

Towards Cellulosic Ethanol: Characterization of Xylose Metabolic Phenotypes in *Saccharomyces* Yeasts

Jared Wenger, Katja Schwartz, and Gavin Sherlock

Department of Genetics, Stanford University

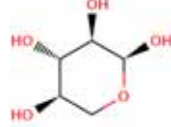


Abstract

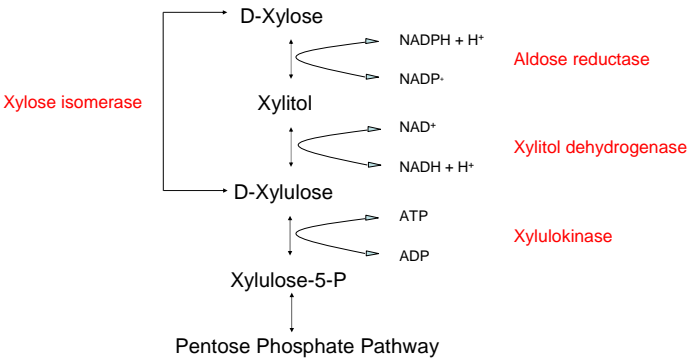
The production of renewable energy is of critical importance to the global economy and for the reduction of atmospheric accumulation of greenhouse gases. A key element of the renewable energy equation is fuel ethanol. Currently, fuel ethanol is produced by fermentation of hexoses found in sugar cane (Brazil), or corn (US), using *Saccharomyces cerevisiae*. However, current practices based on food production models do not maximize energy or green house gas benefits (because they use fossil fuels) and are not economically competitive with fossil fuels at today's energy prices. Production of ethanol from more abundant pentoses such as xylose, which found in hemicellulosic biomass, would have a marked impact on the viability of ethanol as an alternative fuel source. Therefore, we aim to develop *S. cerevisiae* strains with useful phenotypes that can be used towards this end. Here, we describe the phenotypic characterization of strains which showed some ability to utilize xylose and accumulate biomass (see Poster by Katja Schwartz) that were identified as "xylose positive" in a screen of a collection of different *Saccharomyces sensu stricto* yeast strains and species. Using microarrays, we also demonstrate that the "xylose positive" yeasts are funneling xylose into the endogenous pentose phosphate pathway. In the future, we aim to generate both intra- and inter-specific hybrid yeasts and use natural selection to evolve robust xylose fermentation phenotypes.

What is xylose?

Xylose is an aldopentose, considered the second most abundant carbohydrate in nature. It is commonly known as "wood sugar" and is the principle component of hemicellulose. Hemicellulose typically makes up 20-40% of biomass dry weight.



How is xylose metabolized?



Does *Saccharomyces* have a functional pathway?

Xylose Reductase	GRE3, YPR1, YJR096W
Xylitol dehydrogenase	YLR070C, SOR1, YDL246C
Xylulokinase	XKS1

Table 1. *S. cerevisiae* ORFs showing enzyme activity or sequence similarity to members of the xylose pathway. Despite having homologs to each gene in the pathway, *Saccharomyces* is still not efficient at metabolizing xylose.

Characterization of initial "xylose positive" phenotype (see Katja Schwartz for screen details)

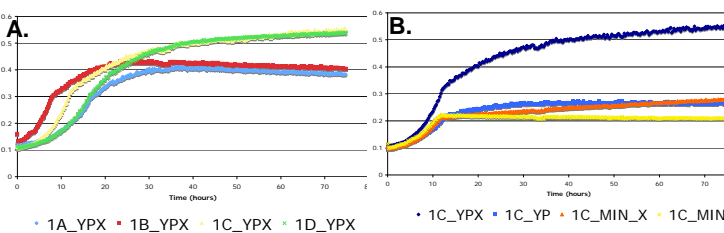


Figure 1. Xylose positive phenotype persists in simi white backcrossed to S288C. A) Growth of one full tetrad from simi x S288C in YP-based media supplemented by 2% xylose, measured by OD595 in TECAN plate reader. A-D correspond to sister spores from the same tetrad. B) "xylose positive" spore grown on YP 2% xylose (YPX), YP no carbon (YP), Minimal 2% xylose (MIN_X), Minimal no carbon (MIN). Phenotype is more robust in YP-based media. See Katja Schwartz's poster for description of screen to identify initial "xylose positives."

Do "xylose positives" use pathway intermediates?

