Genetically Encoded Biosensors for Mitochondrial and Cytosolic Biosynthesis of Branched-Chain Higher Alcohols in *Saccharomyces cerevisiae*

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Project Goals:

The overall goal of the project is to carry out a comprehensive systems biology study of branched-chain higher alcohol (BCHA) production and tolerance in yeast. We will leverage the genetically encoded biosensor of BCHA production described in this presentation to screen yeast genomic libraries to measure the effects of genetic perturbations on BCHA production and tolerance. Introducing this biosensor in strains engineered with optogenetic circuits that control BCHA production with light will enable us to establish a closed-loop control systems to study these metabolic pathways. This includes measuring transcriptomic changes in steady state or dynamic production systems. Ultimately, we will use these genomic and transcriptomic data to discover the key cellular networks involved in BCHA production and tolerance, which will be instrumental in developing better producing strains.

Branched-chain higher alcohols (BCHAs), including isobutanol and isopentanol, have been identified as key biofuels by the Office of Energy Efficiency & Renewable Energy of the U.S. Department of Energy [1]. These alcohols have better fuel properties than bioethanol (including higher energy density and better compatibility with current gasoline infrastructure), and their blends belong to a select group of advanced biofuels with the highest potential to increase spark ignition engine efficiency [1]. Furthermore, BCHAs can be upgraded to jet fuel, making them excellent renewable fuels for ground as well as air transportation.

The yeast *Saccharomyces cerevisiae* is a preferred host organism for BCHA production because of its relatively high tolerance to their toxicity, and the potential to retrofit existing bioethanol plants (most of which use this yeast) with strains engineered to produce these advanced biofuels. Isobutanol and isopentanol are derived from valine and leucine, respectively, which are biosynthesized in a common pathway that has been engineered in either the mitochondria or the cytosol. Efforts to commercialize these biofuels are challenged by limited productivities, as well as their high toxicity. While significant progress has been made in boosting yields and titers, particularly of isobutanol, there are currently no biosensors to enable high throughput screens to accelerate further stain improvement, or study their biosynthesis. In addition, very little is known about the interplay between different cellular networks and BCHA production and tolerance. Our recent study on the relationship between yeast tolerance to isobutanol and its adaptive response to nitrogen starvation begins to shed light into these complex phenotypes [2].

In this presentation I will provide a detailed description of the development, characterization, and application of the first genetically encoded biosensor for BCHA production in *Saccharomyces cerevisiae* [3]. This biosensor is based on the transcriptional regulator of branched chain amino acid biosynthesis, Leu3p. The activity of this transcription factor depends on the intracellular concentrations of α-iosopropylmalate (α-IPM), which is a byproduct and precursor of isobutanol and isopentanol biosynthesis respectively. Therefore, expressing green fluorescent protein (GFP) from a Leu3p-controlled promoter results in a robust biosensor for BCHA biosynthesis. Small modifications make the biosensor specific for either isobutanol or isopentanol production with correlation coefficients of $R^2 = 0.97$ and $R^2 = 0.99$, respectively. With these biosensors, we have been able to develop high throughput screens to isolate high BCHA producing strains, identify hyperactive variants of three enzymes in BCHA biosynthesis, and support the construction and optimization of whole metabolic pathways for isobutanol production in both mitochondria and cytosol.

These biosensors are essential to achieve the goals of the project. We are currently in the process of introducing the isobutanol biosensor into the yeast gene deletion library; this has already enabled the discovery of new genes that, when deleted, enhance isobutanol production. In addition, we are conducting the initial characterization of strains containing both the isobutanol biosensor and optogenetic controls of isobutanol biosynthesis [4], with the objective of developing a closed-loop control system for BCHA biosynthesis. These advances are significant milestones towards our ultimate goal of increasing our basic understanding of BCHA biosynthesis and tolerance, as well as expanding our capabilities to control and improve strains for their production.

References

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