

Co-consumption of mixed sugars through the division of labor (DOL) in a synthetic *Saccharomyces cerevisiae* consortium

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Project Goals: Short statement of goals. (Limit to 1000 characters)

For bioconversion of multiple sugars in lignocellulosic hydrolysates into value-added products, there are two utilization strategies: single ‘superbugs’ (SS) capable of utilizing all sugars, and multiple ‘division of labor’ (DOL) strains using each sugar. Our working hypotheses are that DOL might be favored over SS because of heavy metabolic cost—metabolic burdens and toxicity of a product—for utilizing multiple sugars and that DOL is more adaptive than SS to changing sugar compositions. To test the hypotheses, we constructed a set of engineered *Saccharomyces cerevisiae* (*S. cerevisiae*) strains to implement both strategies and built a multiscale mathematical model to quantitatively elucidate the general rules for designing optimal mixed sugar utilization.

Abstract

Abundant and inexpensive agricultural residues contain mixed sugars—mostly glucose and xylose—which can be utilized by wild-type or engineered microbes for the production of biofuels and chemicals. Previous studies have shown that microbes often exhibit a sequential utilization of the mixed sugars—preferential consumption of glucose over xylose. This resulted in impaired yield and productivity of a target molecule. Here, we dissected glucose and xylose consumption by constructing a yeast consortium with glucose utilizing strain and a xylose utilizing strain. The glucose-utilizing strain (Y_{G1}) was constructed by deleting endogenous hexose transporters (*HXT1-7* and *GAL2*) and introducing a heterologous glucose-specific transporter into *S. cerevisiae*. The xylose-utilizing strain (Y_{X1}) was constructed by deleting endogenous hexose transporters (*HXT1-7* and *GAL2*) and introducing a heterologous xylose-specific transporter into an engineered *S. cerevisiae* expressing xylose reductase (*XYL1*), xylitol dehydrogenase (*XYL2*), and xylulokinase (*XYL3*).

We observed that the consortium consisting of Y_{G1} and Y_{X1} could consume glucose and xylose simultaneously when equal amounts of glucose and xylose are present in a culture medium. However, we observed potential issues in achieving simultaneous consumption of glucose and xylose at various concentrations which are often resulted from different feedstocks. First, while the major hexose transporters were deleted, xylose consumption was still inhibited by glucose in Y_{X1} . In order to prevent glucose repression and eliminate consumption in Y_{X1} , we deleted hexokinases (*HXK1-2*) and glucokinase (*GLK1*) which are involved in glucose repression and metabolism. As a result, Y_{X2} (*HXK1-2* and *GLK1* deleted Y_{X1}) grew well on xylose in the presence of glucose and did not show glucose consumption at all. Second, Y_{G1} and Y_{X2} showed different specific glucose and xylose uptake rates, leading to difficulties in constructing an optimal consortium. If the consumption rates of glucose and xylose are not equally controlled, one of the strains could dominate the consortium. In order to synchronize sugar consumption rates of Y_{G1} and Y_{X2} , a less efficient glucose-transporter was introduced to Y_{G1} to build Y_{G2} and the copy numbers of the xylose-specific transporter in Y_{X2} were doubled to build Y_{X3} . By replacing the glucose sugar transporter and increasing the copy number of the xylose transporter, the glucose and xylose uptake rates of Y_{G2} and Y_{X3} were adjusted to a comparable level. Lastly, as monitoring the populations of Y_{G2} and Y_{X3} during cultivation was difficult, fluorescence proteins (GFP and RFP) were expressed in Y_{G2} and Y_{X3} to enable real-time monitoring of the populations in the consortium. The resulting

Y_{G2f} and Y_{X3f} were employed for studying the division of labor during the fermentation of glucose and xylose. Finally, we co-cultivated Y_{G2R} and Y_{X3R} in mixed sugar conditions of glucose and xylose. As expected, glucose and xylose were simultaneously consumed by a consortium of Y_{G2f} and Y_{X3f} strains. Next, we compared the consumption rates of glucose and xylose by the consortium containing Y_{G2f} and Y_{X3f} and a single strain SR8 which can consume glucose and xylose. The overall sugar consumption rate by the SR8 was higher than that by the DOL consortium under the tested conditions. This was mainly because of the inhibition on xylose utilization by ethanol at high concentrations, suggesting that simultaneous consumption might not be a prominent solution for the production of ethanol from a mixture of glucose and xylose. To improve ethanol production from a mixture of glucose and xylose by a DOL consortium, we switched the order of glucose and xylose utilization. As xylose consumption was severely inhibited by the produced ethanol from glucose consumption, we reasoned that utilization of xylose ahead of glucose consumption might increase overall sugar consumption and ethanol production rate from a mixture of glucose and xylose by a DOL consortium. By controlling the timing of DOL between Y_{G3f} and Y_{X3f} , we observed that early xylose consumption by only Y_{X3f} could avoid ethanol inhibition when Y_{G3f} (D452-2 expressing GFP) was inoculated later. These results suggest that we need to think of the timing of DOL for maximizing the overall sugar consumption and product formation rates.

In summary, we confirmed that fine-tuning consumption rates of glucose and xylose by Y_G and Y_X can be utilized to optimize the simultaneous consumption of glucose and xylose by a DOL consortium in response to different sugar concentrations in cellulosic hydrolysates. Nonetheless, the overall sugar consumption and ethanol production rates by SS were higher than those by DOL. As the performance of DOL was impacted by severe inhibition on xylose fermentation by ethanol produced from glucose fermentation, we controlled the timing of DOL by inoculating a xylose-utilizing strain only and supplementing a glucose-utilizing strain later. As a result, the performance of DOL was comparable to that of SS. Our results demonstrate that the division of labor in ecosystems and controlling the timing of DOL can be applied for mixed sugar fermentation for the efficient production of value-added products.