SYNECHOCOCCUS ELONGATUS UTEX 2973: METABOLIC MODELS AND FLUXOMIC ANALYSIS

Thomas J. Mueller[1*] (tjm5293@psu.edu), Saratram Gopalakrishnan[1*] (sxg375@psu.edu), Mary H. Abernathy[2*] (mary.h.abernathy@wustl.edu), Fangfang Ma[3], Michelle Liberton[4], Whitney Hollinshead[2], Lian He[2], Doug K. Allen[3], **Yinjie J. Tang**[2], **Costas D. Maranas**[1], and **Himadri B. Pakrasi**[2,4]

[1]Department of Chemical Engineering, The Pennsylvania State University, University Park, PA; [2]Department of Energy, Environmental and Chemical Engineering, Washington University, St. Louis, MO; [3]USDA-ARS/Donald Danforth Plant Science Center, St. Louis, MO; and [4]Department of Biology, Washington University, St. Louis, MO

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Project Goals: The overall objective of this project is to use an integrated systems biology approach to develop *Synechococcus* 2973, a fast growing cyanobacterial strain, as a platform organism for photobiological production of advanced biofuels and other useful chemicals. The project aims to improve the understanding of metabolic processes in this microbe through *in silico* analysis followed by experimental validations to enable efficient strain design. Overall the goals are three-fold: (a) to reconstruct a genome-scale metabolic model of *Synechococcus* 2973, (b) to develop a carbon-mapping model of this strain, and (c) to better understand its phenotypical properties by isotopically nonstationary metabolic flux analysis (INST-MFA).

Photosynthetic microbes offer advantages over commonly used heterotrophic microbes such as *E. coli* and *Saccharomyces cerevisiae* due to their ability to harvest light energy and fix carbon dioxide. Researchers have demonstrated that photosynthetic microbes like cyanobacteria have the ability to convert light energy and CO_2 into value-added chemicals. However, there are still key challenges in the use of cyanobacteria as microbial cell factories: low biomass yield and production rates. Recently, *Synechococcus elongatus* UTEX 2973 has been identified in the Pakrasi lab as a unicellular cyanobacterial strain with an extraordinarily fast growth rate under high light and CO_2 conditions.

Modeling and subsequent comparison of the metabolism of *Synechococcus* 2973 and its close (but slower-growing) relative *Synechococcus elongatus* PCC 7942 can provide further insights into these strains and their differing growth rates, along with enabling future design of metabolic interventions. Genome-scale metabolic (GSM) models were developed for both *Synechococcus* 7942 (*i*Syf686) and *Synechococcus* 2973 (*i*Syu627) using a semi-automated method that combined information from UniProt, NCBI Protein Clusters, and the RAST annotation pipeline with the previously developed *Synechocystis* GSM model *i*Syn731. The *i*Syf686 model correctly predicted 232 of 267 non-essential genes and 234 of 338 essential genes present within the model. Experimental measurements for CO_2 consumption were used to constrain the

corresponding fluxes. Differing rates of CO_2 fixation resulted in a growth rate for *Synechococcus* 2973 that was 1.7 times higher than that of *Synechococcus* 7942, which is in the same order of magnitude of the experimental observations (i.e., 3.7 times). In addition, carbon-mapping models can be useful in tracking the flow of carbon within metabolic models. However, the availability of a carbon-mapping model remains the major bottleneck in the genome-scale ^{13}C metabolic flux analysis of cyanobacteria. To this end, we have constructed the genome-scale metabolic mapping (GSMM) model *i*mSyf608 describing reaction atom mapping for all carbon-balanced reactions contained within *Synechococcus* 7942. This model was constructed by appending to the already published *E. coli* mapping model, *i*mEco726, and reaction mapping information generated using our previously developed CLCA algorithm. We found that the 99.8% genomic sequence similarity between *Synechococcus* 7942 and the fast-growing *Synechococcus* 2973 gives rise to a 100% identity of carbon-balanced reactions contained within their respective genome-scale models, resulting in the applicability of *i*mSyf608 for ^{13}C-MFA of *Synechococcus* 2973.

In parallel to these modeling efforts, to better understand the phenotypical properties of *Synechococcus* 2973, pulse-chase experiments and isotopically nonstationary metabolic flux analysis (INST-MFA) were performed. INST-MFA allows for the quantification of autotrophic carbon fluxes through transient ^{13}C-labeling patterns of free metabolites. Using a "deep-frozen" metabolite quenching method followed by ion-pairing LC-MS/MS and ESI-TOF-MS, we efficiently recovered fast-turnover metabolites (including sugar phosphates and organic acids) for the analysis of isotopomer dynamics as well as relative pool size determination. Using such data and the computational platform, INCA, we deciphered the fluxomic topology for photoautotrophic growth of *Synechococcus* 2973. Our research yielded three new insights. First, *Synechococcus* 2973 has no measureable fluxes through the oxidative pentose phosphate pathway. This was further confirmed by the normal growth of a *zwf* inactivation mutant. Second, metabolic channeling from 3PGA towards the TCA cycle is evident, indicating an internal heterogeneous distribution of central metabolic enzymes. Third, compared to *E. coli*, cyanobacterial metabolite pool sizes are much higher for sugar phosphates, while the TCA cycle metabolites and acetyl-coA pools are about 5-10 fold lower. This indicates that *Synechococcus* 2973 is an ideal chassis for producing chemicals derived from glycolysis and the Calvin cycle.

In future work, we will use the genome-scale and carbon-mapping models to derive efficient strain designs for *Synechococcus* 2973, in order to study its growth phenotypes for various carbon sources and thereby pinpoint the key reasons for its fast growth from the metabolic point of view. Furthermore, these models will also be utilized to carry out additional INST-MFA analyses. Ultimately, such studies will help develop this cyanobacterium as a novel chassis for the production of fuels and value-added chemicals.

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