Sustainable production of biofuels by consortia of *Synechococcus elongatus* and *Aspergillus* species.

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The goal of this project is to combine phototrophic and heterotrophic microorganisms as a novel platform for the sustainable production of biofuel and its precursors. Here we study the consortia of *Synechococcus elongatus* cscB$^+$ and *Aspergillus* species. *S. elongatus* cscB$^+$ is providing sucrose and oxygen to the heterotrophic *Aspergillus* species, and in exchange, the fungi are producing CO_2 for *S. elongatus* for carbon fixation via photosynthesis. Synthetic microbial communities of cyanobacterium-fungus pairs were evaluated for productivity through genome-scale metabolic modeling. For this, we manually curated and updated transport capabilities in the model of the cyanobacterium *S. elongatus* PCC 7942 (*i*JB792) and reconstructed new metabolic models for two *Aspergillus* strains, *i.e. A. nidulans* and *A. niger*. Subsequently, two community metabolic models were created by pairing the cyanobacterium model with the new *Aspergillus* models.

The experimental success of working with these communities relies on the characterization of sucrose secretion by the phototroph. For this, we performed ^{13}C-MFA under photoautotrophic conditions for three *S. elongatus* cultures: wild type, wild type + NaCl, and cscB$^+$ + NaCl. ^{13}C-labelled bicarbonate served as tracer to quantify labeling of more than a dozen intracellular metabolites over time. We identified significant changes in intracellular metabolite pool sizes and metabolic fluxes of these three cultures, unveiling that expanding the sucrose sink via overexpressing the *cscB* gene increases carbon fixation in *S. elongatus*. Using the metabolic model for *S. elongatus* we evaluated the solutions space of the three cultures using random sampling. We found that sucrose secretion is linked to a change in the flux through the photosystem, reducing photon absorption. These energetic changes augmented asparagine hydrolysis and decreased glutathione, glutamate, and glycine synthesis. We reconstructed the models of *A. nidulans* (*i*ANid1230) and *A. niger* (*i*ANig1153) using semi-automated algorithms. These knowledge base tools were extensively validated using high-throughput phenotypic growth data on 190 carbon and 95 nitrogen sources for each species.

The highly curated metabolic models of *S. elongatus, A. nidulans,* and *A. niger* enabled the development of two community metabolic models (CM-models). CM-models were integrated using the shared metabolite pool (SMP) approach, which includes metabolites that can potentially be exchanged by each synthetic community. Potential exchanges were refined by high-throughput phenotypic data. Co-cultures were characterized by a) predicting growth rates and population proportions (constraints-based choice to achieve experimental growth rates), b) determining metabolic interactions (theoretical interchange of metabolites, SMP analysis), c) co-culture medium optimization (robustness analysis), and d) syntrophic pathway inclusion (metadata contextualization). The reconstructed CM-models helped elucidating syntrophic interactions between community members. We furthermore deployed the CM-models to evaluate the capability of the consortia to produce organic chemicals and biofuels. Growth and flux distribution predictions will be validated by physiological data, as well as untargeted metabolomics, and gene expression.

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