Metabolic flux analysis of an engineered sucrose-secreting strain of the 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 have been 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.

The capability of cyanobacteria to produce sucrose from CO_2 and light has significant societal and biotechnological impact since sucrose can serve as a carbon and energy source for a variety of heterotrophic organisms. Efforts on strain development and process optimization have taken place since a decade ago, and technology has been advanced significantly. However, most efforts have focused on understanding local pathway alterations that drive sucrose biosynthesis and secretion rather than global flux re-routing that occurs following induction of sucrose production by NaCl treatment. Therefore, we investigated global metabolic flux alterations in a sucrose-secreting ($cscB^+$) strain versus the wild-type *Synechococcus elongatus* 7942. We used ^{13}C metabolic flux analysis (MFA) and Genome-Scale Modeling (GSM) as complementary approaches to elucidate differences in intracellular resource allocation by quantifying metabolic fluxes between these strains. We performed ^{13}C-MFA and GSM for three cyanobacterial cultures – wild-type *S. elongatus* 7942 grown in BG11 minimal culture medium (WT), wild-type *S. elongatus* 7942 grown in BG11 supplemented with additional 100 mM NaCl (WT/NaCl), and *S. elongatus* $cscB^+$ strain grown in BG11 medium supplemented with additional 100 mM NaCl ($cscB^+$/NaCl) – all under photoautotrophic conditions. We quantitatively described the dramatic rewiring of metabolic fluxes in WT/NaCl and $cscB^+$/NaCl relative to that of the WT culture, and identified a potential metabolic bottleneck limiting carbon fixation and sucrose biosynthesis in the engineered $cscB^+$ strain. Our study also demonstrates that combining two complementary approaches, namely ^{13}C-MFA and GSM, is a useful strategy to both extend the coverage of MFA beyond central metabolism and to improve the accuracy of flux predictions using GSM.

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