Title	Analysis of adaptation to high ethanol concentration in Saccharomyces cerevisiae
Author(s)	Dinh, Nho Thai
Citation	大阪大学, 2009, 博士論文
Version Type	VoR
URL	https://hdl.handle.net/11094/27624
rights	
Note	

Doctor Thesis

Analysis of adaptation to high ethanol concentration in *Saccharomyces cerevisiae*

Thai Nho Dinh

Division of Advanced Science and Biotechnology
Graduate School of Engineering
OSAKA UNIVERSITY

2009

Doctor Thesis

Analysis of adaptation to high ethanol concentration in *Saccharomyces cerevisiae*

（出芽酵母 *Saccharomyces cerevisiae* の高エタノール濃度に対する適応現象の解析）

Thai Nho Dinh

Division of Advanced Science and Biotechnology
Graduate School of Engineering
OSAKA UNIVERSITY

2009

Contents of thesis

Page

Chapter 1 1

General introduction

Chapter 3

Chapter 1

General introduction

In recent years, due to the increasing cost of the non-renewable fossil fuels and the increasing concern of global warming, many governments have tried to find new energy sources. One of the promising sources is bio-ethanol, which can be easily used as an alternative energy for transportations, heating and industrial processes [1]. With a natural ability to efficiently convert sugars to ethanol, yeast has been paid attentions in the field of industrial application, particularly, in the production of bioethanol.

The budding yeast, *Saccharomyces cerevisiae* has been used for fermentation processes from several thousand years before [2]. At present, it has become an important species in the industrial applications, such as, brewing, winemaking, baking, and recently, fuel ethanol production. During these production processes, yeast cells are exposed to many stresses, such as osmotic, oxidative, and heat stresses. As a consequence, the production of target product(s) is reduced. Therefore, many researches have been studying the stress response of yeast in order to improve its stress tolerance ability [3-9]. Among these many stresses, the accumulation of high ethanol concentration has been considered as one of the most harmful stresses in the industrial production process using yeast. To study the effect of stresses on the yeast cell physiology and the response of yeast cells to the stresses, many researches have been performed [7, 10-16].

For surviving (or growing) under the stress condition(s), organism has to adapt to the environmental changes by changing its intracellular physiological state. Adaptation is an alteration or change in the structure or functioning of an organism that makes it to become fit to its environment. In general, the adaptation process is related to time. The "short-term" adaptation is defined as "quick" response of cells to stress in within one or several hours after encountering the stress. At this time, cells change their internal condition in order to recover their growth and reproduction. For a longer time such as several days or tens of days after exposing cells to stress, it is referred as the "long-term" adaptation.

For the "short-term" ethanol response, many studies were carried out [7, 10- 12, 17]. The stress was introduced into the culture of yeast and evaluation of dynamic change in intracellular state of yeast cells was performed. In the "short-term" response to stress, it was reported that there was a large changes in the intracellular state of cells, for example, the activation of transportation and certain amino acid biosynthesis pathways and the down-regulation of genes related to protein synthesis. However, after several hours after the addition of ethanol, a certain fraction of these genes recover their original expression levels. Such genes that recover the original expression levels can be regarded as those that are only responsible for the quick stress response. On the other hand, there were very few reports related to the studies on the "long-term" ethanol adaptation of yeast [18, 19].

1.1 Overview of environmental stresses occurred during the fermentation processes of *Saccharomyces cerevisiae*

Several environmental stresses occur during the industrial production processes using yeasts. At the beginning of production, a high gravity of sugars and nutrients causes osmotic stress. Usually for propagation and growth of yeast, the aeration of oxygen is performed and oxidative stress concomitantly affects yeast cells. Then the aeration is switched off in order to achieve anaerobic condition for production. Cultural environment drastically changes during this production process, such as, changing the pH, the exhaust of nutrient, the starvation stress and the ethanol accumulated to high concentration [3, 4].

As described above, yeast cells face many stresses during fermentation process [3, 4]. Yeast cells adaptively respond to these stresses by regulating two major stress response pathways: the general stress response (GSR) and heat shock response (HSR) pathways. It have been reported that GSR is induced by wide variety of stresses caused by heat, osmotic pressure, oxidative damage, change in pH and nitrogen starvation [20, 21, 22]. Using DNA microarray, more than 200 genes were found to be related to GSR [13, 14]. The promoter region of these GSR genes contains the pentameric *cis*-acting sequence CCCCT called stress responsive element (STRE) [21, 22]. When encountering the stress, transcriptional factors (Msn2p/Msn4p) bind to STRE region and induce the expression of ESR gene. However, as reported by Gasch *et al.*, gene expression via GSR pathway is a transient process [13], and Msn2p is degraded following the stress response progression [24].

HSR pathway is activated when cells are exposed to sublethal heat shock. This pathway requires the activation of a specific heat-shock transcription factor (Hsf1p), which binds to the conserved heat shock element (HSE) in the promoter region of the heat shock protein genes (HSPs). The heat shock element (HSE) consists of contiguous inverted repeats of the sequence 5'-nGAAn-3' (where n is any nucleotide) [25]. In nonstressed cells, Hsf1p is constitutively phosphorylated and binds to the conserved heat shock element (HSE). Under heat stress, Hsf1p becomes hyperphosphorylated and adopts an activated conformation resulting in the transcription of target genes [26]. Using DNA microarray, hundreds of genes were found to be related to HSR pathway, these genes are involved in protein folding, detoxification, energy generation, carbohydrate metabolism, and cell wall organization [27].

1.2 Ethanol toxicity on yeast cells

It is well known that during the fermentation process involving yeast, ethanol is accumulated to high concentration. It has been reported that ethanol affects some phenomena of the yeast cells; for example, the inhibition of growth [6, 9, 19], reduced fermentation ability [28], change in morphology [29, 30], increased frequency of petite mutation resulting in respiratory deficiency [32-35]. In addition, ethanol also changes the membrane fluidity, permeability of the yeast cells [36, 37]. The effect of ethanol on the lipid composition of cell membrane will be presented below.

1.2.1 Effect of ethanol toxicity on the lipid composition of plasma membrane

Plasma membrane surrounds the cell and its organelles, separate the cell from the outside environment. Biological membranes consist of a continuous double layer of lipid molecules in which membrane proteins are embedded. Membrane lipids are amphipathic molecules, that is, they have a hydrophilic ("water-loving") or polar end and a hydrophobic ("water-fearing") or nonpolar end. There are three major classes of membrane lipid molecules: phospholipids, cholesterol, and glycolipids [38]. The lipid bilayer is a two-dimensional fluid. The fluidity of a lipid bilayer depends on its composition. The most abundant membrane lipids are the phospholipids (PL). PL has a polar head group and two hydrophobic hydrocarbon tails. The tails are usually fatty acids, and they can differ in length. One tail usually has a kink because of a cis-double bond (i.e., it is unsaturated), while the other tail does not (i.e., it is saturated) [38]. Unsaturated fatty acid has one or more cis-double bonds. This kink structure in the tail of fatty acid preventing the fatty acids from packing together as tightly, thus decreasing the melting point (increasing the fluidity) of the membrane. When the membrane enriched with unsaturated fatty acid, the fluidity of membrane is increased. By comparing the plasma membranes of highly ethanol tolerant yeasts to less tolerant yeast, Chen revealed that, in every case, ethanol tolerant yeasts contain higher percentage of unsaturated fatty acids [39].

Many studies have reported that a primary target of ethanol toxicity is cell membrane surrounding the cell and its organelles [7, 8, 40-45]. Possibly, when ethanol appears in the medium, it enters into the hydrophobic interior region of plasma membrane. Then it increases the polarity of this hydrophobic region and induces a weak interaction with the component of plasma membrane [41], as consequence, ethanol affects to reduce the fluidity and permeability of cell membrane.

Yeast adaptively responds to ethanol by changing the structure of plasma membrane and increasing un-saturated fatty acid in lipid composition of cell membrane [7, 8, 38, 43-45]. The fraction of palmitoleic acid ($C_{16:1}$) and oleic acid ($C_{18:1}$) is increased with a reduced fraction of palmitic acid ($C_{16:0}$) and stearic acid ($C_{18:0}$), when ethanol is added into cultural medium [45, 46]. The importance of unsaturated fatty acid in improvement of ethanol tolerance was reported by Kajiwara *et al.* [47]. The ethanol tolerance ability of yeast was improved by modification of composition of membrane lipid through genetic engineering and unsaturated fatty acid supplementation, and You *et al.* found that oleic acid is one of the determinants of ethanol tolerance of yeast [48].

1.2.2 Effect of ethanol toxicity on proteins and biological function of yeast cells

Ethanol also affects the transport of proteins in the plasma membrane, resulting in negative impact on their function, for example, decreased respiration and nutrient uptake through cellular membrane [49, 50]. Previous studies have shown that ethanol inhibits the activity of key glycolytic enzymes and denatures proteins [6, 50]. In the presence of ethanol, yeast cells exhibit an increase in plasma membrane H^+-ATPase activity [11, 44, 51]. Moreover, the importance of H^+-ATPase in ethanol tolerance was reported by Fujita *et al.,* who found that homozygous diploid deletion mutants lacking genes involved in H^+-ATPase activity were sensitive to ethanol [52].

1.3 Ethanol adaptation and ethanol tolerance of *S. cerevisiae*

Two terms of “ethanol adaptation” and “ethanol tolerance” sometimes are used for the similar meaning. Indeed, the term of “ethanol adaptation” is used when referring to “an alteration or change process in the structure or functioning of an organism that makes it becomes fitted to ethanol stress in its environment”. And the term of “ethanol tolerance" is used when referring to the "ability" of an organism to survive in extreme ethanol stress condition. Through the ethanol adaptation process, to know whether yeast cells can adapt to the extreme ethanol stress environment or not, ethanol tolerance ability of yeast strain was evaluated.

1.3.1 Ethanol tolerance in *Saccharomyces cerevisiae*

Ethanol is a major stress factor that decreases the production of target products in the fermentation processes using yeast cells. To improve the production processes, the response to ethanol stress and the mechanism underlying ethanol tolerance of yeast cells have been widely investigated. Many studies have reported that a primary target of ethanol toxicity is cell membrane: Alterations in the fatty acid composition of the cell membrane affect the ethanol tolerance of yeast and unsaturated fatty acids result in an increase in membrane fluidity and an enhancement of ethanol tolerance [8, 41, 42, 43, 47, 48]. Piper reported that heat shock proteins (HSPs) are synthesised during ethanol stress [53]. Of many heat shock proteins in yeast genome, however, only Hsp104p [54, 55] and Hsp12p [56] are found to be involved in enhancing ethanol tolerant ability of yeast cells. Trehalose, which has been reported to stabilize proteins, are synthesised and accumulated upon ethanol stress [57]. The accumulation of trehalose is a critical determinant of stress resistance in *S. cerevisiae*. It was found that two enzymes are capable of hydrolyzing trehalose: a neutral cytosolic trehalase (NTH) and an acidic vacuolar trehalase (ATH) [5758]. Kim *et al.* reported that increasing intracellular accumulation of trehalose by deletion of *ATH1* improved ethanol stress tolerance [59]. And ethanol stress tolerance of yeast strain is also improved when using the antisense-

RNA technique to decrease the expression of acid trehalase gene *ATH1* [60]. Kajiwara *et al.* found that increases in fatty acid unsaturation via metabolic engineering improved ethanol tolerance of *S. cerevisiae* [47]. Takagi *et al.* reported that enhancement of intracellular proline accumulation improved ethanol tolerance of yeast used for Japanese rice wine [15]. Ethanol tolerance and production of *S. cerevisiae* is also improved by engineering yeast transcription machinery [61]. More recently, Hirasawa *et al.* (2007) has investigated that over-expression of genes related to tryptophan biosynthesis conferred the ethanol stress tolerance to yeast cells [17].

1.3.2 Analysis for understanding adaptation process to high ethanol concentration

As for the "long-term" ethanol adaptation, Ismail and Ali reported that no increase in the tolerance of yeast to a high ethanol concentration was observed after ten successive transfers to an environment containing a high ethanol concentration [18]. In this study, since the environmental condition was suddenly changed to a severe condition, it is thought that the yeast cells could not adapt to the changed environment. Lloyd *et al.* found that yeast previously grown in the presence of 5% ethanol can grow in the medium containing 10% ethanol, whereas yeast inoculated directly into medium containing 10% ethanol fails to grow [19]. Therefore, it is expected that exposing yeast cells to a stepwise increase in the level of ethanol stress from low to high concentration with the repetitive cultivations would be effective in observing the ethanol adaptation process.

In this study, the stepwise adaptation process of *S. cerevisiae* was investigated by carrying out a stepwise increase in ethanol concentration with repetitive cultivations. Evaluation of ethanol tolerance ability of yeast strain was conducted by measuring the specific growth rate (SGR) of yeast strain cultured in medium contained high ethanol concentration. When subjecting yeast cells to the stepwise increase ethanol, the SGR of the ethanol-adapted strain did not change through repetitive cultivation under high ethanol concentration, suggesting that the ethanol-adapted strains, whose SGR is not changed through repetitive cultivation at high ethanol concentrations, could be successfully obtained.

1.4. Study on ethanol adaptation in *S. cerevisiae* using comprehensive analysis systems

We have many tools and methods to study about the entire biological aspects of many organisms. *S. cerevisiae* genome project was completed in 1996 [62], and based on its genome information, comprehensive analysis systems have been developed. These systems enable us to conduct large-scale quantitative experiments on studies about biological properties of yeast cells in molecular levels, such as in the genome (i.e. genome-wide analysis [30, 52, 63, 64]), transcriptome (i.e. using DNA microarray [11-14, 65-68]), proteome (i.e. two-dimensional gel electrophoresis combined with the identification of proteins by peptide mass fingerprinting method [16, 69-72]), and metabolome (measurement of intracellular metabolites by mass spectrometry [73, 74]).

Because these systems are powerful for comprehensive analysis of the cellular state, they have been successfully used to study about the response of yeast cells to stress.

The principle of transcriptome (DNA microarray) and proteome (2-DE) methods I used in my study were summarized in Figure 1-1 and Figure 1-2.

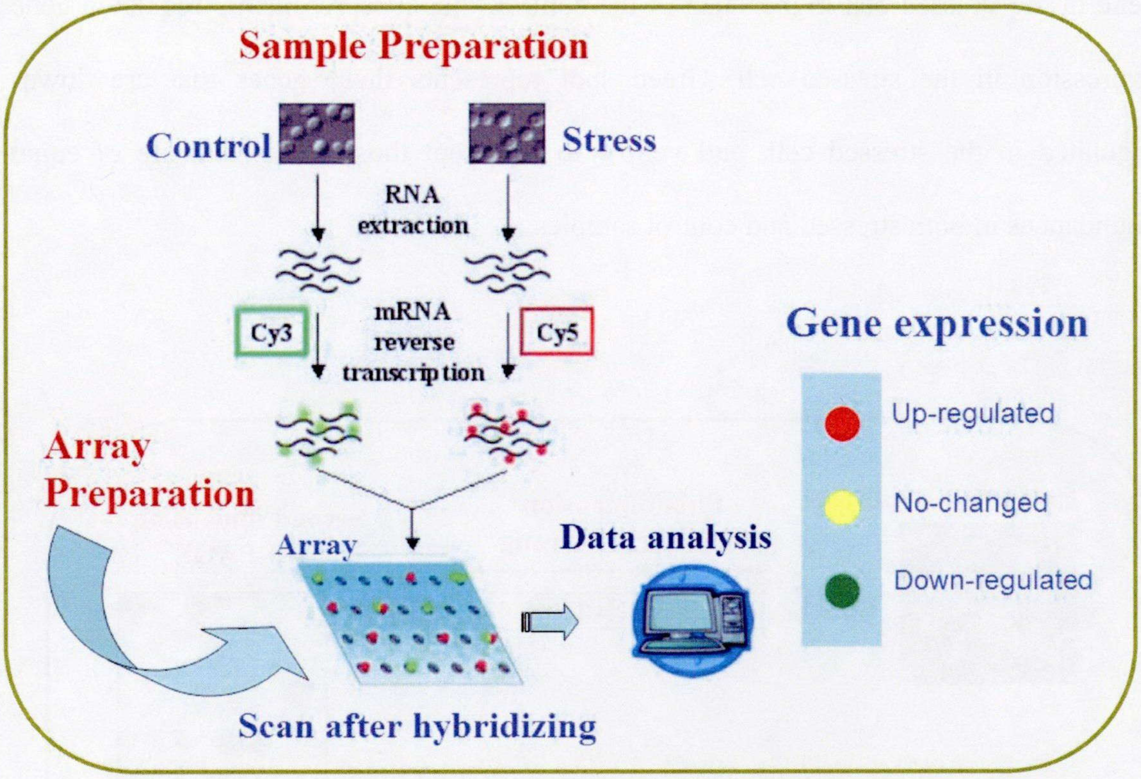


Figure 1-1 Principle of DNA microarray.

