Engineering Robust Hosts for Microbial Biofuel Production

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Project Goals: The goal of this project is to develop tools for improving microbial tolerance of biofuel production conditions. We aim to develop specific strategies for improving tolerance to enhance microbial synthesis of next-generation biofuels. The work is organized around three objectives: (1) Identify novel biofuel tolerance mechanisms from a targeted set of microorganisms. We are focusing on microorganisms that survive in hydrocarbon-rich environments as a source of tolerance genes, looking specifically for strategies that prevent biofuel from compromising membrane integrity and mechanisms for exporting biofuel-like compounds from the cell. (2) Engineer a synthetic feedback loop that responds to biofuel production. To optimize biofuel production yields, cells must balance several competing sources of stress. We are designing and constructing a novel feedback loop that senses biofuel production and turns on export pumps in response. (3) Finally, we are integrating multiple tolerance strategies in a biofuel production strain. In addition to having the potential to greatly enhance biofuel yields, this work will advance understanding of how multiple stress tolerance mechanisms interact within a cell.

Abstract:
Microorganisms can be used to synthesize fuel from renewable materials. Microbial biofuel synthesis is a cost effective and environmentally sustainable way of producing replacements for gasoline, diesel, and jet fuel from lignocellulosic biomass. In a typical production process, biomass is deconstructed into sugars that are metabolized by a microbe engineered to convert sugar into biofuel. In this work, we focus on fuel synthesis—the final stage of biofuel production—and develop engineering tools for increasing the robustness of a biofuel production host.

A major challenge when using microorganisms to produce bulk chemicals like biofuels is that the production targets are often toxic to cells. Biofuel-like compounds are known to reduce cell viability through damage to the cell membrane and interference with essential physiological processes. Thus, cells must trade off biofuel production and survival, reducing potential yields. The majority of microbial biofuel research to-date has focused on engineering metabolic pathways for biofuel production. It is essential that we also engineer strains for biofuel tolerance. This is especially important for the large-scale production environments that will be required for generating cost effective biofuels.

Recent work on ethanol has already demonstrated that engineering strains for end-product tolerance can greatly improve overall yield. Here, we focus on engineering tolerance to advanced biofuels, taking a targeted approach toward improving biofuel tolerance and yield. Our goal is to develop a list of rational targets, rather than employing strategies like adaptation and random mutagenesis, to identify mechanisms that can be easily moved into production strains.

Recent work by the PI has indicated that microorganisms that survive in oil-rich environments are a valuable source of tolerance mechanisms [1-3]. We are testing genes from microbes that have been isolated from environments near natural oil seeps and in the vicinity of oil spills to see if they improve biofuel tolerance when expressed in *Escherichia coli*. Whole genome sequencing efforts have included several of these hydrocarbon-tolerant microbes. We are using this sequence data as a starting point for identifying tolerance genes to heterologously express in a biofuel production host.

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References

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