Lowering Lignin Recalcitrance and Producing Value Bioproducts in Poplar

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Project Goals: Establish the scientific knowledge and new technologies to transform the maximum amount of carbon available in bioenergy crops into biofuels and bioproducts.

Sustaining an economically viable cellulosic biofuel solution requires finely optimized lignocellulosic feedstock to overcome its biomass recalcitrance and to reduce the processing costs. Poplar as a dedicated bioenergy crop offers ample cellulosic resource convertible for biofuels and bio-chemicals. In order to achieve the cost-effective cellulosic biofuel production, as part of JBEI feedstock development program, we employ different synthetic biology strategies to modify the woody biomass of poplar with purpose to lower lignin recalcitrance and to produce value-added specialty chemicals: 1) Stacking monolignol modifying genes to potentially alter lignin structure. The genes encoding monolignol 4-*O*-methyltransferase (MOMT) and monolignol feruloyltransferase (FMT) were co-expressed in poplar. MOMT converts monolignols to the 4-*O*-methylated monomers and FMT transfers ferulate to the monolignols to form ester conjugates. We are investigating whether the combination of two enzymes would lead to the formation and incorporation of 4-*O*-methylated monolignol-ferulate conjugate into lignin polymer, thereby generating the *para*-methylated "dead end" of the polymer, with which to alter lignin structure. 2) Introducing a novel flavonoid tricin biosynthetic pathway in poplar. With recognition of a wide range of monocot grasses to utilize flavone tricin as a natural comonomer with monolignols for cell wall lignification, we are exploring the potential effect of introducing tricin biosynthesis in the dicot poplar on its cell wall lignification. A collection of tricin pathway genes including *CHS, CHI, FNSII (CYP93G1), F3'5'H (CYP75B4)* and *COMT*, driven by the individual xylem specific or preferential promoters, were stacked into an expression vector and transformed into hybrid aspen. 3) Producing industrial platform chemical muconic acid (MA). MA is a dicarboxylic acid used for the production of industrially relevant chemicals such as adipic acid, terephthalic acid, and caprolactam that are widely used in the nylon and thermoplastic polymer industries. The dual expression of plastid-targeted bacterial salicylate hydroxylase (NahG) and catechol 1,2-dioxygenase (CatA) together with plastid-targeted versions of bacterial salicylate synthase (Irp9) and the feedback-resistant 3-deoxy-*D*-arabino-heptulosonate synthase (AroG) were introduced into the hybrid aspen. Biochemical and genetic characterization of the resulted transgenic trees are ongoing.

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