The principle of DNA microarray technology is based on the fact that complementary sequences of DNA can be used to hybridize immobilized DNA molecules. This involves three major multi-stage steps: **(1) Array preparation:** This step involves the availability of a chip or a glass slide with its special surface chemistry, robotics is used for producing microarrays by spotting the DNA (targets) onto the chip or for their *in situ* synthesis. (2) **Sample preparation and array hybridization step:** This step involves mRNA isolation followed by fluorescent labelling of cDNA probes and hybridisation of the sample to the immobilized target DNA. (3) **Image acquisition and data analysis:** Finally, this step involves microarray scanning, and image analysis using sophisticated software programs that allows us to quantify and interpret the data. As shown in Figure 1-1, red spots on the microarray shows that amount of cDNA of this gene in the stressed cell higher than of the control, thus it represents up-regulated gene expression in the stressed cell. Green spot represents those genes that are down-regulated in the stressed cell, and yellow to represent those genes that are of equal abundances in both stressed and control samples.

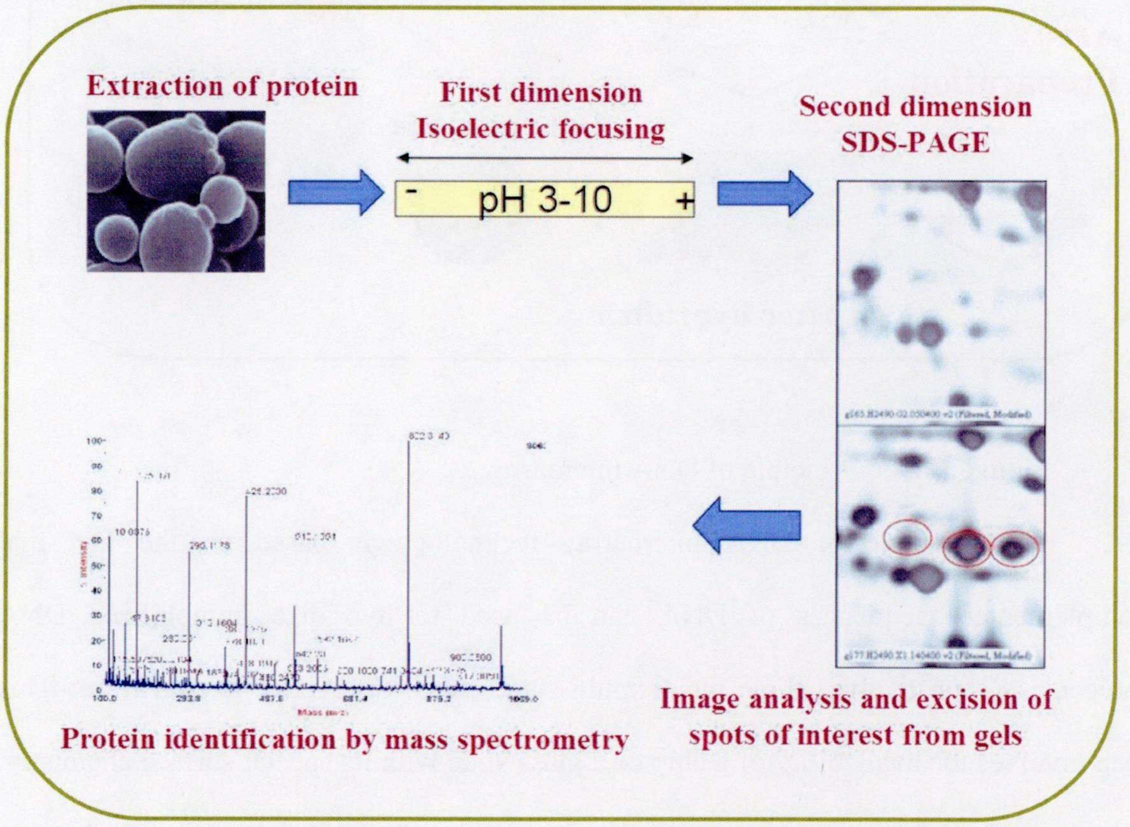


Figure 1-2 The principle of proteome analysis by 2-DE gels.

Proteins, extracted from the cells, are first separated according to their isoelectric points (the first dimension) and subsequently separated according to their molecular weights in SDS-PAGE (the second dimension). Protein spot patterns from different samples are compared and quantified and the protein spots of interest are identified by mass spectrometric methods.

In the viewpoint of transcription level, Alexandre *et al.* (2001) reported the global gene expression of *S. cerevisiae* S288C when adding 7% ethanol to the culture medium at mid-log phase. The expression data was obtained within 30 min after the addition of ethanol, corresponding to the analysis "short-term" response to stress. Yeast cell altered the expression levels of 395 open reading frames (ORFs) in response to ethanol stress. Most of the down-regulated genes are involved in protein biosynthesis, cell growth, RNA metabolism and cellular biogenesis. Genes that are up-regulated by ethanol are mainly involved in energetic metabolism, protein destination, ionic homeostasis, heat shock proteins and the stress response [11]. A consistent result was also reported by Chandler *et al.* 2004 [12]. Since the authors could not identify any induction of genes involved in lipid metabolism or cell wall biogenesis, which are known targets of the ethanol stress response, in these studies about the "short-term" response, it is possible that these changes might be related the "long-term" ethanol adaptation [11].

At present, however, there are no reports on the analysis of "long-term" ethanol adaptation process of yeast using comprehensive analysis systems. The studies of the long-term adaptation process using comprehensive analysis systems are expected to provide us a basis to understand the properties of biological systems that enable growth under ethanol condition.

1.5 Objective of this study

The objective of this study is to understand the adaptation process to high ethanol concentration in *S. cerevisiae*. For this objective, I first performed repetitive cultivation of yeast with stepwise increase in ethanol concentration. Through this repetitive cultivation, I succeeded in obtaining ethanol-adapted yeast strains. To examine the physiological changes in ethanol-adapted strains, cell morphology and fatty acid composition of the obtained ethanol-adapted strains were observed. Moreover, to observe genome-wide physiological changes in transcription and translation in ethanol-adapted strains, transcriptome and proteome analysis of the ethanol-adapted strain using DNA microarray and two-dimensional gel electrophoresis of intracellular proteins, respectively, were conducted. Through this study, I discussed the properties of "long-term" adaptation process to high ethanol concentration in *S. cerevisiae* and application for conferring ethanol torelance to *S. cerevisiae* cells.

1.6 Overview of the thesis

This thesis consists of five chapters.

Chapter 1 deals with the overview of fermentation and ethanol adaptation of yeast. This chapter presents the significance of this work. The "short-term" adaptation to ethanol has been numerously reported and well studied, however the "long-term" adaptation of yeast to high ethanol is not. This work aims to establish the extent understanding of the "long-term" adaptation of yeast to high ethanol concentration.

Chapter 2 deals with the investigatation of adaptation process of *S. cerevisiae* cells to high ethanol concentration though the repetitive cultivation with a stepwise increase in ethanol concentration. To compare yeast cells after ethanol adaptation with that before ethanol adaptation, measurement of changes in cell morphology and fatty acid composition was performed.

Chapter 3 deals with the study on ethanol adaptation mechanism of yeast at the transcription level. DNA microarray was used to monitor the genome-wide gene expression profiles of the ethanol adapted yeast and non-adapted yeast under high ethanol concentration. Certain genes and functional categories that involved in adaptation to high ethanol concentration were investigated.

Chapter 4 deals with the study of ethanol adaptation mechanism of yeast at the translation level. Based on the result of two-dimensional gel electrophoresis combined with identification of proteins by using peptide mass fingerprinting method, the correlation between protein abundance and mRNA expression was investigated. Certain proteins that involved in adaptation to ethanol were identified.

Chapter 5 deals with the general conclution of this research.

Chapter 2

Adaptation of *Saccharomyces cerevisiae* cells to high ethanol concentration and changes in fatty acid composition of membrane and cell size

2. 1 Introduction

The yeast *Saccharomyces cerevisiae* has been used for the production of useful chemical compounds as well as alcoholic beverages. In the industrial production of useful target products using yeast cells, cells have faced a variety of the environmental changes, such as an increase in osmotic pressure, the accumulation of ethanol and carbon dioxide and a decrease in the amount of nutrients [3, 4]. Of these environmental changes, the accumulation of ethanol during cultivation causes stress to yeast cells, leading to a decrease in cell growth and the production of target products. Thus, understanding the adaptation process of yeast under high ethanol concentration is important and this might lead to construction of the yeast strains that can grow well in high ethanol concentration and are highly desired in the field of production of useful compounds using cells. As described in Chapter 1, some researchers have analyzed adaptation phenomena of yeast cells to high ethanol concentration. It is expected that exposing yeast cells to a stepwise increase in the level of ethanol stress will be effective for obtaining the ethanol-tolerant yeast strains.

In this chapter, the results of observing the adaptation process of *S. cerevisiae* to a high ethanol concentration were shown. For this purpose, a repetitive cultivation of *S. cerevisiae* with a stepwise increase in ethanol concentration was performed. By exposing to a stepwise increase in ethanol concentration in the culture medium, cell growth activity defined by specific growth rate (SGR) of the yeast cells did not decreased during repetitive cultivation in high-ethanol-concentration medium. This indicates that the yeast cells successfully adapted to high ethanol concentration. In addition, to examine the phenotypic changes during adaptation process to high ethanol concentration, analyses of fatty acid composition of the cell membrane and cell morphology of the ethanol-adapted strains were also performed.

2.2 Materials and methods

2.2.1 Strain, medium and cultivation conditions

S. cerevisiae FY834 (*MATα his3Δ200 ura3-52 leu2Δ1 lys2Δ202 trp1Δ63*) [75] obtained from the yeast genetic resource center (Osaka University, Japan), was used in this study. Yeast cells were precultured in 5 ml of YPD medium (1% Bacto yeast extract, 2% Bacto peptone, 2% glucose) in a test tube at 30 °C for 24 h. Then, for the main culture, the precultured yeast was cultivated aerobically at 30 °C in a Sakaguchi flask containing 100 ml of YPD medium. The cell density for inoculation was set to about 10^6 cells/ml, because the inoculum size affects the length of the lag time of yeast cell growth as reported by Walker-Caorioglio *et al.* [76].

2.2.2 Repetitive cultivation of *S. cerevisiae* with stepwise increase in ethanol concentration

Repetitive cultivation with a stepwise increase in ethanol concentration was performed as follows (Figure 2-1). The cultivation of FY834 strain was carried out in YPD medium containing ethanol and then the culture was transferred to fresh medium containing the same ethanol concentration. After that, the culture was transferred to medium containing a higher ethanol concentration, followed by repetitive cultivations. The initial ethanol concentration was set at 2.5% (v/v) and it was changed to 5, 6.5, 8, 9 and 10% step by step. In cases of cultivations with 2.5 and 5% ethanol in the medium, the transfer of the broth at the end of the previous culture to medium containing the same ethanol concentration was performed twice. In cases of the cultivations containing 6.5, 8, 9 and 10% ethanol, the transfer of the culture was performed once. I performed four series of such repetitive cultivations from 2.5% of ethanol concentration to 10%, and the obtained culture series were called A1, A2, A3, and A4, respectively.

The adaptation of the yeast was evaluated by measuring the optical density of the culture at 660 nm (OD_{660}) and calculating SGR of all the strains examined using OD_{660} data at mid-exponential phase. The SGR is defined as increasing rate of cell concentration (OD_{660}) divided by cell concentration, by calculating the slope of the logarithmic plot of OD_{660} value as described by Blanch and Clark [77]. For measurement of OD_{660}, spectrophotometer U-2000 (Hitachi High Technologies, Japan) was used.

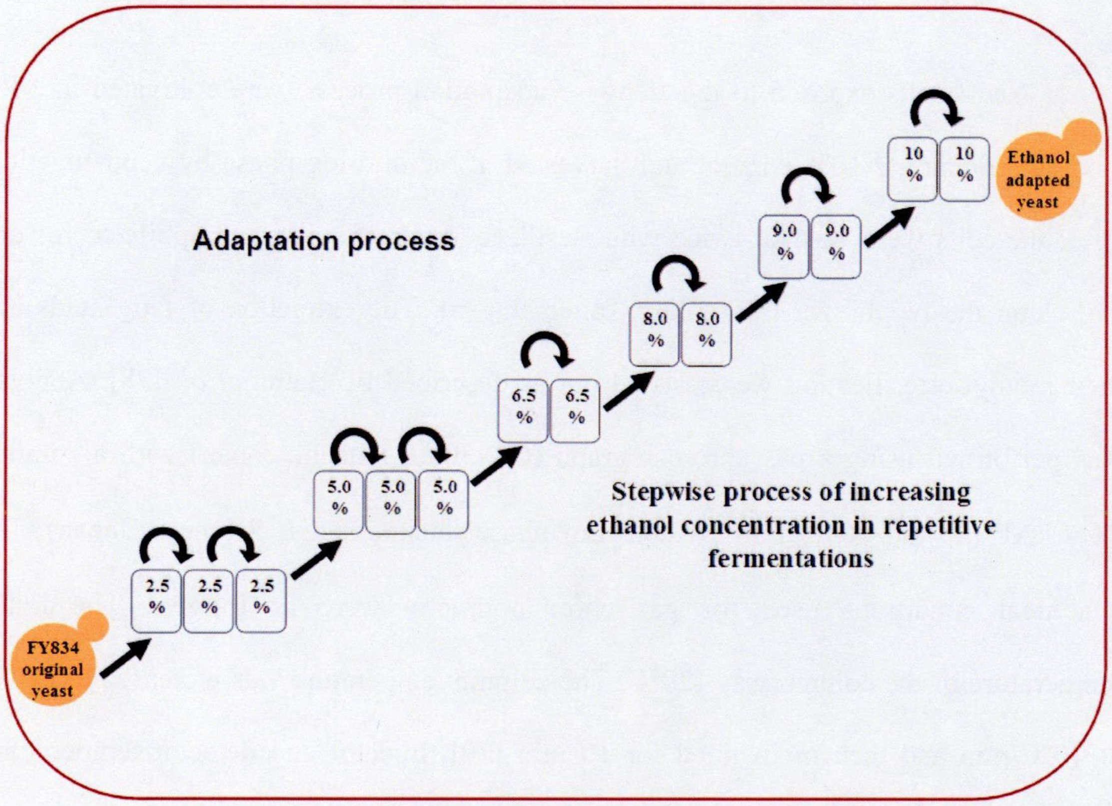


Figure 2-1 Scheme of adaptation process of *S. cerevisiae* to high ethanol concentration.

