Systems biology and pathway engineering enable *Saccharomyces cerevisiae* to utilize C-5 and C-6 sugars simultaneously for cellulosic ethanol production

Z. Lewis Liu and J. Moon

BioEnergy Research Unit, National Center for Agricultural Utilization Research,
USDA-ARS, Peoria, IL USA
ZLewis.Liu@ars.usda.gov

ABSTRACT

Saccharomyces cerevisiae is a traditional industrial workhorse for ethanol production. However, conventional ethanologenic yeast is superior in fermentation of hexose sugars (C-6) such as glucose but unable to utilize pentose sugars (C-5) such as xylose richly embedded in lignocellulosic biomass. In order to efficiently utilizing biomass sugars for lower cost cellulosic ethanol production, a significant effort has been taken worldwide for decades to improve xylose utilization capability of *S. cerevisiae* by genetic engineering. Yet challenges remain strong since the efficiency of the improved C-5 utilization is low and insufficient for low-cost cellulosic ethanol production. Scientists at ARS using tolerant yeast strain NRRL Y-50049 as a mother host created six (6) new genotypes of *S. cerevisiae* NRRL Y-50049-*YXI-XUT4, -XUT5, -XUT6, -XUT7, -RGT2*, and *-SUT4* applying systems biology and pathway engineering approaches. Instead of a commonly used traditional fungal xylose utilization pathway, we introduced a bacterial xylose isomerase pathway into the yeast. We first synthesized a novel sequence of xylose isomerase (*YXI ;* GenBank Accession No. JF261697) containing optimized transcription codons for our yeast expression, and then integrated it into a specific chromosomal locus of the yeast to obtain a high level of constitutive expression, resulting in a daughter host strain NRRL Y-50049-*YXI*. We cloned and characterized six xylose transporter genes from *Scheffersomyces stipitis*, a natural xylose utilization yeast, to aid xylose transport and uptake. These heterologous xylose transporter genes were genetically engineered into the daughter host resulting in a set of new genotypes of *S. cerevisiae*. These newly developed industrial yeast strains are able to grow on xylose as sole carbon source and produce ethanol. When mixed sugars of glucose and xylose were added in the medium, all these new strains displayed a simulteneous utilization of C-5 and C-6 sugars and significantly improved xylose uptake and utilization for ethanol conversion. Among which, genotypes *S. cerevisiae* NRRL Y-50049-*YXI-XUT7, -RGT2*, and *-SUT4* demonstrated superior fermentation capability in utilizing both sugars. In contrast with poor results observed from lab model strains, our research established the first example of using industrial yeast as a host for the next-generation biocatalyst development for advanced biofuels production. All these US patented strains are available for interested parties in collaborative efforts.

Keywords: cellulosic ethanol, genetic engineering, next-generation biocatalyst, xylose utilization, yeast

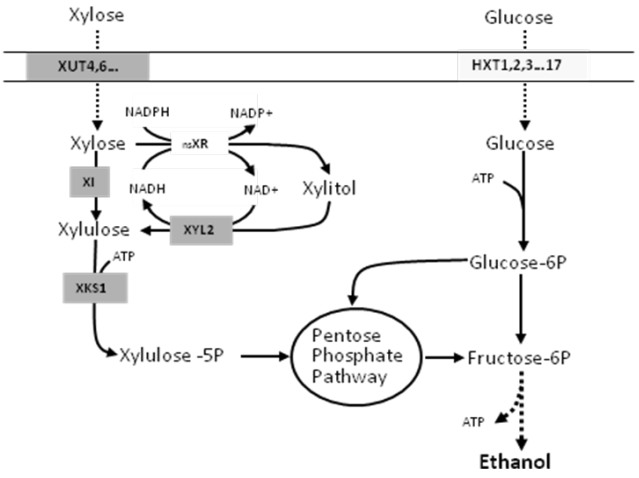

Figure 1. A design of improved xylose metabolic pathways for mixed sugar fermentations. Xylose transport related genes (XUT4 and XUT6) from S. stipitis are incorporated into an inhibitor-tolerant ethanologenic yeast for improved xylose uptake and utilization; a synthesized xylose isomerase gene (YXI) based on codon preference of *S. cerevisiae* is integrated into a defined chromosomal locus as a main xylose utilization route; previously reported xylulokinase gene (XKS1) and xylitol dehydrogenase gene (XYL2) from *S. stipitis* are introduced into the yeast strain for enhanced downstream metabolism and xylitol conversion. Genetically engineered heterologous genes are highlighted. Nonspecific xylose reduction activities by

aldose reductase are marked by nsXR in distinguishing from specific xylose reductase (XR) from *S. stipitis*.

