Additions to the BAHD Acyltransferase Toolbox

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Project Goal: Use ligases and BAHD acyltransferases to modify lignin composition in bioenergy crop species.

Abstract: Plants create a myriad of primary and secondary metabolites through the action of acyl-CoA ligases and acyl-CoA transferases. Acyl-CoA ligases use ATP and an organic acid to form an acyl-CoA donor, whereas the BAHD transferases combine the acyl-CoA donor with an acceptor, typically an alcohol or phenol. The resulting ester-containing metabolites can be used as 'monomers' for lignification, and function as donors to further modify lignin with clip-offs such as *p*-hydroxybenzoate, in biosynthesis of cuticular wax esters, or in many other vital biological reactions.

To expand knowledge of these reactions, we combine phylogenetics, gene synthesis, cell-free protein synthesis, biochemical assays, structure determination, state-of-the art analytical methods, and protein expression *in planta,* microbes or yeast. Wheatgerm cell-free protein synthesis was used to produce previously known *p*-coumaroyl-CoA and feruloyl-CoA monolignol transferases (PMT and FMT) from rice as controls (1). Comparable synthesis of putative PMT and FMT genes from sorghum and switchgrass revealed new enzymes that produced monolignol *p*-coumarate and ferulate conjugates with a breadth of substrate selectivities and relative rates (1). The new FMTs and PMTs were transformed into *Arabidopsis thaliana* (which does not naturally make monolignol conjugates) and the presence of ferulates and *p*-coumarates on the lignin demonstrated enzymatic function within plants. Additional screening of the poplar BAHD acyltransferase superfamily (116 genes) revealed five enzymes capable of producing varying levels of monolignol *p*-hydroxybenzoates (2). Analysis of transcript abundance and *p*-hydroxybenzoate levels in unrelated genotypes of poplar indicated that *pHBMT1* would be the best candidate for studies in transgenics. Three analytical methods showed that an increased level of *p*-hydroxybenzoate, a precursor to parabens used in pharmaceuticals and cosmetics, could be released by saponification from the transgenic poplar. In addition, five uncharacterized paralogous BAHD transferases from clade II genes (*PtCER2-like1* through *7*) were expressed in yeast and PtCER2-like5 produced the highest levels of C28 fatty acids when expressed in the presence of the condensing enzyme AtCER6. Its expression

was localized to the epidermis in GUS-reporter poplar lines, consistent with a role in cuticular wax biosynthesis. We have also found new examples of aromatic acid CoA ligases in bacteria, and these have unique domain architectures, reaction mechanisms, substrate specificities, and rates of reaction relative to ligases from plants. These newly identified genes and enzymes are being further evaluated for potential use in engineering lignocellulosic biomass with increased value for emerging biorefinery strategies.

References:

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