SYSTEMS LEVEL STUDY OF A NOVEL FAST-GROWING CYANOBACTERIAL STRAIN FOR NEXT GENERATION BIOFUEL PRODUCTION

Justin Ungerer[1]* (JustinUngerer@wustl.edu), Cory Knoot[1], Saratram Gopalakrishnan[2], Lin Wang[2], Mary Abernathy[3], Whitney Hollinshead[3], **Yinjie J. Tang**[3], **Costas D. Maranas**[2], and **Himadri B. Pakrasi**[1]

[1]Department of Biology, Washington University, St. Louis, MO; [2]Department of Chemical Engineering, The Pennsylvania State University, University Park, PA; [3]Department of Energy, Environmental and Chemical Engineering, Washington University, St. Louis, MO

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

Project Goal: The overall objective of this project is to use integrated systems biology and synthetic biology approaches 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 and in vivo* analysis followed by experimental validations to enable efficient strain design. Overall the goals are to (a) develop a genetic tool kit that will enable facile metabolic engineering of this strain, (b) measure photosynthetic parameters to identify factors that are critical to rapid growth, (c) reconstruct a genome-scale metabolic model of *Synechococcus* 2973, (d) to develop a carbon-mapping model of this strain, and (e) to better understand its phenotypical properties by isotopically nonstationary metabolic flux analysis (INST-MFA).

Cyanobacteria have great potential as green biofactories because they grow on carbon dioxide and sunlight alone which reduces greenhouse gas emissions and moves society away from dependence on petroleum-based products. Unfortunately, cyanobacteria display growth rates that are much slower than conventionally-used heterotrophic biofactories such as *E. coli* and yeast. This leads to an inherently-lower productivity from the cyanobacteria. The Pakrasi lab have recently identified a cyanobacterial strain, *Synechococcus elongatus* UTEX 2973, with significant industrial potential. *Synechococcus* 2973 exhibits autotrophic biomass productivity comparable to that of heterotrophs such as yeast. Under conditions of high light and high CO_2 this strain has a doubling time of 1.5 hours. Genome sequencing revealed that *Synechococcus* 2973 is a close relative of the slower-growing strain, *Synechococcus elongatus* PCC 7942. The strains differ by 55 SNPs (only 35 of which are in coding regions), a 188 kb inversion and a 7.5 kb deletion.

The Pakrasi lab has developed an advanced CRISPR toolkit for *Synechococcus* 2973 to allow us to investigate the difference between the two strains in more detail. Using CRISPR technology, we performed a mutational analysis of *Synechococcus* 2973 to identify the subset of the 55 SNPs that grant the ability for rapid autotrophic growth. Four mutations were identified as essential for the fast-growth phenotype. Engineering these mutations individually into *Synechococcus* 7942 increases its growth rate; while combinations of the mutations exhibit additive effects. Three of the four mutations that we found to increase growth rate were also identified as factors that may contribute to rapid growth in the genome-scale model (GSM) that was developed by the Maranas group, suggesting that the GSM is highly valid.

The Maranas lab has developed a composite GSM for both *Synechococcus* 7942 and *Synechococcus* 2973 (iSyf686). This model serves as both a foundation for further modeling efforts as well as a means to interrogate possible factors contributing to the fast-growth phenotype. The inclusion of constraints based on experimental measurements of CO_2 uptake resulted in a ratio of the growth rates of *Synechococcus* 2973 to *Synechococcus* 7942 of 2.03, which nearly recapitulates the *in vivo* growth rate ratio of 2.13. The model was also used to identify four ORFs in *Synechococcus* 2973 with SNPs whose associated reactions have higher achievable fluxes, three of which were independently identified experimentally to contribute to the fast-growth phenotype. Additional insights into the biology of the fast-growing phenotype of *Synechococcus* 2973 can be obtained upon quantification of intracellular fluxes using isotopic non-stationary metabolic flux analysis (MFA). The Maranas lab has constructed the mapping models imSyn711 and imSyf608 based on GSMs for *Synechocystis* sp. PCC 6803 (iSyn731) and *Synechococcus* 7942 (iSyf686) respectively, which highlight cyanobacteria-specific carbon skeleton rearrangements. Upon flux quantification via genome-scale non-stationary MFA using imSyn711, we found that the oxidative branch of the pentose phosphate pathway was inactive, leaving photosynthetic light reaction as the sole source of reducing equivalents for anabolic processes. The model also predicts that a fraction of the serine pool was synthesized directly from 3-phosphoglycerate, whereas glycine was synthesized predominantly via photorespiration.

In the Tang lab, isotopically nonstationary metabolic flux analysis (INST-MFA), biomass compositional analysis, and metabolite profiling were performed comparing *Synechococcus* 2973 and *Synechococcus* 7942. The outcomes indicate a highly effective metabolism in *Synechococcus* 2973 compared to other model cyanobacteria. First, the flux maps demonstrate strong Calvin cycle, photorespiration, and pyruvate kinase activity, but minimal flux through malic enzyme and oxidative pentose phosphate pathways. Second, anabolism drains intermediate pools from central pathways under high light conditions, while central metabolism accumulates metabolites under suboptimal light (*i.e.*, energy metabolism, rather than carbon fixation pathways, constrains fast cyanobacterial growth). Third, *Synechococcus* 2973 shows similar genetic background and flux ratios to *Synechococcus* 7942, but exhibited greater carbon assimilatory and photorespiratory flux, less accumulation of glycogen, and potentially metabolite channeling that together result in increased biomass growth. Finally, *Synechococcus* 2973 has weak flux through a linear TCA pathway and small pool sizes of acetyl-CoA/TCA intermediates under all growth conditions. Such metabolic features support a photosynthesis platform to produce valuable products from its sugar phosphate pathways.

We are currently exploring the potential for *Synechococcus* 2973 as a production vehicle for alkane biofuels. All cyanobacteria utilize one of two primary biosynthetic pathways to synthesize C_{15} to C_{19} alkanes or alkenes from fatty acid precursors. We are studying these pathways in *Synechococcus* 7942 and 2973 in order to understand the best means to engineer the cells to produce more of these valuable metabolites. The biochemical and genetic studies are being coupled with insights gained from the GSMs and metabolic flux analyses.

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