The cultivation of FY834 strain was carried out in YPD medium containing ethanol and then the culture was transferred to fresh medium containing the same ethanol concentration (It was indicated as ***circular arrow***). After that, the culture was transferred to medium containing a higher ethanol concentration (It was indicated as ***linear arrow***), followed by repetitive cultivations. The initial ethanol concentration was set at 2.5% (v/v) and it was changed to 5, 6.5, 8, 9 and 10% step by step.

2.2.3 Measurement of fatty acid composition of cell membrane

Yeast cells exposed to the stepwise adaptation process were cultivated in YPD medium containing 10% ethanol and harvested at the mid-log phase by centrifugation. After the cells were washed twice with sterilized water, they were lyophilized for one day using the lyophilizer (VD-500F; Taitec, Japan). The extraction of fatty acids and their methyl esterification were carried out as described by Hama *et al.* [78]. Analysis was performed using a gas chromatograph (GC-3000; Hitachi, Japan) with a column, TC-FFAP (30 m × 0.25 mm i.d., 0.5 μm phase thickness; GL Sciences, Japan). The analytical conditions used for gas chromatography were as follows. The initial temperature of the column was 120°C. The column temperature was increased to 240°C at 10°C/min and then maintained for 10 min [79]. Injector and detector temperatures were 250 and 280°C, respectively. Nitrogen gas was used as a carrier. Fatty acid methyl ester mixtures (Sigma-Aldrich Inc, catalog number 18918) were used as lipid standards. The peaks corresponding to each fatty acid methyl ester in the obtained samples were identified comparing their retention times with those of the lipid standards.

2.2.4 Measurement of the size of yeast cells

Yeast cells exposed to the stepwise adaptation process were cultivated in YPD medium and the cells at the mid-log phase (OD_{660}=about 1) was observed using phase contrast microscope (BX60, Olympus). Microphotographs of yeast cells were taken by a digital camera (Camedia C-5060 Wide Zoom, Olympus). For each strain, long and short diameters of mother and daughter cells from 50 cells were measured. For evaluation of the difference in cell sizes between the ethanol-adapted and non-adapted strains, average and standard deviation of long and short diameters of mother and daughter cells, respectively, from 50 cells were calculated.

2.3 Results and Discussion

2.3.1 Effect of ethanol addition on cell growth of *S. cerevisiae*

To evaluate the effect of ethanol on yeast cell growth, a laboratory strain of *S. cerevisiae* FY834 was directly inoculated into YPD medium containing 2.5, 5, 6.5, 8, 9, or 10% (v/v) ethanol, respectively, and then the cells were incubated at 30°C (Figure 2-2A). After that, the culture was transferred to medium with the same ethanol concentration. The cell density for inoculation was set to about 10^6 cells/ml, because the inoculum size affects the length of the lag time of yeast cell growth as reported by Walker-Caorioglio *et al.* [76].

The SGRs during the repetitive cultivation in which the cells were directly inoculated into medium with a high ethanol concentration are summarized in Table 2-1. In the YPD medium, the SGR of FY834 was 0.45±0.04 h^{-1} and decreased as a result of increase in ethanol concentration. The SGR further decreased in the second cultivation, compared with that in the first cultivation in medium with the same ethanol concentration. This phenomenon was clearly observed when ethanol concentration was more than 6.5%, that is, the difference in SGR between the first and second cultivations became large.

Figure 2-2A

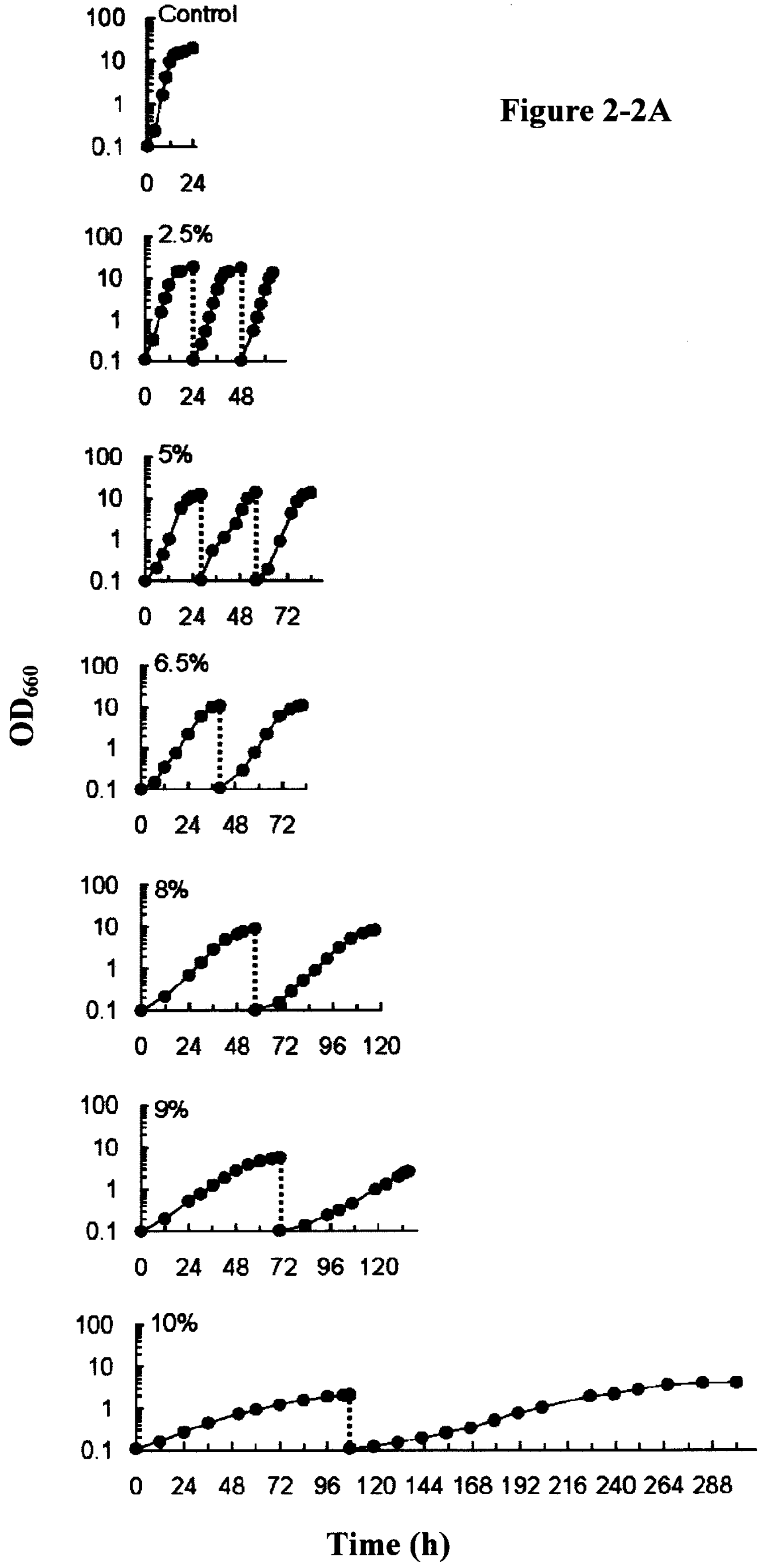

Control
2.5%
5%
6.5%
8%
9%
10%
100
10
1
0.1
0 24 48 72 96 120 144 168 192 216 240 264 288
OD660
Time (h)

Figure 2-2B

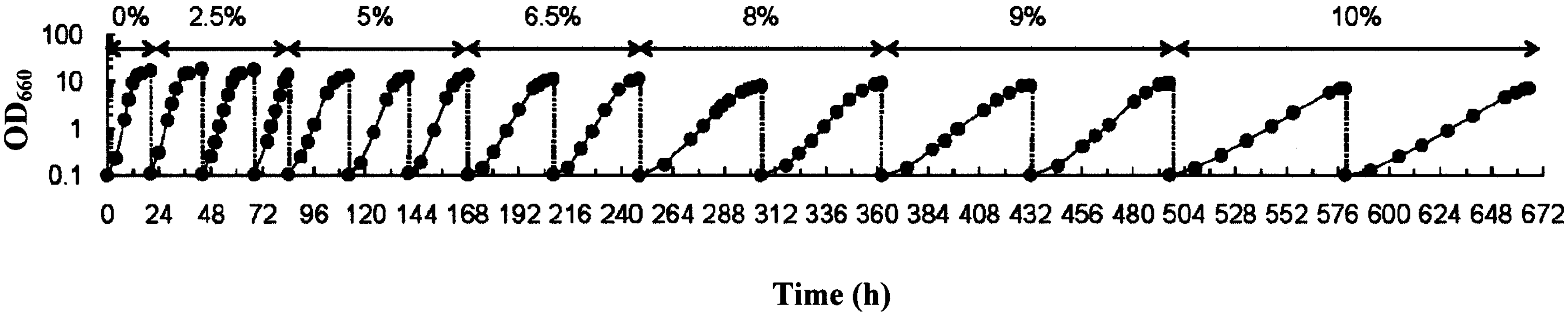


Figure 2-2. Growth characteristics of FY834 under high ethanol concentrations. (**A**) Growth characteristics of FY834 in repetitive cultivations at high ethanol concentrations. FY834 was inoculated into medium containing 2.5, 5, 6.5, 8, 9 or 10% ethanol, respectively, and then the growth of each culture was monitored, measuring at OD_{660}. In each culture, the culture broth was transferred to fresh medium containing the same ethanol concentration and then the growth was observed. The timing of the transfer to fresh medium is shown by the dotted lines. (**B**) Growth characteristics during subjection of FY834 to stepwise increase in ethanol concentration with repetitive cultivations. The method used for subjecting the yeast cells to a stepwise increase in ethanol concentration is described in Materials and methods. The timing of the transfer of culture to fresh medium is shown by the dotted lines. In the experiment shown in this figure, we obtained an ethanol-adapted strain series A2.

Table 2-1 Effect of ethanol addition on growth of *S. cerevisiae* strain FY834

Cultivation	Specific growth rate at each ethanol concentration (h^{-1})						
	0%	2.5%	5%	6.5%	8%	9%	10%
First	0.45 ± 0.04	0.39 ± 0.02	0.27 ± 0.02	0.19 ± 0.03	0.13 ± 0.02	0.09 ± 0.01	0.04 ± 0.01
Second	-	0.38 ± 0.01	0.26 ± 0.01	0.15 ± 0.02	0.11 ± 0.01	0.06 ± 0.02	0.03 ± 0.01
Third	-	0.38 ± 0.01	0.26 ± 0.01	-	-	-	-

Average of SGR ± standard deviation of three independent experiments in each culture condition is shown.

Table 2-2 Specific growth rates of ethanol-adapted yeast strain series during stepwise increase in ethanol concentration with repetitive cultivation

Cultivation	Specific growth rate at each ethanol concentration (h^{-1})						
	0%	2.5%	5%	6.5%	8%	9%	10%
First	0.45 ± 0.03	0.38 ± 0.01	0.26 ± 0.01	0.18 ± 0.01	0.12 ± 0.03	0.08 ± 0.01	0.06 ± 0.01
Second	-	0.37 ± 0.01	0.26 ± 0.01	0.17 ± 0.02	0.12 ± 0.02	0.09 ± 0.01	0.06 ± 0.01
Third	-	0.39 ± 0.01	0.26 ± 0.01	-	-	-	-

Average of SGR ± standard deviation of four ethanol-adapted strain series in each culture condition is shown.

2.3.2 Adaptation of *S. cerevisiae* to high ethanol concentration during stepwise increase in ethanol concentration in culture medium

Adaptation to environmental changes is achieved by the mechanism that a cell adjusts its intracellular physiological conditions to the surrounding environment to grow. Ismail and Ali reported that adaptation does not occur when the environmental changes are extreme [18]. Yeast cells are expected to be able to grow well even in the severe environments, when intracellular physiological conditions can be appropriate for growth in such environments through the stepwise adaptations to the environmental changes. Therefore, the adaptation process of yeast to a high ethanol concentration was analyzed through a repetitive cultivation of *S. cerevisiae* with a stepwise increase in the ethanol concentration.

The FY834 strain was subjected to a stepwise increase in the ethanol concentration of the medium from 2.5 to 10% via 5, 6.5, 8 and 9% through the repetitive cultivation, and the SGR in each cultivation process was measured. Four series of ethanol-adapted strain, named A1, A2, A3 and A4 were independently obtained. As an example, the growth of yeast strain series A2 during stepwise adaptation process is shown in Figure 2-2B. Similar growth properties were observed in the series A1, A3 and A4 to that in series A2 (data not shown). As shown in Table 2-2, the SGR of ethanol-adapted strain series decreased in the first culture with an increase in the ethanol concentration of the medium. Unlike the case of Table 2.1, the SGR of the second cultivation became almost the same as that of the first cultivation. This result suggests that the yeast cells once subjected to certain ethanol concentration are able to adapt to the same condition. Even though the ethanol-adapted strains showed a decrease in SGR with increase in ethanol concentration in the medium, a relatively high SGR was maintained in the yeast cells experiencing the cultivation with stepwise increase in ethanol concentration. Here, the strain showing the phenotype in which the SGR does not decrease through repetitive cultivation with a stepwise increase in ethanol concentration is called the ethanol-adapted strain.

Next, the SGR in the second cultivation of the ethanol-adapted yeast strain series were compared (Table 2-2) with that in the second cultivation of the non-adapted yeast strain (Table 2-1). In case of medium containing more than 6.5% ethanol, the SGR of ethanol-adapted strain became larger than that in non-adapted strain. However, in the case of lower than 6.5%, the difference in SGRs between ethanol-adapted and non-adapted strains in the presence of same ethanol concentration was not significant.

2.3.3 Comparison of fatty acid compositions of cell membrane between non-adapted and ethanol-adapted strains

Some researchers have shown that the cell membrane is one of the primary targets of ethanol: Alterations in the fatty acid composition of the cell membrane affect the ethanol tolerance of yeast and unsaturated fatty acids result in an increase in membrane fluidity and an enhancement of ethanol tolerance [8, 41, 42, 43, 47, 48]. Therefore, the fatty acid compositions of the cell membrane were analyzed and compared them among ethanol-adapted yeast strains in media containing ethanol.

The content of oleic acid ($C_{18:1}$) in FY834 strain grown in the presence of 10% ethanol was higher than that of the FY834 grown in the medium without ethanol addition (Figure 2-3). However, the content of oleic acid in the cell membrane of the ethanol-adapted strains was similar to that of the non-adapted yeast grown in the presence of 10% ethanol, while the fraction of palmitic acid ($C_{16:0}$) fatty acids was lower in ethano-adapted yeast strains than in the non-adapted strain in the presence of 10% ethanol (Figure 2-3). These results suggest that the fatty acid composition in cytoplasmic membrane was changed by exposing yeast cells to high ethanol concentration, but the increased fraction of oleic acid was not observed in the ethanol-adapted strain compared with the non-adapted strain. Decreased fraction of $C_{16:0}$ fatty acid in ethanol-adapted strains was found in the presence of 10% ethanol, suggesting that the content of other fatty acid(s) that could not be measured in this study might be changed during adaptation process to high ethanol concentration.