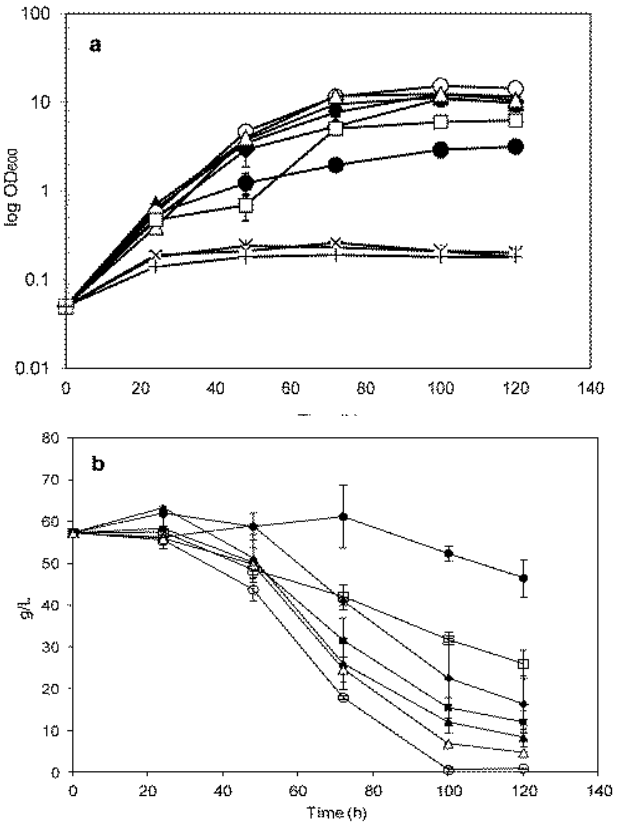

Figure 2. Growth on xylose as sole carbon source. Comparison of cell growth as measured by cell dry mass (g/L) (a) and xylose consumption (b) of S. cerevisiae Y-50049-YXI (filled circle) and its enriched genotypes with varied xylose transporter genes using xylose as sole carbon source under aerobic conditions. Genotypes are labeled as Y-50049-YXI-XUT4 (filled triangle), Y-50049-YXI-XUT5 (filled diamond), Y-50049-YXI-XUT6 (filled square), Y-50049-YXI-XUT7 (open circle), Y-50049-YXI-RGT2 (open triangle), and Y-50049-YXI-SUT4 (open square). Growth of strain Y-50049 without YXI background (star) was evaluated comparing with its transformant derivatives Y-50049-XUT4 (cross) and Y-50049-XUT6 (plus). These three genotypes showed no growth and overlapped at baseline nearly indistinguishable from each other. No xylose consumption was observed from these genotypes (data not shown). Values are means of replications while error bars represent the range.

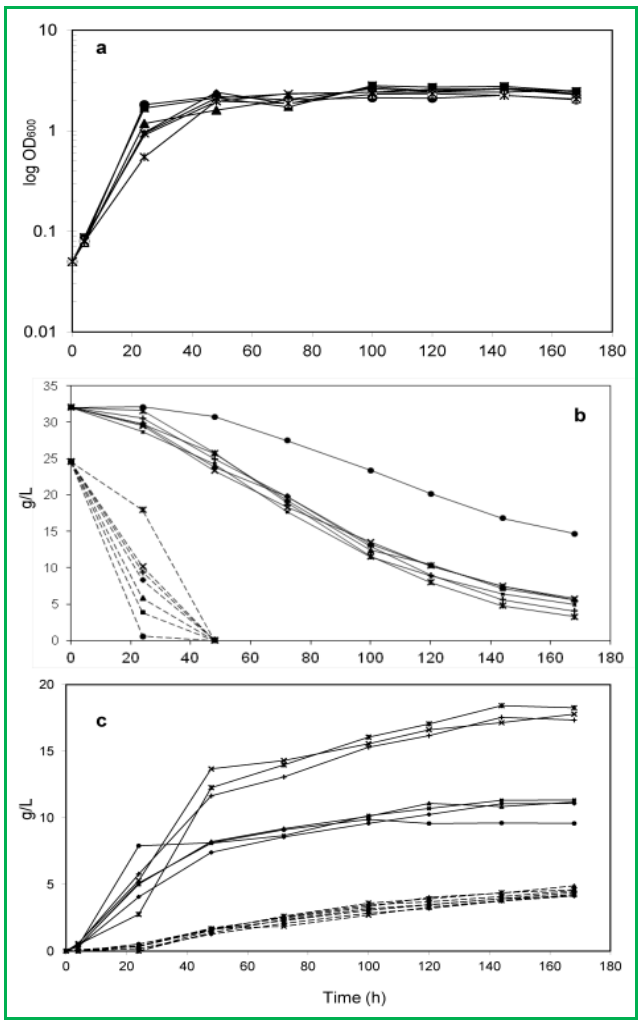

Figure 3. Performance on mixed sugars of glucose and xylose. Comparisons of cell growth as measured by cell dry mass (g/L) (a), sugar consumption (b), and metabolic production (c) for S. cerevisiae Y-50049-YXI (filled circle) and its enriched genotypes with varied xylose transporter genes Y-50049-YXI-XUT4 (filled triangle), Y-50049-YXI-XUT5 (filled diamond), Y-50049-YXI-XUT6 (filled square), Y-50049-YXI-XUT7 (open circle), Y-50049-YXI-RGT2 (open triangle), and Y-50049-YXI-SUT4 (open square) on YP medium supplemented with 24.3 g/L D-glucose and 32.5 g/L D-xylose under oxygenlimited conditions. In panel b, xylose consumption was labeled by solid lines and glucose by dotted lines. In panel c, ethanol production is indicated by a solid line and xylitol, a dotted line. Values aremeans of three replications and error bars removed for visual clarity; however, average percent deviations about the means were: 8.5% for optical density, 5.6% for glucose, 14.7% for xylose, 6% for xylitol, and 6.4% for ethanol.

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