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 glyceral 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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