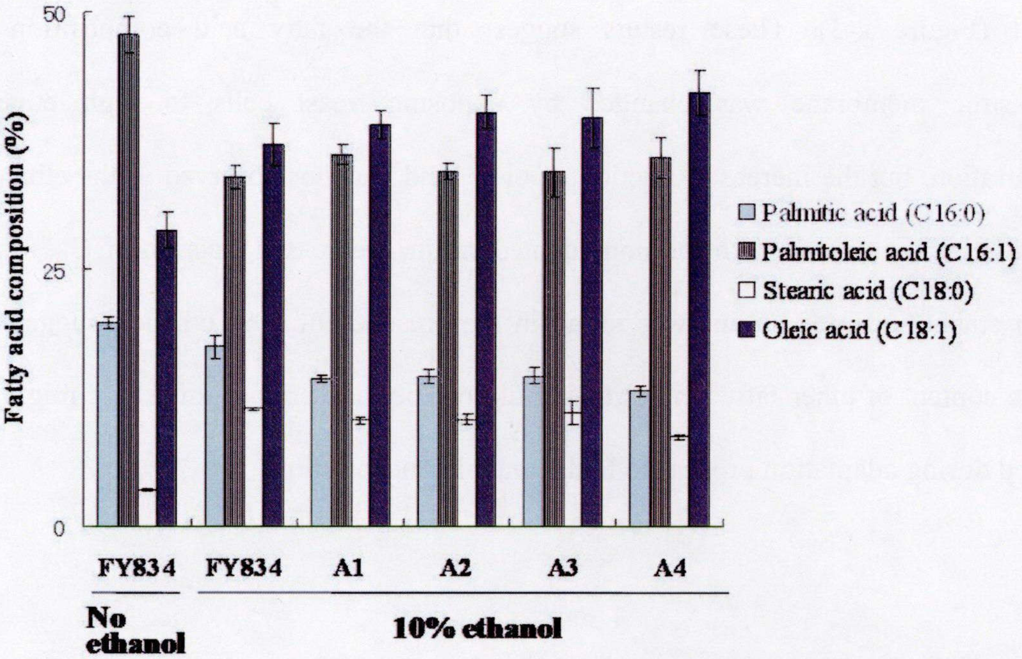


Figure 2-3 Comparison of fatty acid compositions of yeast cell membrane among four ethanol-adapted strains. Ethanol-adapted strain series were cultivated in YPD medium in the presence of 10% ethanol and the fatty acid composition of each strain was determined. Fatty acid compositions of FY834 strain cultured in the presence and absence of ethanol were also measured. Data represented the average values of three independent experiments with standard deviations.

2.3.4 Comparison of cell size between non-adapted and ethanol-adapted strains

Since Kubota *et al.* showed that the size of the cells cultured in the presence of ethanol was enlarged [30], the morphology of ethanol-adapted and non-adapted strains cultured in the presence of 10% ethanol was observed. The yeast cells that finally obtained through a stepwise increase in ethanol concentration in each culture series were inoculated into medium without the addition of ethanol. After reaching the mid-log phase, the morphologies of the cells were microscopically observed. Cells at the mid-log phase were used for microscopic observation because at this phase yeast cells have adapted to new condition of medium. It is thought that during batch cultivation the cells achieve the "steady state" at the mid-log phase. Microscopic observation revealed that the size of cells of the ethanol-adapted strains seemed to be larger than those of in the non-adapted strain (Figure 2-4A).

To quantitatively evaluate the change in cell morphology, the longest and shortest diameters of the cells of ethanol-adapted and non-adapted strains were compared (Figure 2-4B). The shortest diameters of the mother and daughter cells in the non-adapted and ethanol-adapted strains were similar. In contrast, the longest diameter of cells was different between the ethanol-adapted and the non-adapted strains, namely, a difference in the longest diameters of the mother cells between the non-adapted and ethanol-adapted strains was clearly observed. The longest diameter of the mother cells of the ethanol-adapted strains was larger than that of the non-adapted strain. These phenomena were consistent with the results shown by Kubota *et al.* [30].

Figure 2-4

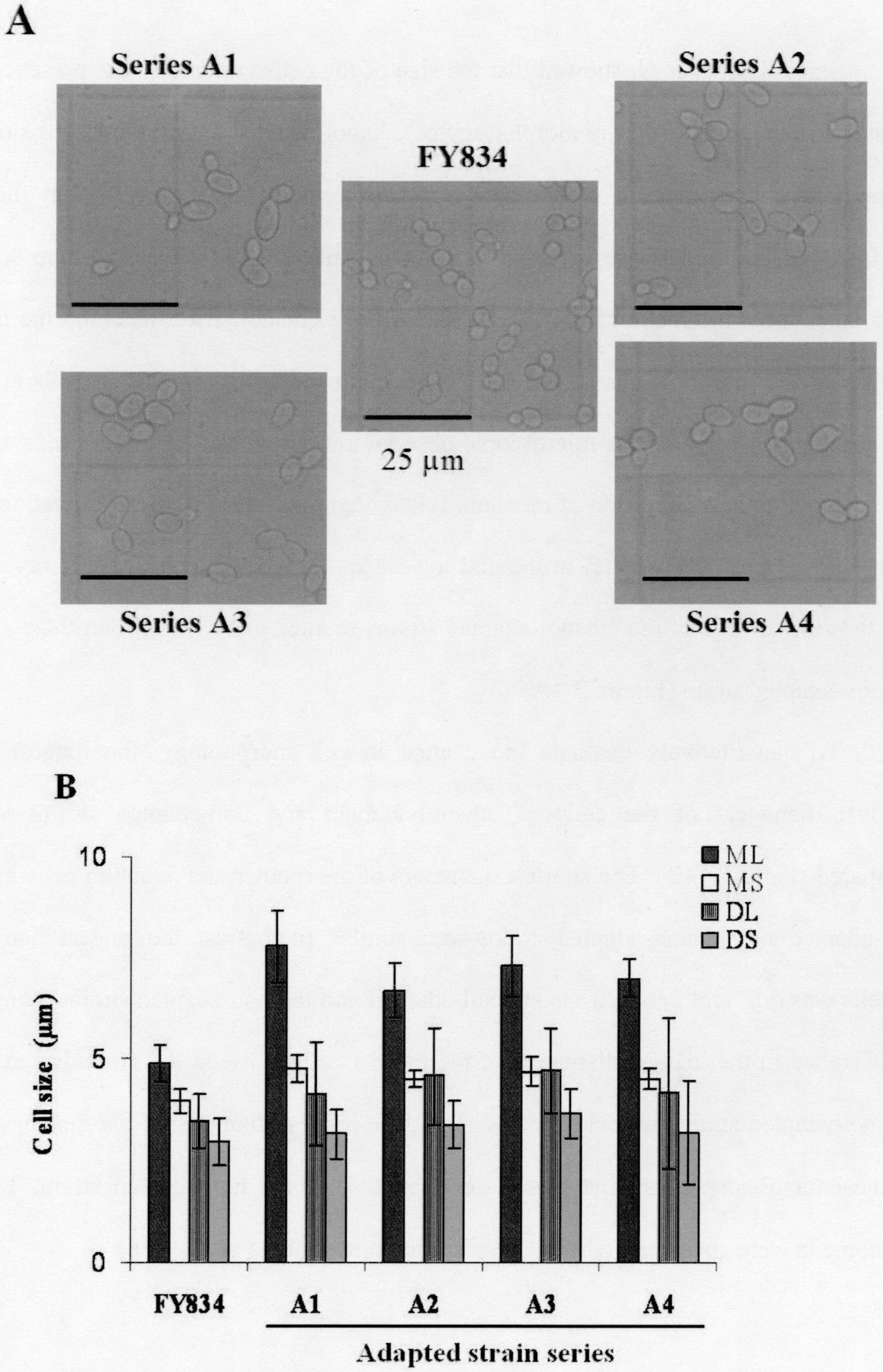

A
Series A1
Series A2
FY834
25 µm
Series A3
Series A4
B
■ ML
□ MS
▥ DL
▨ DS
10
5
0
Cell size (µm)
FY834
A1
A2
A3
A4
Adapted strain series

Figure 2-4 Cell morphology of control and ethanol-adapted yeast strain series. The yeast cells obtained through the repetitive cultivation with a stepwise increase in the ethanol concentration were cultivated in the YPD medium and the cell morphology was observed. **(A)** Microscopic observation of cell morphologies of ethanol-adapted strain serieses A1 - A4 and non-adapted yeast strain (FY834). The bars in each photograph represent 25 μm. **(B)** Comparison of cell size between non-adapted strain and adapted strain series at mid-log phase. ML, the longest diameter of mother cell; MS, the shortest diameter of mother cell; DL, the longest diameter of daughter cell; DS, the shortest diameter of daughter cell. Data represent the average size of counted 50 cells with standard deviations.

In the budding yeast *S. cerevisiae*, it is well known that the cells must achieve the critical cell size [80, 81]. The cell division of *S. cerevisiae* is asymmetric, i.e. one small daughter cell is budded from one mother cell during one cell division event. In principle, the size of the mother cell always achieves the critical cell size, indicating that the size of mother cell is independent on the cell cycle. In contrast, the size of daughter cell is dependent on the cell cycle, because the daughter cell has to enlarge to achieve the critical cell size for initiating cell division during cell cycle progression. Enlargement of the mother cell size of the ethanol-adapted strains shown in this study suggests that the critical cell size of the ethanol-adapted strains might be changed to adapt to high ethanol concentration. The change in cell size is one of the typical phenotype of ethanol-adapted yeast cells.

2.4 Conclusion

In this chapter, the stepwise adaptation process of *S. cerevisiae* was investigated by carrying out exposure to a stepwise increase in ethanol concentration with repetitive cultivations. When subjecting yeast cells to a stepwise increase in the ethanol concentration of the culture medium, the SGR of the ethanol-adapted strain did not change through repetitive cultivation under high ethanol concentration, suggesting that the ethanol-adapted strains, whose SGR is not changed through repetitive cultivation at high ethanol concentrations, could be successfully obtained. Observations of cell morphology and measurement of fatty acid composition of the cell membrane in adapted and non-adapted yeast strains revealed that yeast cells change the intracellular physiological state, such as a change in the composition of fatty acid and the increase in the size of yeast, to adapt to ethanol stress.

From the observation results of the difference in sizes of the mother cell between the non-adapted and ethanol strains, the cell size, cell cycle and adaptation to ethanol are thought to be closely correlated. Enlargement of the mother cell size of the ethanol-adapted strains shown in this study suggests that the critical cell size of the ethanol-adapted strains might be changed to adapt to high ethanol concentration. Moreover, the relationship between the stress response and the cell cycle of yeast has been also suggested [82].

In addition, another factors related to the adaptation to high ethanol concentration should be taken into account, such as fatty acids in the cell membrane that could not be observed in this study, lipids content in the cell membrane and ergosterol that is one of the component of cell surface and responsible for ethanol stress tolerance in *S. cerevisiae* [8, 41-43, 47, 83]. Analyses of these factors as well as cell cycle progression in ethanol-adapted strains will help us to understand the molecular mechanism of adaptation to high ethanol concentration in *S. cerevisiae.*

Chapter 3

Analysis of adaptation to high ethanol concentration in *Saccharomyces cerevisiae* using DNA microarray

3.1 Introduction

As described above, the yeast, *Saccharomyces cerevisiae*, has been used for fermentation processes of alcoholic beverages, and is also important for the production of biofuel. During these production processes, yeast cells are generally exposed to several types of environmental stresses which decrease the productivity of the target products, such as osmotic pressure, low pH, and oxidative stresses [3, 4]. Since ethanol is a major stress factor that decreases the production of target products in the fermentation processes using yeast, the response to ethanol stress and the mechanism underlying ethanol tolerance of yeast cells have been investigated to improve the production processes [47, 84, 85].

The stepwise adaptation process of *S. cerevisiae* was investigated by subjecting yeast cells to a stepwise increase in ethanol concentration with repetitive cultivations, as described in Chapter 2. And the change in the intracellular physiological state of the ethanol-adapted yeasts in comparison with those the control was observed, such as a change in the composition of fatty acid and the increase in the size of yeast. However it is not enough to understand the properties of biological system that enable yeast cells to adapt and grow under ethanol stress condition, further studies are necessary for a detail understanding about ethanol adaptation in *S. cerevisiae*.

DNA microarray is a powerful tool for comprehensively investigating the intracellular state; thus it is used to survey the changes in the intracellular state after exposing yeast cells to ethanol stress. In fact, many studies have revealed changes in characteristics after the addition of ethanol based on genome-wide expressions profiles, such as the activation of transportation and certain amino acid biosynthesis pathways and the down-regulation of genes related to protein synthesis [11, 12, 17, 67]. However, these genome-wide expression analyses have mainly been focused on the "short-term" adaptation process, where the expression data were obtained within several hours after the addition of ethanol [11, 12, 17]. It is known that despite the fact that many genes change their expression levels soon after stress exposure, a certain fraction of these genes recover their original expression levels after cells adapt to the stress and recover their growth [13, 67, 86, 87]. Such genes that recover the original expression levels can be regarded as those that are only responsible for the stress response and are not important for growth under the stress condition. Thus, in order to understand which genes are important for growth under ethanol stress condition, one possible strategy is to screen genes whose expression remains up-regulated after the recovery of cell growth under the ethanol stress condition.

Therefore, I sought to identify the genes and the functional categories of the genes that could be involved in growth under the ethanol stress condition by analyzing the comprehensive expression profile of an ethanol-adapted yeast strain. It is known that when yeast cells are exposed to ethanol stress, the cells often are able to adapt to the stress environment, i.e., change their intracellular state and recover sufficiently to grow under the stress condition [11, 12, 17]. As reported in Chapter 2, it was shown that by cultivating yeast cells over a long period (26 days) under the ethanol stress condition, I could obtain ethanol-adapted strains that exhibited significantly higher growth rate under the stress than did the wild-type strain [88]. However, no detailed analyses of the intracellular state of the ethanol-adapted yeast cells have been performed yet, even though such analysis can provide a better understanding what cellular state enables active growth under ethanol stress. In this chapter, using DNA microarray analysis, comparison of comprehensive expression profiles of two yeast trains (i.e, the previously obtained ethanol-adapted yeast strain and the parental strain as control) under the ethanol stress condition was shown.

3.2 Materials and methods

3.2.1 Strains, medium and cultivation conditions

A laboratory strain of *S. cerevisiae* FY834 (*MATα his3Δ200 ura3-52 leu2Δ1 lys2Δ202 trp1Δ63*) [75] and the ethanol-adapted yeast strain A1 [88] obtained from the FY834 as a parent were used.

Yeast cells were precultured in 5 ml of YPD medium (1% bacto yeast extract, 2% bacto peptone, 2% glucose) at 30 °C for 24 h. Then, for the main culture, the precultured yeast cells were cultivated aerobically at 30 °C in a 500 ml Sakaguchi flask containing 100 ml of YPD medium or synthetic complete medium (0.67% yeast nitrogen base without amino acids, 0.19% yeast synthetic drop-out without uracil, 2% glucose and 7.6×10^{-3}% uracil) adding 10% (v/v) ethanol at the beginning of the main culture. This main culture was repeated twice, where a part of the medium used for the first main culture along with yeast cells were transferred to fresh medium containing 10% ethanol.

The growth of each strain was monitored by measuring the optical density of the culture medium at 660 nm (OD_{660}) with a spectrophotometer, UVmini-1240 (Shimadzu Corporation, Japan). The relationship between the optical density (OD_{660}) and the dry cell weight (DCW) was obtained as follows. Samples of 20-50 ml culture medium containing cells were filtered through previously dried and weighted Whatman filters (N# 111107, 0.4 μm), and then these filters were dried at 60 °C for 48 hours until their weights become constant. The dry cell weight was calculated from the OD_{660} value using the following formulae;

Dry cell weight of the FY834 (g/l) = $OD_{660} \times 0.252$

Dry cell weight of the ethanol-adapted A1 strain (g/l) = $OD_{660} \times 0.261$.

