Interrogating Metabolism via the Mapping of Fluxomic Responses to Gene Knockouts and Adaptive Evolution

Christopher P. Long
Adviser: Maciek R. Antoniewicz
Committee Members: Eleftherios T. Papoutsakis, Wilfred Chen, Mark J. Nelson

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Biological systems have enormous potential for chemical conversion, including otherwise inaccessible syntheses and utilizing renewable feedstocks. Designing production strains through metabolic engineering requires detailed systems knowledge, particularly in the mapping of genotype (where manipulations are most typically made) to the metabolic phenotype (e.g., overproduction of a chemical product). In this thesis, advances were made in metabolic characterization methods, and these methods were then applied to map metabolic responses to gene knockouts and adaptive laboratory evolution. Specifically, novel methods for measuring biomass composition, useful new measurements for ^{13}C metabolic flux analysis (^{13}C-MFA), and strategies for optimal tracer design were developed. These optimized methods were used to comprehensively assess metabolic responses to 45 *E. coli* gene knockouts of enzymes spanning central carbon metabolism. Analysis of flux rewiring in these strains revealed bottlenecks and areas of flexibility in metabolism, a novel reversibility of Enzyme I of the PTS system, and a glucose secretion phenotype. These results constitute a significant new resource for systems biology, particularly for metabolic modeling where they are being directly used in the development of ensemble kinetic models.

Genetic and metabolic flux responses to adaptive laboratory evolution were also characterized, providing new insights into the processes of microbial adaptation and fitness enhancement. Growth recovery (up to 3.6-fold) in an *E. coli* knockout strain of a core glycolytic enzyme (PGI) was enabled by a unique set of mutations which alleviated rate limiting steps in metabolism. In evolved *E. coli* wild-type strains, growth rate enhancements of 50% did not correspond to intracellular flux rewiring, indicating broad and proportional regulatory change. Mutations in both experiments also suggest critical roles for global regulators in adaptation. Finally, the metabolism of *Vibrio natriegens*, a very fast growing and potential next-generation host organism, was elucidated by ^{13}C-MFA. This provides an important baseline of knowledge to facilitate modeling and engineering of this organism. Further investigation into the mechanisms of fast-growth, both natural and evolved, will enable the development of hosts with superior productivity and economic potential.