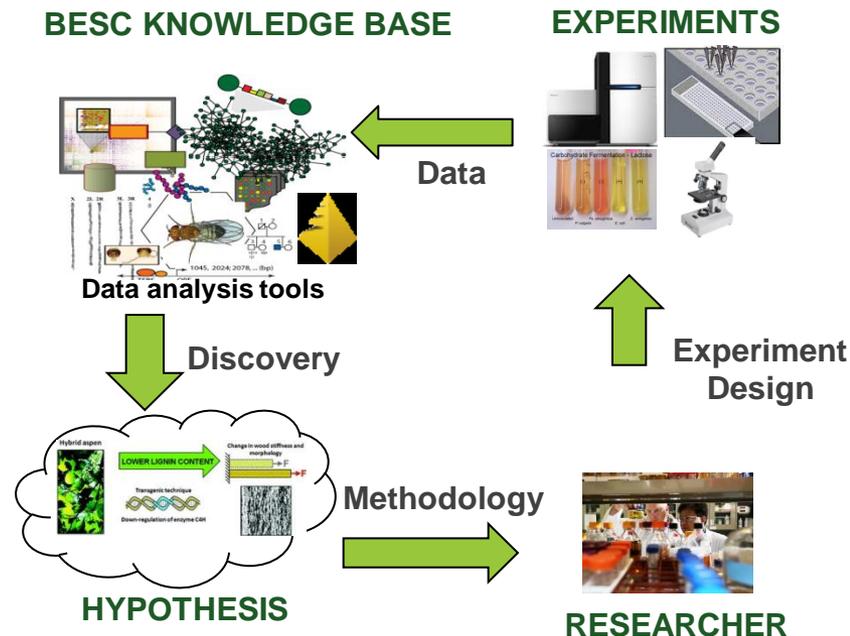
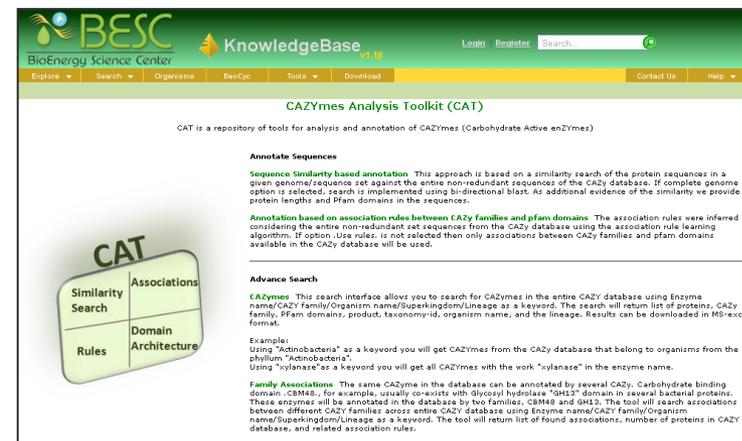
Metabolite tracing



Expression on genome map

BESC Knowledge Base (BESCKB)

- Paper summarizes the discovery and data mining environment for design of experiments.
- The BESCKB is a centralized repository for genomic, phenotypic, metabolic and experimentally generated data by BESC researchers.
- It integrates diverse data types across BESC data and community / reference data such as genomes, pathways, networks, and Omics data in the context of organism systems biology.
- This integration is the key to gain comprehensive, systems level understanding of cellular processes involved in plant biomass formation, degradation and biofuel production.
- Data available through BESCKB include genomic, pathways, Omics data, resequencing data, and various assay results related to biomass properties, structure and composition.
- Tools available through knowledgebase include annotation, visualization, comparative analysis, integration, data mining, and various navigation tools.

The screenshot shows the BESC KnowledgeBase website interface. At the top, there is a navigation bar with options like 'Explore', 'Search', 'Organisms', 'BioCyc', 'Tools', 'Download', 'Contact Us', and 'Help'. Below this is the 'CAZYmes Analysis Toolkit (CAT)' section. The text describes CAT as a repository of tools for analysis and annotation of CAZYmes (Carbohydrate Active enZymes). It includes sections for 'Annotate Sequences' (with sub-sections for 'Sequence Similarity based annotation' and 'Annotation based on association rules between CAZY families and pFam domains') and 'Advance Search'. A 'CAT' logo is also visible, which is a green square divided into four quadrants labeled 'Similarity Search', 'Associations', 'Rules', and 'Domain Architecture'.