3.2.2 DNA microarray experiments

DNA microarray experiments were carried out as described in previous papers [87, 89]. Cells cultivated in YPD medium containing 10% ethanol were harvested by centrifugation at the logarithmic growth phase. After that, cells were immediately frozen in liquid nitrogen, and then stored at −80°C until preparing the total RNA samples. Total RNA was extracted by the hot phenol method [9190] and purified into mRNA using an Oligo dT30<Super> mRNA purification kit (Takara Bio Inc., Japan). Fluorescent-dye-labeled cDNA targets were prepared from purified mRNA (1–2 μg) using Superscript II RNase H^- reverse transcriptase (Invitrogen Co., USA) and fluorescent-dye-labeled nucleotides, Cy3-dCTP and Cy5-dCTP (Amersham Biosciences Inc., UK). Cy3- or Cy5-labeled cDNA targets were prepared from mRNA samples of the adapted and control strains with and without the 10% ethanol addition. The samples were mixed and then hybridized with cDNA probes corresponding to each open reading frame of *S. cerevisiae* on a slide glass-based DNA microarray, Yeast Gene Chip ver. 2 (DNA Chip Research Inc., Japan). The fluorescence intensity of each spot was measured using a Genepix 4000A scanner (Axon Instruments, USA) and software Genepix pro ver. 3.0 (Axon Instruments). The preparation of fluorescent-dye-labeled cDNA targets, hybridization, and washing were carried out as described in instruction manuals supplied by the manufacturer of the DNA microarray.

3.2.3 Data analysis

The fluorescent signal intensities of Cy3 and Cy5 on the DNA microarray were acquired using Genepix pro ver. 3.0 software (Molecular Devices, CA, USA). The signal was normalized by the locally weighted linear regression (Lowess) method [91]. Data analysis accompanied by gene functional category was performed using the data from the Munich information center for protein sequences (MIPS) database (http://mips.gsf.de/genre/proj/yeast/) [92]. I applied a hypergeometric test to evaluate whether or not a functional category was overrepresented by a group of selected genes from total genes in genome by the following formula:

$$f(k; N, m, n) = \frac{\binom{m}{k}\binom{N-m}{n-k}}{\binom{N}{n}}$$

where N is the total number of genes, M is the number of genes related to a functional category from the total genes, n is the number of genes in the selected genes, and k is the number of genes related to a functional category from among the selected genes.

The data analysis was performed using Matlab ver. 2006b (Mathworks Inc., USA) and Microsoft Excel (Microsoft, USA).

3.3 Results and discussion

3.3.1 Ethanol-adapted yeast strain

The adaptation process of yeast cells under the ethanol stress condition has been reported previously in chapter 2 [88]. Briefly, *S. cerevisiae* FY834 strain was repetitively cultivated with a stepwise increase in the ethanol concentration in YPD medium, with the basal concentration being 0%, increased to 2.5, 5, 6.5, 8, 9%, and finally, 10% ethanol. By this method, the yeast cells adapted to a high ethanol concentration, and the growth rate under the ethanol stress condition significantly increased. In this study, I used the yeast strain A1 adapted to 10% ethanol. Figure 3-1 showed the growth curves of the ethanol-adapted strain and its parent strain as a control in the presence of the 10% ethanol condition. As shown in the figure, the growth rate of the ethanol-adapted strain was significantly higher than that of the control strain under high ethanol concentration. Since the control strain FY834 has auxotrophy for amino acids and uracil, I examined the effect of the auxotrophy on the growth behavior under ethanol stress condition. I confirmed that the higher growth rate in the adapted strain was also observed in synthetic medium with the addition of 10% ethanol (Figure 3-2), suggesting that the difference in growth rates between the two strains is independent of the choice of culture media.

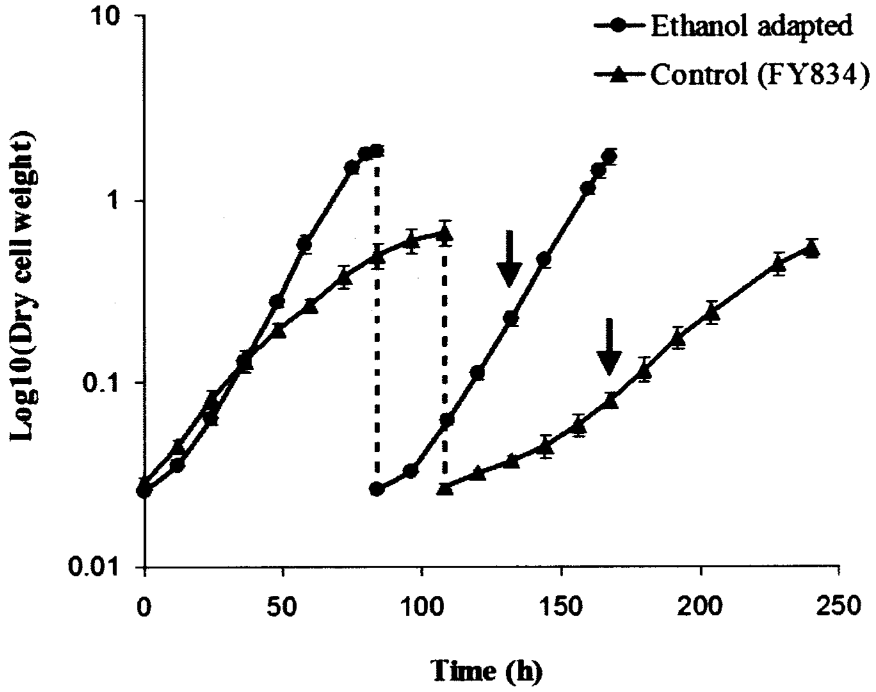


Figure 3-1 Growth characteristics of the ethanol-adapted strain A1 and the control strain FY834 under high ethanol concentration. Yeast strains were cultivated aerobically at 30 °C in YPD medium containing 10% ethanol. I repeated this culture, where a part of the first culture medium along with the yeast cells was transferred to a fresh medium containing 10% ethanol. The timing of the transfer to the fresh medium is indicated by the dotted lines. The time of cells harvest for RNA extraction is indicated by the arrows. Average and standard deviation of dry cell weights in three independent experiments were shown.

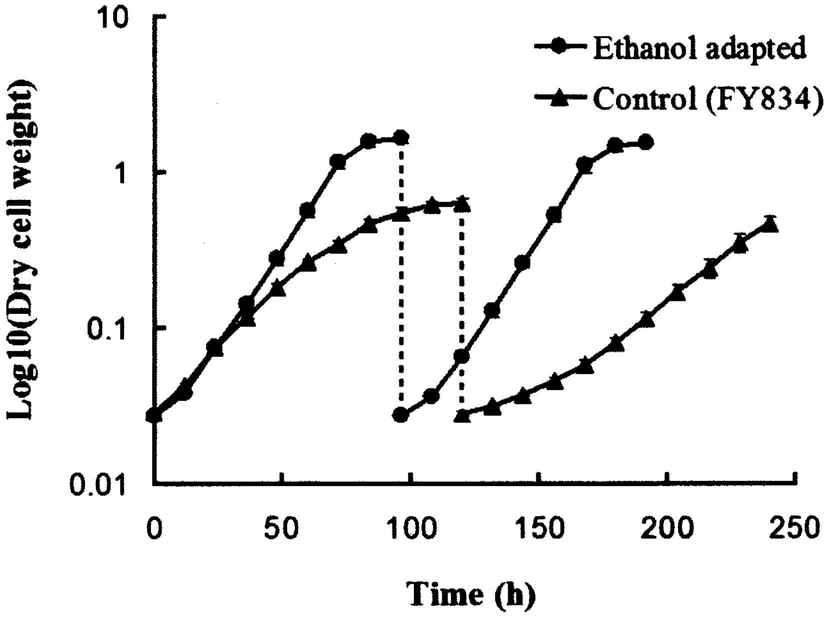


Figure 3-2 Growth characteristics of the ethanol-adapted strain A1 and control strains (FY834 and S288c) under high ethanol concentration. Yeast strains were cultivated aerobically at 30 °C in synthetic complete medium (0.67% yeast nitrogen base without amino acids, 0.19% yeast synthetic drop-out without uracil, 2% glucose and 7.6×10-3% uracil) adding 10% (v/v) ethanol. I repeated this culture, where a part of the first culture medium along with the yeast cells was transferred to a fresh medium containing 10% ethanol. The timing of the transfer to the fresh medium is indicated by the dotted lines. Average and standard deviation of OD_{660} in three independent experiments were shown.

3.3.2 Transcriptional analysis of the ethanol-adapted and control yeast strains under ethanol stress

The gene expression profiles of the ethanol-adapted and control yeast strains under the ethanol stress condition were analyzed using DNA microarray. Cy5-labeled cDNA targets were prepared from mRNA isolated from the ethanol-adapted and control strains cultivated in YPD medium containing 10% ethanol, while Cy3-labeled cDNA targets were obtained from mRNA isolated from the control strain cultivated in YPD medium without the addition of ethanol. In this study, I analyzed the expression profiles of the adapted and control strains in the logarithmic phase of the second culture (denoted by arrow in Figure 3-1), where the growth rate was significantly different between these two strains. As reported previously, an expression ratio of more than 2 and less than 0.5 between two samples can be regarded as a significant change in this microarray analysis [87].

Using the microarray data, I screened genes whose expression levels were significantly up-regulated under the ethanol stress condition in comparison with the non-stress condition. I classified genes into three classes as follows: (1) up-regulated in the adapted strain only, (2) up-regulated in both the adapted and control strains, and (3) up-regulated in the control strain only. The number of genes in each class is shown in Table 3-1 and the list of up-regulated or down-regulated genes is presented in Supplemental data, Table I–VI. It is expected that, the genes in classes (1) and (2) would include those genes that were important for the recovery of growth of the adapted strain under the ethanol stress condition. In contrast, genes in class (3) are not essential for the growth under ethanol although they might play a role in the ethanol stress response. Then, to characterize the up-regulated genes in each class, I further classified these genes into functional categories using the MIPS database [92]. I screened the functional categories in which the genes classified above were significantly overrepresented by using a hypergeometric test. The list of these functional categories with $p < 0.01$ is shown in Table 3-2.

Table 3-1 Numbers of genes changing their expression levels under ethanol stress condition.

	Number of genes			
	Up-regulated	Not changed	Down-regulated	Total
(1) Ethanol-adapted strain only	473	789	462	1724
(2) Both control and ethanol-adapted strains	512	3028	315	3855
(3) Control strain only	494	1084	373	1951
Total	1479	4901	1150	

Table 3-2 Representative functional categories of overrepresented genes which were up-regulated in the ethanol-adapted yeast or control strains or both

MIPS functional category	*p* value	No. of up-regulated genes	No. of total genes
Up-regulated genes only in the ethanol-adapted strain			
12.01.01 ribosomal proteins	6.90E-05	36	229
42.16 mitochondrion	4.61E-04	26	161
Up-regulated genes in both control and ethanol-adapted strains			
01.05.01 C-compound and carbohydrate utilization	5.24E-03	47	370
02 Energy generation	8.90E-11	68	350
02.11 electron transport and membrane-associated energy conservation	9.60E-15	27	56
02.13 respiration	2.33E-12	39	131
14.13.04 lysosomal and vacuolar protein degradation	2.68E-03	7	23
20.01 transported compounds (substrates)	2.20E-06	81	562
20.09.04 mitochondrial transport	6.22E-03	17	101
32.01 stress response	5.98E-07	67	422
32.01.01 oxydative stress response	3.35E-07	18	54
34.01.01.03 homeostasis of protons	3.94E-03	10	44
Up-regulated genes only in the control strain			
01.01 amino acid metabolism	2.55E-08	46	233
01.01.03 assimilation of ammonia, metabolism of the glutamate group	8.44E-06	14	44
01.01.03.02 metabolism of glutamate	1.20E-03	7	21
01.01.03.05 metabolism of arginine	8.63E-04	7	20
01.01.09.04 metabolism of phenylalanine	8.42E-03	5	16
01.01.09.05 metabolism of tyrosine	6.21E-03	5	15
01.05 C-compound and carbohydrate metabolism	1.91E-05	67	482
02.10 tricarboxylic-acid pathway (citrate cycle, Krebs cycle, TCA cycle)	3.39E-03	8	31
32.01 stress response	1.39E-05	61	422
42.16 mitochondrion	1.96E-03	25	161

Table 3-3 Representative functional categories of overrepresented genes which were down-regulated in the ethanol-adapted yeast or control strains or both

MIPS functional category	*p* value	No. of down-regulated genes	No. of total genes
Down-regulated genes only in the ethanol-adapted strain			
01.01 amino acid metabolism	1.38E-04	35	233
01.01.06 metabolism of the aspartate family	4.36E-03	12	65
01.01.09.01 metabolism of glycine	2.09E-03	4	8
01.01.09.02 metabolism of serine	9.52E-04	6	16
01.01.11 metabolism of the pyruvate family (alanine, isoleucine, leucine, valine) and D-alanine	2.63E-03	6	19
01.03.01 purine nucleotide metabolism	2.90E-03	12	62
10.03.03 cytokinesis (cell division) /septum formation	6.35E-03	12	68
16.21 complex cofactor/cosubstrate binding	3.80E-03	11	56
42.10 nucleus	6.24E-03	19	130
Down-regulated genes in both control and ethanol-adapted strains			
01.01 amino acid metabolism	3.34E-03	23	233
01.01.06.05.01 biosynthesis of methionine	5.38E-03	4	14
01.01.09.07 metabolism of histidine	1.59E-03	5	17
01.03.01 purine nucleotide metabolism	1.91E-05	13	62
02.07 pentose-phosphate pathway	1.93E-04	7	24
11.04.01 rRNA processing	1.85E-04	21	165
12.04.01 translation initiation	7.68E-04	8	38
12.10 aminoacyl-tRNA-synthetases	3.04E-06	11	38
16.03.03 RNA binding	2.59E-03	19	175
16.19 nucleotide binding	2.38E-03	21	200
16.19.03 ATP binding	3.61E-03	18	167
20.01.21 RNA transport	3.01E-04	13	80
20.09.01 nuclear transport	1.85E-03	11	74
Down-regulated genes only in the control strain			
01.03.04 pyrimidine nucleotide metabolism	7.83E-03	8	46
11.04 RNA processing	2.11E-09	55	375
11.04.01 rRNA processing	5.11E-14	40	165
11.06 RNA modification	7.98E-06	15	64
11.06.02 tRNA modification	1.29E-03	9	43
12.01 ribosome biogenesis	2.09E-06	40	290
12.04 translation	7.61E-04	14	84
16.03 nucleic acid binding	4.17E-03	33	322
16.03.03 RNA binding	2.72E-04	24	175

With respect to the genes up-regulated in the adapted strain only, I found that these genes were significantly concentrated in the category of "ribosomal proteins", as shown in Table 3-2. This result suggests that the adapted yeast strain enhances the activity of protein synthesis under the ethanol stress condition. It is worth noting that in the control strain, the expression of the genes related to protein synthesis, i.e. "translation" and "ribosomal biogenesis", was significantly down-regulated as shown in Table 3-3. Such difference suggests that the recovery of protein synthesis activity plays an important role in growth recovery under high ethanol concentration.

In the case of genes up-regulated in both strain, I found that genes related to "energy generation" (for example, ATP synthase) were significantly overrepresented ($p< 10^{-10}$, Table 3-2). In this functional category, which is involved in ATP synthesis in mitochondria, the genes encoding the subunits of F-type ATP synthase (*ATP14*, *ATP18* and *ATP19*) were up-regulated specifically in both adapted and control strains. This fact indicates that ATP production is activated under the ethanol stress condition in comparison with the non-stress condition. Under the ethanol stress condition, the increase in ATP synthesis might be responsible for the activation of protein synthesis as mentioned above and as also for proton transportation through plasma membrane [11]. Furthermore, I found that genes related to "oxidative stress response" to be significantly overrepresented. This result is consistent with the evidence that reactive oxygen species are produced in yeast cells exposed to ethanol [4, 93].

