Title: Monochromatic control of bacteria/yeast consortia for fuel and chemical production

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Project Goals: The overall goal of this project is to develop optogenetic controls over the population dynamics and composition of microbial consortia. This includes unidirectional monochromatic controls of one strain in the consortia; bidirectional monochromatic controls of two strains; and multidirectional polychromatic controls of two or more strains. We will apply these new capabilities to optogenetically control various consortia for biofuel and chemical production, using systems that showcase different advantages of co-culture fermentations. We will show how varying light duty cycles can optimize microbial populations throughout co-culture fermentations to maximize chemical production. Thus, this work addresses the long-standing challenge of stabilizing and optimizing the population composition of microbial consortia for chemical production.

Abstract Text: Engineered microorganisms have enormous potential to produce biofuels, chemicals, and other valuable products from renewable substrates and waste. However, the genetic and metabolic burden that exogenous biosynthetic pathways impose on engineered strains negatively impact their robustness and productivity. In addition, containing a full biosynthetic pathway in a single strain (i.e., in a monoculture) prevents metabolic engineers from separately optimizing each of its components (or modules), forcing them instead to optimize the entire pathway in a single cell by making compromises that often limit productivity. This shortcoming is magnified when different modules compete with or inhibit each other. These factors greatly contribute to the insufficient productivities of most bioprocesses, and prevent them from being competitive with fossil fuels and non-sustainable manufacturing processes.

Using co-cultures of more than one organism, instead of monocultures, has been proposed as a powerful solution to address these challenges. This approach draws inspiration from natural microbial processes (e.g., biomass decay, or food digestion), which are almost always carried out by microbial communities. Splitting biosynthetic pathways across multiple microorganisms would reduce the burden on each individual member and prevent negative interactions between different metabolic modules. Furthermore, the efficiency of each module would be significantly improved by optimizing them separately in specialized strains (akin to the way different parts of a chemical process are optimized in separate reactors in a chemical plant). However, stabilizing the population composition of engineered microbial consortia has proven to be a formidable challenge, with the fastest growing members usually dominating the consortia and turning them into futile monocultures. Controlling inoculation ratios and engineering clever biological

orthogonalities or synthetic symbioses between members have done much to improve co-culture operations, but these strategies have still been insufficient to advance engineered consortia to commercial processes.

In this proof-of-principle study, we demonstrate that optogenetics is an effective strategy to dynamically control populations in microbial co-cultures. Using a new optogenetic circuit we call OptoTA, we regulate an endogenous toxin-antitoxin system, enabling tunability of *Escherichia coli* growth using only blue light. With this system we can control the population composition of co-cultures of *E. coli* and *Saccharomyces cerevisiae*. When introducing in each strain different metabolic modules of biosynthetic pathways for isobutyl acetate or naringenin, we found that the productivity of co-cultures increases by adjusting the population ratios with specific light duty cycles. This study shows the feasibility of using optogenetics to control microbial consortia populations and the advantages of using light to control their chemical production.

References/Publications

1. Lalwani MA, Kawabe H, Mays RL, Hoffman SM, **Avalos JL**. Optogenetic control of microbial consortia populations for chemical production. *ACS Synthetic Biology* **10** (8), 2015-2029 (2021).

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