Engineering Bacteria to Produce Branched-chain Fatty Acid Derived Advanced Biofuels

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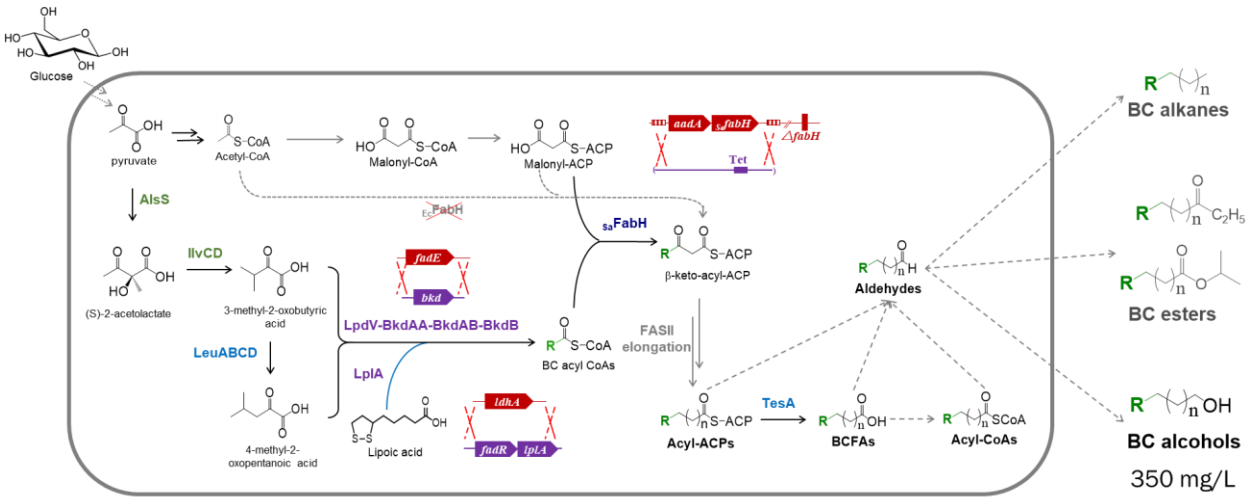
Project Goals: Extensive research has focused on engineering the fatty acid (FA) biosynthetic pathway for biofuel production because free FAs can be converted to fatty acid esters and alkanes that have similar energy content and cetane number to that of petroleum-derived diesel fuel. Most biological systems naturally produce straight-chain fatty acids (SCFAs). However, diesel fuels derived from SCFAs have undesirable freezing points and cold flow, limiting their practical use in low temperatures. On the other hand, petroleum-derived diesel contains a significant amount of branched hydrocarbons, which offer better cold flow properties. The overall goal of this project is to develop microbial systems for the production of branched-chain fatty acids (BCFAs) and their derived biofuels with improved physical and combustion properties. Our previous work focused on testing novel BCFA pathways in model bacterium E. coli. Our current aim is to transfer the pathway to a non-model bacterial species *Rhodococcus opacus* to enable production of BCFA-derived advanced biofuels from lignocellulose.

Abstract text.

BCFAs are important precursors of advanced biofuels with improved cold-flow properties. We developed metabolic pathways and a series of strategies to produce BCFAs and their derived biofuels in *E. coli* in high percentage. We first replaced the acetyl-CoA-specific *E. coli* FabH with one of branched-chain-acyl-CoA specific FabHs. Screening the most active branched-chain-acyl-CoA specific FabHs resulted in a 81-fold enhancement in BCFA-production compared to a strain containing *E. coli* FabH[1]. Next, we found that the position of the branch can be controlled by changing the metabolic pool of branched-chain α-keto acids. Supplementing different α-keto acids allowed us to produce specific BCFAs in high purities[1]. Meanwhile, we discovered a key bottleneck in BCFA production: overexpression of BKD is toxic to *E. coli* due to the depletion of protein lipoylation capacity. Engineering a complementary protein lipoylation pathway alleviated the toxicity and improved BCFA production to 276 mg/L and 85% of total free fatty acids[2]. Finally, an α-keto acid biosynthetic pathway was engineered and coupled with the rest of the BCFA pathway, the resulting strain produced BCFAs from glucose at 181 mg/L and 72% of total FFA[2].



Using this system, we introduced and tested four different downstream pathways that convert BCFAs to branched long-chain fatty alcohols (BLFLs). Furthermore, by engineering the α-keto acid biosynthetic pathways and balancing the expression levels of three different modules, we achieved BLFL titers of up to 350 mg/L from glucose, and BLFL percentages up to 79%[3]. We are currently exploring our engineering strategy in *R. Opacus* PD630 to produce BCFA-derived advanced biofuels from lignocellulose.

References

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