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Transcriptomics to Develop Biochemical Network Models in Cyanobacteria

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Cyanobacteria-derived biofuels hold promise for sustainably producing drop-in fuels that reduce our reliance on fossil fuel. Due to its rapid growth rate and suitability for genetic engineering, the cyanobacteria Synecococcus elongatus sp UTEX 2973 (henceforth, UTEX 2973) was the focus of this research. Our overall goal is to understand the complex relationship between gene expression and metabolite production.

UTEX 2973 is a novel strain of cyanobacteria whose metabolism has not been well-studied. This project sought to characterize UTEX 2973 growth and metabolite production under a number of environmental conditions, including the following: ideal (40°C, 200µmol/sec-m2, 3% CO2), light-limiting (40°C, 100µmol/sec-m2, 3% CO2), and carbon-limiting (40°C, 200µmol/sec-m2, atmospheric CO2). Samples from tubular photo-bioreactors were collected during mid-exponential phase and stationary phase. Total lipid, glycogen, and biomass production rates, as well as fatty acid profiles, were determined, and mRNA was extracted, transcribed to DNA, and sequenced for each condition.

Optimal doubling times, 3.9±0.8 hours, were observed under the ideal condition. Metabolite analysis demonstrated the effect of growth environment on metabolite production: cellular lipid content reached a maximum of 12% of the dry cell weight under carbon-limitation, while maximum cellular glycogen content, 35% of the dry cell weight, occurred under dim light in the stationary phase.

We next performed RNASeq to determine the effect of growth condition on UTEX 2973 gene expression. Differential gene expression indicates that many more genes are up-regulated during stationary phase when grown under a stress condition compared to the ideal condition. By clustering genes with similar expression profiles, we seek novel insights into the complex relationships between gene and metabolite production. Initial principal component analysis shows a pronounced difference in gene expression between the exponential phase of the carbon-limiting condition and all other conditions. Applying weighted gene correlation network analyses reveals that genes with similar expression profiles do not belong to similar metabolic pathways, but have many diverse metabolic functions, indicating the complexities of gene regulation in UTEX 2973. Further results from these gene network and association analyses will be presented to identify specific genes that have major influences on metabolite production.

By integrating the metabolite data with gene expression data, we will begin targeted, rational genetic modification in UTEX 2973, in order to optimize the production of biofuel precursors.