Lignin Valorization: Biosynthesis and Bioengineering of an Ideal Lignin

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Project Goals: The Center for Bioenergy Innovation (CBI) vision is *to accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain.* CBI will address strategic barriers to the current bioeconomy in the areas of: 1) high-yielding, robust feedstocks, 2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and 3) methods to create valuable byproducts from the lignin. CBI will identify and utilize key plant genes for growth, composition and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to specialty biofuels (C4 alcohols and C6 esters) using CBP at high rates, titers and yield in combination with cotreatment or pretreatment. CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

Natural lignins are generally composed of *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units, that are biosynthesized by polymerization of three primary monolignols, p-coumaryl, coniferyl, and sinapyl alcohols, respectively. C-lignin is a polymer of caffeyl alcohol, found in the seed coats of a number of exotic plant species [1,2]. C-lignin exists as a linear homopolymer with only one type of benzodioxane linkage, with promising properties for generation of carbon fibers and high value chemicals [3-5].

In the seed coats of the ornamental plant *Cleome hassleriana*, lignin composition switches from conventional G-lignin to C-lignin during development. Bioinformatic analysis of RNA sequencing data identified a complete set of lignin biosynthesis genes for *Cleome*. Transcript analysis, coupled with kinetic analysis of recombinant enzymes in *E. coli*, revealed that the switch to C-lignin formation was accompanied by down-regulation of transcripts encoding functional caffeoyl CoA- and caffeic acid 3-O-methyltransferases (CCoAOMT and COMT) and a cinnamyl alcohol dehydrogenase (CAD) isoform with preference for coniferaldehyde as substrate, and up-regulation of another CAD isoform with preference for caffealdehyde. Blockage of lignin monomer methylation by down-regulation of both OMTs appears to be the major factor in diversion of flux to C-lignin in the *Cleome* seed coats, although the change in CAD specificity also contributes to the shift in lignin composition based on transgenic manipulation of CAD.

Based on this information, we started to engineer the C-lignin biosynthesis pathway in the model bioenergy crop poplar. Since the *ccoaomt comt* double mutant shows severely retarded growth in *Medicago truncatula* and *Arabidopsis*[6], we have designed to downregulate CCoAOMT or COMT, and at the same time, overexpress the CAD with preference for caffealdehyde in

Medicago hairy roots. Our preliminary results show that it is necessary to downregulate both methyltransferases to direct lignin pathway to C-lignin precursor since completely blocking COMT alone while overexpressing CAD does not lead to the C-lignin accumulation. We are also generating transgenic plants to express C-lignin pathway specific transcription factors and genes related to lignin polymerization. At the same time, we started to generate transgenic poplar plants in which the lignin biosynthesis pathway genes are altered through CRISPR gene-editing technology. These plants will be used for gene pyramiding later so that carbon flux will be redirected to the C-lignin precursor, the caffeyl alcohol and C-lignin biosynthesis.

References

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