# Metabolic Pathway Recruiting through Genomic Data Analysis for Industrial Application of *Saccharomyces cerevisiae*

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**Abstract**

In this paper, present status and result of a project for a process development based on genomics going on in our groups is explained. Goals is to build object-oriented metabolic pathway models which describe metabolic flux change due to the cultivation conditions changesuch as from normal one to high osmotic pressure (sugar concentration), and also to apply the model for effectively designing an improved strain producing useful compounds which can play important roles for green chemistry. The model will be developed using the data of transcriptome, proteome and metabolites, including metabolic flux analysis online NMR analysis. Practically, profiling data of transcriptome and proteome have been taken at high osmolarity condition. And a better-improved strain based on the analysis was obtained.

"@As a model case, we have been focused on the molecular breeding for high osmotic pressure tolerant strain. Both strains of S. cerevisiae, laboratory and brewing strains, were cultivated in YPD medium and 1 or 0.5 M of NaCl was added to the culture medium 5 hours after the start of the cultivation. Then the gene expression data before and after the addition of NaCl using DNA microarray in each strain were compared. The degree of change of the gene expression level of the laboratory strain was larger than that of the brewing one. Response of the gene expression for the 0.5 M of NaCl stress was faster than that for the 1 M stress.

These up-regulated genes are able to characterize osmotic stress-tolerance of the brewing strain.

Judging from the results of DNA microarray experiments under high osmotic pressure condition, biosynthetic pathways for glycerol, trehalose and acetate were transcriptionally responded to high osmotic pressure in S. cerevisiae. Furthermore, glycerol is well known as one of the protecting agent for high osmotic pressure in yeast. Thus, we first try to construct a strain of S. cerevisiae whose glycerol synthetic pathway is improved, that is, a glycerol overproducing strain. For construction glycerol over producing strain, we cloned by PCR the GPD1 gene from the FY834 strain into the downstream of ADH1 promoter on the vector plasmid pAUR123, which is used for constitutive expression and then introduced the resulting plasmid, named pGPD1-4, into the laboratory strain of S. cerevisiae FY834.

First, we observed the growth of two strains, FY834/pAUR123 (vector) and FY834/pGPD1-4 (GPD1-overexpressing) under the condition for 1 M of NaCl addition. In the FY834/pAUR123 strain, length of the lag of growth after NaCl addition was 4 hours. On the other hand, in the FY834/pGPD1-4 strain, the lag was 3 hours, decreased 1 hour by expressing the GPD1 gene constitutively. It means that the target gene found by transcriptome analysis, is partially responsible for osmotic pressure tolerant. However, complete recovery of growth was not observed by expression of GPD1 gene.

More information from data mining of the gene expression data cooped with proteome will be integrated to modeling of the metabolic pathway for describing the high osmotic pressure characteristics. And the approach will be extended to strain improvement method for high ethanol concentration tolerant strain. Finally the methodologies of cyclic approach to get a metabolic pathway modeling based on genomics will be validated through this practical example.