108. A Strategy for Genome-scale Design, Redesign, and Optimization for Ethylene Production in *E. coli*

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Project Goal: This project aims to apply state-of-the-art synthetic and systems biology tools to design and optimize *E. coli* for sustained production of biofuels. Chassis biofuel strains, optimized for production based on predictive design and systems biology knowledge, will serve as the framework for high throughput genome re-design. Using targeted genome-scale and multiplex genome-engineering technologies, strains with improved production will be selected for, and gene-to-trait mapping will identify key factors for further optimization. Herein, we will focus on the construction of an *E. coli* prototype chassis strain for the production of ethylene by optimization of protein expression, growth medium compositions, and metabolic pathway flux. Moreover, we are evaluating "landing pads" in *E. coli* suited for integration of genes/pathways for biofuel production, as well as developing high throughput strategies for selection of strains with increased biofuel/precursor levels.

Abstract
Ethylene is the most highly utilized organic compound for the production of plastics and chemicals, and its catalytic polymerization to alkane fuels has been demonstrated. At present, global ethylene production involves steam cracking of a fossil-based feedstock, representing the most CO_2-emitting process in the chemical industry. Biological ethylene production has the potential to provide a sustainable alternative while mitigating CO_2 emission. The expression of a single gene found in some bacteria and fungi, ethylene-forming enzyme (EFE), can catalyze ethylene formation (1). However, its efficient biotechnological application requires a more in-depth understanding of the interactions between the EFE reaction and other metabolic pathways in the cell, which will be afforded by genome-scale synthetic biology approaches.

Construction of the first generation chassis strain is based on *E. coli* MG1655 as the host and the *efe* gene from *Pseudomonas syringae* (*Ps*). EFE has been postulated to catalyze ethylene production according to the equation (2):

$$3\ \alpha\text{-ketoglutarate} + \text{Arginine} + 3O_2 \rightarrow 2C_2H_4 + \text{Succinate} + 7CO_2 + 3H_2O + \text{guanidine} + \text{P5C}^*$$

However, low yield and EFE protein insolubility are key challenges at present. Our initial focus is on optimization of EFE protein expression levels, improvement of its solubility and stability, and analysis of interactions between the EFE reaction and other metabolic pathways by nutrient and genetic alterations. Rates of ethylene production are improved by three-fold when EFE is expressed from a medium vs. high copy number plasmid, yet most of the EFE protein is in the insoluble inclusion bodies based on Western data. To improve solubility, we co-expressed the GroES/EL chaperones and observed a further 7-fold improvement in ethylene productivity, concurrent with an increase in the level of soluble EFE. We are currently exploring the addition of solubility tags to EFE to further improve its solubility. To improve ethylene production based on the above reaction, we explored media composition (rich versus defined), addition of exogenous substrates (α-ketoglutarate [AKG] and arginine), and the incorporation of predicted genetic modifications to improve flux to AKG and

arginine in the TCA cycle (Figure 1), the findings from which will be reported. Lastly, work is ongoing to verify "landing pads" for the safe integration of pathway genes necessary for both ethylene and isobutanol production strains, as well as to develop methods for high-throughput selection of strains with increased production of key intermediates and/or ethylene from pooled libraries.

*P5C: L-Δ^1-pyrroline-5-carboxylate

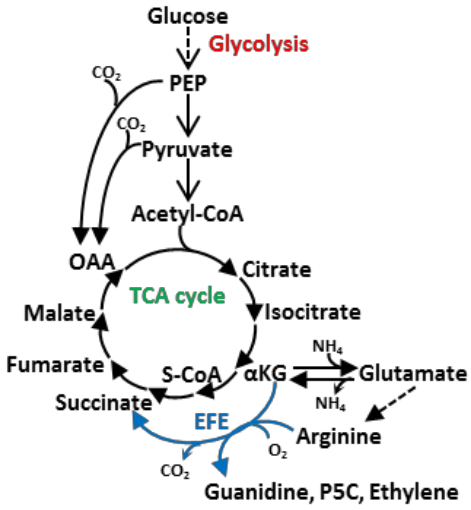

Figure 1. Putative metabolic scheme for ethylene production in *E. coli*. EFE: ethylene-forming enzyme. P5C: L-Δ^1-pyrroline-5-carboxylate.

References

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