L-malic Acid Production from Xylose by Engineered *Saccharomyces cerevisiae*

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Project Goals: As *Saccharomyces cerevisiae*, a Crabtree-positive yeast, produces ethanol exclusively during glucose fermentation, the reduction of ethanol without growth defects is necessary to efficiently produce value-added products. In this study, we developed a metabolic engineering strategy which enabled a high titer production of malic acid from xylose by engineered *S. cerevisiae*.

With increasing environmental concerns and decreasing oil supply, there are growing interests in developing technologies that utilize renewable sources for the production of fuels and chemicals. Microbial conversion of lignocellulosic biomass into biofuels and chemicals can be a substitute for petroleum-based industry [1]. As hydrolysates of lignocellulosic biomass mainly contain glucose and xylose, it is necessary to develop a xylose-utilizing microorganism [2]. *Saccharomyces cerevisiae* is one of the most promising microbial strains for bioconversion of lignocellulosic biomass because there have been many efforts to make *S. cerevisiae* consume xylose by introducing the xylose isomerase and oxidoreductive pathways.

L-malic acid is widely used in the food and chemical industries. Here, we report on production of malic acid from xylose by engineered *S. cerevisiae*. To enable malic acid production in a xylose-assimilating *S. cerevisiae* with the oxidoreductase pathway, we employed the cytosolic reductive TCA (rTCA) pathway. We overexpressed *PYC1* and *PYC2,* coding for pyruvate carboxylases, a truncated *MDH3,* coding for malate dehydrogenase, and *SpMAE1,* coding for a *Schizosaccharomyces pombe* malate transporter. Additionally, *GPD1* and *GPD2,* coding for glyceraldehyde-3-phosphate dehydrogenase, were deleted to completely block the glycerol production pathway. The metabolic pathway responsible for ethanol production was partially blocked through deleting *PDC1* and *ADH1*, because complete deletion of the ethanol pathway could lead to severe growth defects due to the limited synthesis of acetyl-CoA, an important precursor of cell growth [3-5]. The resulting strain produced malic acid from both glucose and xylose, but it produced much higher titers from xylose. Interestingly, the engineered strain had higher malic acid yield from lower xylose concentrations (10 g/L), with no ethanol production, than from higher xylose concentrations (20 g/L and 40 g/L). As such, a fed-batch culture maintaining xylose concentrations below 10 g/L was conducted, and 61.2 g/L of malic acid was produced with a productivity of 0.32 g/L·h.

These results represent successful engineering of *S. cerevisiae* for the production of malic acid from xylose and therefore confirm that xylose offers the efficient production of various biofuels and chemicals by engineered *S. cerevisiae*.

References

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Funding statement: *This work was funded by the DOE Center for Advanced Bioenergy and Bioproducts Innovation (U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Number DE-SC0018420). Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the U.S. Department of Energy.*