Development of a genetic toolbox for metabolic engineering of *Issatchenkia orientalis*

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Project Goals: To develop precise genetic tools for efficient metabolic engineering of non-model yeast *Issatchenkia orientalis* for organic acid production.

Abstract

There is a growing interest in engineering microbial cell factories for the production of chemicals and fuels from renewable feedstock. Among the twelve platform chemicals identified by the US Department of Energy (DOE), most of them are organic acids. Biosynthesis of these molecules leads to a multistress condition such as low pH, high temperature and accumulation of inhibitory products. *Issatchenkia. orientalis* is acid tolerant, ethanol tolerant, and thermotolerant, and is thus a multistress tolerant yeast and an ideal heterologous host for the organic acid production. However, lack of effective genome editing tools as well as nonavailability of its transcriptomics, proteomics, metabolomics, and genome-scale model have hindered its widespread application in biomanufacturing of industrially relevant molecule. In this study, an efficient genome editing method was developed for *I. orientalis* using a CRISPR-Cas9 system. We have constructed a single plasmid system, carrying 20 bp spacer, scaffold sequences, the gene for Cas9 protein expression, and 100 bp homology arm. The *ADE2* gene was deleted with the efficiency of more than 95%. Further, we have shown the application of these tools for the experimental validation of a genome-scale metabolic model of *I. orientalis*.

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