148. Use of Systems Biology Approaches to Develop Advanced Biofuel-Synthesizing Cyanobacterial Strains

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

Project Goals: This project aims to develop new tools for biofuel production in photosynthetic bacterial hosts. Our studies include the identification of a novel, fast- growing, mixotrophic, transformable cyanobacterium. This strain has been sequenced and will be made available to the community. In addition, we have developed genome-scale models for a family of cyanobacteria to assess their metabolic repertoire. Furthermore, we developed a method for rapid construction of metabolic models using multiple annotation sources and a metabolic model of a related organism. This method will allow rapid annotation and screening of potential phenotypes based on the newly available genome sequences of many organisms.

A new, fast-growing, mixotrophic, transformable cyanobacterium

Photosynthetic microbes are of considerable interest in biotechnological applications due to their ability to use sunlight to convert CO_2 into fuels and other useful chemicals. Important and desirable traits for production organisms include fast growth and amenability to genetic manipulation. Synechococcus elongatus UTEX 2973, a novel cyanobacterial strain showed rapid growth rates, with a doubling time of less than two hours under optimal conditions, while other commonly used cyanobacteria (Synechocystis sp. PCC 6803, Synechococcus sp. PCC 7942 and Synechococcus sp. PCC 7002) in biofuel production have considerably longer doubling times. We have determined that this strain can grow mixo- and photoheterotrophically in the presence of 30 mM fructose. Synechococcus elongatus UTEX 2973 can be readily transformed by conjugation and fully segregated mutants can be generated more quickly than with other model cyanobacteria such as Synechocystis sp. PCC 6803.

The genome of this strain was sequenced and compared to its close relatives Synechococcus elongatus PCC 6301 and PCC 7942. Significant differences were found, including a large deleted genomic region that may be related to the unique attributes of this strain. Future aims include using Synechococcus UTEX 2973 for long-chain alkane overproduction and gene function studies, as well as developing a genome-scale metabolic model for this organism.

Genome-scale model development

Genome-scale models allow researchers to both analyze an organism's metabolism and make predictions about how genetic engineering might change that metabolism. However, the reconstruction of quality genome-scale metabolic models of organisms with limited annotation resources remains a challenge that often requires a time-consuming manual approach. To mitigate this challenge, we developed a workflow that combines annotation information from multiple sources: the Universal

Protein Resource (Uniprot); NCBI Protein Clusters; Rapid Annotations using Subsystems Technology (RAST); and a previously developed reference model for the cyanobacterial genus Cyanothece (the iCyt773 model for Cyanothece ATCC 51142) to create genome-scale metabolic reconstructions of new sequenced strains with limited manual effort. Models were created for five Cyanothece strains, namely Cyanothece sp. PCC 7424, 7425, 7822, 8801 and 8802. All five models include fully traced photosynthesis reactions and respiratory chains, as well as mass and charge-balanced reactions and gene-protein-reaction (GPR) associations. Meeting these stringent criteria for model quality makes the models far more useful for phenotype prediction and for guiding metabolic engineering.

Upon examination, the reactions shared between these five models match the known phylogenetic relationships between the organisms. These models also allow for the assessment of the bio-production potential of the modeled species. The non-fermentative pathway for alcohol production is found only in Cyanothece 7424, 8801, and 8802, while the fermentative pathway for butanol production exists in varying levels of completion within the five models. The models also highlight other metabolic differences, such as in arginine catabolism.

The workflow that we have developed expedites construction of curated metabolic models for organisms that, while not yet developed as model systems, have sequenced genomes, reviewed gene annotations, and are related to an organism with a curated metabolic model. Models created from this workflow can be used to develop strategies for targeted metabolite overproduction or to gain insight into the metabolic differences between organisms.

Alkane production by Synechocystis 6803

Nearly all known cyanobacteria produce n-alkanes in the C_{17} range. While initial reports of these compounds date back to the 1960's, the genes responsible were only identified recently, and the biological function of these compounds remains a mystery. Although these compounds are produced at relatively low levels by biotechnology standards (~0.1% dw), they make up a significant portion of the cell membranes of cyanobacteria, and are produced during all phases of cell growth and under a wide variety of growth conditions at relatively constant cellular concentration, suggesting that they play a critical role in normal cellular function.

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Publications

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