Contacts: (Oak Ridge National Laboratory) Ed Uberbacher (ube@ornl.gov, 865-241-3490)

Funding Source: DOE Office of Science BioEnergy Science Center

Citation: Mustafa H. Syed; Tatiana V. Karpinets; Morey Parang; Michael R. Leuze; Byung H. Park; Doug Hyatt; Steven D. Brown; Steve Moulton; Michael D. Galloway; Edward C. Uberbacher, Bioinformatics 2012; doi: 10.1093/bioinformatics/bts016

Metabolic map reconstruction tool in BESCb (BeoCyc)

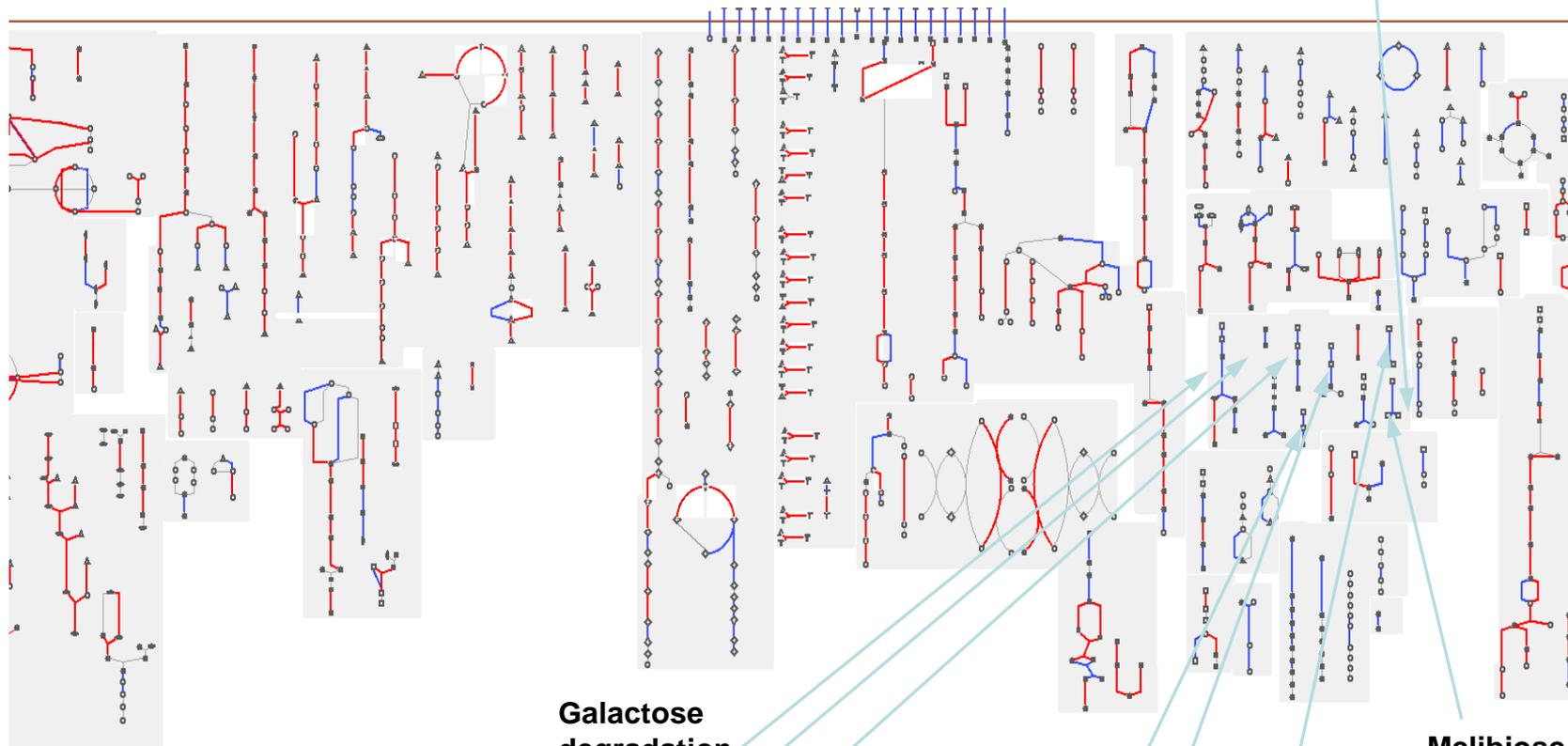
T. thermosaccharolyticum specific reactions are in blue;
reactions shared with *C. thermocellum* are in red