In the case of genes that were specifically up-regulated in the control strain, the functional categories of several amino acid metabolisms were screened (Table 3-2). It is known that genes related amino acid biosynthesis changes their expression levels significantly during the response to several stresses such as ethanol and osmotic pressure [11, 12, 17, 87]. However, the results of this study suggests that, at least as for the glutamate, arginine, phenylalanine and tyrosine, the up-regulation of the genes responsible for the biosynthesis of these amino acids involve only in the response to ethanol stress, but are not important for growth under this stress.

Martinez *et al.* found that 127 genes of *S. cerevisiae* were up-regulated in a stationary phase in comparison with a logarithmic growth phase [94]. Of these 127 genes, 74 % were up-regulated in the control strain in the presence of ethanol, suggesting that the expression profile of the control strain under the ethanol stress condition is similar to that in the stationary phase, although the sample was collected in the mid-exponential phase. In the stationary phase, the yeast cells alter their gene expression such as an increase in expression of genes related to the response to environmental stress and amino acid metabolism and a decrease in expression of genes related to translation, ribosome biogenesis and protein synthesis; further, the growth rate is reduced [95]. Our results agree with the suggestion of Marks *et al.* [96] that ethanol concentration rather than nutrient starvation appears to be responsible for the entry of yeast cells into the stationary phase. Under the high ethanol concentration, the control strain receives signal(s) to enter the stationary phase, but the ethanol-adapted strain does not. This fact might suggest that there is a difference in the signal transduction mechanism between the ethanol-adapted and control yeasts.

Powers and Water, (1999) found that Rapamycinsensitive TOR-signaling pathway regulates the ribosomal biogenesis [97]. Further, Martin *et al.* (2004) investigated that the signal transduction TOR regulates ribosomal protein gene expression via PKA and the Forkhead transcription factor FHL1, and the two cofactors IFH1 (a co-activator) and CRF1 (a co-repressor). TOR, via PKA, negatively regulates YAK1 and maintains CRF1 in the cytoplasm. Upon TOR inactivation, activated YAK1 phosphorylates and activates CRF1. Phosphorylated CRF1 accumulates in the nucleus and competes with IFH1 for binding to FHL1 at RP gene promoters, and thereby inhibits transcription of ribosomal protein genes [98]. In this chapter, gene expression of YAK1, IFH1 and CRF1 were up-regulated in the control strain only. The up-regulated YAK1 activates CRF1 and as consequence described above, the transcription of ribosomal protein was inhibited because CRF1 bind with FHL1 at promoter [98]. In the case of the ethanol-adapted yeast, gene expression of YAK1, IFH1, FHL1 and CRF1 did not change and might be the gene expression of ribosomal proteins was maintained. However, the mechanism how do ethanol adapted yeast maintain the expression of these gene is remained unknown.

Oleic acid (un-saturated) is formed in S. cerevisiae by the oxygen- and NADH-dependent desaturation of stearic acid (C18:0), catalyzed by a single integral membrane desaturase encoded by the *OLE1* gene. Result of DNA microaary showed that the expression of gene related oleic acid production (*OLE1*) did not change in both the ethanol-adapted and the control strains. One possibility is that the post transcription in oleic synthesis is effective to increase the oleic acid production, although it has been reported that transcriptional upregulation of *OLE1* is effective to increase amount of unsaturated fatty acids [47].

Other fatty acids biosynthesis, I found that only in the ethanol adapted yeast, the expression of gene related very long chain fatty acid biosynthesis was increased. The expression of *SUR4* and *TSC13* (very-long-chain fatty acid metabolism) were up-regulated in the ethanol-adapted strain only. The content of very-long-chain fatty acid that could not be measured in Chapter 2 might be changed during adaptation process to high ethanol concentration.

3.3.3 Comparison of gene expression analysis with gene deletion analysis

As mentioned above, the comparison of expression profiles between the ethanol-adapted and control yeast strains enables us to screen the genes that can be important for growth under the ethanol stress condition. To further support the results of DNA microarray analysis, I compared the microarray results to the results of comprehensive phenotypic analysis of single gene-deletion strains under ethanol stress condition [30, 52, 63, 64]. To investigate the relationship between effect of gene deletion and expression analysis of genes in the ethanol adapted and control strains, first I defined a set of genes whose deletion exhibit ethanol growth defect as the sum of all genes screened in these four previous studies [30, 52, 63, 64]. Furthermore, I removed the genes whose deletion strain showed growth defect also under non-stress condition from the analysis, to focus on the effect of gene deletion specifically under ethanol stress condition. For the data of growth defect under non-stress condition, I referred ref. [64], where growth rates of all single-gene deletion strains were measured in YPD medium without ethanol stress. As result of this data analysis, I screened 494 genes whose deletion results growth defect under ethanol stress condition specifically (the list of these 494 genes are presented in Supplemental data, Table VII). Then, I checked the relationship between above genes and results of microarray analysis of the ethanol-adapted and control strains. As result, it was found that, among 494 genes whose deletion strains showed the growth defect under the ethanol stress condition, 51 genes were specifically up-regulated in the adapted strain under ethanol stress condition. An important point here is that, the ratio (51/494) is significantly larger than that expected from random selection, confirmed by a hypergeometric test ($p < 0.05$). These genes were concentrated in functional category such as those concerning ribosomal proteins, and they were up-regulated in the adapted strain only; moreover, their deletion strains showed growth defect under the ethanol stress condition (data not shown). In contrast, the genes which are up-regulated in both strains and specifically up-regulated in the control strain did not exhibit such overrepresentation. This result indicates the genes which are specifically up-regulated in the adapted strains are more important for the growth under ethanol stress condition than other classes, i.e., genes which are up-regulated in both strains and in the control strains only.

3.4 Conclusion

Comprehensive gene expression analysis of the ethanol-adapted and control strains revealed certain genes and functional categories that are possible involved in growth under the ethanol stress condition. Although there are several studies as for transcriptome analysis under ethanol stress condition in *S. cerevisiae* [11, 12, 17, 61, 67, 68], the most significant difference between our study and earlier studies is the stage of ethanol adaptation process to be investigated. In the previous studies, the measurements of growth behavior and transcriptome analysis were generally done within several hours or tens of hours after ethanol stress addition. In contrast, our previous study presented in [88] showed that the longer adaptation process as it takes several tens of days results in significant improvement of growth under the ethanol stress, and in the present study I investigated the transcriptomic response in such long adaptation process. The transcriptome analysis of the long-term adapted strain can provide a basis to understand the properties of biological systems that enable growth under ethanol stress condition. For example, our results showed that the expression of the genes encoding ribosomal proteins was specifically up-regulated in the adapted strain under the ethanol stress condition, suggesting the increase in protein synthesis activity in the adapted strain. The importance of such genes that were specifically up-regulated in the adapted strain was supported by comparing the result of single-gene deletion analysis under the ethanol stress condition. Further, I found that genes related to ATP synthesis in mitochondria and oxidative stress response were significantly up-regulated in both the adapted and control strains. This suggests that these functions are also involved in growth under the ethanol stress condition. In contrast, I found that genes related to amino acid biosynthesis are up-regulated specifically in the control strain under the ethanol stress condition, indicating that such genes are not involved in the growth recovery under the ethanol stress condition. A relationship between the expression profile of the control strain under the ethanol stress condition and that at the stationary phase was also suggested. So far, it is unclear whether the phenotypic differences between the adapted and control strains are due to mutations elsewhere in the yeast genome or due to adaptive changes in the intracellular state without mutations. However, in either case, I expect that the differences in the gene expression levels reflect different phenotypes with respect to the growth rate under ethanol stress condition. I expect that these results of the comprehensive expression analysis of long-term adaptation process to high ethanol concentration will provide the basis for a better understanding of ethanol tolerance of yeast cells.

Chapter 4

Proteomic analysis of adaptation to high ethanol concentration in *Saccharomyces cerevisiae*

4.1 Introduction

Saccharomyces cerevisiae, the brewing yeast, has been widely used in food fermentation and brewing industry (such as beer and sake wine) and recently in the bio-ethanol production [3, 4, 99]. In these fermentation processes, ethanol is produced and gradually accumulated to high concentration which affects on the growth, metabolic activities, and other functions of yeast cells [6, 19, 28, 30]. Therefore, making yeast strains that have high ethanol tolerance is desirable in order to optimize industrial processes.

Environmental stress conditions elicit responses of *S. cerevisiae* at proteomic and transcriptomic levels. Some researchers revealed the potential of proteomes as a measure of global change in protein abundance during fermentation processes [16, 69-72, 101-104]. For instance, Kolkman *et al.* (2005) performed analysis of the global changes of proteins of yeast, however, only the enzymes involved in the central carbon metabolisms of yeasts displayed a significant change in steady-state expression under limitation of glucose or ethanol [72].

As shown in Chapter 2, adaptation process of yeast to high ethanol concentration in repetitive cultivation was investigated [88]. In Chapter 3, using DNA microarray, the global gene expression of ethanol-adapted strain under high ethanol concentration was performed [100] in which genes and functional categories specifically up-regulated or down-regulated in the adapted yeast strain were identified, suggesting that such genes and categories are involved in the adaptation of cell to high ethanol stress condition. However, several previous studies revealed that the expression levels of mRNAs as obtained from microarray analysis does not necessarily correspond to the expression levels of the corresponding proteins [101-103], as the efficiency of translation and the protein degradation rate can be different among proteins. Thus, analyzing only mRNA expression profile can fail to detect the change of protein expression levels influencing the ethanol tolerance of the adapted strain. To measure the protein expression levels directly and then analyze the difference in the protein expression levels between the ethanol-adapted and control strains are desirable to characterize the molecular mechanism of ethanol adaptation process.

In this chapter, the intra-cellular protein expression levels of yeast strains was analyzed by two dimensional gel electrophoresis (2-DE) combined with peptide mass fingerprinting method (matrix assisted laser desorption /ionisation time-of-flight mass mpectrometry, MALDI-TOF MS) for identification of the proteins. First, the correlation between changes in mRNA expression levels and protein expression levels of yeast strains was analyzed to evaluate the availability of protein expression data obtained by 2-DE. Then, the functions of proteins whose expression levels were up-regulated in the ethanol-adapted yeast strain were discussed.

4.2 Materials and Methods

4.2.1 Strain, medium and cultivation condition

A laboratory strain of *S. cerevisiae* FY834 (*MATα his3Δ200 ura3-52 leu2Δ1 lys2Δ202 trp1Δ63*) [75] and the ethanol-adapted yeast strain A1 obtained from FY834 as the parent were used as reported in Chapter 2 [88].

Yeast cells were precultured in 5 ml of yeast extract-peptone-dextrose (YPD) medium (1% bacto yeast extract, 2% bacto peptone, 2% glucose) at 30 °C for 24 h. Then, for the main culture, the precultured yeast cells were cultivated aerobically at 30 °C in a 500 ml Sakaguchi flask containing 100 ml of YPD medium containing 10% (v/v) ethanol at the beginning of the main culture. I repeated this main culture twice, where a part of the medium used for the first main culture along with yeast cells were transferred to a fresh medium containing 10% ethanol. The growth of each strain was monitored by measuring the optical density of the culture medium at 660 nm (OD_{660}) with a spectrophotometer, UVmini-1240 (Shimadzu Corporation, Japan).

4.2.2 Preparation of yeast cells sample and protein extraction

Yeast cells were collected at the mid-log phase by centrifugation. After washing by cold distilled water, the cell pellet was immediately frozen in liquid nitrogen and was kept at –80°C until use.

To extract the proteins from the cells, the pellet was suspended in 150 μl of lysis buffer consisting of 10 mM Tris-Cl (pH 8.0), 2 mM $MgCl_2$, 1% protease inhibitor cocktail for fungi (SIGMA), followed by addition of an equivalent volume of glass beads prewashed by sulfic acid. Cells were disrupted by vigorously shaking with glass beads. The sample was centrifuged, and the supernatant was transferred to vivaspin 500 (Sartorious) for ultrafiltration. The protein solution was transferred into a new 1.5 ml tube and 2 μl of Benzonase nuclease (Novagen) was added to digest DNA and RNA for

30 min on ice. Then digested DNA was removed using vivaspin 500, and the protein sample was collected. Protein concentration was determined by Bradford method using Protein assay solution (Bio-Rad).

4.2.3 Two-dimensional electrophoresis

Isoelectric focusing was performed using a Multiphor II electrophoresis unit (GE Healthcare UK, Buckinghamshire, UK) and a commercially available immobilized pH gradient (IPG) strip (Immobiline drystrip, p*I* range 3–10, 13 cm, linear gradient; GE Healthcare UK) [106]. 30 μg of protein in 250 μl of rehydration buffer (6.25 M urea, 2% 3-[(3-cholamidopropyl)dimethylammonio]propane sulfonate, 1% protease inhibitor, 2% IPG buffer [GE Healthcare UK], 0.96% destreak reagent [GE Healthcare UK], 0.01% bromophenol blue) was loaded onto the IPG dry strip, which was then rehydrated for 15 h. Isoelectric focusing was carried out at 20 °C as follows: 50 V for 30 min, 200 V for 3.5 h, linear increase to 3500 V for 1.5 h, and 3500 V for 6 h.

For the polyacrylamide gel electrophoresis (PAGE) in the second dimension, the focused IPG strip was equilibrated in 10 mol of buffer A (50 mM Tris-Cl [pH8.8], 6 M urea, 30% glycerol, 2% sodium dodecyl sulfate [SDS], 10 μg/ml dithiothreitol, 0.01% bromophenol blue) for 15 min and then in buffer B (dithiothreitol in equilibration buffer A was replaced with 25 μg/ml of iodoacetamide). The equilibrated IPG strip was applied onto 12.5% polyacrylamide gel for standard SDS-PAGE (160 mm × 160 mm × 1 mm) and the proteins in the IPG strip were separated [105]. Electrophoresis was performed at 15 mA for 15 min and then at 30 mA until the bromophenol blue buffer front reached the end of the gel using a slab gel electrophoresis apparatus (TMN-5S; Taitec, Saitama, Japan).

The gel was stained with SYPRO red protein gel stain (Invitrogen, Carlsbad, CA) [107], according to the manufacturer's protocol and scanned using Typhoon 9210 (GE Healthcare) to detect the protein spots on the gel. Image Master 2-D Elite software (GE Healthcare) was used to quantify the volume of each spot.

For staining the gels with SYPRO red gel stain (Invitrogen, Carlsbad, CA), one side of the glass was coated with bind silane solution (0.1% bind silane in 80% ethanol and 2% acetate) before preparing the gel.

4.2.4 Peptide mass fingerprinting for protein identification

Protein spots were identified by peptide mass fingerprinting using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) [108, 109]. The 2-D PAGE gels were stained with Coomassie brilliant blue R-250 by the standard method and SYPRO red by the method described in the preceding. Silver staining and negative staining were performed using Silver stain MS kit (Wako Pure Chemicals Inc., Osaka, Japan) [110], and Negative gel stain MS kit (Wako Pure Chemicals Inc.), respectively, according to the manufacturers' protocols. For the silver-stained gel pieces, pretreatment of the gel was required according to the supplied instruction manual.

