

Revealing the Molecular Interactions in Phototroph-Heterotroph Co-Cultures for Bioenergy Through Development of an In Silico Platform for Design and Discovery

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Abstract: Lichens are nature's solar cells, harnessing energy from sunlight and converting it along with CO₂ into biochemicals. They achieve this goal through a complex web of interactions between a photobiont, typically a cyanobacteria or eukaryotic algae, with heterotrophic species. Unfortunately, nature's lichens are not optimized for fast growth and prolific biosynthesis of biomass or bioproducts. Therefore, our team established autotrophic-heterotrophic partnerships to serve as a novel platform for biofuels production. The engineered photobionts use the sunlight energy and CO₂ and provide substrates to the heterotrophs, which produce lipids and other metabolites as biofuels precursors. What is missing from this project is an understanding of the nature of the molecular interactions beyond simply the provision of carbon sources to each co-culture participant. Indeed, we have observed enhanced growth and improved fitness for cyanobacteria in the presence of a heterotrophic partner which must involve complex metabolic interactions beyond carbon substrates and perhaps include changes in gene expression. Also, economical production of bio-products requires redirection and optimization of carbon flux into target molecules. Unfortunately, the metabolic modeling and associated analytic characterization of microbial co-culture systems remains a challenge and a bottleneck to our improved understanding of these complex interacting biosystems. Consequently, our goal is to develop and validate a pipeline for advanced understanding of molecular interactions in co-culture systems. This information will be applied to implement an In-Silico Platform for Design and Discovery for optimizing production of potential biofuels and co-products. The challenge to the in-silico models is the lack of useful data to test the constraints and validity. This limitation will be addressed through advanced analytical tools available in the EMSL and JGI followed by species-resolved transcriptional network analysis and next generation genome-scale reconstruction of a model autotrophic-heterotrophic co-culture of a cyanobacterium *Synechococcus elongates* with the bacterium *E. coli*. Our first Aim will be to characterize the major physiological parameters, identify extracellular carbon metabolites and collect transcriptome data of the axenic cultures to validate the existing genome-scale reconstructions. Then, in our second Aim, we will apply valuable tools from EMSL and JGI to characterize the physiological response and metabolic adjustments in microorganisms when co-cultivated together. Specifically, we will undertake metabolome analyses using NMR and MS in concert with transcriptome profiling to decipher interactions and communication between photobiont and its heterotrophic partner. In addition, we will use ¹³C Metabolic Flux Analysis to determine the distribution of carbon flux in co-cultures. Ultimately, insights drawn from these analytical investigations will be incorporated into metabolic reconstruction of this model co-culture system to better understand the complex relationships inherent in symbiotic partnerships. The implemented reconstruction will be used to optimize this novel biofuel production platform with increased functionality and diversity based on molecular interactions and capabilities present in our co-culture. Importantly, our principal goal is to implement and validate a pipeline of analytical tools for improving genome-scale models. This program will provide a tested sequence and collection of methodologies that can be used to characterize and interpret the complex interactions occurring in a variety of multispecies populations.