Engineering of the Non-Model Yeast *Issatchenkia orientalis* to Produce Organic Acids

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Project Goals: The goal of this research is to engineer a non-model yeast, *Issatchenkia orientalis,* to produce organic acids in high titers. 3-Hydroxypropionic acid is one of the key sugar-based building block chemicals that can be produced in biorefineries. The ability to produce 3-hydroxypropionic acid from glucose or other renewable carbon sources would provide a biosustainable alternative to acrylic acid production from fossil resources.

The nonconventional yeast *Issatchenkia orientalis* is a potential platform microorganism for production of organic acids thanks to its unusual ability to grow in highly acidic conditions. However, the lack of efficient genetic tools, including a stable episomal plasmid and precise genome editing tool, prevented metabolic engineering of this organism. We previously developed this genetic toolbox to efficiently engineer this non-model yeast to produce value-added chemicals.[1,2] Here we present the production of 3-hydroxypropionic acid (3HP) from *I. orientalis.* There are multiple pathways to produce 3HP; however, only two are generally used, the malonyl Co-A pathway and the β-alanine pathway. We have generated both pathways on a plasmid to determine preliminary production quantities of 3HP in shake flasks as well as the dependence of pH on the production. Our highest production of 3HP thus far is ~1.5 g/L from 50g/L glucose as the carbon source over 5 days fermentation from the β-alanine pathway. The malonyl Co-A pathway yielded only ~700 mg/L. Further experiments will be done utilizing the β-alanine pathway to further improve production by improving the expression of the beta alanine pyruvate amino transferase as well as genome engineering of the base strain.

References

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2. Cao, M., Fatma, Z., Song, X., Hsieh, P.H., Tran, V., Lyon, W.L., Sayadi, M., Shao, Z., Yoshikuni, Y., Zhao, H. *Metabolic Engineering*, 2020, 59:87-97.

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