

164. Systems Level Study of a Novel Fast-Growing Cyanobacterial Strain for Next-Generation Biofuel Production

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<http://pages.wustl.edu/photo.synth.bio/>; <http://maranas.che.psu.edu/>; <http://tang.eece.wustl.edu/>

Project Goals: The overall objective of this project is to develop a fast growing cyanobacterial strain as a platform organism for photobiological production of advanced biofuels and other chemicals.

Cyanobacteria are oxygenic photosynthetic microbes that use light and CO₂ as feedstocks. A key issue in cyanobacterial biofuel production is the growth rates of these microbes. Compared to other photosynthetic organisms, many cyanobacterial strains have superior growth properties. However, they grow significantly slower than heterotrophic microbes such as *E. coli* and yeast that are commonly used in biofuel research. We have identified *Synechococcus elongatus* UTEX 2973, a unicellular cyanobacterium that has the ability of rapid photoautotrophic growth comparable to that of *Saccharomyces cerevisiae*. In this project, we are pursuing a systems approach to develop *Synechococcus* 2973 as a versatile and useful model cyanobacterial host to be used by the bioenergy research community during the coming era.

Identification of *Synechococcus* 2973 as a cyanobacterial strain with rapid growth properties (Pakrasi group): *Synechococcus* 2973 exhibited a 1.9 hours doubling time at 41°C under 3% CO₂ and 500 μmole photons m⁻²s⁻¹. This growth rate was more than 2 times faster than that of its closest related strain, *Synechococcus elongatus* PCC 7942, a widely studied model cyanobacterial strain¹. Interestingly, *Synechococcus* 2973 was isolated from Austin, Texas, whereas *Synechococcus* 7942 was isolated from Oakland, California. Between the genomes of these two organisms, there were a total of 55 SNPs and indels. Among these 55 differences, 39 were in chromosomes and 16 were in plasmids. Among the 39 differences in chromosomes, 28 were in protein coding regions, plus a 5 kb deletion and a 188 kb inversion. Among the 16 differences in the plasmids, 7 were in protein coding regions.

Synechococcus 2973 can be readily transformed by conjugation, and fully segregated genetic mutants can be acquired more quickly than with *Synechocystis* sp. PCC 6803, another model workhorse cyanobacterial strain that we have studied for a long time. During this project period, we are generating a genome wide single gene knockout library of mutants in *Synechococcus* 2973. Such mutant strains are then being deployed for advanced metabolite analysis to aid development and refinement of a genome scale model (see below).

We have also developed new insights into the physiological function of a promising candidate biofuel molecule in cyanobacteria. All cyanobacterial membranes contain diesel- range C₁₅-C₁₉ hydrocarbons at concentrations similar to chlorophyll. Recently, two universal but mutually exclusive hydrocarbon production pathways in cyanobacteria were discovered. We engineered a mutant strain of *Synechocystis* 6803 with its alkane production pathway inactivated. This mutant grew poorly at low temperatures. We analyzed this growth defect in the mutant by assessing the redox kinetics of Photosystem I, a central hub in reductant production by the photosynthetic electron transfer pathway. The mutant exhibited enhanced cyclic electron flow (CEF), especially at low temperatures. CEF in photosynthesis raises the ATP:NADPH ratio produced and allows photoautotrophs to balance the reductant requirements of

biosynthesis with maintenance of the redox poise of the electron transport chain. An in silico flux balance analysis showed that growth rate reached a distinct maximum for an intermediate value of CEF equivalent to recycling of 1 electron in 4 from PSI to the plastoquinone pool. Based on this analysis, we conclude that the lack of membrane alkanes necessitates higher CEF for maintenance of redox poise, perhaps because of reduced membrane fluidity and electron carrier mobility. In turn, increased CEF reduces growth by forcing the cell to use less energy- efficient pathways, and in effect lowering the quantum efficiency of photosynthesis. This study highlights the unique and universal role of medium-chain hydrocarbons in cyanobacterial thylakoid membranes: they regulate redox balance and reductant partitioning in these oxygenic photosynthetic cells under stress conditions.

Genome Scale Modeling (Maranas Group): The genome-scale metabolic model for *Synechococcus* 2973 is currently under development based on (1) an updated version of the iSyn731 model of *Synechocystis* 6803 containing 109 additional reactions, (2) 106 annotations with associated EC numbers for *Synechococcus* 2973, and (3) gene annotations from the closely related *Synechococcus* 7942. Using the GSM model as a starting point, reaction atom mapping information is generated using the MetRxn database and our novel atom mapping algorithm CLCA. For approximately 55% of the iSyn731 reactions, detailed atom mapping information is already in the MetRxn database. CLCA leverages number theory (i.e., prime factorization) to identify a one-to-one mapping between the vertices of reactant and product molecular graphs. Reaction atom mapping information will be used in conjunction with ¹³C tracer data for metabolic flux elucidation for the wild type and also a comprehensive set of single gene mutants. Metabolic flux information for a number of genetic perturbations will then be used to estimate kinetic parameters of the reactions in central metabolism using an Ensemble Modeling paradigm. This modeling infrastructure has recently been leveraged by the C.D. Maranas group to construct a large-scale kinetic model of *E. coli* core metabolism spanning 138 reactions, 93 metabolites and 60 substrate-level regulatory interactions. Model parameterization was carried out using flux data for seven separate mutants.

Development of fluxomics tools (Tang group): We are developing new non-stationary MFA protocols for the analysis of metabolism in *Synechococcus* 2973 wild type and mutant strains. Nonstationary ¹³C-MFA during photoautotrophic growth of cyanobacteria has been pioneered by Jamie Young's group. We have made several modifications in their cultivation and sampling protocols. First, to avoid metabolic disturbances (light/carbon) caused by culture volume changes during continuous sampling from photobioreactors, we are using an alternative approach. We prepare a mother culture, and then divide it into smaller shake flasks (~25 mL).

Each flask is then pulsed with 1 mL ~40 g/L NaH¹³CO₃, and sampled as one time point (seconds to hours). We use liquid nitrogen to chill the quenching solution (10 mL methanol) and quickly add the shake flask culture to it. The total sampling time can be as fast as 10 s. Secondly, our experiments are also modified using an inverse labeling approach to probe cell metabolism using gaseous CO₂. The mother culture is grown in ¹³C-bicarbonate, and then the fully labeled culture is pulsed with ¹²CO₂ for differential labeling. Thirdly, we use methanol-chloroform method for metabolite extraction. We are employing both traditional GC-MS and advanced LC-MS to analyze metabolite labeling. Once fully standardized, this method will be used for rapid analysis of a large set of *Synechococcus* 2973 mutant strains generated during this project period (see above).

1. Yu, J., Liberton, M., Cliften, P. F., Head, R. D., Jacobs, J. M., Smith, R. D., Koppenaal, D. W., Brand, J. J. and Pakrasi, H. B. (2015) *Synechococcus elongatus* UTEX 2973, a fast growing cyanobacterial chassis for biosynthesis using light and CO₂. *Nature Scientific Rep.*, 5: 8132.

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