Metabolic Engineering of Yeast for Xylose Uptake and Fermentation

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Fuel ethanol production from plant biomass hydrolysates by the baker’s yeast *Saccharomyces cerevisiae* is of great economic and environmental significance. Cost-effective production of ethanol from plant biomass should not only be based on the readily fermentable starch and sucrose fractions of plant carbohydrates, but also on the much more resistant lignocellulosic fractions. The utilization of xylose, the second abundant monosaccharide in lignocelluloses, is essential for the efficient bioconversion of lignocellulosic fractions of biomass, yet still a technical barrier to the commercial fermentation and a major challenge in metabolic engineering.

Previous work has established feasible xylose metabolism pathways in *S. cerevisiae*. Fermentation of xylose in *S. cerevisiae* has been greatly improved by recent metabolic and evolutional engineering studies, but still remains significantly less efficient than that of glucose by these strains. Since *S. cerevisiae* takes up xylose through its glucose transporters, which have much lower affinity for xylose, the trans-membrane xylose transport is likely to be one step that limits the xylose uptake.

For the study on xylose uptake in yeast, two heterogeneous xylose metabolism pathways were introduced to a lab *S. cerevisiae* strain. A series of key enzymes for pentose metabolism were also overexpressed to accelerate xylose fermentation. Furthermore, strain evolution based on specific growth rate on xylose medium was carried out for the selection of recombinant strains with high xylose uptake rate. The resulting strains were characterized and used as platform in order to isolate and identify heterogeneous xylose transporters from native xylose-metabolizing yeasts, via rational and combinatorial approaches. The verified transporters will undergo several rounds of mutagenesis and screening to elevate its efficiency and affinity for xylose transport, and eventually be properly expressed in engineered *S. cerevisiae* to facilitate the xylose uptake and fermentation to ethanol.