Systems Biology of Autotrophic-Heterotrophic Symbiosis for Biofuel Production

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Project goals: The goal of this work is to build and combine autotrophic and heterotrophic organisms as a novel sustainable symbiotic platform for growth and generation of lipids for biofuels. Genome-scale reconstructions of autotrophic-heterotrophic co-cultures will be deployed to decipher and predict metabolic interactions and will lay the foundation for optimizing system performance for growth and biofuel precursor production.

Organisms do not exist in isolation in the environment but form stable communities. For example, lichens form a well-established and beneficial relationship between two types of organisms; a phototroph, which uses light as energy source and a heterotroph, which utilizes organic material. In order to get insights into these natural communities, we are designing and constructing synthetic stable co-cultures experimentally and computationally. One of these co-cultures consists of the phototroph *Chlorella vulgaris* and the heterotroph *Saccharomyces cerevisiae*. Genome-scale metabolic models for *C. vulgaris* and *S. cerevisiae* are generated to delineate computationally possible metabolic interactions between these organisms in co-culture. A model for *S. cerevisiae* already exists, while the model for *C. vulgaris* has been recently reconstructed. Initial work involves the reconstruction, validation, and application of a genome-scale metabolic model for *C. vulgaris* UTEX 395, *i*CZ842. The reconstruction consists of six compartments: the cytoplasm, mitochondrion, chloroplast, thylakoid, glyoxysome, and the extracellular space. It contains 842 out of 7,100 annotated genes (around 12%), delineating 1,763 metabolites and 2,280 reactions. *C. vulgaris* can grow under different trophic conditions (e.g. photoauto-, hetero-, and mixotrophic). Each of these growth conditions is represented mathematically through different biomass objective functions (BOFs). Every equation contains the stoichiometric coefficients for the most important metabolites of the biomass. Here, the lipid, protein, carbohydrates and ribose (RNA) contents were determined experimentally. The reconstruction represents the most comprehensive model for any eukaryotic photosynthetic organism to date based on genome size and number of genes included in the reconstruction. The highly curated model was validated against experimental data.

The model accurately predicts growth rates under photoauto-, hetero-, and mixotrophy. Flux distributions under different trophic conditions show that not only central carbon metabolism but also amino acid, nucleotide, and pigment biosynthetic pathways are impacted when the microalgae is under nitrogen starvation. Furthermore, prediction of growth rates under various medium compositions using *i*CZ842 suggested an increased growth rate with the addition of tryptophan and methionine, which was experimentally verified. This effort lays the foundation for generation of synthetic co-culture models going forward. Initial work and issues to address for building and integrating complementary heterotrophic models will also be described.

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