Engineering a Synthetic *Escherichia coli* Methylotroph for Conversion of Methanol to Fuels and Chemicals

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Abundant and recoverable natural gas supplies have prompted considerable interest in developing synthetic methylotrophic microorganisms that possess the ability to biologically convert the primary component of natural gas, methane, into liquid fuels and chemicals. Methane, along with its oxidized product, methanol, which can be synthesized by biological or chemical means, are promising feedstocks for industrial fermentative bioprocesses, as these one-carbon compounds are more reduced than traditional lignocellulosic sugars, thus providing the opportunity to achieve increased product titers and yields. Although native methylotrophs naturally metabolize these one-carbon compounds, these microorganisms are currently not well-established for industrial application, resulting from their limited genetic engineering toolbox and the fact that many are obligate aerobes and excrete only a few select metabolites, often at low titers. Therefore, development of synthetic methylotrophs, or non-methylotrophic platform microorganisms that have been engineered to metabolize one-carbon compounds, specifically methane and methanol, is of considerable interest.

To support this effort, *Escherichia coli*, a facultative anaerobe and well-established industrial host, was engineered for synthetic methanol utilization in this study. Incorporation of a heterologous methanol dehydrogenase from *Bacillus stearothermophilus* and the ribulose monophosphate pathway from *Bacillus methanolicus* successfully imparted synthetic methanol utilization capabilities in engineered *E. coli* strains. Resulting methylotrophic *E. coli* strains successfully converted methanol-derived carbon into biomass components, liquid fuel molecules and precursors and commodity and specialty chemicals. Throughout this study, several limitations were identified and subsequently alleviated to achieve improved methylotrophic phenotypes. Specifically, an improved methanol dehydrogenase variant was achieved via protein engineering, carbon flux through the pentose phosphate pathway was improved via metabolic engineering and the methanol tolerance of *E. coli* was improved via adaptive evolution. All of these improvements resulted in methylotrophic *E. coli* strains exhibiting improved conversion of methanol-derived carbon into biomass precursors.