Investigating Metabolic Flux Rewiring in Adaptively Evolved and Dysregulated *E. coli*

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The emergence of various omics techniques in biotechnology research has enabled investigators to study altered phenotypes of microorganisms, including the workhorse microbe, *Escherichia coli*, at the molecular level. This includes the development of ^{13}C-isotopic tracing methods and ^{13}C-metabolic flux analysis (^{13}C-MFA), which facilitates the interrogation of *in vivo* metabolic fluxes. Additionally, tools have been developed to more easily genetically engineer *E. coli* strains with diverse metabolic phenotypes, and adaptive laboratory evolution (ALE) has been increasingly employed as part of these bioengineering strategies. Yet, the principles guiding ALE remain poorly articulated and further basic studies into how metabolic changes take place over the course of ALE are needed.

This Thesis seeks to investigate and describe metabolic flux rewiring following ALE in various *E. coli* strains. First, ALE was applied with the goal of engineering methylotrophic *E. coli* strains with improved methanol-utilization capabilities. This was undertaken to develop *E. coli* synthetic methylotrophs that can convert methanol or other reduced C1 compounds to useful platform chemicals. In the first case, a methanol-auxotrophic *E. coli* methylotroph that requires glucose as a co-substrate was designed and optimized through ALE. Another synthetic *E. coli* methylotroph was adaptively evolved on threonine and methanol to tune its ability to employ methanol in biosynthesis. ^{13}C-tracing methods were employed to quantitatively interrogate changes in methanol metabolism following ALE in both engineered *E. coli* methylotrophs.

Next, in order to improve basic understanding of how *E. coli* metabolism is regulated, ^{13}C-MFA was performed on 21 *E. coli* knockout strains – 6 strains which contain one transcription factor knockout each and 15 strains containing two transcription factor knockouts – all grown at the exponential phase in aerobic conditions and abundance of glucose. These 6 transcription factors exert significant regulatory effect on the central carbon metabolism of *E. coli* and were hence selected for this study. The data generated here adds to a set of metabolic fluxes previously generated from 45 *E. coli* central carbon metabolism knockout strains (CCK strains).

To better understand the dynamics of how metabolic fluxes are rewired after ALE, additional multi-omics analyses were performed on 5 CCK strains that were subjected to ALE by growing them at the exponential phase in aerobic conditions and excess glucose. These 5 CCK strains, Δ*pfkA*, Δ*rpe*, Δ*aceEF*, Δ*acnB*, and Δ*sucB,* were specifically selected as the introduction of these knockouts dramatically altered the metabolic phenotype of these strains with respect to

wild-type *E. coli*. Following ALE, all strains demonstrated an improvement in growth rate. ^{13}C-MFA revealed significant changes in metabolism following ALE. The associations between changes in metabolic phenotypes and mutations accumulated over the course of ALE were revealed by evaluating genomic sequencing data of multiple strains evolved in parallel. The comprehensive fluxomic data sets from this study were then harmonized together with a previous data set involving evolved Δ*pgi* strains to reveal broad characteristics of *E. coli* metabolism that emerge when data from multiple CCK strains are analyzed together. It is envisioned that further systematic studies, with even larger data sets, will build toward a more complete understanding of *E. coli* metabolism and the impact of ALE. Knowledge from such endeavors will benefit future *E. coli* strain designs. Additionally, similar analytical methods and approaches can also be translated to other organisms of interest.