Microbial Synthesis of Biodiesel

Investigator
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Objective
This project aims to genetically engineer E. coli bacteria to significantly increase production of fatty acids, or biodiesel, from a biological matter feedstock. This potentially low-carbon fuel could replace fossil hydrocarbons currently used in on-road vehicles and aircraft. The major research goals include increasing the activity of key control enzymes that regulate the production of fatty acids, engineering biodiesel biosynthesis so it does not lead to concomitant accumulation of waste glycerol, and synthesis of tailored fuels that have desirable properties for a range of applications.

Background
Liquid fuel derived from biomass feedstock has been considered as a low-carbon substitute for fossil hydrocarbons in vehicles, but many of the existing approaches are encountering significant challenges. Commercial biodiesel is currently composed of fatty acid methyl (or ethyl) esters, which are derived via a transesterification process that utilizes plant-derived oils (triglycerides) and methanol (or ethanol) as raw materials. Since plant oils are often derived from seeds, biodiesel currently has a low yield relative to the amount of land, water, and farming energy required. Cellulosic ethanol could have higher yield if the proper conversion organisms are developed, but it has the drawback of being miscible with the aqueous broth required for these organisms and requires a separation step. Also, the low energy density of alcohols make them less attractive for fueling aircraft, where fuel is already a large portion of the vehicle's weight and volume. Direct biodiesel production from biomass feedstock using engineered microorganisms would both have a potentially high yield, naturally separate from the aqueous broth, and have the high energy density of a hydrocarbon.

Approach
This project seeks to engineer the most well understood organism in biology, Escherichia coli, as a microbial factory for fatty acid production. To achieve this goal, the investigator is taking a multi-pronged approach that addresses the problem at three different levels. First, the carbon flux to fatty acid biosynthesis will be increased by using genes from other organisms, which in turn should increase the specific productivity of fatty acids via the fermentation process. Second, different types of fatty acid analogs will be evaluated as potential fuel sources. The choices of compounds will be based on meeting or exceeding existing fuel criteria, while at the same time improving productivity and/or recoverability of product from the fermentation broth. Finally, by decoupling fatty acid analogue biosynthesis from triglyceride biosynthesis, it will be possible to produce biodiesel in microorganisms without the concomitant accumulation of glycerol waste.

In E. coli, lipids are produced from acetyl-CoA by a biosynthetic pathway that can broadly be divided into two stages: malonyl-CoA biosynthesis and fatty acid biosynthesis. The biosynthesis of malonyl-CoA, as illustrated in Figure 1, is the subject of tight transcriptional and post-transcriptional control. In order to circumvent this control, acetyl-CoA carboxylase genes from
other organisms can be expressed in E. coli. A second approach will be to altogether bypass acetyl-CoA to synthesize malonyl-CoA directly from exogenous malonate using a malonyl-CoA synthetase and a malonate transporter from Rhizobium organisms. This enzyme has been functionally expressed in E. coli in past work and can lead to high levels of malonyl-CoA synthesis.

The biosynthesis of fatty acids in E. coli is catalyzed by a multi-enzyme system called the fatty acid synthase. As illustrated in Figure 2, an n-carbon long fatty acid molecule is synthesized from n/2 malonyl-CoA building blocks. In E. coli, when the ACP-bound fatty acid chain reaches its full length, it is directly harnessed for phospholipid biosynthesis by the glycerol-3-phosphate acyl transferase (G3PAT). Direct transfer of the fatty acyl chain to glycerol-3-phosphate ensures that free fatty acids do not accumulate as intermediates in lipid biosynthesis in E. coli. In contrast, fatty acids in plants are first released from the ACP via hydrolysis; this reaction is catalyzed by a thioesterase enzyme (TE). Expression of plant TE in E. coli results in a significant (>5-fold) increase in lipid biosynthesis and a concomitant increase in accumulation of free fatty acids in the medium. The investigator is attempting to express fatty acid thioesterases from other organisms in E. coli and evaluate the resulting strains for lipid productivity.

A variety of fatty acid analogues can be produced by expressing selected enzymes in E. coli such as the stearoyl-ACP desaturase from sunflower, engineered polyketide synthase or fatty acid reductases from other microorganisms. In each case, the fuel compounds will be purified, modified semi-synthetically into methyl esters (if relevant), and submitted to a commercial testing laboratory. By developing fermentation strategies where biodiesel is directly separated from the producing culture, it may be possible to reduce the toxic effects of biodiesel overproduction, thereby enabling the productive phase of the fermentation process to be sustained.