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

Anindita Banerjee[1]* (anindita@wustl.edu), Deng Liu[1], Zi Ye,[1] John I. Hendry[2], Costas D. Maranas[2], Maciek R. Antoniewicz[3] and **Himadri B. Pakrasi**[1]

[1]Washington University in St. Louis, MO; [2]Pennsylvania State University, University Park, PA, [3]University of Michigan, Ann Arbor, MI

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. 5) Establish a stable consortium between *Anabaena* 33047 and a heterotroph for cost-effective bioproduction.

Abstract

Anabaena sp. ATCC 33047 is heterocystous cyanobacterium that thrives under very high light intensities and exhibits fast growth both in the presence and absence of fixed nitrogen, traits that make this strain an attractive platform for the cost effective production of nitrogen-rich compounds. The strain was known to be genetically intractable and hence not utilized in molecular and synthetic biology studies. During the course of this project we developed a genetic manipulation system that enabled us to make targeted modifications in its genome. We further characterized one of the modified strains, which shows greatly enhanced rates of nitrogen fixation. We also developed a genome scale metabolic model, *i*AnC892, for *Anabaena* 33047 to identify genetic interventions to overproduce valerolactam and caprolactam.

Phycobilisomes (PBS) are large antenna protein complexes in cyanobacteria that harvest light and funnel the energy to the photosynthetic reaction centers. When subjected to nitrogen deficient growth conditions, heterocystous cyanobacteria exhibit transient PBS degradation mediated by the NblA protein. As nitrogen fixation commences, PBS is resynthesized back to their normal levels in vegetative cells, but their abundance remains low in

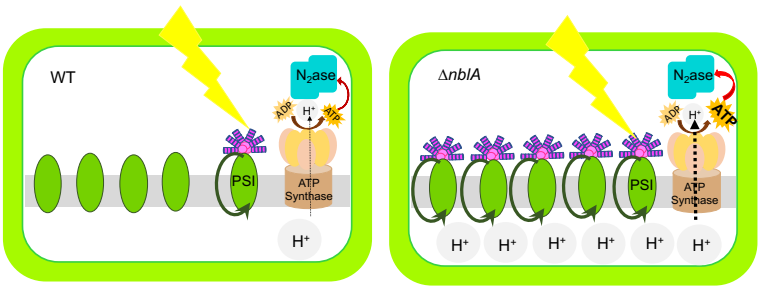

Figure 1. Schematics depicting the differences in the heterocysts of the WT and Δ*nblA* strains that contribute to enhanced nitrogenase activity. Higher abundance of phycobilisomes in the mutant heterocyst and their association with PSI centers leads to higher ATP generation and nitrogenase activity.

heterocysts. Earlier studies implicating a role for PBS in energy transfer to photosystem I (PSI) in heterocysts [1,2], instigated our efforts to investigate the effect of antenna modification on nitrogenase activity in the high light tolerant *Anabaena* 33047. To this end, we generated a Δ*nblA* mutant of *Anabaena* 33047 that retains large amounts of PBS in its heterocysts. Intriguingly, when subjected to high light the mutant exhibited 2.5 folds higher rates of nitrogen fixation compared to the WT. Analysis of the mutant indicated, increased cyclic electron flow, possibly resulting from higher PBS mediated energy transfer to PSI. This contributes to increased ATP synthesis and enhanced nitrogenase activity in the mutant (Figure 1) [3]. Thus the Δ*nblA* mutant of *Anabaena* 33047 offers an improved platform for the production of nitrogen rich compounds.

Genome-scale metabolic models (GSM) facilitate in-silico engineering of microbial metabolism for over production of target chemicals [4]. We developed a genome scale metabolic model, *i*AnC892, for *Anabaena* 33047 to identify genetic interventions to overproduce valerolactam and caprolactam [12]. The model was constructed by retrieving annotations from multiple databases: KEGG [5], MetaCyc [6] and ModelSEED [7] and a recently published model for the closely related *Anabaena* 33047 [8]. *i*AnC892 contains 953 unique reaction representing the annotation of 892 genes. The diazotrophic life cycle of *Anabaena* 33047 is captured by accounting for both vegetative and heterocyst cell types. This is achieved by creating super-compartments that reflect the metabolic differences and interactions between these two cell types. The model was used alongside the strain design algorithm, OptForce [9], to identify genetic interventions that would lead to overproduction of caprolactam and valerolactam. The production of valerolactam and caprolactam were enabled by adding 5-aminovalerate pathway [10] and Adipyl-CoA pathway [11] respectively into *i*AnC892. The identified strategies recapitulated several of the previously reported genetic interventions that enhance the target compound production [12].

