Metabolic Analysis of Cyanobacteria Carboxysome Mutant Indicates a More Flexible lux Network for Bio-manufacturing

Mary H. Abernathy[1], Jeffery J. Czajka[1*], Doug Allen[2,3], **Jeffrey C. Cameron[4,5,6]** and **Yinjie J. Tang[1]**

[1]Department of Energy, Environmental and Chemical Engineering, Washington University in St. Louis, MO 63130; [2]Donald Danforth Plant Science Center, St. Louis, MO 63132, USA; [3]United States Department of Agriculture, Agricultural Research Service, St. Louis, MO 63132, USA; [4]Department of Biochemistry, University of Colorado Boulder, Boulder, CO 80309; [5]Renewable and Sustainable Energy Institute, University of Colorado, Boulder, CO 80309; [6]National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO 80401

Presenter email: jjczajka@wustl.edu

Project goals:

1. Investigate cyanobacterial metabolic network and reveal its regulations for fast biosynthesis.
2. Elucidate effects of micro-compartmentation on cyanobacterial metabolisms.

Abstract

Cyanobacterial carboxysomes encapsulate carbonic anhydrase and ribulose-1,5-bisphosphate carboxylase/oxygenase and are key organelles that promote CO_2 concentration and fixation. Genetic deletion of the major structural proteins encoded within the ccm operon in *Synechococcus* sp. PCC 7002 (Δ*ccmKLMN*) disrupts carboxysome formation and significantly affects cell physiology. In this study, we employed both metabolite pool size analysis and isotopically nonstationary metabolic flux analysis (INST-MFA) to examine metabolic regulation in cells lacking carboxysomes. Under high CO_2 environments, the Δ*ccmKLMN* mutant had similar growth rates as the control strain and maintained a similar flux distribution through the central metabolism, with the exceptions of moderately elevated protein synthesis and photorespiration activity. Metabolite analyses indicated that the Δ*ccmKLMN* strain had larger pool sizes of pyruvate, UDPG, and aspartate as well as higher levels of secreted malate and succinate. Under photomixotrophic conditions, both the control strain and the Δ*ccmKLMN* mutant metabolized acetate and pyruvate. Provision of acetate promoted carboxysome mutant growth when light and CO_2 were insufficient. The results suggest that the Δ*ccmKLMN* mutant is able to minimize changes in fluxes (except for elevated photorespiration) and instead reorganizes its metabolism through significant changes in intracellular metabolite pool concentrations. The removal of microcompartments may loosen the flux network regulation and allow for redirection of central metabolites to competing pathways (e.g., lactate production). This study provides important insights into both metabolic regulation via enzyme compartmentation and the compensatory responses in cyanobacterial phototrophic metabolism.

Publications

1. Abernathy, et al. Cyanobacteria carboxysome mutant reveals the influence of enzyme compartmentalization on cellular metabolism and metabolic network rigidity. *Submitted*

Funding Statement

This work was partially supported by the Department of Energy, DESC0012722.