Crabtree-like aerobic xylose fermentation through increased metabolic flux and altered sugar signaling pathways in *Saccharomyces cerevisiae*

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Project Goals

We aimed to better understand how to enhance the conversion of xylose into fermentative end-products by yeast through directed evolution and engineering.

Abstract

Plant-based biofuel is considered as sustainable and renewable energy. Xylose, which composes up to 40% of the sugar present in plant cell walls, cannot be fermented into biofuels, such as ethanol, by native *Saccharomyces cerevisiae*, the most common biofuel-producing organism. Xylose contrasts with glucose, which *S. cerevisiae* has evolved to ferment at such high rates that it will do so aerobically (Crabtree Effect) and preferentially in the presence of other carbon substrates (glucose-repression). Despite extensive knowledge of the regulatory networks controlling carbon metabolism, little is known about how to reprogram *S. cerevisiae* to ferment xylose at rates comparable to glucose. Previously, we discovered that loss-of-function mutations in *ISU1*, *HOG1*, *GRE3* and *IRA2* enabled *S. cerevisiae* strain engineered with xylose metabolism enzymes to respire xylose aerobically and ferment xylose anaerobically. Still, however, these genetic changes do not confer xylose conversion rates similar to that for glucose. Here, we report on our approach to enhance the rate of xylose fermentation by converting our engineered, xylose-respiring yeast strain into one that ferments xylose into ethanol aerobically. First, we deleted *COX15*, which is essential for respiration and rendered the strain unable to grow aerobically on xylose. We then evolved the strain to grow aerobically on xylose, subsequently isolating two independent clones with the abilities to grow on and convert xylose into ethanol aerobically. Evolved strains expressing (or retransformed with) *COX15* metabolize xylose aerobically and anaerobically faster than the unevolved parent. Whole genome sequencing of these clones identified overlapping duplications in Chromosomes IV and XVI; overlapping regions included the site where the xylose metabolism enzymes were engineered and the pentose phosphate pathway enzyme *TKL1*. Engineered duplications of the xylose metabolism enzymes and *TKL1* in the unevolved parent strain enabled greater fermentation of xylose-to-ethanol aerobically, suggesting the greater metabolic flux of xylose is a major requirement for aerobic xylose fermentation. However, the rationally-engineered strain did not ferment xylose to the

same extent as the evolved strains, indicating that additional genetic differences lead to their Crabtree-like traits or phenotype for xylose. Together, our findings identify genetic changes that may allow for faster conversion of xylose from plant biomass into biofuels.

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