Metabolite Excretion and Metabolic Flux Analysis in *Chromochloris zofingiensis*, an Emerging Model Green Alga for Sustainable Fuel Production

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https://sites.google.com/view/czofingiensis/home

Project Goals: Our overarching research goal is to engineer the green alga *Chromochloris zofingiensis* for the production of biofuels. Our strategy involves large-scale multi-omics systems analysis to understand the genomic basis for energy metabolism partitioning as a consequence of carbon source. Enabled by cutting-edge synthetic biology and genome-editing tools, we will integrate the systems data in a predictive model that will guide the redesign and engineering of metabolism in *C. zofingiensis*.

C. zofingiensis is an emerging model system for the production of biofuels and bioproducts. It is an especially attractive system because it produces astaxanthin along with a large amount of lipids. Astaxanthin is a high value product (~$7,000 per kilogram) with uses in the pharmaceutical, nutraceutical, and cosmetic industries. It also demonstrates high levels of triacylglycerol accumulation and low photosynthetic productivity when additional organic carbon sources are provided[1], making it ideal for metabolic or genetic engineering focused on increasing algal lipid production.

First, we created a genome-scale metabolic model of this organism, iCre1925[2], using a new computational algorithm, Rapid Annotation of Photosynthetic Systems[3] (RAPS). The results of flux balance analysis (FBA) studies conducted using this model predicted the excretion of lactate, among other products when *C. zofingiensis* is grown on glucose. This prediction was later affirmed with experimental data on extracellular metabolites in *C. zofingiensis* cultures.

To investigate intracellular flux distributions during *C. zofingiensis* growth on glucose, an isotopically assisted metabolic flux analysis (MFA) experiment was performed. A model of central metabolism was created by pulling 140 reactions from iCre1925. Atom transitions were assigned for each reaction, and this network was then simplified by combining series of linear reactions to ease computational load in running simulations. A combination of ^{13}C labeled glucose tracers was chosen for this experiment based on isotopic tracer simulations performed on central metabolism. *C. zofingiensis* cultures were grown in the presence of this labeled substrate until isotopic steady state was reached, at which point the culture was harvested and samples quenched for metabolite analysis. Amino acids, organic acids, and sugars were derivatized from labeled biomass and analyzed via GC-MS to determine mass isotopomer distributions (MID) for relevant fragments. This MID data was used along with experimentally determined uptake and excretion fluxes and the central metabolic network model to calculate intracellular fluxes using the isotopomer network compartmental analysis (INCA) software package. Results of this analysis indicate that glucose is transported to the plastid after uptake and is either directed into starch biosynthesis or

utilized by the pentose phosphate pathway (PPP). This high flux through the PPP seems to indicate a need for reducing equivalents in the plastid, which is consistent with the high energy requirements for astaxanthin synthesis. Future MFA studies will be needed to continue probing intracellular flux distributions under various perturbations.

To generate additional phenotypic data for model refinement, we conducted a *C. zofingiensis* time-course experiment, by analyzing its metabolome and measuring alterations in media composition resulting from algal growth. During the heterotrophic growth of *C. zofingiensis*, we observed a decreased concentration of intermediates of the tricarboxylic acid cycle and amino acids, indicating a possible redirection of energy flux toward triglyceride synthesis. Our results showed that *C. zofingiensis* secreted more diverse exometabolites during its heterotrophic growth stages when supplemented with glucose. We find that *C. zofingiensis* has a clear nutrient (carbon/nitrogen source) preference order and recaptures secreted metabolites when the exogenous glucose is limited. To examine this observed diauxic growth in more detail, we screened 15 carbohydrates, including pentose, hexose, disaccharide, trisaccharide, to investigate their abilities to suppress algae photosynthesis and support heterotrophic growth. From this found that four hexoses: glucose, fructose, galactose, and mannose are able to serve as organic carbon sources for heterotrophic growth yet not all of these sugars were observed to inhibit algal photosynthesis.

The metabolic model that has been developed, used in combination with this extensive data set, has great potential to provide insights into this organism's metabolism and elucidate dramatic metabolic shifts within the organism. This will enable informed strain engineering strategies to maximize lipid productivity in this organism.

References

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