Assembly of a Lignin Modification Toolbox

**Investigators**
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**Objective**
The objective of this project is to fundamentally alter the chemistry of the lignin polymer in such a way that it radically alters the ease with which it can be removed from biofuel crops, thus substantially increasing yields of cell wall-derived sugars and the efficiency of their subsequent fermentation without compromising the viability of the plants themselves. This proposal focuses on establishing an enzymatic toolbox for the production of lignin modification molecules (LMMs). These will be created by manipulation of genes and gene sequences of enzymes from different classes present in the plant genome. The component objectives of the project include:

1. Identification of P450s active against phenylpropanoid pathway intermediates and related molecules for the production of LMMs.
2. Assignment of function to SCPL acyltransferases.
3. Analysis of BAHD acyltransferase substrate specificity.
4. Generation of chimeric LMM biosynthetic enzymes with novel catalytic properties.
5. Development of an Arabidopsis model for LMM deployment.

**Background**
Cytochrome P450 dependent mono-oxygenases (P450s) represent a superfamily of heme-containing proteins, most of which catalyze NADPH- and O₂-dependent hydroxylation reactions. The analysis of a number of mutants and transgenic plants has demonstrated the role that P450s play in determining lignin monomer composition. The isolation of additional P450s that lead to unique hydroxylation patterns in monolignols could generate lignins with novel chemical properties that may improve lignin degradability. The group of proteins known as BAHD acyltransferases use Coenzyme A (CoA) thioesters as activated donors in acyltransferase reactions that generate dimeric phenylpropanoid conjugates.

![Figure 1: Upper panel shows an image of the cross-sectional area of an Arabidopsis stem with lignin rich areas stained purple. Lower panel shows a possible LMM with hydrolysable sites in red.](image-url)
These are acyltransferases that use sinapoylgucose for the synthesis of sinapic acid esters including the LMM candidate 1, 2- disinapoylgucose. The hope is that the expression of SCPL acyltransferases in lignifying tissues will generate LMMs with internal ester linkages that will be incorporated into the lignin polymer and vastly improve the ease with which lignin can be degraded.

**Approach**

Assembly of the LMM enzymatic toolkit is based upon several attributes that these enzymes have in common. As a result of their involvement in many secondary metabolic pathways, P450s as well BAHD and SCPL acyltransferases can be found in a wide range of plants, and exhibit broad catalytic diversity. A common feature of all three protein families is that the genes that encode them can be identified by their sequence alone allowing the straightforward identification of likely candidates. Heterologous expression of these unknown proteins and assays using a spectrum of metabolites to determine their substrate specificity and suitability for LMM synthesis will be carried out.

**Figure 2:** Schematic of steps in chimeric protein generation showing: **a.** selection of suitable recombination sites; **b.** generation of new gene sequences by swapping regions of two different genes, denoted by yellow and blue, at recombination sites; and **c.** the final 3D structure of the chimeric proteins showing which regions correspond to cyp73 (yellow) and cyp84 (blue).

**Chimeric protein generation for the production of novel catalysts**

LMM synthesizing enzymes from a range of plant species will be identified using high-throughput methods that are described schematically in Figure 2. Although the genes encoding these enzymes may be of direct utility in the improvement of biomass crops, it is possible that the ideal LMM-synthesizing enzyme would have characteristics exhibited by no single enzyme found within the screen. In this case, it would be ideal to modify these enzymes or combine the characteristics of two or more enzymes that have been identified to generate the ideal LMM. Thus protein engineering via the generation of protein chimeras within the families of extant LMM-synthesizing enzymes will be used to develop novel enzymes capable of producing LMM’s. The engineered enzymes will be employed in an Arabidopsis model for early-stage testing of their applicability to crop plants.