

## Identification of Target Genes Conferring Ethanol Stress Tolerance to *Saccharomyces cerevisiae* Based on DNA Microarray Data Analysis

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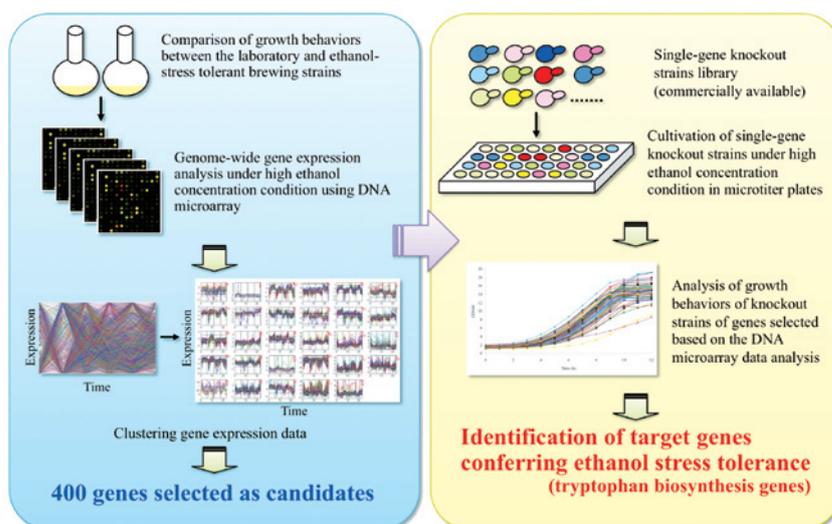
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The yeast *Saccharomyces cerevisiae* has been used in fermentation or brewing industries and fuel ethanol production from biomass resources such as cellulose and starch. Yeast cells are usually exposed to some environmental changes during the course of production process, such as increase in osmotic pressure or accumulation of ethanol and/or carbon dioxide. Ethanol is one of the main stress factors for yeast cells in industrial production process using yeast, yeast strains that achieve high growth activity under ethanol stress condition are highly desired.

To identify the target gene(s) for constructing ethanol stress tolerant yeast strains, we obtained the whole gene expression profiles of two strains of *S. cerevisiae*, namely, a laboratory strain and a strain used for brewing Japanese rice wine (sake), in the presence of ethanol, using DNA microarray. For the selection of target genes for breeding ethanol stress tolerant strains, clustering of DNA microarray data was performed. Clustering analysis was performed using integration method of batch-learning self-organizing map with hierarchical clustering. For further selection, the ethanol sensitivity of the knockout mutants in each of which the gene selected by DNA microarray analysis is deleted, was also investigated. The DNA microarray data and the ethanol sensitivity data of knockout strains suggests that tryptophan biosynthesis might be related to the response to ethanol stress in *S. cerevisiae* and the enhancement of expres-



sion of genes related to tryptophan biosynthesis was expected to confer the ethanol stress tolerance to yeast cells. Indeed, overexpression of tryptophan biosynthesis genes, addition of tryptophan to the culture medium, and overexpression of tryptophan permease gene showed a

stress tolerance to 5% ethanol. Our methodology for the selection of target genes for constructing ethanol stress tolerant strains, based on the data of DNA microarray analysis and phenotypes of knockout mutants, was validated.

## Fabrication of Cellular Multilayers with Nanometer-Sized Extracellular Matrix Films

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Tissues and organs consist of a complex, closely balanced assembly of different types of cells, extracellular matrix (ECM), and special signaling molecules. The creation of such structures in the laboratory, perhaps for transplantation into patients, has remained an unmet challenge. We have successfully fabricated multilayer architectures from layers of cells and nanometer-sized ECM films (Figure a). A nanometer-thick film made of fibronectin and gelatin was produced covered the layer of cells by layer-by-layer assembly technique. It was seen that thin 6 nm of fibronectin/gelatin film can act as a suitable cell adhesive surface similar to the natural ECM. Another layer of cells could then be placed onto this film. We were thus able to produce a structure with a total of four layers of cells. This layered structure was so stable that it could be removed from its support without any damage at all.

By using the same method, the five-layered architecture of blood vessels consist of human endothelial cell and smooth muscle cell layers was reproduced (Figure b). Building on the foundation of our technique, it should be possible to grow artificial tissues, such as human blood vessels, skins, and skeletal muscle tissues, in the lab.

