

Xylose Metabolic Engineering of *Issatchenkia orientalis* for 3-hydroxypropionic Acid Production from Cellulosic Hydrolysate Without Nutrient Supplementation

Background/Objective

Bioconversion of lignocellulosic biomass offers a promising route to high-value platform chemical production. However, inefficient xylose utilization and toxic compounds in cellulosic hydrolysate limit microbial fermentation, as the hydrolysate contains substantial amounts of xylose in addition to glucose. To address these challenges, the non-model yeast *Issatchenkia orientalis* was engineered to produce 3-hydroxypropionic acid (3-HP), a key precursor for numerous industrial products, directly from sorghum hydrolysate under low-pH conditions.

Approach

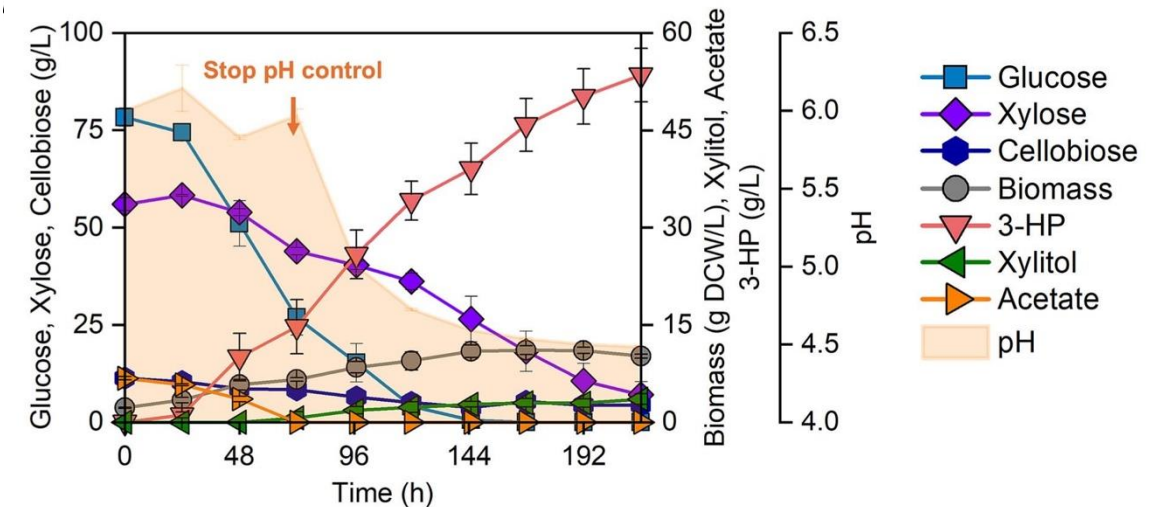
An efficient xylose utilization pathway consisting of *XYL1*, *XYL2*, and *XYL3* from *Scheffersomyces stipitis* was introduced into an engineered 3-HP producing strain, enabling efficient conversion of xylose to 3-HP. To optimize fermentation in bioreactors and mitigate hydrolysate-associated toxicity, pH was initially maintained at pH 6.0 to enhance cell growth and eliminate the lag phase under low-pH conditions.

Results

The engineered *I. orientalis* strain produced 46.8 g/L 3-HP from sorghum hydrolysate, without additional nutrient supplementation. Implementation of the partial pH control strategy increased 3-HP productivity by 55% (from 0.20 to 0.31 g/L·h), while maintaining low-pH conditions. Further improvement of xylose metabolism was achieved by introducing an additional copy of *XYL2*, which increased 3-HP titer to 53.5 g/L and the yield by 33% (from 0.30 to 0.40 g/g sugars), with pH reaching 4.5 at the end of fermentation.

Significance/Impacts

This work reports one of the highest 3-HP titers and yields from cellulosic hydrolysate without additional nutrient supplementation. Overall, the study highlights the strong industrial potential of non-conventional yeasts, like *I. orientalis*, for the production of platform chemicals from feedstocks.



Fermentation profile (mean of two independent runs) of *I. orientalis* 1APX-X2 strain using sorghum hydrolysate, with pH 6.0 for first 3 days

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