Systems analysis of a fast growing N_2-fixing cyanobacterium for production of advanced biofuels and nitrogen-containing petrochemical replacement compounds

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https://sites.wustl.edu/photosynthbio/anabaena_33047/

Project Goals:
The overall objective of this project is to use an integrated systems biology approach to develop the filamentous cyanobacterium *Anabaena* sp. PCC 33047 as a model fast-growing, photosynthetic, diazotrophic production platform. The specific goals for this project are: 1) Construct a genome-scale metabolic model and predict genetic alterations that optimally direct fixed CO_2 and N_2 into target products. 2) Apply 13C and 15N assisted metabolomics and metabolic flux analysis to dissect the metabolism of the strain. 3) Develop an efficient genetic toolkit. 4) Demonstrate production of caprolactam and valerolactam in engineered *Anabaena* 33047.

Abstract

Anabaena sp. ATCC 33047 is a fast-growing heterocystous cyanobacterium that utilizes high light and exhibits the highest recorded conversion rate of CO_2 into biomass in an oxygenic photosynthetic organism[1]. Unlike most diazotrophic strains, this cyanobacterium has the ability to maintain similar growth rates both in the presence and absence of combined N_2 sources and displays a doubling time as short as 3.8 h under photoautotrophic and N_2-fixing conditions. These characteristics render *Anabaena* 33047 an attractive platform for cost effective production of nitrogen-rich compounds. However, this strain was known to be genetically intractable and hence not widely studied. In the initial phase of our project we developed a genetic manipulation system that enabled us to make targeted modifications in its genome. We also focused on the development of a genome scale metabolic model for *Anabaena* 33047.

Nitrogenase activity in heterocystous cyanobacteria rely on ATP generated by PSI-mediated cyclic phosphorylation in the heterocysts and light is essential for this process. Under N_2 limited growth conditions, cyanobacteria exhibit an initial degradation of phycobilisomes (PBSs), the major light harvesting complex. This helps to maintain a more balanced C/N ratio under N_2 limitation by reducing photosynthetic activity of the cells and also ensures mobilization of cellular nitrogenous resources until the heterocysts engage in fixing nitrogen. PBS is resynthesized in the vegetative cells after fixed nitrogen becomes available but its fate in heterocysts remains poorly understood. The degradation of PBS is mediated by the NblA protein. In the *nblA* deficient strain of *Anabaena* sp. PCC 7120, nitrogen step down does not trigger PBS degradation and does not affect growth[2]. To assess the effect of this deletion on N_2 fixation in a high light tolerant strain, we engineered a Δ*nblA* mutant of *Anabaena* 33047. Intriguingly, under high light intensities, the Δ*nblA* mutant exhibited up to 2.5-fold higher rates of nitrogenase activity compared to the wild

type. Spectroscopic analysis shows higher PSI activity in the mutant possibly aided by higher levels of PBS in the mutant heterocysts. Thus, retaining higher levels of PBS appears to be an effective strategy to enhance rates of N_2 fixation in this high light-tolerant, fast growing diazotrophic strain.

We have also developed a comprehensive genome scale metabolic model for *Anabaena* 33047 by mining annotation from diverse data bases such as KEGG[3], MetaCyc[4] and ModelSEED[5] and a recently published model for the closely related *Anabaena* 7120[6]. A species-specific biomass equation was formulated based on biomass composition of *Anabaena* 7120[7]. To account for the heterocystous lifestyle of this species, the model accounts for two different cell types: vegetative cell (950 reactions) and heterocyst (942 reactions). The model accounts for 892 genes and 953 unique reactions. The vegetative cell performs photosynthesis but lacks the nitrogenase enzyme. The heterocyst performs N_2 fixation but lacks the capacity to perform oxygenic photosynthesis. The model was able to predict the growth rate under N_2 fixing and N_2 sufficient conditions. Under both the conditions, the PSI/PSII flux ratio predicted by the model was above 1.2 as observed for cyanobacteria in general. The model is being used to design metabolic engineering strategies for the overproduction of nitrogen containing compound valerolactam.

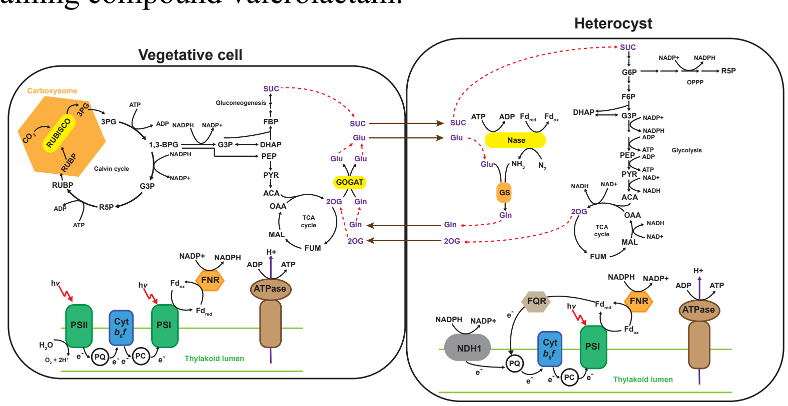

Figure 1: Two cell model of *Anabaena* 33047. The genome scale metabolic model, iAnC915, has two super-compartments, the vegetative cell and the heterocyst, in-order to capture the diazotrophic and heterocyst forming life style of this cyanobacterium.

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