Biosynthesis:
cofactors, nucleosides and nucleotides cell structures

Biosynthesis:
fatty acids

**Energy generation/
Fermentation**

Degradation/Utilization
sulfate reduction IV
(dissimilatory)



Galactose degradation

Fructose

Arabinose degradation

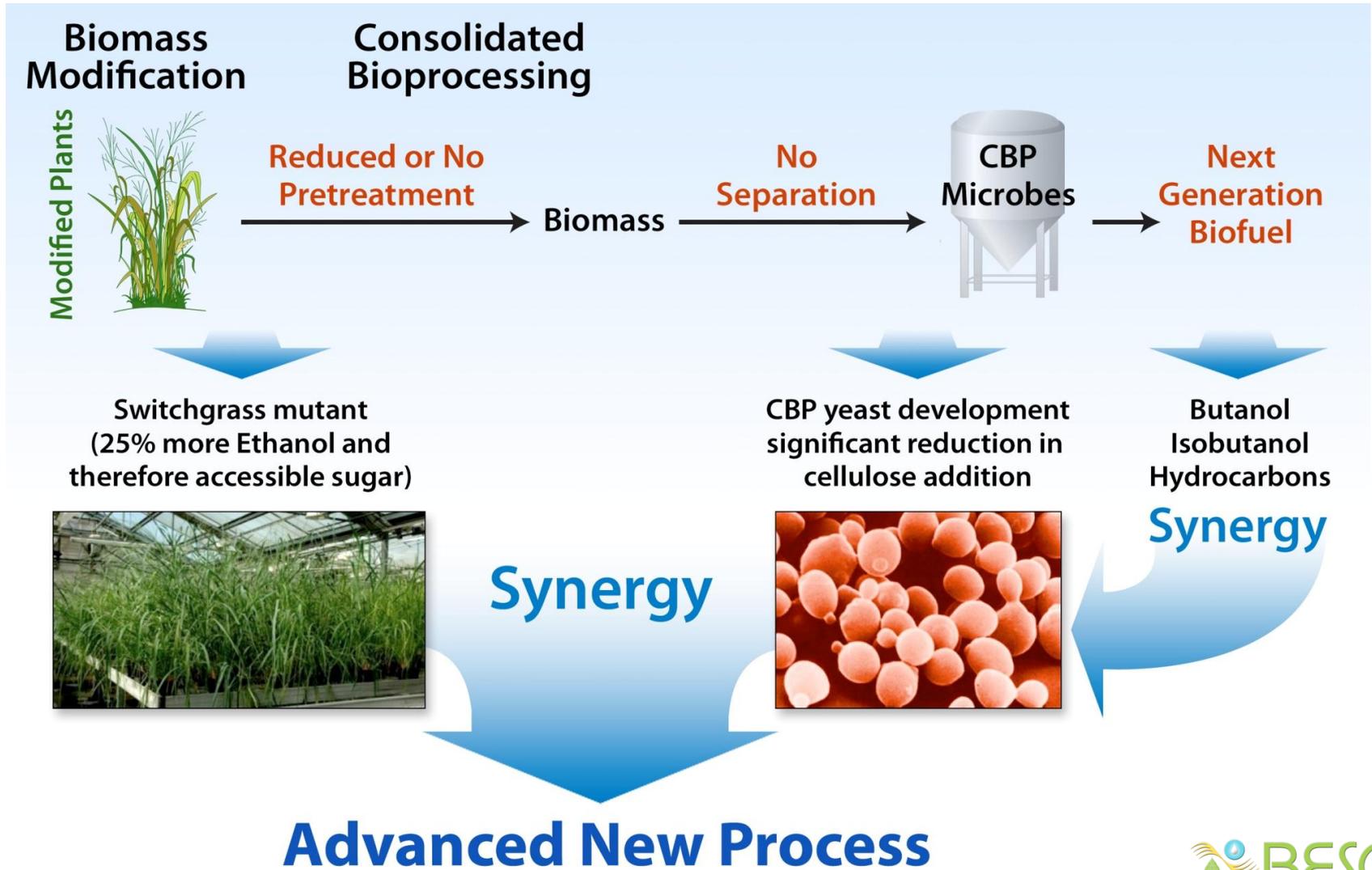
Xylose

Rhamnose

Lactose

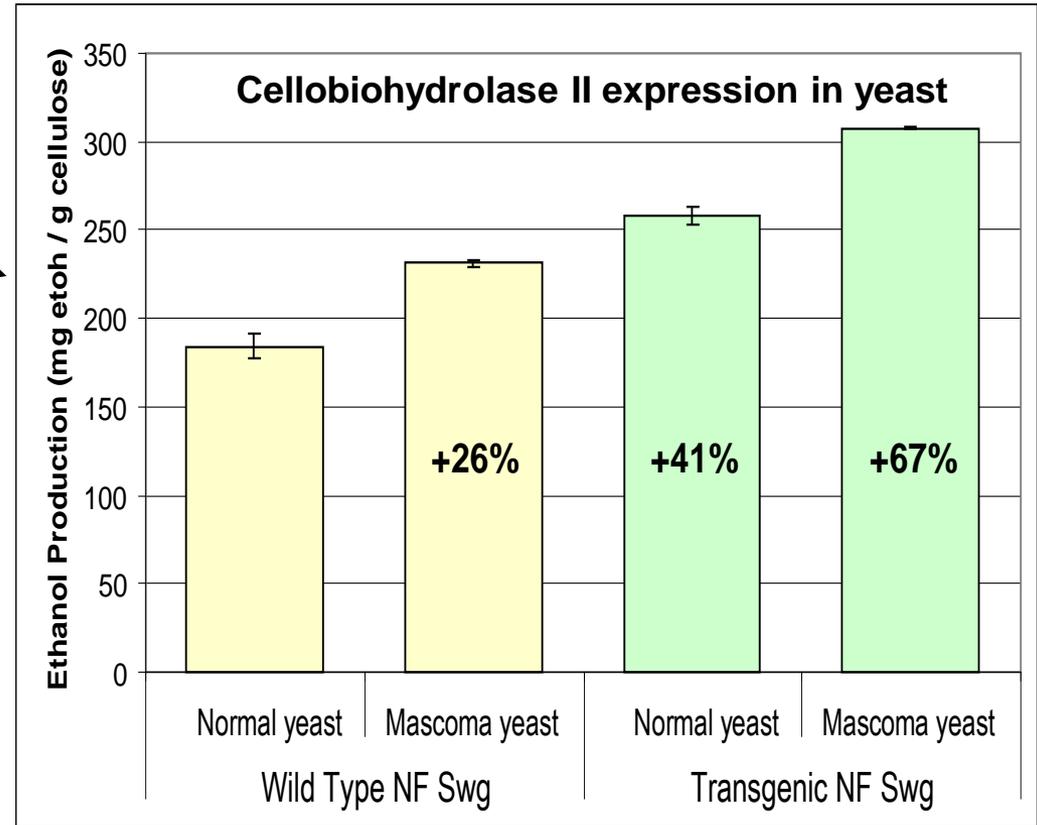
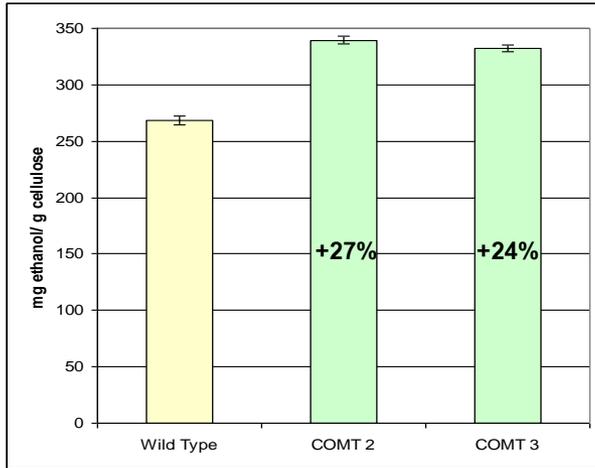
Melibiose

