Rapid Prototyping of Novel Bioproduct Pathways in an Acetogen through Integrated Computational Modeling and High-throughput Candidate Screening

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Project Goals: The Clostridia Foundry for Biosystems Design (cBioFAB) pulls together new capabilities in computational modeling, cell-free metabolic engineering (CFME), genetic engineering of *Clostridium*, and laboratory automation to streamline the development of industrial bioprocesses for next-generation bioproducts from gasified carbon substrates. In one aim of this project, we set out to develop a comprehensive set of enzymatic parts using methods for automated identification pulling from a wide net of databases and genome-scale models. A tailored set of enzyme parts is then applied towards the generation of novel metabolic pathways to targeted bioproducts. High-throughput assembly and characterization of predicted pathways allows for screening data to be fed back into the computational framework to optimize and predict improved novel pathways.

Mono-ethylene glycol (MEG) is a chemical used predominantly as a building block in the synthesis of polyethylene terephthalate (PET), a polymer with major applications in the plastics and textiles industries. MEG derived from gas fermentation would have several advantages over traditional chemical synthesisincluding reduced carbon emissions and the ability to utilize low-cost waste carbon feedstocks. Currently the only known biological route to MEG occurs through the metabolism of C5 sugars, which is less attractive from the perspective of carbon recycling and feedstock cost, and has not scaled commercially. *Clostridium autoethanogenum*, an acetogen used in the industrial production of chemicals, is being investigated as an engineered host strain for MEG production from synthesis gas (1). To accomplish this, we are employing CFME alongside high-throughput *in-vivo* screening to rapidly prototype novel pathways to MEG that were computationally predicted by the BNICE framework (2). Enzymes are produced via cell-free protein synthesis (CFPS) in *E. coli* cell extracts and then combined *in vitro* to recapitulate predicted routes to MEG. High-performing candidates are assembled in large combinatorial plasmid libraries with varying promoter strengths and transformed into *C. autoethanogenum* to be screened on a high-throughput automated biofoundry platform (3). This approach allows us to rapidly test, optimize, and rank novel predicted pathways. Using these methods, we were able to generate strains demonstrating production of MEG in small-scale gas fermentation.

References/Publications

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