Title: Population phenomics of *Issathenkia orientalis* for creation of a safer and more robust chassis for next-generation industrial biotechnology

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Project Goals: To alleviate reliance on chemical synthesis and shift into the bioeconomy regime, it is imperative to develop a biological chassis for producing organic acids from renewable biological resources. *Issatchenkia orientalis*, a nonconventional acid-tolerant yeast, has been characterized, engineered, and employed as a production chassis for several organic acids, including succinate acid[1] and D-lactic acid[2]. In addition to tolerating acid, *I. orientalis* has extraordinary tolerance to other stressors, including heat, lignocellulosic inhibitors, and antifungal drugs, although the biological mechanisms for these tolerances are still unknown. These innate tolerances and the limited information on their underlying mechanisms arouse safety concerns, making use of *I. orientalis* as a production chassis a double-edged sword. To help alleviate these concerns, we elucidated genotype-phenotype relationships by analyzing populations obtained by collecting *I. orientalis* strains from around the globe. This effort will provide a knowledge database to help design and build a safer and more robust *I. orientalis* chassis for industrial biotechnology.

Abstract Text: The bioeconomy revolves around efforts to use bio-based resources and products to help tackle grand societal challenges such as climate change, nature conservation, and food security. Microbial chassis mediate the bioeconomy scheme, serving as molecular factories that convert various biological resources into a broad spectrum of chemicals, fuels, and materials. Among these products, organic acids are value-added platform molecules that have gained attention in the food, pharmaceutical, and chemical industries. Producing them microbially using bioenergy crops such as miscanthus and switchgrass as feedstocks is a viable way to reduce reliance on chemical synthesis and avoid its environmental burdens. Tolerance of final organic acids, low pH, and inhibitory compounds present in lignocellulosic hydrolysates are prerequisites in order for any organic acid production chassis to be cost-effective and environmentally friendly. A nonconventional yeast, *Issatchenkia orientalis*, is well aligned with the requirements because of its renowned tolerance to multiple stresses, including various organic acids[1], low pH, phenolic compounds, furan aldehydes, and salts. However, it also resists antifungal drugs, potentially raising concerns (most pathogenic fungi are opportunistic, even *Saccharomyces cerevisiae*, or baker's yeast)[3]. These potential concerns can be alleviated by following laboratory practices appropriate for *I. orientalis*'s assessment at biosafety level 1, the lowest security level. Nevertheless, considering that *I. orientalis* would typically be genome engineered to optimize it as a production chassis, it is important to identify genotype-phenotype relationships that might bring to light opportunities to engineer robust but safer properties. In this study, we focused on expanding knowledge of *I. orientalis* through population phenomics with associated statistical analyses. We developed a high-throughput phenotyping assay method that allowed us to measure growth profiles of respective strains on an agar plate in various chemical compounds and under various environmental conditions. We used the assay to analyze 160 *I. orientalis* strains across 58 representative conditions. Analysis of the correlation between conditions indicated a trend in population-level behavior, suggesting that significantly (anti-)correlated cellular responses likely occur through a mechanism that affects the phenotypes and no significantly correlated pairs may not arise from a common mechanism. Therefore, we highlighted specific paired conditions in the context of generating a safer and more robust chassis. We also compared the geographical origins of each isolate with the phenotypic behaviors to investigate potential evolutionary effects of surrounding environments. In addition, a genome-wide association study (GWAS) identified potential genetic loci, and deletion of these loci allowed us to select for a genetic locus that affects phenotypic changes. Our study showcases the utility of this population growth assessment strategy for evaluating phenotypic changes for further statistical analyses. The knowledge database we developed will offer potential opportunities to engineer safer and more robust chassis for biotechnological applications.

References

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