Revealing Metabolic Exchange and Optimizing Carbon Transformation in Co-Culture for Applications to Sustainable Biosynthesis

Pavlo Bohutskyi,[1] Jackson Jenkins[2]* (jjenki71@jhu.edu), Foteini Davrazou,[3] Jon Magnuson,[1] Michael Guarnieri,[3] and **Michael Betenbaugh**[2]

[1]Pacific Northwest National Laboratory, Richland, WA; [2]Johns Hopkins University, Baltimore, MD; [3]National Renewable Energy Lab, Golden, CO

Project Goals: The goal of this project is to develop and characterize synthetic lichen communities of autotrophic and heterotrophic microbes as a novel sustainable symbiotic platform for the production of biofuels and biochemicals. Carbon-fixing autotrophs provide oxygen, organic carbon substrates and other metabolites to their heterotrophic neighbors, which in turn generate carbon dioxide and diverse interemediate compounds. By optimizing and enhancing these interactions, we can create a robust, sustainable and more efficient synthetic lichen community to transform carbon dioxide into bioproducts. Multi-omics driven genetic engineering will enhance metabolite exchange and product generation capabilities with the microbial co-culture.

Lichens are communities that collect sunlight and carbon dioxide and apply it to power the group's activities. They potentially 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. Understanding the metabolite exchange between photoautotrophic and heterotrophic members of co-cultures is a complex but crucial task in optimizing a synthetic lichen for more efficient bioproduction. Two major challenges include recognizing the species origin of secreted metabolites and uncovering key rapidly-consumed exchange metabolites that may only be present in trace concentrations. To overcome these challenges, we implemented a membrane-separated photobioreactor (mPBR) allowing co-cultivation of photoautotrophic and heterotrophic microbes and capturing of critical exchanged metabolites. Profiling of extracellular metabolome in different compartments of the mPBR provided insights into the origin of exchange metabolites and direction of their flux, and suggested exchanged compounds crucial for a mutualistic microbial relationship. In addition, to explore the potential of improved carbon utilization in heterotrophs, our team has engineered an industry-relevant yeast *Yarrowia lipolytica* strain to grow on sucrose as the only carbon source by constitutively expressing invertase, the enzyme responsible for sucrose hydrolysis. Interstingly, invertase expression improved the utilization of several other carbon substrates compared to the wild-type strain. Improving the range of consumable substrate in heterotrophic co-culture partners opens the potential for more resilient and flexible synthetic lichens allowing generation of a wide array of biochemicals.

Supported under award DE-SC0019388, Biological and Environmental Research