Investigation of the Functional Role of Aldose 1- Epimerase in Engineered Cellulose Utilization

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**Fermentation assays of the AEP overexpressing strains**

Cellobiose-utilizing BY4741 strain was constructed by introduction of the pRS425-gh1-1-cdt1 plasmid. pRS423-AEP plasmids were introduced to establish AEP overexpressing strains. Resultant yeast cells were grown in CSM medium containing 20 g/L of glucose to prepare inoculums for cellobiose fermentation. Cells at the mid-exponential phase were harvested and inoculated after washing twice by sterilized water. All of the flask fermentation experiments were performed using 50 mL of YP medium containing 40 g/L of cellobiose in 250 mL flask at 30 °C with initial OD$_{600}$ of ~ 1.0 and under oxygen limited conditions.

Cell growth was monitored by optical density (OD) at 600 nm using UV-visible Spectrophotometer (Biomate 5, Thermo, Rochester, NY). Sugars and ethanol concentrations were determined using Shimadzu HPLC equipped with a Bio-Rad HPX-87H column (Bio-Rad Laboratories, Hercules, CA) and Shimadzu RID-10A refractive index detector following the manufacturer’s protocol. The HPX-87H column was kept at 65 °C using a Shimadzu CTO-20AC column oven. 0.5mM sulfuric acid solution was used as a mobile phase at a constant flow rate of 0.6 mL/min.

**Enzymatic assay of the AEP overexpressing strains**

Cell cultures were grown in culture tubes filled with 5 mL YP medium supplemented with cellobiose. The cells were grown at 30 °C at 250 rpm for 48 hours, and then resuspended in Y-PER Extraction Reagent (Thermo Scientific, Rockford, IL). Supernatants were then collected for measurement of protein concentration and AEP activity. To determine the total protein concentration, BCA Protein Assay Reagent (Thermo Scientific, Rockford, IL) was used. A Synergy 2 Multi-Mode Microplate Reader was used to measure the absorbance change at OD$_{580}$. 
Total protein concentration was calculated following the manufacturer’s instructions. To
determine the AEP activity, the conversion between α-glucose and β-glucose is coupled to the
oxidation of β-glucose catalyzed by β-D-glucose dehydrogenase and the reduction of NAD\(^+\)). An
assay mixture containing 0.34 mM NAD\(^+\), 0.5 U of β-D-glucose dehydrogenase and 50 mM
Tris/HCl buffer, pH 7.2 was prepared. 820 µL of the mixture was pipetted into a UV cuvette and
then 130 µL AEP-containing solution was added. The reaction was initiated by the addition of
50 µL 166 µM freshly prepared α–glucose, and the increase in absorption at 340 nm was
recorded for 3 minutes.
Figure S1. Comparison of cellobiose fermentation by the cellobiose-utilizing *S. cerevisiae* BY4741 strains overexpressing *GAL10*, *YHR210C*, and *YNR071C*, respectively. Symbols: control (●), *YHR210C* (▲), *YNR071C* (■), and *GAL10* (◆). In all fermentation results, values are the mean of four independent fermentations, and error bars represent the standard deviations.
**Figure S2.** Specific AEP activity of the BY4741 AEP overexpression strains grown on cellobiose. One unit of AEP activity is defined as the amount of enzyme converting 1 µmol of α-glucose to β-glucose in 1 min in addition to the non-enzymatic rate at 22 °C. Values are the mean result of two activity assays, and error bars represent the standard deviations.