Protein spots excised from the gel were transferred in the Treff tube (Treff AG, Degersheim, Switzerland). The excised spots were destained by incubation in 100 µl of destaining solution consisting of 50% methanol and 40 mM ammonium bicarbonate for 30 min with vigorous shaking, and then dried at 60 °C with evaporation. 20 µl of trypsin solution (4 nM Trypsin [SIGMA] in 40 mM NH_4HCO_3) was added to each sample tube and it was incubated on ice for 45 min. Then the enzyme solution was replaced with 20–

40 μl of 10% acetonitrile in 40 mM NH_4HCO_3 and the gel was incubated at 37 °C for 14–16 hours.

Extraction of the trypsin-digested protein was performed by sonication and the supernatant after each ultrasoinication steps were collected as sequence: 10 min in 100 μl of 0.1% trifluoroacetate (TFA), 10 min in 100 μl of 0.1% TFA in acetonitrile, 10 min in 50 μl of 0.1% TFA in 50% acetonitrile, and 10 min in 80 μl of 0.1% TFA in acetonitrile. Samples were then collected and concentrated with evaporation by 3 steps; 20 min at room temperature, 20 min at 40°C, and at 60°C until 30 μl of solution was remained in the tubes.

The extracted peptides were purified with ZipTipC18 (Millipore, Billerica, MA) using water containing 0.1% TFA, acetonitrile containing 0.1% TFA and 50% acetnitrile containing 0.1% TFA, according to the manufacturer's protocol. 2 μl of matrix solution (α-cyano-4-hydroxy-cinnamic acid-buffered 33% acetonitrile containing 0.1% TFA) and then spotted onto a target plate for MALDI-TOF MS.

For the MALDI-TOF MS, Autoflex (Bruker Daltonics, Bremen, Germany) was used in the measurement range of 800–4600 (*m/z*) [110]. Calibration was achieved using a peptide calibration standard (Bruker Daltonics) containing angiotensin II (average *m/z*, 1047.19 Da), angiotensin I (1297.49 Da), substance P (1348.64 Da), bombesin (1620.86 Da), ACTH clip 1-17 (2094.43 Da), ACTH clip 18-39 (2466.68 Da), and somatostatin 28 (3149.57 Da). The *m/z* data from the detected peptides were used to search for the *S. cerevisiae* genome in the NCBInr database with Mascot 2.0 (Matrix Science Inc., Boston, MA). Proteins identified by peptide mass fingerprinting were evaluated and compared with each molecular mass and the p*I* calculated from the amino acid sequence.

4. 3 Results

4.3.1 Experimental design for analyzing the difference in protein abundance between two gel images obtained by 2-D gel electrophoresis

As shown in Figure 4-1, 400 protein spots could be detected in my proteomic experiments with SYPRO red staining.

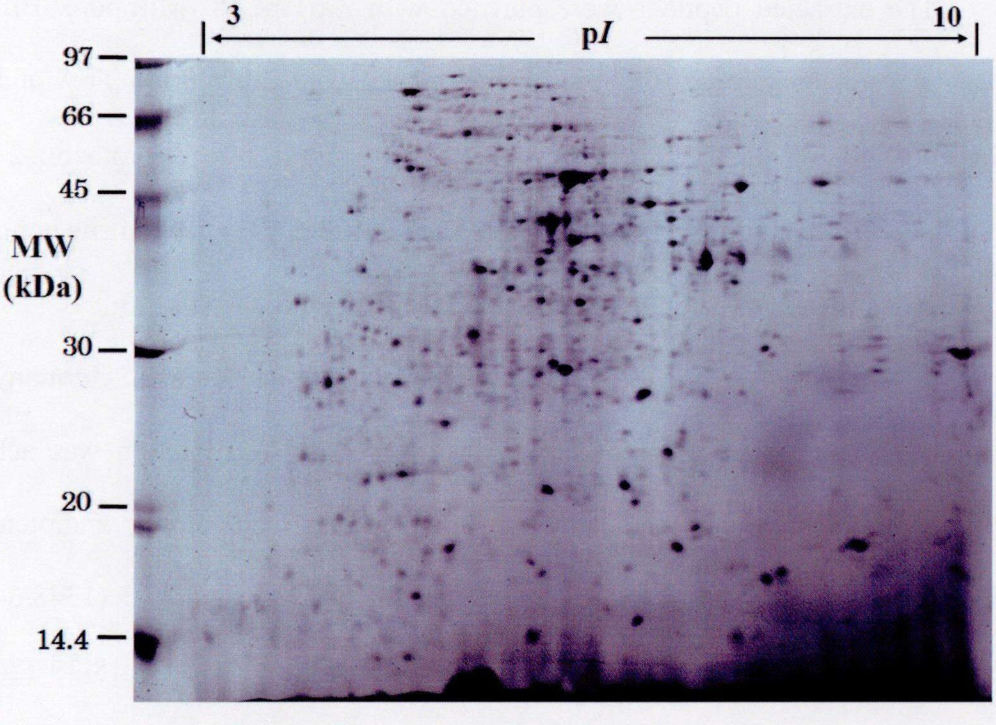


Figure 4-1 SYPRO red stained gel images of proteins separated by two-dimensional (2-D) polyacrylamide gel electrophoresis.

Using the same protein sample, reproducibility of two dimensional gel electrophoresis experiments was examined. For comparison of the patterns of proteins obtained from two gel images, each spot volume was normalized as the summation of the volumes of all detected spots. To evaluate any significant change in protein amount, the same samples were independently applied to 2-D gel electrophoresis and volumes of spots on one gel were compared with those of the corresponding spots on the other gel. Ratios of the spot volumes of more than 99% proteins were in the range between 3 and 0.33 (= 1/3), suggesting that the ratio of intensity of protein spot with more than 3 fold or less than 1/3 was significant (Figure 4-2).

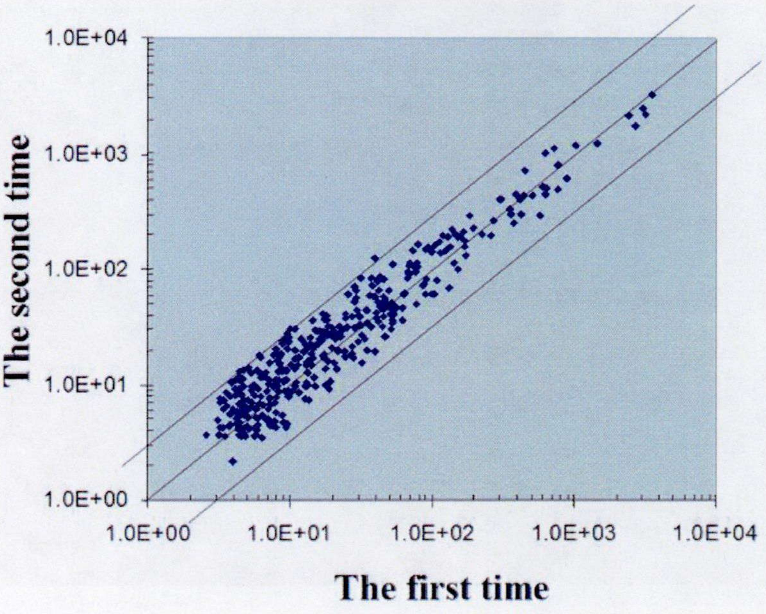


Figure 4-2 Reproducibility of proteomic experiment. X and Y axes represent the protein spot intensities obtained from 1st and 2nd proteomic experiments, respectively, in which the same protein sample was used.

Using MALDI-TOF MS, 36 proteins and two fragments of proteins (Eno1 and Eaf5, as indicated with asterisk (*)) could be identified and the proteome reference map of the FY834 strain was made (Figure 4-3). The molecular weight of Eaf5 protein is 31.64 kD with p*I*=8.9, however as shown in Figure 4-3, the spot which was identified as Eaf5 located in 2-DE gel with molecular weight about 25.4 kD and p*I* about 4.5 - 4.7. Thus it suggested that Eaf5p was degraded and I found the fragment of Eaf5p.

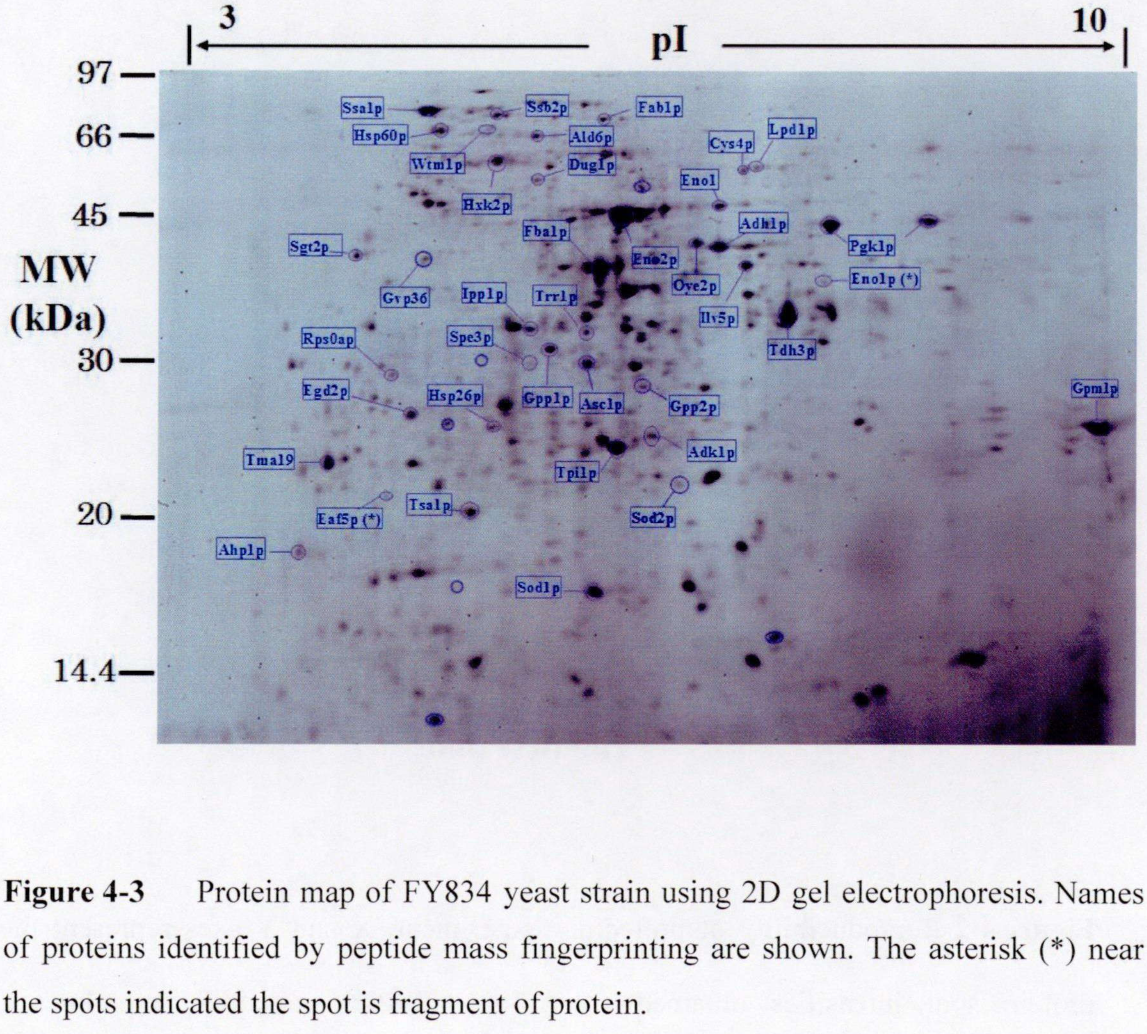


Figure 4-3 Protein map of FY834 yeast strain using 2D gel electrophoresis. Names of proteins identified by peptide mass fingerprinting are shown. The asterisk (*) near the spots indicated the spot is fragment of protein.

4.3.3 Proteome analysis of the ethanol adapted strain of *S. cerevisiae*

The A1 strain was grown in YPD medium containing 10% of ethanol, and at mid-log phase, the cells were collected for 2-D gel electrophoresis. The proteomic data was simultaneously obtained from the FY834 grown in the YPD medium without adding ethanol and compared with that obtained from the same strain cultured in YPD medium containing 10% ethanol. The growth behavior and sampling point of the A1 strain were shown in Figure 4-4.

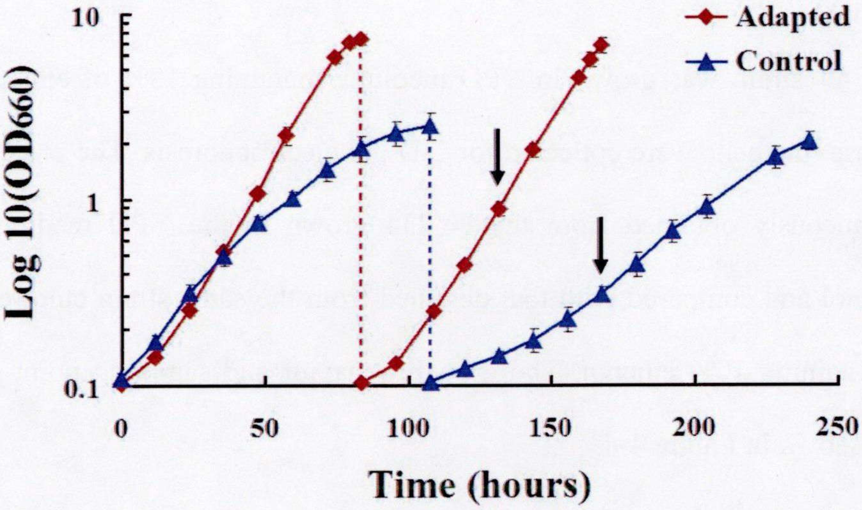
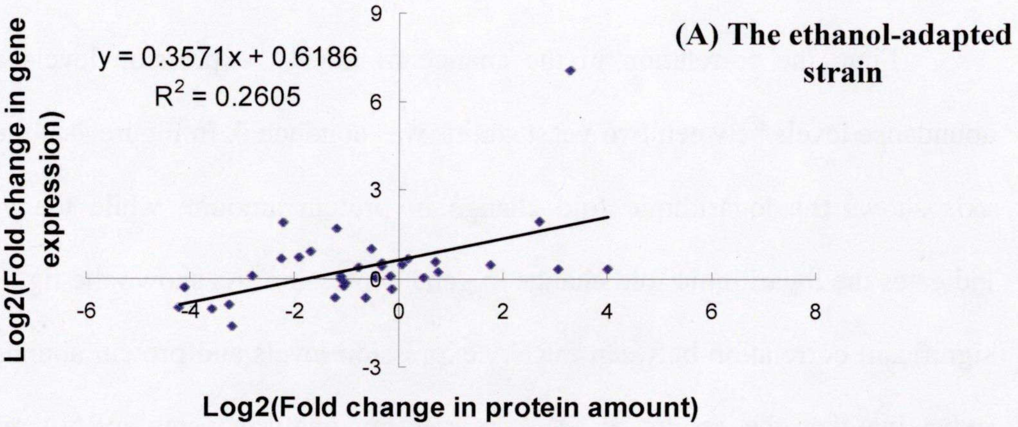


Figure 4-4 Growth characteristics of the ethanol-adapted strain A1 and the control strain FY834 under high ethanol concentration. Yeast strains were cultivated aerobically at 30 ℃ in YPD medium containing 10% ethanol. I repeated this culture, where a part of the first culture medium along with the yeast cells was transferred to a fresh medium containing 10% ethanol. The timing of the transfer to the fresh medium is indicated by the dotted lines. The time of cells harvest for protein extraction is indicated by the arrows. Average and standard deviation of OD_{660} in three independent experiments were shown.

