

115. Engineering Robust Hosts for Microbial Biofuel Production

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Project Goals: The goal of this project is to enhance microbial synthesis of next-generation biofuels by developing tools for improving microbial tolerance of biofuel production conditions. Research is organized around three objectives: (1) Identify novel biofuel tolerance mechanisms from microorganisms that naturally thrive in hydrocarbon-rich environments. (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) Integrate 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 tolerance mechanisms interact within a cell.

Abstract:

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 project, we 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 cells¹. Biofuel-like compounds are known to reduce cell viability through damage to the cell membrane and interference with essential physiological processes^{1,2}. Thus, cells must trade off biofuel production and survival, reducing potential yields. In addition, residual chemicals that remain after biomass pretreatment can inhibit cell growth, further reducing yields. Recent studies have shown that strains engineered to increase tolerance can improve biofuel production yields¹.

Recent work by the PI has indicated that microorganisms that survive in oil-rich environments are a valuable source of tolerance mechanisms³. We are using a transgenic screening approach, building libraries from the genomic DNA of microbes that have been isolated from environments near natural oil seeps and in the vicinity of oil spills, and screening for biofuel tolerance in *Escherichia coli*. We are focusing specifically on libraries from *Pseudomonas* species and the alkane-metabolizing microbe *Alkanivorax borkumensis*.

Expression of transport proteins called efflux pumps can increase tolerance to biofuels and pretreatment chemicals by pumping toxins out of the cell and improving fuel yields. However, overexpression of efflux pumps can compromise the cell, creating a trade-off between chemical toxicity and pump toxicity^{4,5}. Research has suggested that certain combinations of efflux pumps can confer additional tolerance compared to the individual pumps themselves. But, the functional form of the combination of the tolerance provided by each pump and the toxicity due to their simultaneous activity is unknown. We approach this problem using a combination of mathematical modeling and experiments. Using differential equations, we developed a growth model incorporating the trade-offs between toxicity of inhibitors and tolerance provided by efflux pumps to describe the dynamics of bacterial growth under these conditions. By analyzing each inhibitor and efflux pump independently

through a series of biological experiments and mathematical models, we determine the functional form of their combined effect on growth rate, with the long-term goal of increasing biofuel yields. Modeling predictions are tested experimentally to quantify the combined effect of expressing multiple tolerance mechanisms.

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