Genome-scale design and engineering approach towards optimizing ethylene production in *E. coli*

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Project Goal:

This project aims to apply rational design and state-of-the-art synthetic and systems biology tools to 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 and thermal stability will be selected for further optimization. Herein, we will focus on the construction of an *E. coli* prototype chassis strain for the production of ethylene and the development of a selection strategy for gene-to-trait mapping at single nucleotide resolution to identify key factors for optimizing ethylene production. Moreover, we are 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 largest 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, 2). 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 (3):

3 α-ketoglutarate + Arginine + $3O_2$ → $2C_2H_4$ + Succinate + $7CO_2$ + $3H_2O$ + guanidine + P5C*

The two key substrates α-ketoglutarate (AKG) and arginine are tightly controlled by an intricate regulatory network that coordinates carbon and nitrogen metabolism (Figure 1). We conducted genetic modifications to rewire central carbon metabolic flux and improved ethylene production by 2.3-fold (4). This chassis strain will serve as the framework to guide genome-scale redesign and optimization to further boost ethylene production using CRISPR enabled trackable genome engineering (5). Succinate is a byproduct of the EFE reaction. We generated a succinate auxotroph in *E. coli* and showed that it must rely on an active heterologous EFE pathway yielding succinate to afford growth. EFE is not stable above 30 ℃. We thereby screen for thermal stable EFE by expressing an *efe* mutant library in the succinate auxotroph and select for growth at 37 ℃. We identified key mutations of *efe* mapped to semi-conserved residues in EFE homologues with its outcomes unraveling the catalytic mechanism of EFE. Work is also ongoing to construct high-throughput sensors to screen for AKG and ethylene, *in situ*. As such, current work from our groups at the National Renewable Energy Laboratory and the University of

Colorado at Boulder seeks to improve ethylene production by combining traditional metabolic engineering strategies with synthetic biology-enabled evolutionary approaches involving the high-throughput construction of genome-scale libraries. Coupled with novel screens and selections, these methods will identify strains with increased production of key intermediates and/or ethylene.

*P5C: L-Δ^1-pyrroline-5-carboxylate

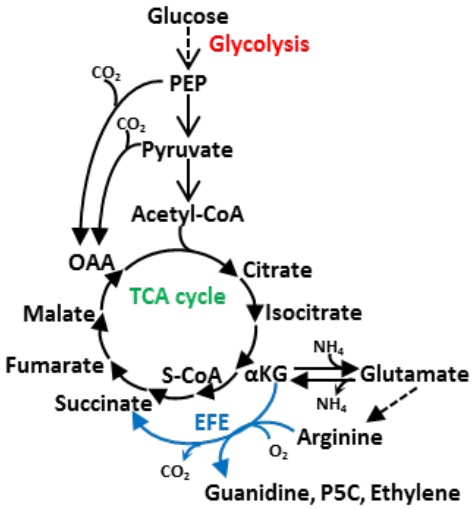

Figure 1. Putative metabolic scheme for ethylene production in *E. coli*. EFE: ethylene-forming enzyme. P5C: L-Δ^1-pyrroline-5-carboxylate.

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