Simultaneous 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 natural carbon sources composed of multiple substrates, there are two utilization strategies: single 'superbugs' (SS) capable of simultaneously utilizing all of these substrates and the division of labor (DOL) among strains with each specializing in one substrate. Our working hypotheses are that heavy metabolic cost, including burdens and toxicity, of substrate utilization favors DOL over SS and that DOL is more adaptive than SS to changing substrate compositions. To test the hypothesis, we will engineer a set of *Saccharomyces cerevisiae* strains to implement the both strategies and also build a multiscale mathematical model to quantitatively elucidate the general rules for designing substrate co-utilization.

Abstract

Abundant and inexpensive agricultural residues contain mixed sugars, such as glucose, xylose, and arabinose, that can be utilized by wild-type and engineered microbes for chemical production. Studies have shown that, during consumption, microbes often follow a sequential fashion for mixed sugars—preferential consumption of glucose over other sugars—which results in impaired yield and productivity of target molecules. Here, we dissect glucose and xylose consumption by constructing a yeast consortium of a glucose utilizing strain and a xylose utilizing strain. The former (Y_{G1}) was constructed by deleting endogenous hexose transporters (*HXT1-7* and *GAL2*) and introducing a heterologous glucose-specific transporter in a wild-type *Saccharomyces cerevisiae*. The latter (Y_{X1}) was constructed by deleting endogenous hexose transporters (*HXT1-7* and *GAL2*) and introducing a heterologous xylose-specific transporter in an engineered *S. cerevisiae* expressing xylose reductase (*XYL1*), xylitol dehydrogenase (*XYL2*), and xylulokinase

(*XYL2*).

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 major hexose transporters (*HXT1-7* and *GAL2*) were deleted, Y_{X1} could consume glucose after lengthy cultivations on glucose due to activation of minor hexose transporters (*HXT8-17*). In order to prevent glucose consumption of Y_{X1}, we deleted hexokinases (*HXK1-2*) and glucokinase (*GLK1*) which are responsible for glucose phosphorylation so that glucose consumption can be completely eliminated even after the activation of minor hexose transporters. Indeed, Y_{X2} (*HXK1-2* and *GLK1* deleted Y_{X1}) 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 sugars were 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}. As a result, the glucose and xylose uptake rates of Y_{G2} and Y_{X3} were comparable. Lastly, monitoring the populations of Y_{G2} and Y_{X3} during cultivation was difficult. As such, fluorescence proteins (GFP and RFP) were expressed in Y_{G2} and Y_{X3} to monitor each population using a real-time fluorescence image of the consortium. The resulting Y_{G2f} and Y_{X3f} were employed for studying the division of labor during fermentation of glucose and xylose via DOL.

In summary, we confirmed that the inoculation ratio and controlled sugar consumption rate of Y_{G2} and Y_{X3} can be used as tuning parameters to optimize simultaneous consumption of glucose and xylose at various concentrations. Our results demonstrate that the division of labor in ecosystems could be applied to consume mixed sugars and develop biochemical processes for efficient conversion of mixed sugars into value-added products.