Novel Pathway Design for Biopolymer Building Block Production

by

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Abstract

The carbon and energy intensity associated with plastics production from petroleum, combined with the accumulation of plastics waste in the environment, necessitates the development of technologies for the production of renewably-derived, degradable alternatives. Microorganisms can be metabolically engineered to convert renewable feedstocks to plastic building blocks. This thesis aims to design and implement metabolic pathways to industrially relevant hydroxy acids (HAs) and diols with the ultimate goal of using them for sustainable plastics production.

We began by prioritizing bioaccessible HAs for bio-production. Our analysis identified 182 bioaccessible HAs. We prioritized monomers from this list based on novelty, ease of chemical polymerization, maximum theoretical yield, and potential to improve material properties in a biopolymer. 3-Hydroxyisobutyric acid (3HIB) and 3-hydroxy-2-methylbutyric acid (3H2MB) were prioritized based on their high molecular weight polymerization and ability to reduce thermal degradation when incorporated into a biopolymer.

Next, we designed a novel pathway to 3HIB and 3H2MB. This pathway involves the conversion of glucose to various branched acyl-CoAs and ultimately to 3HIB or 3H2MB. As proof of concept, we engineered *E. coli* for the production 3HIB and 3H2MB from glucose at titers as high as 66 ± 5 mg/L and 290 ± 40 mg/L, respectively. To our knowledge, this is the first report of 3H2MB bio-production from glucose. We optimized this pathway for 3H2MB production by deleting competing pathways and developing a byproduct recycle. Finally, we investigated mutagenesis of a pathway enzyme to expand the product range of this pathway. Future work should investigate these mutants for the production of other biopolymer building blocks.

Finally, the feasibility of biological pathways to 3-methyl-1,5-pentanediol (3MPD) was investigated. 3MPD is an attractive monomer for the production of degradable, diol-diacid copolyesters. The feasibility determining step in this pathway involves the hydroxylation of 3-methylpentanol (3MP) to 3MPD by AlkBGT from *P. putida*. Despite optimizing our system for *alkBGT* expression, strain growth, and substrate transport, no 3MP conversion to 3MPD was observed. Future work should probe other hydroxylation enzymes.

Overall, this thesis demonstrates the utility of novel pathway design to reach HAs and diols that lead to biopolymers with improved industrial application.

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