Optimizing Carbon Metabolism in Co-Culture for Applications to Sustainable Biosynthesis

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Project Goals: The goal of this project is to develop synthetic lichen communities of autotrophic and heterotrophic microbes as a novel sustainable symbiotic platform for the production of biofuel and its precursors. Carbon-fixing autotrophs provide oxygen and organic substrates to their heterotrophic neighbors, which in turn produce carbon dioxide. By optimizing and enhancing these interactions, we can create a robust, sustainable synthetic lichen community. Multi-omics driven genetic engineering will improve metabolite exchange and product generation capabilities with the microbial co-culture.

Lichens are communities of auto- and heterotrophic microbes that collect sunlight and carbon dioxide and apply it to power the group's activities. They also represent a novel biotechnology platform that can transform CO_2 and sunlight into valuable energy-related biochemicals, eliminating the need for costly substrate feeding. Unfortunately, natural lichens have slow growth rates, making them impractical for most industrial applications. In this project, our goal is to enhance the exchange of metabolites between autotrophs and heterotrophs, creating superior synthetic lichens able to generate useful products of interest to the energy and chemical industries. Key metabolite excretion bottlenecks will be identified in each partner, then the organisms will be modified as appropriate in order to share particular metabolic intermediates with their heterotrophic partners for channeling into key metabolic pathways, thus generating energy-related precursors of biochemicals or biofuels with high commercial value. To achieve these goals, we have screened several photoautotrophs and heterotrophs to find mutualistic coculture partners. One such photoautotroph, *Picochlorum renovo*, exhibits a rapid growth rate and is tolerant to a wider range of temperature and salinity than many other microalga. Genetic engineering toolboxes have been created to improve coculture applications with this organism. Additionally, we have engineered overexpression of the invertase enzyme in *Yarrowia lipolytica*, allowing for the consumption of sucrose and other less-utilized organic carbon sources. Other coculture partnerships with heterotrophic fungi and yeast (including *R. glutinis* and filamentous *Aspergillus* fungi) have been successfully conducted with cyanobacteria including *Nostoc* and engineered sucrose-secreting *S. elongatus*.

We have further explored the growth of *S. elongatus/R. glutinis* cocultures in continuous conditions using a custom-built, feedback-controlled photobioreactor equipped with a radial LED-based irradiation system. Since the organisms are cultured together, it is challenging to assign the origin of most essential metabolites to a particular specie in both the extracellular and

intracellular domains. To address this issue, we tested a new co-culture incubation strategy through incubation of cells using membrane segregation, hydrogel matrix immobilization, and other segregation techniques.. We also developed an analytical pipeline allowing investigators to identify extracellular and intracellular metabolites, estimate their fluxes and to assign them to the originating species.

References

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