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 rational design and 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 and a selection strategy aimed to improve thermal stability of the key enzyme involved in 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 CO₂-emitting process in the chemical industry. Biological ethylene production has the potential to provide a sustainable alternative while mitigating CO₂ 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 + 3O₂ → 2C₂H₄ + Succinate + 7CO₂ + 3H₂O + 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. 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 °C. We thereby screen for thermal stable EFE by expressing an efe mutant library in the succinate auxotroph and select for growth at 37 °C. We identified key mutations of efe mapped to semi-conserved residues in EFE homologues with its outcomes unveling the catalytic mechanism of EFE. Work is also ongoing to construct high-throughput sensors to screen for AKG and ethylene,
*P5C: L-Δ¹-pyrroline-5-carboxylate

Figure 1. Putative metabolic scheme for ethylene production in *E. coli*. EFE: ethylene-forming enzyme. P5C: L-Δ¹-pyrroline-5-carboxylate.

References


Funding Statement

Grant title: A Platform for Genome-scale Design, Redesign, and Optimization of Bacterial Systems; Project grant number (DE-SC008812) and FWP number (ERWER44) for NREL.