Title: Developing the thermotolerant yeast *Kluyveromyces marxianus* as a host for next generation bioprocessing

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Project Goals: This systems and synthetic biology project seeks to understand and engineer the native stress tolerance phenotypes of the yeast *Kluyveromyces marxianus* with the goal of developing a new synthetic biology chassis for fuel and chemical production.

Kluyveromyces marxianus is a promising nonconventional yeast for biobased chemical production due to its rapid growth rate, high TCA cycle flux, and tolerance to low pH and high temperature. Unlike *S. cerevisiae*, *K. marxianus* grows on low-cost substrates to cell densities that equal or surpass densities in glucose, which can be beneficial for utilization of lignocellulosic biomass (xylose), biofuel production waste (glycerol), and whey (lactose). This project seeks to understand and exploit these native traits to create a new thermotolerant eukaryotic host chassis for biofuel and biochemical products. Our efforts include developing genome-wide mutagenesis and regulation strategies to identify essential genes and the genotype-phenotype relationships underpinning the novel traits. The new systems-level data created in these experiments will inform metabolic engineering to increase the yield of acetyl-CoA derived products such as triacetic acid lactone (TAL) and ethyl acetate. To date, we have created and used a multiplexed CRISPR interference (CRISPRi) system to understand and engineer ethyl acetate synthesis on glucose, increasing production by upward of 3.8-fold.[1] We have also engineered TAL biosynthesis, with the highest titers achieved in xylose at 37 °C. The ~1.0 g/L titer achieved in mL-scale cultures shows promise for high titer, high yield production once scaled to bioreactor conditions. Ongoing work utilizes our newly developed CRISPR tools to modify native and heterologous pathways to increase the levels of the acetyl-CoA and malonyl-CoA, precursors to ester and TAL biosynthesis. Functional genetic screens to understand the origins of high thermal and acid tolerance are also ongoing.

References

1. Löbs AK, Schwartz C, Thorwall S, and Wheeldon I. Highly multiplexed CRISPRi repression of respiratory functions enhances mitochondrial localized ethyl acetate biosynthesis in *Kluyveromyces marxianus*. *ACS Synthetic Biology*, 2018;7:2647−2655.References are not required but should be placed here if needed.

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