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 interspecific hybrid yeasts and use natural selection to evolve robust xylose fermentation phenotypes.

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

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.

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 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, 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.

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.

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GO Term | P-value | Gene(s) annotated to the term
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pentose-phosphate shunt | 1.10E-06 | TKL2:SOL4:SOL3:GND1:TAL1
alcohol catalytic process | 2.87E-06 | TKL2:DAK2:SOL4:SOL3:GND1:TAL1:GCV1
NADPH regeneration | 4.71E-06 | TKL2:SOL4:SOL3:GND1:TAL1
NADP metabolic process | 1.43E-05 | TKL2:SOL4:SOL3:GND1:TAL1

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Figure 3. Fold changes for pentose phosphate pathway and potential xylose pathway members. The values listed are fold change between xylose and no carbon (+ = xylose positive, - = xylose negative).

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