To explore the possibility of establishing a stable consortium between *Anabaena* 33047 and a heterotroph, several *E.coli* strains were co-cultured with this cyanobacterium under different growth conditions. Our study revealed that *Anabaena* 33047 can support the growth of *E.coli* Top10 cells when the strains are co- cultured in nitrogen free medium. Our preliminary results indicate that *Anabaena* 33047 can be a suitable partner in a synthetic photoautotrophic-heterotrophic consortium.

References

1. Cardona, T, Magnuson A: **Excitation energy transfer to Photosystem I in filaments and heterocysts of *Nostoc punctiforme***. *Biochim Biophys Acta* 2010, **1797**: 425-433.
2. Watanabe, M, Semchonok, DA, Webber-Birungi, MT, Ehira, S, Kondo, k, Narikawa, R, Ohmori, M, Boekema, E, Ikeuchi, M: **Attachment of phycobilisomes in an antenna-photosystem I supercomplex of cyanobacteria**. *Proc Natl Acad Sci U S A* 2014, **111**: 2512-2517.
3. Bandyopadhyay, A, Ye, Z, Benedicty, Z, Trtilek, M, Pakrasi, HB: **Antenna modification leads to enhanced nitrogenase activity in a high light tolerant cyanobacterium**. (Submitted) 2021.
4. Simeonidis E, Price ND: **Genome-scale modeling for metabolic engineering**. *J Ind Microbiol Biotechnol* 2015, **42**:327-338.
5. Kanehisa M, Goto S, Kawashima S, Okuno Y, Hattori M: **The KEGG resource for deciphering the genome**. *Nucleic Acids Res* 2004, **32**:D277-280.
6. Caspi R, Billington R, Fulcher CA, Keseler IM, Kothari A, Krummenacker M, Latendresse M, Midford PE, Ong Q, Ong WK, et al.: **The MetaCyc database of metabolic pathways and enzymes**. *Nucleic Acids Res* 2018, **46**:D633-D639.
7. Henry CS, DeJongh M, Best AA, Frybarger PM, Linsay B, Stevens RL: **High-throughput generation, optimization and analysis of genome-scale metabolic models**. *Nat Biotechnol* 2010, **28**:977-982.
8. Malatinszky D, Steuer R, Jones PR: **A comprehensively curated genome-scale two-cell model for the heterocystous cyanobacterium Anabaena sp. PCC 7120**. *Plant Physiol* 2017, **173**:509-523.

9. Ranganathan S, Suthers PF, Maranas CD: **OptForce: An optimization procedure for identifying all genetic manipulations leading to targeted overproductions**. *PLOS Computational Biology* 2010, **6**:e1000744.
10. Zhang J, Barajas JF, Burdu M, Wang G, Baidoo EE, Keasling JD: **Application of an Acyl-CoA ligase from *Streptomyces aizunensis* for lactam biosynthesis**. *ACS Synth Biol* 2017, **6**:884-890.
11. Turk SCHJ, Kloosterman WP, Ninaber DK, Kolen KPAM, Knutova J, Suir E, Schürmann M, Raemakers-Franken PC, Müller M, de Wildeman SMA, et al.: **Metabolic engineering toward sustainable production of Nylon-6**. *ACS Synthetic Biology* 2016, **5**:65-73.
12. Hendry, JI, Dinh, HV, Sarkar, D, Wang, L, Bandyopadhyay, A, Pakrasi, HB, Maranas CD: **A genome-scale metabolic model of *Anabaena* 33047 to guide genetic modifications to overproduce nylon monomers.** (Submitted) 2021.

Funding statement: This study is being supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomic Science Program under Award Number DE-SC0019386