Using Gene Editing and an Accumulated Bioproduct as a Reporter for Genotypic and Phenotypic Heterogeneity in Growth-vs-Production for *Methylobacterium extorquens* Conversion of Aromatics to Butanol

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https://marxlab.org/doe-biosystems-project/

Project Goals: With a unique capacity to assay growth and production – for either a tremendous number of genotypes in a mixture, or for individual cells – we will provide an unprecedented view of the critical tradeoff between growth and production. This will be used to guide development of *M. extorquens* as a novel platform for conversion of methoxylated aromatics to butanol. We will accomplish this work through the following aims:

1. Engineer/evolve improved use of methoxylated aromatics in M. extorquens

2. Explore growth-vs-production tradeoffs for genetic and phenotypic variation in PHB production

3. Combine improvements in substrate use and production capacity

4. Exchange PHB synthesis for butanol synthesis to test best genotypes

Abstract. Over the past year we have made progress on several fronts in order to move towards achieving our project's goals. First, we have made significant progress on the development of the genetic tools for introduction of genome-edited gene clusters from *Vibrio* into *Methylobacterium*. Second, we have developed the capacity to grow *Methylobacterium* in patterned microfluidic devices to permit growth dynamics and PHB accumulation to be ascertained. Third, we have examined PHB accumulation across a variety of substrates, growth phases, and nitrogen levels. This allows comparison of image cytometry, HPLC analysis of total levels, and flow cytometry. Fourth, these PHB accumulation studies have been conducted for a variety of natural strains, revealing interesting diversity in both the mean and variation of PHB produced. Fifth, we have nearly completed genetic and physiological analysis of the novel pathway for aromatic utilization that we have found in *Methylobacterium*. Finally, we have evolved strains to effectively utilize novel methoxylated aromatic compounds. All of these steps forward move us toward our ultimate goal to develop *M. extorquens* for conversion of methoxylated aromatics to butanol, while simultaneously demonstrating a novel approach that combines the advantages of gene editing and

deep-sequencing, an internally-accumulated product as a proxy, and an analysis of phenotypic heterogeneity for both growth and production.

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