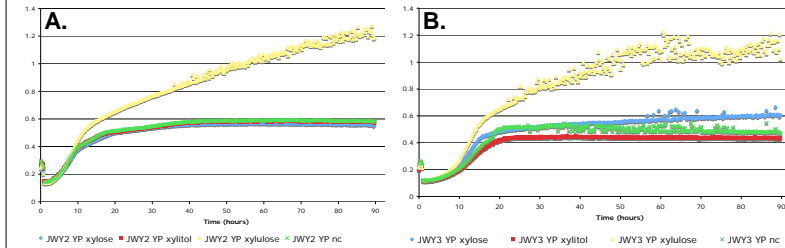


Figure 2. "Xylose positives" can utilize xylulose but not xylitol. All strains grown in YP-based media, with 1% intermediate (1% xylose, 1% xylitol, 1% xylulose, or no carbon). Growth measured by OD595 in TECAN plate reader. A) "xylose negative" *S. cerevisiae* (Simi white x S288C), spore 1B from Figure 1. B) "xylose positive" *S. cerevisiae* (Simi white x S288C), spore 1C from Figure 1.

Our strains' ability to utilize xylulose indicates the downstream pentose phosphate shunt should be functional. However, the failure of Simi white to utilize xylitol indicates two probable possibilities, either xylitol dehydrogenase is the limiting step or xylitol is not transported into the cell. It is unlikely that there is a xylose isomerase functioning.

Expression microarrays confirm "xylose positives" are forcing xylose into the pentose phosphate shunt

GO Term	P-value	Gene(s) annotated to the term
pentose-phosphate shunt	1.10E-06	TKL2:SOL4:SOL3:GND1:TAL1
alcohol catabolic process	2.87E-06	TKL2:DAK2:SOL4:SOL3:GND1:TAL1:GCY1
NADPH regeneration	4.71E-06	TKL2:SOL4:SOL3:GND1:TAL1
NADP metabolic process	1.43E-05	TKL2:SOL4:SOL3:GND1:TAL1

Table 2. Significant GO annotations show enrichment for PPP in xylose positives, in the presence of xylose. Selecting genes that have a 10-fold increase in RNA level between the presence and absence of xylose, in the xylose positive strain, we see significant enrichment for the PPP and redox balancing.

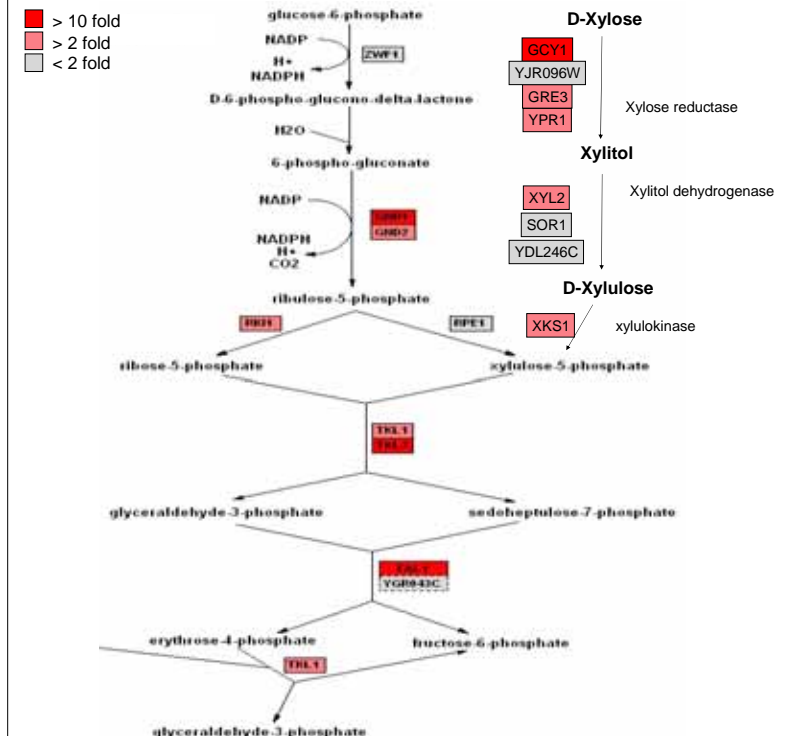


Figure 3. Fold changes for pentose phosphate pathway and potential xylose pathway members. The genes in the table are known or suspected to have activities in the xylose metabolic pathway. The values listed are fold change between xylose and no carbon (+ = xylose positive, - = xylose negative).

Classification	Gene/ORF	Fold Change (+)	Fold Change (-)
xylose reductase	YJR096W	1.9	0.9
	GRE3	6.8	1.1
	YPR1	2.8	0.9
aldo-keto reductase	GCY1	36.0	1.6
xylitol dehydrogenase	XYL2	4.0	2.2
	SOR1	1.6	2.1
	YDL246C	1.1	1.6
xylulokinase	XKS1	4.1	1.4