Synthetic lichens as a sustainable platform for biosynthesis

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The goal of this project is to combine autotrophs and heterotrophs as a novel sustainable symbiotic platform for the production of biofuel and its precursors. The photosynthetic microorganisms in these synthetic lichens are providing substrates and oxygen to the heterotrophs, which in exchange produce CO_2 for carbon fixation via photosynthesis. Synthetic microbial communities of cyanobacterium-bacterium or cyanobacterium-fungus pairs were evaluated through genome-scale metabolic modeling. For this, we manually curated and updated transport capabilities in the model of the cyanobacteria *Synechococcus elongatus* PCC 6803 (*i*JB792) and *Yarrowia lipolytica* (*i*YO844). Three community metabolic models were created by pairing the cyanobacteria model with the models of *Yarrowia lipolytica*, and the two *Escherichia coli* strains W and K-12.

Symbiotic partnership between heterotrophs and phototrophs has promising biotechnology applications. The experimental success of working with these communities relies on the correct selection of the photobiont and the heterotroph partner as well as on our ability to predict the partners' fitness and responses to environmental changes and genetic stability. Here, community metabolic models were reconstructed in order to guide strain selection for a fruitful lichen-like partnership. We paired the photobiont *Synechococcus elongatus* with the heterotrophs *Yarrowia lipolytica, Escherichia coli* W *and* K-12, respectively. Three community metabolic models (CM-models) were reconstructed after updating individual genome-scale metabolic 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 defined using BIOLOG data. The Constraint-Based Reconstruction and Analysis of Communities (COBRA-C) toolbox has been expanded in the framework of this project. COBRA-C allows obtaining single model statistics and provides tools for the reconstruction of community models. Additionally, COBRA-C 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); and e) gene essentiality (knock-out analysis for population)

The reconstructed CM-models helped elucidating syntrophic interactions between community members, while sustaining the heterotroph using CO_2 as only carbon source. We also studied the bioproduction capabilities of organic chemicals and amino acids by the synthetic lichens. Furthermore, we validated our growth and flux distribution predictions using physiological data, untargeted metabolomics, and expression data.