

## Creating Synthetic Lichen Platforms for Sustainable Biosynthesis of Biofuel and Biochemical Precursors

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**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 microbes that collect sunlight and carbon dioxide and apply it to power the group's activities, allowing the autotrophic member to optimize photosynthesis and metabolite generation while their heterotrophic fungal partners produce biochemical compounds for the community. Lichens can thrive in the harshest environments on earth, and they represent a robust model for a novel biotechnology platform that can transform CO<sub>2</sub> and sunlight into valuable energy-related biochemicals, eliminating the need for costly substrate feeding.

To create this novel platform, we are investigating multiple co-culture communities to determine optimal synthetic lichen partnerships. Several autotrophs, including engineered sucrose-secreting *S. elongatus+cscB* and other known extracellular polysaccharide (EPS)-secreting *Nostoc 7413* and *Nostoc 6720* were examined for their ability to secrete organic carbon into the culture media. Heterotrophic partners are under evaluation, including filamentous fungi *A. nidulans* and *A. niger*, yeast species *S. cerevisiae*, *R. glutinis*, and *C. curvatus* with the goal of generating energy-related precursors of biofuels or biochemicals of commercial value. We first examined the growth of these microbes in axenic culture and evaluated the sucrose and EPS production of the autotrophic partners, then used the spent media to successfully grow heterotrophs on the carbon produced. We also evaluated combinations of these organisms in co-culture, which showed symbiotic benefits to both partners. Results also show a shift in the cellular fatty acid profile, indicating potential interactions between the partners. Ongoing work with co-cultures will combine insights from community metabolic models, multi-omics profiling, and metabolic flux analysis to promote a fundamental understanding of synthetic lichen communities, to clarify interactions between co-culture partners, and to provide engineering targets for improved metabolic interaction and biofuels and bioproducts generation. The resultant findings will aid in the elucidation of fundamental mechanisms governing microbial symbioses and will in turn generate fundamental design rules and dynamic network properties of microbial systems for rational biosystems engineering.

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