BESC will revolutionize how biomass is processed and converted

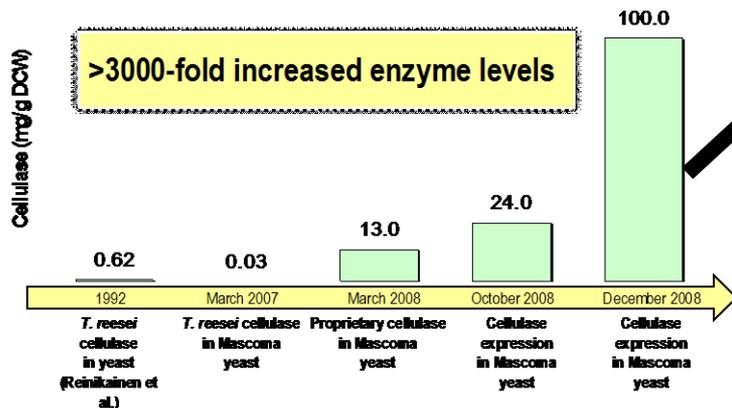


Improved switchgrass and new fermentation yeast dramatically improve biofuel production

COMT Transgenic Switchgrass Produced More Ethanol

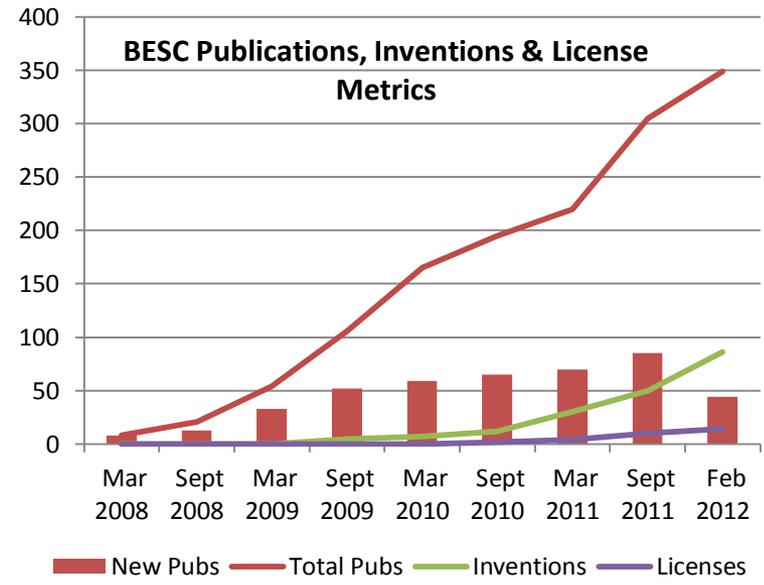


Mascoma development of a CBP yeast



Translating Discoveries to the Scientific Community

- **349+** Scientific publications (~61% multi-institutional)
 - *Cited 1500+ times in peer review journals*
- **2000+** Scientific presentations at meetings and conferences worldwide
- **250+** Workshops and seminars for BESC researchers and graduate students
- **86+** Inventions disclosed under evaluation by the BESC Commercialization Council
- **14+** Patents in process
- **300+** Presentations to Stakeholders (Secretary, Under Secretaries, Congressmen and Staff Members, Businessmen, etc.)
- **200+** Television, Print, and Radio Interviews
- **34000+** Students reached through an Education program with the Creative Discovery Museum in Chattanooga, Tennessee with a Biofuels Outreach Lesson



