SYNTHETIC METHYLOTROPHY: ENGINEERING METHANOL METABOLISM IN A NONNATIVE HOST

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Methylotrophy is the ability of microorganisms to utilize one carbon compounds such as methane and methanol for growth and energy generation. The recent discovery of abundant natural gas reserves has prompted considerable interest in utilizing these compounds as substrates or co-substrates in industrial fermentations of chemicals and fuels. Increased biomass and product yields are expected from these compounds since both are more reduced than lignocellulosic sugars. Native methylotrophic microorganisms are poor industrial host organisms since many are strict aerobes, produce few metabolites and lack genetic engineering tools. Therefore, the development of synthetic methylotrophy in nonnative host organisms is of considerable interest. Here, the development of *Escherichia coli* as a platform microorganism for methanol metabolism is presented. Incorporation of native methylotrophic enzymes confers methylotrophic properties to *E. coli*, allowing the nonnative metabolism of methanol into biomass and metabolites. For example, as shown in Figure 1, methanol supplementation provides a 50% improvement in biomass yield on yeast extract in engineered *E. coli*. Further discussion will be given on how to overcome the challenges involved in synthetic methylotrophy, including unfavorable methanol oxidation, methanol toxicity, carbon conservation and regulatory limitations. Specifically, a high-throughput fluorescence activated cell sorting assay was developed to identify engineered methanol dehydrogenase mutants exhibiting improved methanol oxidation characteristics. Separately, chemical mutagenesis and directed evolution led to the isolation of a mutant methylotrophic *E. coli* strain exhibiting improved methanol tolerance. The importance of the pentose phosphate pathway during nonnative methanol metabolism in *E. coli* and its regulatory tuning for optimal methanol assimilation will also be discussed. Finally, data demonstrating the use of methanol as a substrate for cell growth, energy generation and metabolite production in *E. coli* will be presented.

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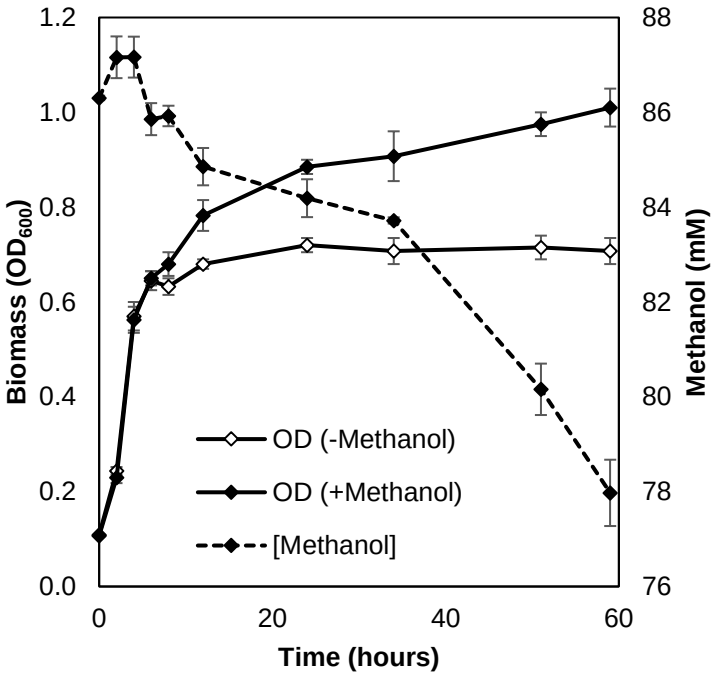

Figure 1. Methylotrophic E. coli growth.