Systems Biology of Autotrophic-Heterotrophic Symbionts for Bioenergy: Constraint-Based Community Modeling Reveals Condition-Dependent Interactions

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The goal of this proposal is to combine autotrophs and heterotrophs as a novel sustainable symbiotic platform for the production of biofuel and its precursors. Photosynthetic microorganisms are providing substrates and oxygen to the heterotrophs, which in exchange will produce CO_2 for carbon fixation. Synthetic communities of cyanobacterium-bacterium, cyanobacterium-fungus, and fungus-algae pairs were studied through genome-scale metabolic modeling. As part of this project, we also reconstructed the model for the algae *Chlorella vulgaris* UTEX 395 (*i*CZ843), which was experimentally validated.

Six co-culture metabolic models for all proposed co-cultures were constructed and used to predict growth phenotypes. The models enabled the systematic characterization of co-cultures and provided insight into the interaction of the autotrophic and heterotrophic symbiotic communities. Defined growth conditions changes or genetic manipulations yielded improved metabolic phenotypes for these co-cultures. Furthermore, the metabolic models allow elucidation of the nature of specific interactions between individual members of the co-culture (e.g. commensalism, mutualism, competition, predator-prey, etc). In order to validate the predicted results, studies were designed to vigorously examine the predictions. Based on experimental observations, we corroborated our predictions about culture medium and genetic modifications for the co-culture pair of *Chlorella vulgaris* UTEX 395 and *Saccharomyces cerevisiae* S288c. The co-culture model was reconstructed using individual genome-scale metabolic models integrated into a combined model and by including a shared metabolite pool (SMP). The community model *i*CZ-Cv-Sc(1748) contains 14 unique compartments (cytoplasm, mitochondria, peroxisome, nucleus, endoplasmic reticulum, vacuole, Golgi apparatus, glyoxysome, chloroplast, thylakoid and extracellular space). Metabolites that can potentially be shared by *C. vulgaris* and *S. cerevisiae* were defined using BIOLOG data. The Constraint-Based Reconstruction and Analysis of Communities (COBRAcom) toolbox was developed in the framework of this proposal. COBRAcom allows obtaining single model's statistics, and provide tools for the reconstruction of community models. Additionally, COBRAcom contains test functions for several co-culture characterization applications, such as a) prediction and fitting of growth rates and population proportions (constraints-based choice to achieve experimental growth rates); b) determination of possible interactions (theoretical interchange of metabolites, SMP analysis); c) co-culture medium optimization (robustness analysis); d) syntrophic pathway inclusion (metadata contextualization); e) gene essentiality (knock-out analysis for population); and f) gene interactions within the population members.

The model successfully predicts growth rates observed by experiments. While *S. cerevisiae* uptakes O_2 and provides CO_2 when nitrate is the nitrogen source in medium, both members split the available glucose

and grow as mutualists. When NH_4 was added to the culture medium *S. cerevisiae* dominated the co-culture and outcompeted *C. vulgaris,* changing the type of interaction.

The SMP analysis showed that under mutualistic co-culture conditions, the growth of *S. cerevisiae* and *C. vulgaris* is mediated by exchange of built-in metabolites. Validation of these metabolites exchange by targeted metabolomics is under way. We also evaluated the effect of 1,748 single gene deletions for monocultures and the co-culture. The experimental growth rates match with predictions, e.g. yeast's mutant alters both Chlorella and yeast growth rates. When another gene is deleted, differential effects on yeast and Chlorella growth are observed. In some cases, deleting a gene can offer a growth advantage in co-culture, including an improved growth phenotype for the participating species.

These examples demonstrate how co-culture metabolic models can accurately predict the behavior of heterogeneous co-culture pairs thus improving production phenotype for bioproduction.

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