Isoprenoid Biofuels Research at JBEI

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Project Goals: The Joint BioEnergy Institute (JBEI) aims to produce a chemically diverse suite of biofuels from lignocellulosic biomass. Among the biofuels compounds, isoprenoids have low freezing temperature and high octane number, which makes them promising biofuels. Mevalonate (MVA) pathway is one of the major biosynthetic pathways of isoprenoids biofuels, and the engineering of this pathway is a key approach to achieve higher production of these biofuels. JBEI's approaches to improve isoprenoids biofuel production involves understanding pathway enzymes, identifying bottlenecks and optimizing the pathway, which has been accelerated by development of various engineering strategies, analytical tools and mathematical models.

Isoprenoids are the largest and most diverse group of natural products. They are commonly used for medicinal purposes but their high energy density and low freezing point (due to methyl branching and cyclic structure often found in their carbon skeleton) also make them good candidates for gasoline, diesel and jet fuel replacements. Various isoprenoids are all synthesized from the two universal C_5 building blocks: isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Using an engineered *Escherichia coli* strain for the overproduction of IPP and DMAPP via the mevalonate pathway, we have produced isopentenol (C_5), limonene, pinene (C_{10}), cineole (C_{10}) and bisabolene (C_{15}), achieving more than 2 g/L of isopentenol, 1.1 g/L of bisabolene, and 0.5-0.6 g/L of limonene and 1,8-cineole. These improved titers were achieved by various strategies and analytical tools including omics analysis (e.g. proteomics and metabolomics to quantify key enzymes and intermediate metabolites), mathematical models to interpret the data. In addition, modified IPP-bypassing MVA pathways have been developed to reduce intrinsic energy demands of the MVA pathway and subsequently the aeration cost in larger scale fermenters, which occupies a significant portion of overall operational cost. We are currently developing Design-Build-Test-Learn (DBTL) cycle based on rational engineering approaches used for improving isoprenoid biofuels production in JBEI, and the optimized DBTL cycle will also be applied to optimize new target biofuel pathways and molecules.

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