SYNECHOCOCCUS ELONGATUS UTEX 2973, A NEW CYANOBACTERIAL STRAIN THAT EXHIBITS RAPID AUTOTROPHIC GROWTH

Justin Ungerer[1]* (JustinUngerer@wustl.edu), Kristen E. Wendt[1], Rajib Saha[1], **Yinjie J. Tang**[2], **Costas D. Maranas**[3], and **Himadri B. Pakrasi**[1,2]

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

http://pages.wustl.edu/photo.synth.bio

Project Goal: 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. An aim is to develop a genetic tool kit that will enable facile metabolic engineering of this strain. Another aim is to generate a genome wide library of transposon mutants that will be made available to researchers for future studies. We intend to use this library to identify essential genes for specific metabolic characteristics such as sugar utilization, high light tolerance, or rapid growth. Finally, we will measure photosynthetic parameters to identify factors that are critical to rapid growth.

Photosynthetic microbes are of considerable interest for applications in carbon sequestration, photosynthetic production of fuels and other valuable chemicals. The advantage of using cyanobacteria as biofactories is that they can grow on CO_2 and sunlight alone, which reduces greenhouse gas emissions and moves society away from dependence on petroleum-based products. Unfortunately, commonly used cyanobacterial strains exhibit growth rates that are much slower than conventional heterotrophic biofactories such as *E. coli* and yeast. This leads to inherently lower productivity from the cyanobacteria. We have recently identified a cyanobacterial strain, *Synechococcus elongatus* UTEX 2973, that has significant industrial potential. *Synechococcus* 2973 exhibits autotrophic biomass productivity that is comparable to that observed in heterotrophs such as yeast. Under high light and high CO_2 conditions, this strain exhibits a doubling time of 1.9 hours [1]. Genome sequencing revealed that *Synechococcus* 2973 is a close relative of the slower growing and widely used strain, *Synechococcus elongatus* PCC 7942. The two strains differ by only 55 SNPs, a 188 kb inversion, and a 7.5 kb deletion.

Synechococcus 2973 has proven resistant to natural transformation. Recent research efforts have aimed to develop a facile genetic system for this strain. We have been successful in introducing a replicating plasmid containing a fructose uptake system and engineering an *nblA* deletion mutant using conjugation [1, 2, 3]. The strain with the fructose uptake system actively transports fructose and exhibits increased growth rates under mixotrophic conditions. Strains with the *nblA* knockout show the non-bleaching phenotype that is characteristic of this deletion. Additionally, we have also set out to develop a CRISPR/Cas9 genome editing system for *Synechococcus* 2973. As a proof of concept for this system, we have generated a markerless Δ*nblA* strain, exhibiting the expected non-bleaching phenotype.

We have developed a high efficiency transposon mutagenesis system and demonstrated that it inserts randomly across the genome. We are currently using this transposon along with next

generation sequencing technology to generate a library of mutants of *Synechococcus* 2973 that will be made publicly available. Several of the most interesting mutants will be selected from the library for MFA analysis by the Tang lab. Data collected from these mutants will be used to further refine the metabolic models developed by the Maranas lab. The refined models will be exploited to generate hypotheses regarding efficient strain designs as well as fluxomic states under given conditions. These findings will then be tested in the Pakrasi and Tang labs. Using NGS we will screen the library for all mutants that have a changed fitness value under selected conditions. This will allow us to assign essentiality to any gene needed for the process we are querying. Conditions that are being initially studied include light tolerance, CO_2 utilization, and mixotrophic growth.

We are also characterizing photosynthetic parameters for *Synechococcus* 2973, and comparing them with those for the slower growing *Synechococcus* 7942. Such studies will identify differences in photosynthesis that differentiate the fast growing strain from the slower growing strain and will point to adaptations that can be reconstructed in other organisms to increase their photosynthetic output.

References:

1. Yu, J., et al., *Synechococcus elongatus UTEX 2973, a fast growing cyanobacterial chassis for biosynthesis using light and CO_2.* Scientific Reports, 2015. **5**: 8132.
2. Ungerer, J., B. Pratte, and T. Thiel, *Regulation of Fructose Transport and Its Effect on Fructose Toxicity in Anabaena spp.* Journal of Bacteriology, 2008. **190**: 8115-8125
3. Collier, J.L. and A.R. Grossman, *A small polypeptide triggers complete degradation of light-harvesting phycobiliproteins in nutrient-deprived cyanobacteria.* The EMBO Journal, 1994. **13**: 1039-1047.

These studies have been supported by funding from the Office of Biological and Environmental Research in the DOE Office of Science to HBP, YJT and CDM.