System biology approach to determine differences between acetic acid tolerant S. cerevisiae 424A(LNH-ST) – AAR and original S. cerevisiae 424A(LNH-ST) during glucose/xylose fermentation

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Introduction

Bio-ethanol has gained much attention due to its economical benefits as a renewable energy for replacing petroleum, as well as its potential to reduce greenhouse gas emissions. A significant resource for producing renewable biofuels is lignocellulosic biomass. Lignocellulose is composed of cellulose, hemicellulose, and lignin. Several compounds that can inhibit the fermentation step are released from the biomass during pretreatment and hydrolysis, such as furan derivatives, some weak acids, and phenolic compounds. Developing an industrial, robust strain that can be resistant to the inhibitors can improve the ethanol yield and make the bio-ethanol industry more economical.

Acetic acid is one of major inhibitors in lignocellulosic fermentation. Its concentration can range from 1 – 15 g/L depending on the source of biomass. It especially has strong inhibitory effect at pH below 5.0. The acetic acid can enter the cell by diffusion in the form of acetic acid protonated and then dissociates into proton and anion inside the cell which has neutral pH. This results in decreasing the cytosolic pH and the accumulation of anions inside the cells. Eventually it becomes toxic to yeast and inhibits cell function or even leads to apoptosis.

Our lab has genetically engineered a Saccharomyces cerevisiae strain 424A(LNH-ST) that can efficiently co-ferment glucose and xylose. Our previous work has shown that acetic acid under process relevant conditions does not significantly affect glucose fermentation. However xylose utilization is significantly affected, especially at low pH environment (< 5.5) and high acetic acid concentration (> 10 g/L). Therefore, we have developed an acetic acid-resistant yeast strain that is a derived from original 424A (LNH-ST) strain, 424A (LNH-ST) – AAR (acetic acid-resistant). Small-scale fermentation (110 ml YEP containing 120 g/L glucose + 85 g/L xylose + 1.3 g/L galactose, with 10 g/L acetic acid) at starting pH 6.0 has shown that the new strain can utilize more than double of xylose compared to the original strain (64.4% to 22.5%). In this study, a systems biology approach, including transcriptomic and metabolomic analyses, were completed to understand gene expression and metabolic fluxes in this improved strain as compared to the original strain.

Material and Methods

Yeast strains: – S. cerevisiae 424A (LNH-ST) – AAR (acetic acid-resistant), developed and funded by US Department of Energy Biomass Program, Contract DE-FC02-07ER16017.

Fermentation: – Medium: 110 ml YEP + 120 g/L glucose + 85 g/L xylose + 13 g/L galactose + 10 g/L acetic acid, adjusted with ammonium hydroxide to pH 6.

Microaerobic fermentation at 28 °C with 200 rpm in 300 ml side-arm flask.

Fermentation Profil

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Fermentation Profil

Results and Discussion

The new strain Saccharomyces cerevisiae 424A(LNH-ST) – AAR can ferment xylose more efficiently, both in total xylose consumed and xylose consumption rate, compared to the original strain Saccharomyces cerevisiae 424A during glucose/xylose co-fermentation. The ethanol yield is also higher when the new strain is used. However, in both strains, galactose is not utilized (data not shown) under our experimental conditions (Figure 1). Transcriptomics: – Relative gene expression level of 424A (LNH-ST) – AAR compared to 424A (LNH-ST) from the six stages during fermentation are analyzed, and the genes are categorized according to their functions. Most of these up- or down-regulated genes are still unknown proteins or putative proteins. Also, many genes that are involved in metabolism or transport and in steroid/terp/acid biosynthesis are also up- or down-regulated in 424A (LNH-ST) – AAR. (Table 1)

Microarray analysis: – Relative gene expression level of 424A (LNH-ST) – AAR compared to 424A (LNH-ST) from the six stages during fermentation are analyzed, and the genes are categorized according to their functions. Most of these up- or down-regulated genes are still unknown proteins or putative proteins. Also, many genes that are involved in metabolism or transport and in steroid/terp/acid biosynthesis are also up- or down-regulated in 424A (LNH-ST) – AAR. (Table 1)

Microarray signal intensities of various genes that are involved in glycolysis and pentose phosphate pathway from 424A (LNH-ST) – AAR and 424A (LNH-ST) – AAR are compared in this figure. Results from stage 1 to 6 during fermentation are shown. As expected, most of the genes analyzed are not changed significantly. However, a few genes, such as GND2, TKL2, ADH2, and PDC6 seem to have expression higher level in the original strain. On the contrary, HKGL2, GMP3, and RPS1 are shown having higher expression level in the acetic acid-resistant strain.

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