Microbial Synthesis of Biodiesel
Final Report

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Abstract
The goals of our project were: (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. During this 3-year project period, we made significant progress along the following directions:
(i) By introducing multiple genetic changes into the E. coli genome, we engineered an efficient producer of fatty acids. Under scalable fed-batch fermentation conditions, 4.5 g/L/day fatty acids were produced by this metabolically engineered E. coli strain.
(ii) We 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. Using this system, the strong dependence of fatty acid synthesis on malonyl-CoA availability and several important phenomena in fatty acid synthesis were verified. Results from this cell-free system were confirmed via the generation and analysis of metabolically engineered strains of E. coli.

Introduction
Two types of biofuels are approaching commercial utility at the present time – fatty acid methyl esters (a.k.a. biodiesel) from plants, and ethanol from microbial fermentations. However, neither biofuel has emerged as a clear winner and further improvements in both product and process are likely to be incremental in either case. The goal of this GCEP-sponsored project was to develop a fundamentally novel and practically useful fuel derived via a microbial fermentation route. Specifically, we sought to engineer the most well understood organism in biology, *Escherichia coli*, into a microbial factory for overproduction of biodiesel.

Background
The biocatalytic systems that is germane to the understanding of our studies – the fatty acid biosynthetic pathway in bacteria – is summarized in Figure 1.

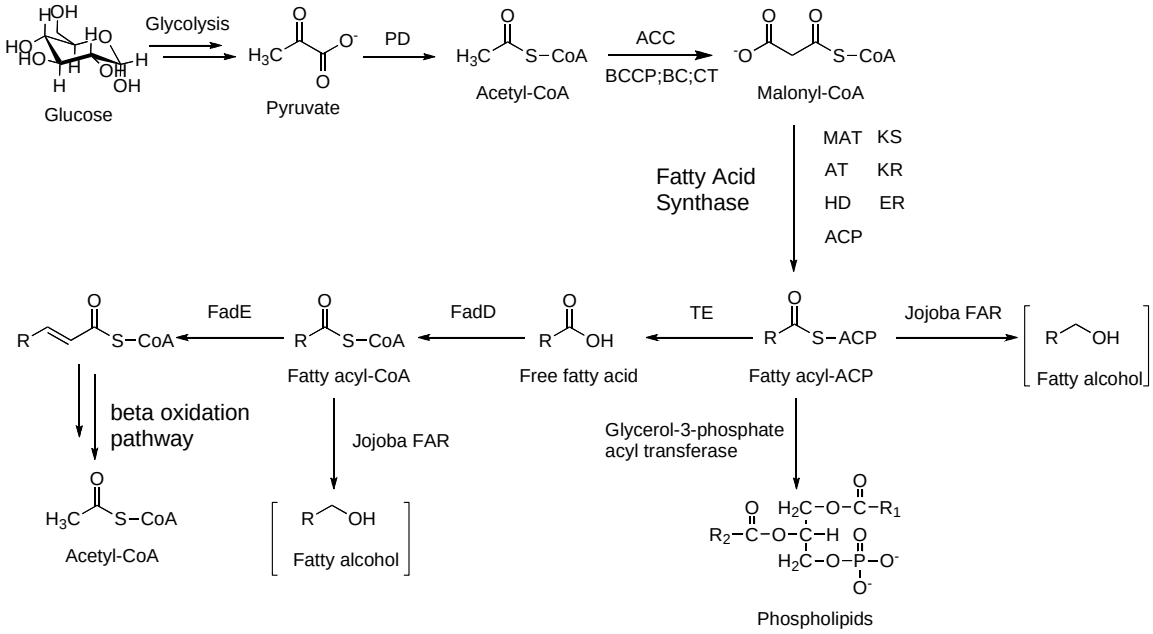

Figure 1. The biosynthetic pathway for fatty acids and their derivatives in *E. coli*. BCCP: biotin carboxyl carrier protein; BC: biotin carboxylase; CT: carboxyltransferase ⟨, ® two subunits; MAT: malonyl-CoA:ACP transacylase; KS: ®-ketoacyl-ACP synthase; AT: acetyl transacylase; KR: ®-ketoacyl-ACP reductase; HD: ®-hydroxyacyl-ACP dehydratase; ER: enoyl-ACP reductase. ACP: acyl carrier protein; FAR: fatty acyl-CoA reductase; R: fatty carbon chain.

Results

(i) By introducing multiple genetic changes into the E. coli genome, we engineered an efficient producer of fatty acids. Under scalable fed-batch fermentation conditions, 4.5 g/L/day fatty acids were produced by this metabolically engineered E. coli strain. The results of these studies were published in publication # 1 below.

(ii) We 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. Using this system, the strong dependence of fatty acid synthesis on malonyl-CoA availability and several important phenomena in fatty acid synthesis were verified. Results from this cell-free system were confirmed via the generation and analysis of metabolically engineered strains of E. coli. The results of these studies were published in publication # 2 below.

Conclusions

Bacterial production of biodiesel from renewable raw materials represents an attractive option for biofuel production.

Publications

1. Lu, X., Vora, H., and Khosla, C. (2008) Overproduction of free fatty acids in E. coli: implications for biodiesel production, *Metabolic Eng 10*, 333-339.

2. Liu, T., Vora, H., Khosla, C. Quantitative analysis and engineering of fatty acid biosynthesis in E. coli. *Metabolic Eng.* In press (2010)

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