# Leveraging Population Genomics Analysis of *Issatchenkia orientalis* for Engineering a Better Strain for the Production of Lignocellulosic-biomass-based Bioproducts

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## Project Goals:

*Issatchenkia orientalis* is an ideal chassis for the organic acid production from lignocellulosic biomass because of its stress tolerance to low-pH and lignocellulosic inhibitors. However, the mechanisms underlying its multi-stress tolerance remain elusive. This project aims to identify the genetic variation contributing to the stress tolerance of *I. orientalis* by population genomics analysis and use the knowledge gained to engineer a better strain for the production of lignocellulosic-biomass-based bioproducts.

## Abstract:

*Issatchenkia orientalis* is a non-model ascomycetes yeast with exceptional ability to tolerate extremely low pH, high concentrations of organic acids, and high concentrations of lignocellulosic inhibitors[1-5]. These unique characteristics make *I. orientalis* an attractive chassis for producing organic acids directly from lignocellulosic hydrolysates. Understanding how *I. orientalis* evolved to tolerate multiple stresses may allow engineering of a strain more suitable for industrial use than natural isolates are. We performed a population genomics study of 162 strains collected from various habitats and identified 305,435 single nucleotide polymorphism (SNPs), 16,177 insertions and deletions (InDels), and other genetic variations, including polyploidy and gene copy number variation. The genome-wide association study identified a putative membrane transporter gene associated with the tolerance to hydroxymethylfurfural (HMF) and phenolic inhibitors. We further engineered loss-of-function mutants of this transporter gene from the industrial SD108 strain, and found that the mutant strains are more susceptible to HMF when missing this transporter gene. Therefore, overexpression of this transporter gene is expected to enhance the HMF tolerance in SD108 strain. Moreover, we use machine-learning analysis to determine genetic variants associated with the unfavorable fluconazole resistance. Based on the machine learning analysis and experimental validation, we found that deleting an ABC transporter gene in the new benchmark strain IO21 can generate a desirable strain with decreased fluconazole resistance and increased HMF tolerance. In sum, our study reveals genes involved in HMF and fluconazole stress tolerance and provides ample genomics resources for the communities.

**References/Publications**


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