Robust Non-Oxidative Glycolysis in *Escherichia coli*

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Project Goals: This project seeks to construct strains of *Escherichia coli* to rely solely on Non-oxidative glycolysis (NOG) for glucose catabolism during aerobic growth, fundamentally rewiring the central metabolism to favor carbon conservation in acetyl-CoA biosynthesis. This work will also engineer the constructed strains for energy applications. Specifically, we aim to produce an advanced biofuel, n-butanol, with close to complete carbon conservation. Hydrogen or formate will be provided to supply reducing equivalents needed to drive the NADH dependent pathway.

Acetyl-coenzyme A (acetyl-CoA) is a two carbon metabolite and important metabolic precursor to a variety of industrially relevant compounds including biofuels. An ultimate limitation of acetyl-CoA derived biochemical production is the inherent carbon loss when forming acetyl-CoA. Most organisms use some glycolytic variation, commonly the Embden-Meyerhof Pathway (EMP), to initially degrade sugar into pyruvate. Pyruvate, a C3 metabolite, is then decarboxylated to form acetyl-CoA, losing carbon to the environment. This decarboxylation limits the carbon yield to only two molecules of acetyl-CoA from one molecule of hexose, thus inhibiting the economics of any associated bioprocess. A synthetic sugar catabolism pathway, termed non-oxidative glycolysis (NOG), was recently developed to address this problem, as it uses a combination of phosphoketolase dependent cleavage of sugar phosphates and a carbon rearrangement cycle to directly generate three C2 units per hexose in a redox neutral manner. To further expand the applications using NOG, an *Escherichia coli* strain was constructed to rely solely on NOG for sugar catabolism in this work. Therefore, the resulting strain offers significant potential to be engineered for the production of a variety of acetyl-CoA derived compounds. To implement NOG as a growth pathway, all native sugar degradation pathways, including the EMP, ED and methylglyoxal bypass, were removed, eliminating the cell's ability to grow on sugar as a sole carbon source. In addition, the glyxoylate shunt and gluconeogenesis pathways, which are necessary for the production of essential metabolites using NOG, were upregulated. Following the chromosomal integration of phosphoketolase, an *in vitro* pathway assay was used to identify limiting core enzymes for C2 metabolite production in cell lysates. Following the overexpression of these enzymes as well as a PTS independent glucose uptake system on an inducible plasmid, the engineered strain was able to develop an improved growth phenotype relative to an uninduced control in minimal glucose media supplemented with acetate. A time course analysis of the growth cultures verified that the induced strain was able to consume glucose while the uninduced strain could not. Additionally, under fermentative conditions this strain was able to produce acetate from glucose at yields approaching the theoretical maximum. Future work on the project will involve understanding the necessity of acetate supplementation for growth in minimal media in order to engineer the cell to grow solely on sugar.

Grant number: U.S. Department of Energy DE-SC0012384