

Microbial Synthesis of Biodiesel

3rd Year Progress Report

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Our laboratory is currently in the 3rd year of this GCEP-funded project. The goals of our ongoing efforts are two-fold: (i) To identify and overcome barriers to efficient fatty acid biosynthesis in *E. coli*; and (ii) To exploit the resulting engineered bacterium for the biosynthesis of new types of energy-dense biofuels.

To these ends, we have made significant progress along the following directions: (i) We have established a cell-free system from *E. coli* that converts acetyl-CoA and related molecules into fatty acids. This system enables us to identify kinetic and regulatory bottlenecks that control fatty acid biosynthesis in a manner that is far more rapid and quantitatively accurate than conventional metabolic engineering approaches; and (ii) By transferring a membrane-bound fatty acid reductase from the waxy jojoba plant into *E. coli*, we have demonstrated the in vivo conversion of endogenous fatty acids into fatty alcohols. Without optimization, the resulting strain produces >150 mg/L/day of a mixture of C₁₂-C₁₈ saturated and unsaturated fatty alcohols in a small-scale fed-batch fermentation.

Future plans

Our primary focus for the remainder of this project period is to prepare publication-quality manuscripts based on our ongoing work on cell-free fatty acid synthesis and on the plant FAR. As time permits, we plan to translate some of our findings from the cell-free system into metabolic engineering strategies for obtaining further increases in fatty acid productivity in *E. coli*.