Microbial Synthesis of Biodiesel
2nd year Progress Report

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The overall goal of our efforts is to develop a microbial process for biodiesel synthesis. *E. coli* was selected as a target bacterium for this project. At the end of the first year, we reported that fatty acids could be markedly overproduced in *E. coli* by genetic manipulation of the fatty acid biosynthetic and degradative pathways. During the past year, our efforts have been focused on three follow-on goals:

(i) Developing a fundamental understanding of the factors that limit the efficiency of fatty acid biosynthesis in *E. coli*;
(ii) Bioconversion of fatty acids into biofuel-like molecules; and
(iii) Engineering *E. coli* to directly fix CO_2 into fatty acids.

Below we summarize our results in each of these directions.

Results:

1. Understanding the factors that limit the efficiency of fatty acid biosynthesis in *E. coli*:

The first step was to develop a scaleable process for the production of fatty acids using the overproducing *E. coli* strain. A high-cell-density fed-batch fermentation was performed, and fatty acid productivity was monitored. The final titer was about >3 g/L total fatty acid with more than 60% of this total accumulating as free fatty acids. To systematically study the factors that limit the yield and productivity of fatty acids in *E. coli*, we sought to develop a defined minimal medium. Assays were developed to determine the flow of carbon into the three major sinks: biomass, carbon dioxide and fatty acid. Cultures were grown in shake flasks in a minimal media with glycerol as the major carbon source. The data showed that glycerol carbon consumption was attenuated in the fatty acid overproducing strain. However, this strain had a higher specific productivity and also a 3-5 fold improved stoichiometric efficiency.

2. Bioconversion of fatty acids into biofuel-like molecules in *E. coli*:

Conventional plant oil derived biodiesel is typically a mixture of fatty acid methyl esters. We sought to establish whether conventional biodiesel or closely related compounds (fatty acid ethyl esters) could be synthesized from fatty acids overproduced by *E. coli*. As an alternative, we also sought to bioconvert the fatty acids into fatty alcohols. This work is still in progress.

3. Engineering *E. coli* to directly fix CO_2 into fatty acids:

To date, all technologies for the production of fuels and chemicals from metabolically engineering *E. coli* depend on "pre-fixed" carbon sources such as sugars. The cost of such carbon sources is the dominant factor that ultimately limits the commercial viability of these bioprocesses. We seek to engineer *E. coli* for direct fixation of carbon dioxide into fatty acids. Recently, a new metabolic pathway has been discovered in archaea that fixes atmospheric CO_2 into acetyl-CoA. Because fatty acids are exclusively derived from acetyl-CoA, this pathway could be used to fix atmospheric CO_2 into fatty acids. Efforts in this direction are under way in our lab.