Incorporation of protocatechuaic acid (3, 4-dihydroxybenzoate) conjugates into the lignin of transgenic poplar

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Project Goals:

The goal of the Bioenergy Plant Design team is to enhance plant biomass deconstruction, composition, and value. As part of this team, one of our specific project goals is the modification of lignin content and composition to improve biomass utilization in poplar. The strategy employed herein was the deviation of carbon upstream of the phenylpropanoid pathway; more specifically, we expressed the bacterial gene *QsuB* targeted to the plastid in attempts to produce protocatechuate. The overproduction of protocatechuate resulted in a reduction in total lignin content and the incorporation of protocatechuate conjugates into the lignin of transgenic poplar.

Current efforts to improve processing efficiency of lignocellulosic biomass are increasingly focused on modifying the lignin of economically and ecologically important plant species. Gain of function approaches that not only reduce cell wall recalcitrance but also add value to the biomass have become a core strategy in the development of more cost-effective processing methods. Lignin is a phenolic polymer found in the secondary cell walls of xylem vessels and fibres, where it plays a key role in facilitating water transport and providing structural support to the plant. Principally, three canonical *p*-hydroxycinnamoyl alcohols (monolignols) compose lignin: *p*-coumaryl, coniferyl, and sinapyl alcohols. However, it is now known that lignin can also include monomeric units outside the traditional monolignols, such as monolignol ester conjugates: *p*-hydroxybenzoate, *p*-coumarate, or ferulate, as well as caffeyl alcohol and 5-hydroxyconiferyl alcohol, and phenolic compounds derived from outside the phenylpropanoid pathway such as flavonoids and hydroxystilbene glucosides. Recently, xylem targeted expression of a bacterial *3-dehydroshikimate dehydratase* (*QsuB*) in Arabidopsis led to the conversion of 3-dehydroshikimate, a precursor in the shikimate pathway, into protocatechuate (3, 4-dihydroxybenzoic acid). This modification specifically diverts carbon away from the phenylpropanoid pathway, resulting in a reduction in total lignin and improved saccharification

efficiency (Eudes *et al*., 2015). Based on this initial study, our research focused on examining the effects of xylem targeted expression of *QsuB* in hybrid poplar (*P. alba* x *grandidentata*). We showed that hybrid poplar expressing *QsuB* accumulated significant amounts of soluble protocatechuaic acid (PA), but largely in the glycosylated form. Additionally, transgenic trees displayed significantly decreased total lignin, and altered monomeric composition with an increased accumulation of H subunits and a concurrent alteration in S and G unit ratio. Moreover, the saccharification yields of QsuB poplar increased by as much as 40% compared to wild-type trees. Interestingly, in-depth analysis of the *QsuB* transgenic lignin showed that conjugated forms of PA were polymerized to the lignin as ester linked pendant groups, as alkaline hydrolysis (saponification) of extracted cell wall material released both phenolic glycosides and free protocatechuic acid conjugates. These protocatechuate conjugates may therefore also be incorporated in the backbone of the lignin polymer forming a new "zip lignin".

References

1. Eudes A, Sathitsuksanoh N, Baidoo EE, George A, Liang Y, Yang F, Singh S, Keasling JD, Simmons BA, Loqué D. (2015) Expression of a bacterial 3-dehydroshikimate dehydratase reduces lignin content and improves biomass saccharification efficiency. Plant Biotechnol J.13(9):1241-50.

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