Cas9-Based Metabolic Engineering of *Issatchenkia orientalis* for Enhanced Utilization of Cellulosic Hydrolysates

CABBI
CENTER FOR ADVANCED BIOENERGY AND BIOPRODUCTS INNOVATION

Background/Objective

- *Issatchenkia orientalis*, a yeast exhibiting high tolerance against harsh environmental conditions, is a promising metabolic engineering host for producing fuels and chemicals from cellulosic hydrolysates containing fermentation inhibitors under acidic conditions. However, existing genetic tools require auxotrophic mutants so that the selection of a host strain is limited.
- We developed a drug resistance genome-editing method for engineering any *I. orientalis* strain, and then we engineered strains for xylose fermentation.

Approach

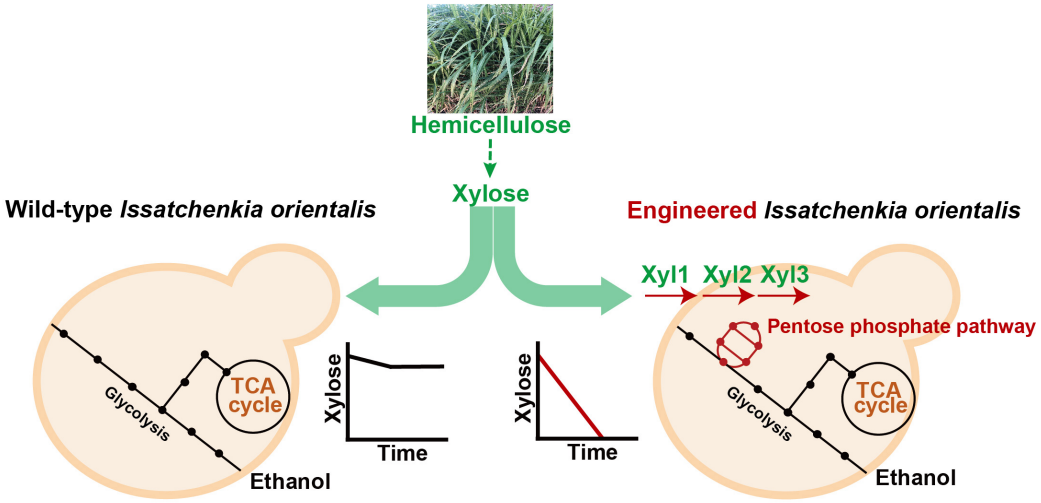
A gene cassette containing xylose reductase (*Xyl1*), xylitol dehydrogenase (*Xyl2*), and xylulokinase (*Xyl3*) from *Scheffersomyces stipitis* were integrated into four *I. orientalis* wild-type strains (SD108, IO21, IO45, and IO46) using Cas9-based genome editing.

Results

The resulting strains (SD108X, IO21X, IO45X, and IO46X) efficiently consumed xylose derived from cellulosic and hemicellulosic hydrolysates even though the pH adjustment and additional nitrogen source were not provided.

Significance/Impacts

These results highlight the potential utilization of various *I. orientalis* strains to produce fuels and chemicals using cellulosic hydrolysates.



Engineered *I. orientalis* strains containing *Xyl1*, *Xyl2*, and *Xyl3* demonstrated efficient xylose consumption, while the wild-type strains can not consume xylose derived from hemicellulose.

Lee, Y.G., Kim, C., Kuanyshev, N., Kang, N.K., Fatma, Z., Wu, Z.Y., Cheng, M.H., Singh, V., Yoshikuni, Y., Zhao, H., Jin, Y.S. Sep. 19, 2022. "Cas9-Based Metabolic Engineering of *Issatchenkia orientalis* for Enhanced Utilization of Cellulosic Hydrolysates." *Journal of Agriculture and Food Chemistry*. DOI: 10.1021/acs.jafc.2c04251.