Metabolic Flux Analysis of Sucrose-Secreting Cyanobacterium *Synechococcus elongatus*

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The goal of this project is to combine autotrophs and heterotrophs as a novel synergistic and symbiotic platform for the production of sustainable biofuel precursors. Photosynthetic microorganisms fix sunlight and CO_2 and provide organic carbon source and oxygen to the heterotrophs that are prolific producers of complex metabolites. Synthetic microbial communities of cyanobacterium-fungus will be studied through genome-scale metabolic modeling and ^{13}C metabolic flux analysis. Our current focus is to evaluate the metabolic response of the cyanobacterium *Synechococcus elongatus* PCC 7942 to osmotic stress and sucrose secretion.

Cyanobacterial strains that are capable of secreting sucrose to support growth of heterotrophs in a co-culture system have gained significant interest from the biotechnology community. Efforts on strain development and process optimization have taken place since a decade ago, and the technology has been advanced significantly. However, most efforts have been focused on investigating local pathways that are closely related to sucrose biosynthesis and secretion, and the global intracellular metabolism, which is crucial for detecting bottlenecks in the network, has not yet been investigated.

In this study, we investigate the global metabolic fluxes in the sucrose-secreting ($cscB^+$) strain versus the wild type. *Synechococcus elongatus* PCC 7942 synthesizes sucrose under NaCl stress as a means of coping with osmotic pressure, while overexpression of CscB, a sucrose transporter, confers the secretion of sucrose out of cells. We use two complementary approaches, *i.e.*, ^{13}C metabolic flux analysis (MFA) and Genome-Scale Modeling (GSM), to elucidate the difference of intracellular resource allocation by quantifying metabolic fluxes between these strains. ^{13}C-MFA uses ^{13}C-labelled compound as tracer to displace the ^{12}C atom of intracellular metabolites, and the metabolic fluxes can be quantitatively mapped through tracking the labelling status of these metabolites along a time course and computational modeling. Constrain-based GSM combine genome-scale network reconstructions with detailed biochemical and physiological information to provide *in silico* estimation of intracellular metabolic flux under a condition-specific set of constraints. ^{13}C-MFA and GSM can be applied to decipher network regulations and hence guide metabolic engineering.

We performed ^{13}C-MFA and GSM for three cyanobacterial cultures – [wild type], [wild type + NaCl], and [$cscB^+$ + NaCl] – under photoautotrophic conditions. For ^{13}C-MFA, we use ^{13}C-

labelled bicarbonate as tracer, and use GC-MS and LC-MS/MS to quantify labeling trajectories of more than a dozen of intracellular metabolites along a time course. We use INCA software to simulate the flux and generate the quantitative flux map. For GSM, we evaluated the solutions space using random sampling, using experimentally observed sucrose secretion rates as constrains in the model of the cyanobacteria to validate growth phenotypes and resource allocation under various growth conditions. This unbiased assessment provides a distribution of all possible flux distributions of the network at given conditions. Additionally, we evaluated osmotic stress conditions by increasing the salts concentration five times from 28 mM to 130 mM. Predicted flux distributions across the solution space were compared for all three conditions, showing significant changes in transport and carbohydrate metabolism.

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