

## **Expression of S-adenosylmethionine Hydrolase in Tissues Synthesizing Secondary Cell Walls Alters Specific Methylated Cell Wall Fractions and Improves Biomass Digestibility**

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**Project Goals: The most abundant organic material on earth is lignocellulosic biomass or non-food plant material. JBEI's mission is to convert biomass to biofuels. The goal is to provide the nation with clean, renewable transportation fuels identical to gasoline, diesel and jet fuel. Building a successful lignocellulosic biofuels industry depends, in part, on developing specialized biofuel crops or feedstocks that are optimized for deconstruction into sugars and fermentation into biofuels and bioproducts.**

**Abstract:** Plant biomass is a large source of fermentable sugars for the synthesis of bioproducts using engineered microbes. These sugars are stored as cell wall polymers, mainly cellulose and hemicellulose, and are embedded with lignin, which makes their enzymatic hydrolysis challenging. One of the strategies to reduce cell wall recalcitrance is the modification of lignin content and composition. Lignin is a phenolic polymer of methylated aromatic alcohols and its synthesis in tissues developing secondary cell walls is a significant sink for the consumption of the methyl donor S-adenosylmethionine (AdoMet). In this study, we demonstrate in *Arabidopsis* stems that targeted expression of S-adenosylmethionine hydrolase (AdoMetase, E.C. 3.3.1.2) in secondary cell-wall synthesizing tissues reduces the AdoMet pool and impacts lignin content and composition. In particular, both NMR analysis and pyrolysis gas chromatography mass spectrometry of lignin in engineered biomass showed relative enrichment of non-methylated p-hydroxycinnamyl (H) units and a reduction of dimethylated syringyl (S) units. This indicates a lower degree of methylation compared to that in wild-type lignin. Quantification of cell wall-

bound hydroxycinnamates revealed a reduction of ferulate in AdoMetase transgenic lines. Biomass from transgenic lines, in contrast to that in control plants, exhibits an enrichment of glucose content and a reduction in the degree of hemicellulose glucuronoxylan methylation. We also show that these modifications resulted in a reduction of cell wall recalcitrance, because sugar yield generated by enzymatic biomass saccharification was greater than that of wild type plants. Considering that transgenic plants show no important diminution of biomass yields, and that heterologous expression of AdoMetase protein can be spatiotemporally optimized, this novel approach provides a valuable option for the improvement of lignocellulosic biomass feedstock.

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