Discovering genes related to biomass recalcitrance using a combination of high throughput and high resolution plant cell wall characterization methods

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

Samuel Roberts Noble Foundation
National Renewable Energy Laboratory
Brookhaven National Laboratory
University of California–Riverside
Cornell University
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University of Minnesota
North Carolina State University
Virginia Polytechnic Institute
University of California–Los Angeles

Oak Ridge National Laboratory
University of Georgia
University of Tennessee
Dartmouth College
Georgia Institute of Technology
West Virginia University
ArborGen, LLC
Ceres, Incorporated
Mascoma Corporation
Verenium Corporation

322 people in 20 institutions
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 multidisciplinary approach

<table>
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<tr>
<th>Time frame</th>
<th>Planned</th>
<th>Actual</th>
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<tr>
<td>Modified plants to field trials</td>
<td>Year 5</td>
<td>Year 4</td>
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<tr>
<td>New or improved microbes to development</td>
<td>Year 4–5</td>
<td>Year 3–4</td>
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<td>Analysis and screening technologies</td>
<td>Year 3 on</td>
<td>Year 2 on</td>
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• **Targeted cell wall synthesis approach:**
  * Test known putative recalcitrance genes in *Populus* and switchgrass transgenics (TP)
  * Basic research to identify unknown genes and decipher how they effect recalcitrance

• **Discovery-based natural variation approach:**
  * Identify natural variation in recalcitrance
  * Identify gene responsible
  * Test via *Populus* and switchgrass transgenics (TP)
  * Activation tagging
Need for High Throughput Screening Systems

Plant Biosynthesis

✓ Targeted Plant Genes and Transformation Pipeline
  • 342 knockdown and/or overexpression constructs

Switchgrass

✓ Association and Field Trials
  • ~4000 samples
✓ QTL Studies
  • ~2000 samples

Populus

✓ Association Studies
  • ~1100 trees replicated in 3 common gardens
✓ Activated Tagging Studies
  • ~1000 Anonymous Tagged Lines
✓ QTL studies
  • ~700 segregating BC1 progeny
Analytical Pyrolysis of Low Lignin Alfalfa
36 minutes of analysis for 6 (x3) samples

Current scheme for the biosynthesis of monolignols. (Hoffmann et. al. (2004)
Plant Cell 16, 1446–1465.)

Arrows are major peaks assigned to lignin pyrolysis fragments.
Molecular Beam Mass Spectrometry provides a rapid estimate of cell wall chemistry

Scatter plot of lignin variability as a function of nitrogen treatment for 1500 *Populus* trees

- ~ 350 samples/day
- Identifies unique outliers
- Intensities are used as phenotypes
- Provides a snapshot of CW chemistry

Novaes et al, University of Florida
Cell wall properties can be estimated quickly from the mass spectra.

\[ \Sigma \text{(intensity of S monomers)} \]
\[ \Sigma \text{(intensity of G monomers)} \]

- Screening for cell wall chemistry changes after transgenic modification
- Rapid assessments of cell wall chemistry information for gene discovery
179 QTL intervals were detected across 3 pedigrees

49 co-localized into common genetic loci

27 of these loci are pleiotropic and are associated with multiple phenotypes

5360 potential candidate genes occur within these QTL interval

- 50% of predicted genes are unknown, hypothetical or putative
- 90+% are computational predictions and have not been experientially verified
High Throughput Screening of Sugar Release

- **Initial concept designed and validated** (Studer et al, UCR)
  - Aluminum base plate with free-standing hastelloy cups
  - Same footprint as standard 300 μL well plate
  - 250 μL reaction volume

- **Concept refined and implemented** (Decker et al, NREL)
  - Screen 300-400 biomass samples per day
    - Rapidly screen 1000s of genetic or environmental variants
    - Pretreatment- steam reactor
      - Hot water
      - Dilute acid (currently limited #s)
    - Enzyme Digestion- 3 days
      - Extent of digestibility, not rate
    - mg glucose/xylose released per mg biomass
      - HTP analysis via enzyme-linked glucose/xylose assays
Mining variation to identify key genes in biomass composition and sugar release

Collected 1300 samples for *Populus* association and activation-tag study

- **Existing collections (N = 500; 1–2 trees/site)**
- **New collections (N = 500; 140–160 trees/site)**

Analyzed cell wall chemistry and sugar release using high-throughput characterization pipeline

Screen showed high level of natural diversity

- Hot water as pretreatment only
- Sugar release varies from 25% to >90% of theoretical value
Steam improves repeatability

- Glucan and xylan yields from poplar pretreated using steam.
- Yields from sand and steam heated tubes are comparable BUT the curves from steam heated tubes are much smoother.
- This makes it easier to identify qualitative trends.
Cross Section Study: Results

- Significant within tree variability in sugar release
- Increasing glucose release from pith to bark
- No clear trend in xylose release across section; however, it does vary
- Similar trend in sugar release pattern for both sets of annual rings
- Bulks 3 and 2 material without bark always performs significantly better than bulk 1 material with bark
Detailed analysis of transgenic alfalfa lines

Nakashima et al. 179 (2008) 738
Detailed analysis of transgenic alfalfa lines reveals changes in lignin molecular weight

$^{13}$C CP/MAS NMR

- Control
- C3H
- HCT

Increasing H content leads to lower MW

$H/G/S$ content of extracts and residues

Molecular weight profiles - isolated ball milled lignin

F. Chen, Nobel Foundation

A. Ziebell, NREL

D. Johnson, NREL
Summary

- HTP measures glucan, xylan, lignin S/G ratio and glucan and xylan release after pretreatment and enzymatic hydrolysis

- Reduces analytical time and sample size

- Ten 5g samples in 2 weeks reduced to 50 mg and >8000 samples/month

- Screens allow gene identification from large natural populations followed by validation using high resolution analytical techniques
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