Fabrication of Biofuels With a Multi-Strain System

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This year, the Arizona State University iGEM team has taken on a project that involves creating biodiesel in a new and efficient way using *Escherichia Coli*. Sustainability has become somewhat of a buzzword here at ASU, and the worldwide demand for renewable fuel sources has been growing in response to increasing concerns about dwindling oil reserves. Modern science has succeeded in genetically engineering bacteria that can metabolically synthesize a form of "biofuel" that is structurally similar to the fuel used in jet engines. Despite these revelations, however, bacterially-produced biofuels have not advanced to the point of being a commercially viable fuel source. There is much work that still needs to be done in making the production of biofuels both efficient and cost-effective.

A wax esterase exists that, when produced in bacteria, can react with the naturally produced ethanol and fatty acyl-CoA to produce an energy-dense biofuel. However, E. coli uses the same intermediate products to produce both fatty acids and ethanol, which reduces the efficiency of this process. The end goal heading into this project was to improve upon an existing idea by fixing this stoichiometric inefficiency. Our proposed solution is to create colonies of bacteria where multiple strains of bacteria coexist in the same isolated environment.

The basic idea is this: with two strains of bacteria, there will be no need for the cells to delegate resources towards producing both fatty acyl-CoA and ethanol at the same time. One strain of bacteria (hereby known as Strain 1) will focus on producing the ethanol, while the other (Strain 2) can devote its energy to the fatty acyl-CoAs. Ethanol, being the smaller molecule, can then diffuse into the extracellular fluid and into the Strain 2 cells. The Strain 2 bacteria will also be transformed with DNA to produce the wax esterase that is needed to catalyze the final reaction to produce the biodiesel. In this way, the synthetic process can be compartmentalized.

A wide variety of genetic engineering parts is being used to create this bacterial system. Pdc and adhb are together responsible for the production of the ethanol in Strain 1. We have obtained experimental results confirming that these parts increase natural ethanol production. An acc and TesA (thioesterase) plasmid has been ligated as well as an attempt to kickstart fatty acyl-CoA production. The first steps that we've been taking towards realizing this goal generally involve maximizing the production of the biofuel's "ingredients".

In order to be successful in improving the efficiency of the system, we must further analyze the stoichiometry of the equation. The reaction between fatty acyl-CoA and ethanol is 1:1, so ideally we would want to be producing roughly the number of molecules of each. Since they're very different molecules in a structural sense, a single strain of engineered cells wouldn't be able to produce equal amounts due to the differences in metabolic cost. In the future, we hope to be able to explore different avenues of controlling the population numbers for the different strains in an attempt at balancing the chemical reaction. Quorum sensing is a promising concept that, with further research, could potentially allow the system regulate itself without any outside interference. There is still a lot of work to do towards making biofuel a successful industry.