Co-culture ^{13}C-Metabolic Flux Analysis: a Novel Approach to Elucidate the Metabolism of Multi-microorganism Systems.

Nikodimos A. Gebreselassie

In nature, microorganisms don't exist in isolation; rather, they are part of complex and interacting systems. However, current understanding of microbial metabolism is mainly constrained to mono-culture systems because of limitations in analytical technologies. This has limited the scope of our knowledge of cellular metabolism to less than 1% of microbes that are culturable in laboratory conditions, and hampered efforts to better understand microbial communities in important areas such as the human microbiome. Furthermore, recent efforts in metabolic engineering have focused on taking advantage of unique properties of multi-microorganism systems to enhance product yields, titers, and productivities. In particular, the modularity of co-culture systems makes them a convenient platform for easy optimization and assembly of biosynthetic pathways. Despite the relevance of multi-microorganism systems both in natural ecosystems and metabolic engineering, quantitative characterization tools used to elucidate their metabolism are still lacking. Particularly, ^{13}C-metabolic flux analysis (^{13}C-MFA), a widely-used approach for measuring metabolic flux in pure cultures, has not been developed and applied towards multi-microorganism systems.

In ^{13}C-MFA, metabolic fluxes are elucidated by culturing cells in the presence of an isotopically labeled substrate. The resulting isotopic labeling patterns of metabolites are then measured using mass spectroscopy techniques. Metabolic fluxes are then estimated by fitting the measured labeling data to a network model containing the known metabolic pathways of the organism. In the few previous examples where ^{13}C-MFA has been applied to co-cultures, it has required species-specific isotopic labeling data in which the labeling measurement of a purified protein was used as a proxy for species-specific labeling data.

In this thesis, I present a novel approach for performing ^{13}C-MFA in co-cultures. I demonstrate for the first time that it is possible to determine metabolic flux distributions in multiple species simultaneously without the need for physical separation of cells or proteins, or overexpression of species-specific products. Instead, metabolic fluxes for each species in a co-culture are estimated directly from isotopic labeling of the total metabolite labeling obtained using conventional mass spectrometry approaches such as GC-MS. Through carefully selected isotopic tracer experiments, I demonstrate a novel multi-scale model fitting framework that allows for the elucidation of steady state intracellular fluxes, population composition, inter-species metabolite exchange (e.g. cross-feeding of molecules between cells), and dynamic reactor level growth and metabolite profiles. The methodology was experimentally validated using a mixed culture of two *E. coli* knockout strains and then applied to three different co-culture systems.

First, co-culture flux analysis was performed in an *E. coli – S. cerevisiae* co-culture, an industrially relevant system in which the two organisms consume the same substrate but have different biosynthetic pathways, and products. Next, metabolic fluxes in a co-culture of *E. coli* knockouts *Δpgi* (knockout of glycolysis pathway) and *Δzwf* (knockout of pentose phosphate pathway) were fully elucidated. This co-culture system is the most challenging to deconvolute as it consists two organisms with the same metabolic network, consuming and producing the same substrate and products. Finally, I successfully applied the methodology to study a cross-feeding synthetic co-culture consisting of a glucose consuming wild-type *E. coli* strain and a glucose non-consuming *ΔptsI Δglk E. coli* strain. Here, the *ΔptsI Δglk* strain relied on acetate produced by the wild-type *E. coli* for growth. Using the co-culture ^{13}C-MFA framework, I characterized the intracellular fluxes in each species and deconvoluted the time dependent culture-level metabolite concentrations. In addition, through the three model co-culture systems presented in this thesis, I discuss various aspects of the experimental design and important considerations for performing co-culture ^{13}C-MFA.

In the future, this work should support both metabolic engineering efforts in multi-microorganism systems as well as scientific efforts aimed at exploring consortium metabolism. Particularly, it lays the foundation for more detailed studies of complex interacting microbial systems, including systems consisting of more than two species.