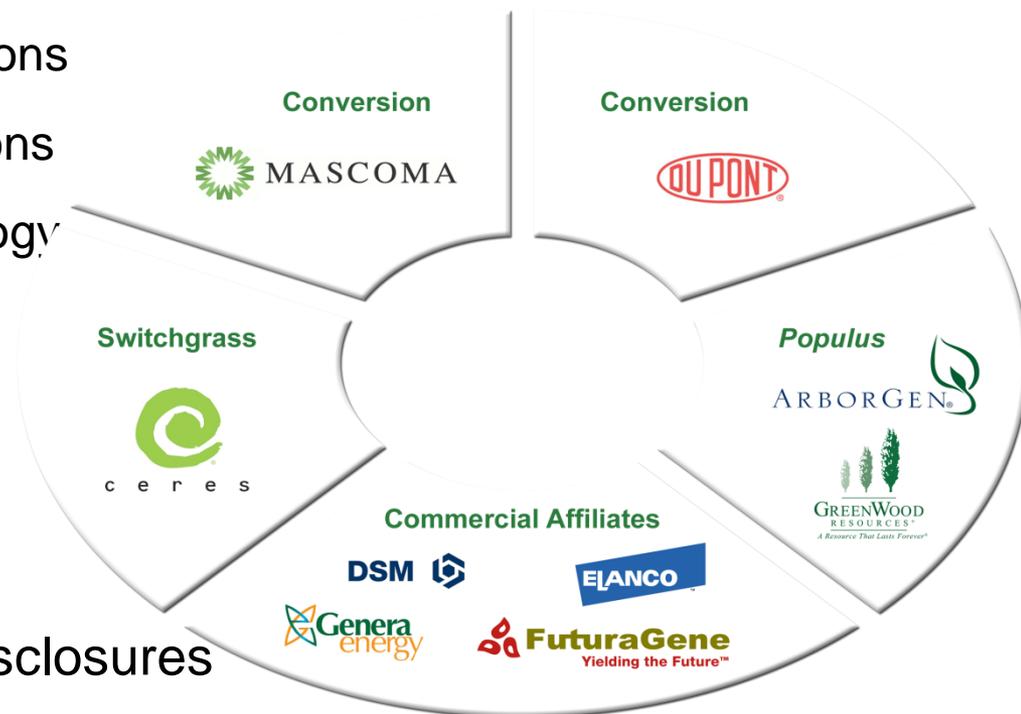
Industry program facilitates strategic commercialization

- 13 Inventions Transferred to Industry

- 7 focused on feedstock inventions
- 3 focused on microbial inventions
- 3 focused on enabling technology inventions

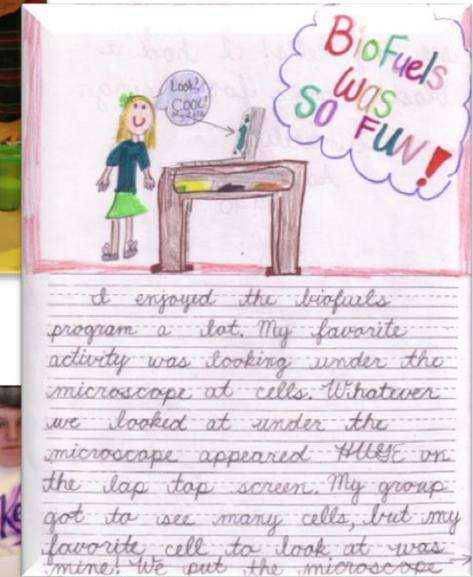
- 23 Patent Applications Filed

- 83 BESC-Funded Invention Disclosures



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

- BESC developed a set of hands-on lesson plans aimed at students in fourth, fifth and sixth 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 35,000 students, teachers and parents.
- Accomplished by partnering with museums and centers in Tennessee, Georgia, Texas, Michigan, Illinois, Florida, New York and Arizona.



**CREATIVE
DISCOVERY
MUSEUM**

Discussion for the future – BESC and sustainability

- BESC will provide improved biofeedstocks for conversion
 - Both transgenic and natural variants
 - Limited agronomic data and field tests are part of plans for down-selecting lines.
- BESC will provide improved CBP biocatalysts for scale-up and optimization
 - Limited technoeconomic evaluation
- Enabling technologies
 - Data sets – mostly system biology data
 - Improved screening and deep characterization tools
- Needs for collaboration?
 - Improved evaluation of impacts of the above?
 - Use of materials in other programs and field trials
 - Other?

Thank you



Bioscience and biotechnology for sustainable mobility at ORNL

