154. Engineering Robust Hosts for Microbial Biofuel Production

Tim Tomko *1(ttomko@uvm.edu), Jessica Lindle1, Will Turner1, Mary J. Dunlop1

1 University of Vermont, School of Engineering, Burlington, VT 05405

Project Goals: The overall 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 advances understanding of how multiple tolerance mechanisms interact within a cell.

Abstract:
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. Recent studies have shown that strains engineered to increase tolerance can improve biofuel production yields.

Microorganisms that survive in oil-rich environments are a valuable source of tolerance mechanisms. Using a transgenic screening approach, we are building fosmid-based libraries using genomic DNA from microbes that have been isolated from hydrocarbons-rich environments. Here, we present data from a transgenic screen using a library from Marinobacter aquaeolei, a hydrocarbon-degrading microorganism isolated from an offshore oil rig. The transgenic library was screened for biofuel tolerance in Escherichia coli using bio jet fuels limonene and pinene. After 4 days of exposure to the biofuels with serial dilutions every 12 hours, we saw marked improvements in tolerance relative to the control strain without the library. We then isolated the fosmids and identified a single 40kb sequence responsible for improving tolerance. Using the fosmid DNA, we constructed a smaller plasmid library and used this to isolate the specific gene responsible for tolerance.

In addition to identifying novel tolerance mechanics, we are designing control systems for efflux pumps known to export biofuel. Pump overexpression inhibits cell growth, suggesting a trade-off between biofuel and pump toxicity. To counter this, we are using the protein MexR, native to Pseudomonas aeruginosa, as a biosensor because it detects oxidative stress such as that caused by the introduction of biofuels. In the feedback loop design, MexR represses the expression of an efflux pump derived from M. aquaeolei by binding to a synthetic promoter region. We developed a library of synthetic promoters, which vary the number and location of MexR binding sites, and screened these for tolerance to pinene, a known pump substrate. The screen tests both constant and dynamic biofuel environments. The dynamic environment is important for selecting a sensor that performs well in both the presence and absence of biofuel. Our experimental findings are further supported by a mathematical model describing the dynamic sensor selection.

We also studied whether expressing multiple pumps in combination could further increase biofuel tolerance. With multiple pumps, the combined impact of pump toxicity and benefits from increased tolerance are unclear. To address this, we measured tolerance of E. coli to pinene in one-pump and two-pump strains. To support our experiments, we developed a mathematical model describing toxicity due to

biofuel and overexpression of pumps. We found that data from one-pump strains can accurately predict the performance of two-pump strains. This result suggests that it may be possible to dramatically reduce the number of experiments required for characterizing the effects of combined biofuel tolerance mechanisms.

Publications

1. W. J. Turner, M. J. Dunlop. "Trade-offs in Improving Biofuel Tolerance Using Combinations of Efflux Pumps." ACS Synthetic Biology, DOI: 10.1021/sb500307w, 2014.
2. M. Frederix, K. Hutter, J. Leu, T. S. Batth, W. J. Turner, T. L. Ruegg, H. Blanch, B. A. Simmons, P. D. Adams, J. D. Keasling, M. P. Thelen, M. J. Dunlop, C. J. Petzold, A. Mukhopadhyay. "Development of a native Escherichia coli induction system for ionic liquid tolerance." PLoS One, 2014.
3. M. E. Harrison, M. J. Dunlop. "Synthetic Feedback Loop Model for Increasing Microbial Biofuel Production Using a Biosensor." Frontiers in Microbiology 3:360, 2012.
4. M. J. Dunlop. "Engineering Microbes for Tolerance to Next-Generation Biofuels. ”Biotechnology for Biofuels 4:32, 2011.

This research was supported by the University of Vermont and the Office of Science (Biological and Environmental Research), US Department of Energy.