ANALYSIS OF THE EXPRESSION OF THE GENES ENCODING SOME OF THE MAJOR ENZYMES INVOLVED IN COFERMENTING GLUCOSE AND XYLOSE TO ETHANOL

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Introduction

Recent studies have proven ethanol to be an ideal liquid fuel for transportation, using renewable sugars from the hydrolysis of cellulosic biomass (such as corn stover, rice straw, sugarcane bagasse, corn fiber, softwood, hardwood, grasses, etc.) are D-glucose and D-xylose. The efficient fermentation of such xylose-rich hemicellulose hydrolysates would significantly improve the economics of large-scale fuel ethanol production from lignocellulosic feedstocks. The challenge now is to develop the most effective microorganisms to convert this widely available cellulosic feedstock to ethanol.

Microorganisms that are capable of fermenting a mixture of xylose and glucose have proven to be safe, effective and user-friendly microorganisms for large-scale production of industrial ethanol from glucose-based feedstocks. However, these yeasts cannot metabolize xylose. Our group has succeeded in developing genetically engineered yeasts, such as Saccharomyces cerevisiae 424A(LNH-ST) containing multiple genes (TDH2, TDH3, ENO2, PFK1, PYK1, G3PDH, ADH2, PGK, GPM1) that encode enzymes involved in xylose transport and metabolism. These engineered yeasts have proven to be highly improved in terms of their capability to co-ferment glucose and xylose. We hope to achieve our goal through enhancing the expression of some of the glycolytic genes and improving xylose transport.

Material and Methods

• Strains - Genetically engineered Saccharomyces cerevisiae 424A(LNH-ST) containing multiple copies of a heterologous xylose transport gene (XDG), and a xylose import gene (XD), and xylulokinase gene (XK) stably integrated into the host chromosome.
• Experimental conditions - Co-fermentation of glucose/xylose mixture by S. cerevisiae 424A(LNH-ST); Samples for preparation of total RNA were taken at 0, 2.5, 5, 10, 15, 25 hours during fermentation at 30°C under complete aeration.
• DNA array – GeneChip Yeast Genome S98 Array (Affymetrix, Inc).
• Software – GeneSpring (Silicon Genetics).

Result and Conclusion

Microarrays generate huge datasets and require special methods for organization, presentation, and analysis. One of the methods is to perform multidimensional scaling and clustering. Figure 1 shows a scatter plot of the expression profile of genes involved in glycolysis and glucose/xylose transport during glucose-xylose co-fermentation with S. cerevisiae 424A(LNH-ST). The results demonstrate that the genes involved in the glycolysis pathway were highly expressed during glucose fermentation and their expression decreased during xylose fermentation. However, the genes involved in glucose/xylose transport were highly expressed during xylose fermentation and their expression decreased during glucose fermentation. This indicates that our engineered yeast strain is capable of co-fermenting glucose and xylose.

We believe that carefully and thoroughly analyzing the co-fermentation of glucose and xylose will help us identify the genes involved in the regulation of the co-fermentation process. For example, the expression of the gene encoding the xylose transport protein HXT5 was increased during the co-fermentation of glucose and xylose.

Figure 1. Scatter plot of the expression profile of genes involved in glycolysis and glucose/xylose transport during glucose-xylose co-fermentation with S. cerevisiae 424A(LNH-ST).

Figure 2. K-means clustering by using Pearson correlation to group together expression patterns of genes.

Figure 3. Number plot of the expression profile of genes encoding some of the major enzymes involved in glycolysis.

Figure 4. Scatter plot of the expression profile of genes encoding some of the major enzymes involved in glycolysis.

Figure 5. The expression profile of the genes in upper part of glycolysis during co-fermentation of glucose/xylose by S. cerevisiae 424A(LNH-ST).

Figure 6. The expression profile of the genes in lower part of glycolysis during co-fermentation of glucose/xylose by S. cerevisiae 424A(LNH-ST).

Figure 7. The expression profile of the genes encoding some of the major enzymes involved in glycolysis.

Figure 8. The specific activities of ADH, PDC, PGK, PYK, G3PDH enzymes during fermentation of mixture glucose/xylose by S. cerevisiae 424A(LNH-ST).

Figure 9. The expression profile of genes encoding some of the major enzymes involved in glycolysis.