Alteration of Cell Wall Ultra-Structure and Digestibility via an Engineered Monolignol Methyltransferase

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Wood is one of the major carbon reservoirs on Earth. Wood cell walls consist of a compound middle lamella and secondary wall layers that are composed of a cellulose/hemicellulose network impregnated with lignin. Previously, we evolved a set of monolignol 4-*O*-methyltransferases (M4OMTs) via protein structure-based iterative saturation mutagenesis. The created enzymes confer significant *para*-methylation activity to lignin precursor monolignols. We then expressed one of M4OMT variants in poplar and discovered that the expression resulted in more than 50% reduction of the thioacidolytic-cleavable, liable S-lignin units in transgenic cell walls, compared to that of the wild types. NMR analysis further confirmed the decrease in S-lignin units and the relative increase in the condensed cross-linkages (e.g., β-5), pointing to a substantial alteration in lignin composition and structure, and an apparent increase of lignin condensation in the transgenic poplar wood tissues. However, surprisingly, the efficiency of simple sugar release from transgenic wood biomass with cellulase treatment increased about 62% and thus the digestibility of wood biomass showed a substantial improvement. These apparently paradoxical data implicate a potential changes in cell wall ultra-structure. Subsequently, polarization synchrotron FT-IR was employed to explore the organization and orientation of cellulose, hemicellulose, and lignin polymers in transgenic cell walls. Our preliminary study revealed that the orientation and spatial arrangement of cell wall biopolymers in relation to the wood fibre axis were drastically altered in the transgenic wood tissues. These data suggest that condensation of lignin does not necessarily correlate with poplar wood biomass digestibility; and M4OMT-mediated alteration of lignin composition might globally affect the cell walls' ultra-structure and the interactions between cell wall polymers in transgenic poplar. Such created transgenic plants may provide an advanced cellulosic feedstock for agro-industrial applications and biofuel production.