

## Targeting plant cell walls at JBEI (advancing cellulosic biofuels)

Joshua Heazlewood Feedstocks Division





## The Joint BioEnergy Institute, CA

- Six Partners
  - Lawrence Berkeley National Lab
  - Sandia National Lab
  - Lawrence Livermore National Lab
- UC Berkeley
- UC Davis
- Carnegie Institute
- Four Science and Technology Divisions
  - Feedstocks
  - Deconstruction
  - Fuels Synthesis
  - Technologies
- Single location
  - Emeryville, CA







## Feedstocks

Cell Wall Synthesis

**Director** Henrik Scheller



Team Members Yuzuki Manabe Chithra Manisseri Ai Oikawa Lan Yin Ulla Christensen Michelle Truong Eva Knoch Carsten Rautengarten Yves Verhertbruggen Anongpat Suttangkakul 4/29/2009 Cell Wall Engineering Director Dominique Loque



Team Members Fan Yang Parul Tomar Prajakta Pradhan Kejian Zheng Jin-Sun Kim Aymerick Eudes Ling Zhang





Team Members Laura Bartley Patrick Canlas Peijian Cao Dawn Chinquy Kihong Jung Miguel Vega-Sanchez Brian Conlin Maw Sheng Chern Xuwei Chen Wing Hoi Sze-To



Director Joshua Heazlewood



Team Members Katy Christiansen Jason Ito Harriet Parsons Michelle Smith Sherry Chan







## How is hemicellulose synthesized?





Hemicellulose and pectin (20% of a plant mass)

- Synthesized by large family of redundant proteins (450 to 800)
- highly complex
- contain large fractions of hard-to-ferment sugars (e.g. xylose)
- contain phenolic crosslinks
- contain acetyl esters that inhibit deconstruction and fermentation

# Only few of the enzymes involved in their biosynthesis are known!





## jbci CESA-CSL superfamily responsible for synthesis of cellulose and hemicelluloses





#### **CsID** proteins are mannan synthases



- In two assays, CsID5 shows mannose mannosyltransferase activity •
- Mannan may potentially replace xylan for improved deconstruction • and fuel synthesis 4/29/2009







csld2/csld3/csld5 triple mutant

Csld5 + (d3 or d2) required for plant growth

As predicted from genetic analysis and 'Split YFP' analysis, CsID2, CsID3, and CsID5 are redundant

Analysis of double and triple mutants and overexpressors is in progress

Co-immunoprecipitation experiments in progress







### Prioritization of rice GTs through bioinformatics

#### 769 rice GT gene models were identified and classified into 40 families







## 282 putative rice-specific GTs in 24 families

(no orthologs in Arabidopsis, Populus, Medicago, Ricinus)

Family	No. (Total)	Family	No. (Total)	Family	No. (Total)
GT1	85(224)	GT24	2(3)	GT47	25(51)
GT2	29(63)	GT28	3(12)	GT48	11(16)
GT4	13(54)	GT29	2(5)	GT58	3(4)
GT5	4(26)	GT31	26(58)	GT61	20(39)
GT8	9(49)	GT34	3(10)	GT64	1(3)
GT10	1(5)	GT37	13(22)	GT66	1(3)
GT14	5(14)	GT41	1(3)	GT75	1(5)
GT20	9(21)	GT43	5(15)	GT77	10(19)

#### 33 genes prioritized based on expression levels in stem/leaf

Peijian Cao Laura Bartley Pam Ronald





## Cell wall enzyme database



#### http://ricephylogenomics.ucdavis.edu/cellwalls/gt/





## **Cell Wall Engineering**



#### Can we overcome lignin recalcitrance?













## Replacement of Ether by Ester and Amine bonds









#### **Plasma membrane transport?**

#### "These data, produced with the best microscopy tools that are available today, support a model where unknown membrane transporters, rather than Golgi vesicles, export monolignols."

Lacey Samuels (Kaneda et al., 2008 Plant Physiology)





## Screening for the lignin transporter



**Monolignols and derivates** 



## **Systems Biology**







## Can we use 'big biology' to further cell wall biosynthesis and fast track targets?





### **Subcellular Characterization of Golgi**



Many complex sugars destined for the plant cell wall are synthesized at the Golgi apparatus

**Distinct Golgi sub**compartments by FP





GT2-YFP

**GUT1-YFP** 

Yumiko Sakuragi University Copenhagen

The Golgi appears to be sub-compartmentalized, potentially a crucial feature of hemicellulose design





## High purity Golgi from plants







#### **Comparative Golgi Proteomics**



- Do cell wall biosynthetic compartments from different tissues contain the same protein and sugar content?
- Can we correlate proteomics and metabolomics with cell wall structure in tissues, mutants and ecotypes?

# jbei Grass Genetics – Transforming Switchgrass



1.1 - 3.6% {50x less efficient compared to rice}





- Functional characterization of GT's involved in hemicellulose biosynthesis
- Targeting monocot (grass) specific GT's involved in hemicellulose biosynthesis
  - Golgi proteomics to identify new targets involved in hemicellulose biosynthesis and comparative studies
  - Attempt lignin replacement for improved deconstruction
  - Identify lignin transporter to for replacement strategies









- Understand & modify polysaccharide biosynthesis
  - Focus on hemicellulose
- Modify lignin to aid deconstruction
  - Introduction of cleavable linkages
- Systems approaches to cell wall biology
- Rice and Arabidopsis as model plant systems to apply to switchgrass

