A Systems Biology and Pond Culture-Based Understanding and Improvement of Metabolic Processes Related to Productivity in Diverse Microalgal Classes for Viable Biofuel Production.

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Project Goals: Understand the fundamental molecular mechanisms involved in maximizing growth rate and lipid accumulation in diverse classes of microalgae under authentic diurnal conditions to enhance production capabilities for biofuels.

Microalgal mass culture in open ponds can provide feedstock for biofuels production. However, major gaps exist in the understanding of the effect of metabolic topology on cellular carbon partitioning and its regulation with regards to productivity. Our research develops two emerging platform green algal species for biofuels production as model systems: *Acutodesmus (Scenedesmus) obliquus* and *Coelastrella* sp. Our Systems Biology approach includes genomics-based investigations with climate-simulated pond culturing to identify species specific versus general green algal mechanisms underlying performance in realistic biofuels production scenarios.

For *A. obliquus*, a draft genome was obtained in collaboration with Dr. Starkenburg (Los Alamos National Laboratory) and Dr. Magnuson (Pacific Northwest National Laboratory). The genome is publicly available at https://greenhouse.lanl.gov/greenhouse/organisms/ (the Greenhouse page of LANL). We annotated the genome and created a metabolic map for the carbon core metabolism of *A. obliquus*, which was used as a foundation for identification of potential bottlenecks and regulatory mechanisms regarding carbon partitioning in this green alga. Based on comparative genomics studies including information from higher plants, we identified several potential bottlenecks and regulatory steps within the carbon core metabolism that could be relevant for biomass productivity and carbon partitioning. One example is the chloroplast localized Triose Phosphase Isomerase (TPI) for which only one gene exists in many green algae, but due to a whole genome duplication (WGD) two gene copies were found in *A. obliquus*. This is the first report for a WGD in a green alga and possibly valuable regarding green algal productivity, because ancient WGDs in higher plants resulted in gene duplications that underlie important productivity traits in crop species. Our broader comparative genomics analysis regarding the TPI of species from different clades within in green algae strongly indicated major differences in carbon flux topology within the cells, which can be linked to the number of TPI isoenzymes and their cellular localization. In addition, pathways were identified for biosynthesis of Trehalose and Fructose-2,6BP, both essential regulatory metabolites in carbohydrate metabolism. Transcriptional studies are ongoing to determine links between specific gene

expression with daily productivity changes in pond cultures. *Omics* data aquired for *A. obliquus* are now being compared to *Coelastrella* sp., for which a draft genome was obtained and annotated.

Regarding pond mass cultures, an important result is that during the linear growth phase already in the late afternoon biomass productivity in ponds was strongly reduced in both green algae *A. obliquus* and *Coelastrella* sp. Analysis of transcriptomic data (dawn versus dusk) in both algae corroborated up-regulation of genes coding for enzymes involved in glycolysis and the Krebs cycle versus down-regulation of genes coding for the Calvin-Benson-Bassham cycle before onset of the dark period. Our results confirm at the molecular level that light-limitation resulting in respiratory losses of biomass already occurring during the light period is a major factor that has to be addressed for improvement of metabolic processes if increases in productivity of green algae are to be achieve through metabolic engineering. Concurrent creation (UV-mutagenesis) and testing of mutants of both algae for biomass productivities resulted in novel strains that demonstrated higher productivities in laboratory cultures.

In summary, our comparative systems approach regarding carbon core topology allowed us to identify the TPI as a general molecular factor in determining cellular carbon flux among different classes of green algae. In green algae, the TPI is an example on class-wide design differences regarding processing of carbon. We also determined that in pond cultures *A. obliquus* performed significantly better than *Coelastrella* sp., but concomitantly in both species early onset of respiratory activity in the late afternoon is preventing higher daily biomass productivities for both species. Our carbon core metabolic maps will enable us to perform a comparative investigation into regulation of gene expression of carbon core enzymes in *A. obliquus* and *Coelastrella* sp. in pond cultures. Such systems analysis should allow linking of phenotypic biomass productivities of both algae to similarities and differences in their carbon flux topology.

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