Engineering and Evolution of *Yarrowia lipolytica* for Producing Lipids from Lignocellulosic Hydrolysates

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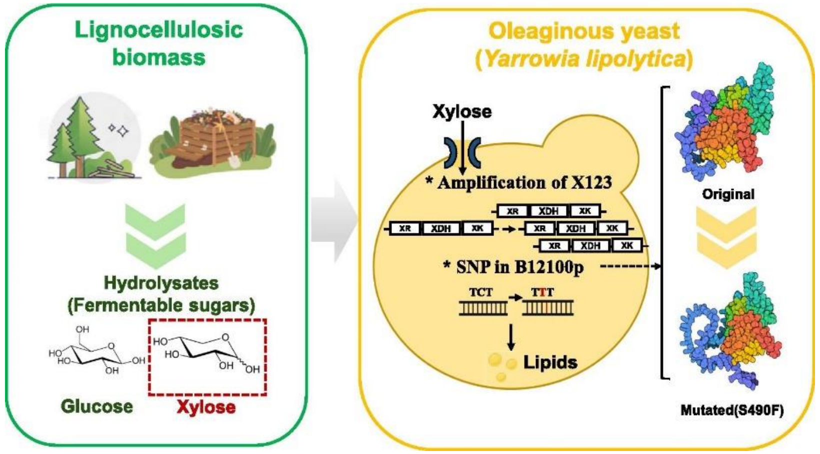
Background/Objective

Y. lipolytica, an oleaginous yeast strain, shows promise for industrial fermentation of biomass due to its robust acetyl-CoA flux and well-developed genetic engineering tools. However, its lack of active xylose metabolism restricts its use to convert cellulosic sugars to valuable products. A PO1f strain was engineered with genes from *Pichia stipitis* to enable xylose metabolism, but xylose utilization was still too slow for industrial applications. We aimed to improve its xylose utilization.

Approach

The genes XR, XDH, and XK from *P. stipitis* were overexpressed in *Y. lipolytica* PO1f to create a xylose-utilizing strain (X123). Adaptive laboratory evolution (ALE) improved xylose assimilation, yielding the evolved strain XEV, whose genetic variations were analyzed by whole-genome sequencing and reverse engineering. Finally, sugar utilization of mutant strains was evaluated by fermenting lignocellulosic hydrolysate obtained from hydrothermally pretreated sorghum.



An illustration of xylose, an abundant sugar in lignocellulosic biomass (left), used as a feedstock for fermentation by *Y. lipolytica*-consuming xylose (right).

Results

Y. lipolytica XEV efficiently converted xylose into lipids at at 2.3 times the rate of the control (1.94 g/L) using lignocellulosic hydrolysate from sorghum. Also, the genetic variations for enhanced xylose assimilation were identified: the amplification of XR, XDH, and XK is essential, and a point mutation in the YALI0B12100g gene can further xylose assimilation.

Significance/Impacts

This study enhances the understanding of xylose metabolism in *Y. lipolytica* and its industrial application in converting cellulosic sugars.

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