First, the correlation in the change of mRNA expression levels and protein abundance levels between two yeast strains was conducted. In Figure 4-5, the horizontal axis shows the logarithmic fold change in protein amount, while the vertical axis indicates the logarithmic fold change in gene expression. As shown the figure, there is a significant correlation between mRNA expression levels and protein abundance levels, indicating that the results of DNA microarray analysis using mRNA reflected the changes in protein amount. The correlation coefficient values of these two data are 0.51 and 0.53, respectively, which were statistically significant ($p<0.01$). However, these correlations are not perfect, indicating that there is some difference between the change of mRNA expression levels and protein abundance levels, as depicted by the previous studies [101-103].



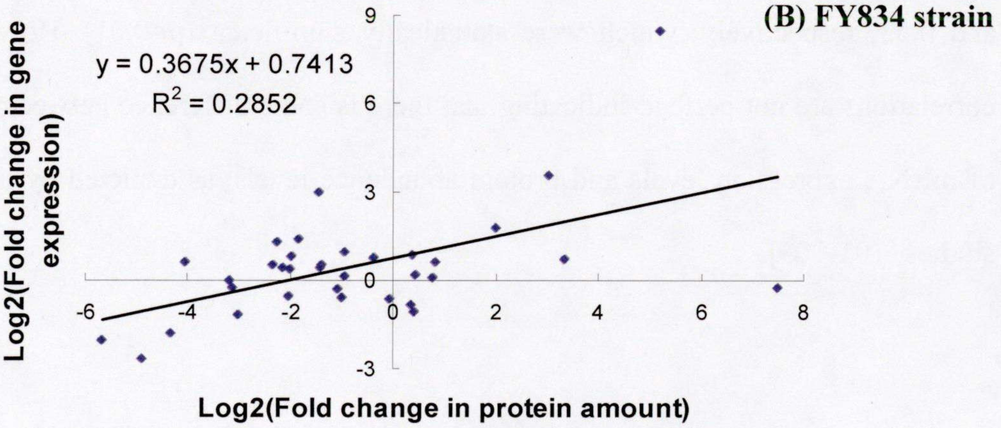


Figure 4-5 Correlation between the abundance of the identified proteins and mRNA expression levels of corresponding genes in the ethanol-adapted (A) and control (B) strains under high ethanol concentration condition. The gene expression data reported in chapter 3 and protein amount data of 34 identified proteins (of the ethanol-adapted and control strains) were used for examining correlation.

SYPRO red stained gel images of proteins separated by two-dimensional (2-D) polyacrylamide gel electrophoresis from the yeast samples of the control and ethanol-adapted strains grown in medium contained 10% ethanol was shown in Figure 4-7. Comparison of the intensity of protein spots of two yeasts (i.e., ethanol-adapted A1 and the control FY834 strains grown under high ethanol stress) was shown in Figure 4-6 and Table 4-1. Amount of five proteins (Ssb2p, Wtm1p, Hxk2p, Gvp36p and Egd2p) of the ethanol-adapted A1 strain were significantly higher than those of the control FY834 strain. In contrast, amount of two proteins (Hsp26p and fragment of Eaf5p) of the control were significantly higher than those of the ethanol-adapted A1 strain.

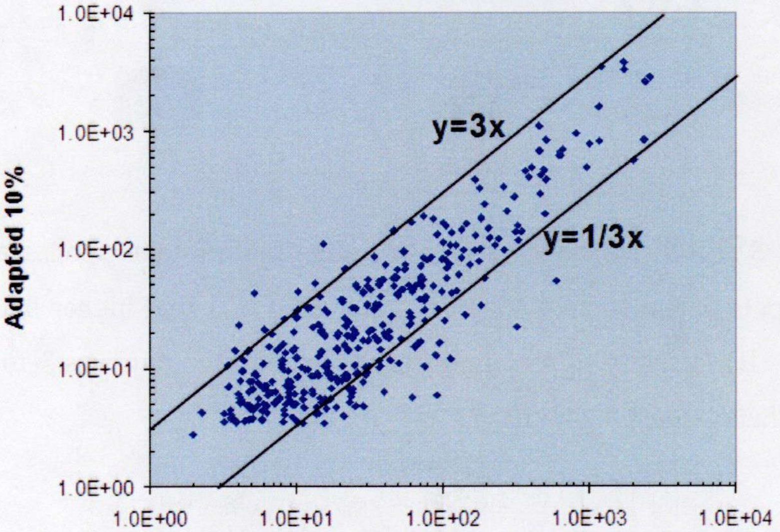


Figure 4-6 The scatter plot of intensity of protein spots in 2-DE gels in comparison between the FY834 (X axis) and ethanol adapted yeast (Y axis) grown in YPD medium containing 10% ethanol.

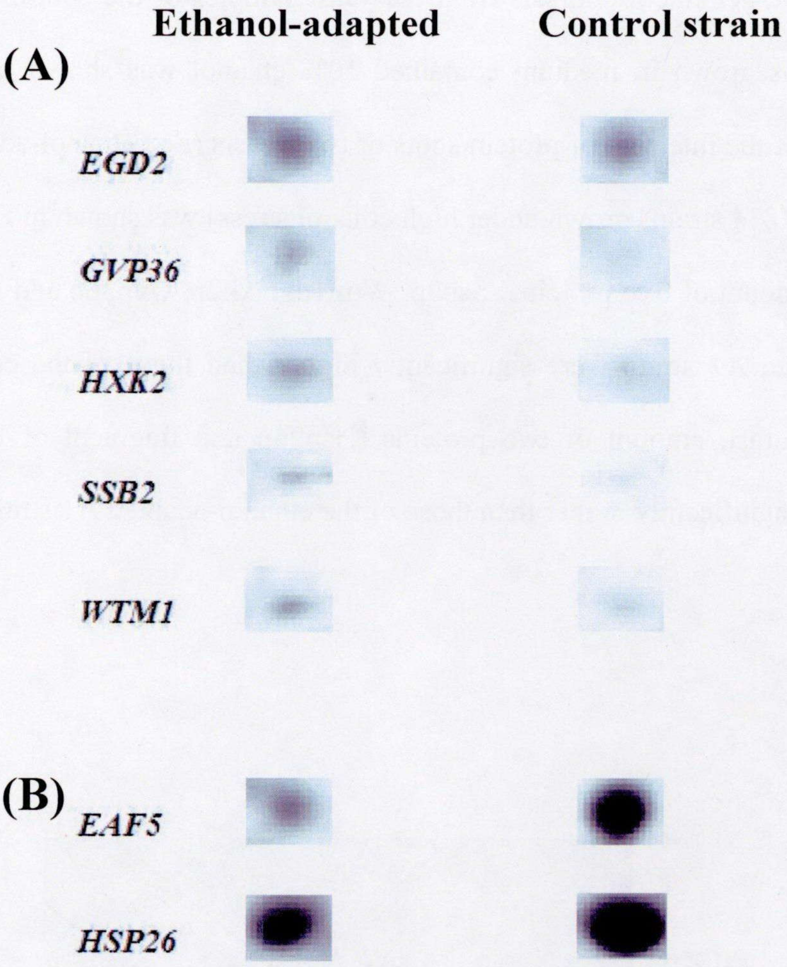


Figure 4-7 SYPRO red stained images of identified proteins spots. (A) Intensity of five protein spots of the ethanol-adapted strain A1 was 3-fold higher than those of the control strain. (B) Intensity of protein spots of the control strain was 3-fold higher than those of the ethanol-adapted strain.

Table 4-1 The protein amount ratio of each identified protein spot of the ethanol-adapted yeast to those of FY834 yeast under the stress condition (10% ethanol). A10/C10 indicated the protein ratio of each identified protein of the adapted strain to those of FY834, the asterisk (*) indicated a main biological process of the protein.

Systematic name	Name	A10/ C10	Biological process
YAL005C	*SSA1*	2.07	protein biosynthesis
YNL209W	*SSB2*	4.27	protein biosynthesis
YLR259C	*HSP60*	1.56	protein folding*
YFR019W	*FAB1*	2.07	phosphoinositide phosphorylation
YOR230W	*WTM1*	3.01	protein import into nucleus
YPL061W	*ALD6*	1.02	acetate biosynthesis
YGR155W	*CYS4*	1.31	cysteine biosynthesis
YFL018C	*LPD1*	0.37	glycine catabolism*
YGL253W	*HXK2*	3.17	replicative cell aging*
YFR044C	*DUG1*	1.75	glutathione catabolic process
YGR254W	*ENO1*	0.84	glycolysis*
YHR174W	*ENO2*	1.86	glycolysis*
YCR012W	*PGK1*	2.59	glycolysis*
YHR179W	*OYE2*	0.64	biological process unknown
YOL086C	*ADH1*	0.98	fermentation
YOR007C	*SGT2*	1.13	glutathione catabolic process
YIL041W	*GVP36*	4.16	vacuole organization and endocytosis
YKL060C	*FBA1*	1.41	glycolysis*
YLR355C	*ILV5*	0.94	mitochondrial genome maintenance*
YGR192C	*TDH3*	1.96	glycolysis*
YBR011C	*IPP1*	1.19	phosphate metabolism
YDR353W	*TRR1*	0.90	regulation of cell redox homeostasis
YPR069C	*SPE3*	0.71	pantothenate biosynthesis*
YMR116C	*ASC1*	1.09	glucose mediated signaling
YIL053W	*RHR2*	0.51	response to osmotic stress*
YER062C	*GPP2*	0.75	response to osmotic stress*
YDR050C	*TPI1*	0.98	glycolysis
YDR226W	*ADK1*	0.83	nucleotide metabolism
YBR072W	*HSP26*	0.27	response to stress*
YKL056C	*TMA19*	1.30	response to oxidative stress
YHR193C	*EGD2*	3.65	'de novo' cotranslational protein folding
YEL018W	*EAF5*	0.09	DNA repair
YML028W	*TSA1*	2.15	response to oxidative stress*
YLR109W	*AHP1*	1.21	regulation of cell redox homeostasis*
YJR104C	*SOD1*	0.41	zinc ion homeostasis*
YHR008C	*SOD2*	0.80	replicative cell aging*
YKL152C	*GPM1*	0.85	glycolysis*

4.4 Discussion

As shown in previous chapter 2 and Fig 4-4, the growth rate of the ethanol-adapted strain A1 was higher than of the control strain grown under high ethanol condition. In Chapter 3, using DNA microarray, the global gene expression of the ethanol-adapted strain under high ethanol concentration was conducted. To examine the protein expression in the ethanol-adapted strain, proteome analysis of the ethanol-adapted A1 and FY834 strains grown in medium containing high ethanol concentration was also performed. Futcher *et al.* (1999) investigated that there was a relatively high correlation ($r=0.74$) between protein abundance and mRNA expression levels [104]. In contrast, Gygi *et al.* (1999) found that even similar in mRNA expression levels could be accompanied by a wide range of protein abundance level, and vice versa [101]. I found that there is a significant correlation between mRNA expression levels (in Chapter 3) and protein abundance levels (in this chapter). The correlation coefficient values of these two data are 0.51 and 0.53, respectively, which were statistically significant ($p<0.01$). However, these correlations are not perfect, indicating that there is some difference between the change of mRNA expression levels and protein abundance levels, as depicted by the previous studies [101-103]. This fact means that there are several proteins whose abundant amount changes cannot be detected by DNA microarray experiments, but are detectable by 2-DE experiments. Such proteins might be involved in the adaptation process to the high ethanol stress condition.

The amount of 2 proteins, Hsp26p and fragment of Eaf5p in the ethanol-adapted strain A1 were significantly lower than that in the control strain FY834 grown in 10% ethanol. Hsp26p is a cytosolic member of small heat shock protein (sHSP) family of molecular chaperones. I found that the Hsp26 protein is expressed with a low amount in unstressed cells but it is strongly induced by high ethanol stress. Under the stress condition, *HSP26* expression is up-regulated by the transcription factors Hsf1p and Msn2p/Msn4p which binds to heat shock elements and stress responsive elements, respectively, in the *HSP26* promoter [111]. Since Carmelo *et al.* previously reported that Hsp26p accumulates when cells enter stationary phase growth [112]. And in this chapter, it was found that Hsp26p amount of the control strain was significantly higher than that of the ethanol-adapted strain. This fact confirmed the conclusion in Chapter 3 that there is a relationship between the expression profile of the FY834 control strain under the ethanol stress condition and that at the stationary phase. In this chapter, fragment of Eaf5p of the control strain was identified with significant higher amount than of the ethanol-adapted strain. Eaf5p is involved in DNA repair process [113], thus further investigation of the change of Eaf5p is needed to understand the effect of ethanol on yeast cell.

The production of five proteins, Ssb2p, Wtm1p, Hxk2p, Gvp36p and Egd2p, of the ethanol-adapted strain A1 was 3-fold higher than those of the control strain FY834. Ssb2p is a chaperone protein that is member of SSB (Stress-Seventy subfamily B), a subfamily of cytosolic heat shock proteins 70 (HSP70s) [114]. The main function of HSP70s is to serve as molecular chaperones that binds with newly-translated proteins to assist proper folding and to prevent aggregation. Wtm1p is a transcriptional modulator that involves in regulation of meiosis, silencing, and expression of RNR (RiboNucleotide Reductase) genes [115]. Hxk2p, a hexokinase isoenzyme 2 that catalyzes phosphorylation of glucose in the cytosol is a predominant hexokinase during growth on glucose [116]. Gvp36p, a BAR (Bin/Amphiphysin/Rvs) domain-containing protein that localizes to both early and late Golgi vesicles, it is required for adaptation to varying nutrient concentrations, fluid-phase endocytosis, polarization of the actin cytoskeleton, and vacuole biogenesis [117]. Egd2p is an alpha subunit of the heteromeric nascent polypeptide-associated complex (NAC), which involves in protein sorting and translocation, associated with cytoplasmic ribosomes [118]. The induction of production of these five proteins might be important for the yeast cells to adapt and grow in the medium containing high ethanol concentration. Three of them, Ssb2p, Wtm1p and Egd2p are involved in translation, these proteins might have an important impact on enhancing the activity of protein synthesis during the adaptation to high ethanol concentration.

4.5 Conclusion

As shown in Chapter 2, adaptation process of yeast to high ethanol concentration in repetitive cultivation was investigated. In Chapter 3, using DNA microarray, the global gene expression of the ethanol-adapted strain under high ethanol concentration was performed. In this chapter, a further study about the intracellular proteins of the ethanol-adapted strain was investigated using 2-D electrophoresis to identify the differential protein expression under ethanol adaptation.

The correlation between the change of mRNA expression levels and protein abundance levels between two yeast strains was conducted. Even though there is a significant correlation between mRNA expression levels and protein abundance however, these correlations are not perfect, indicating that there is some difference between the change of mRNA expression levels and protein abundance levels.

In the control strain, the amount of Hsp26p and Eaf5p were significantly high, suggesting a relationship between the expression profile of the FY834 control strain under the ethanol stress condition and that at the stationary phase. On the other hand, an increase in abundance of five proteins, Ssb2p, Wtm1p, Hxk2p, Gvp36p, and Egd2p, was found in the ethanol-adapted yeast when compare to the control strain. Three of them, Ssb2p, Wtm1p and Egd2p are involved in translation, these proteins might have important impacts on enhancing the activity of protein synthesis and growth recovery of the ethanol-adapted strain during the adaptation to high ethanol concentration.

Chapter 5

General conclusion

There have been many previous studies on the “short-term” ethanol adaptation of *Saccharomyces cerevisiae.* However few studies about the “long-term” ethanol adaptation have been reported. It is well known that despite the fact that many genes and proteins change their expression levels soon after stress exposure, certain fractions of them recover to their original expression levels after cells adapt to the stress and recover growth [13, 67, 86, 87]. Thus, the “long-term” expression of genes/proteins in response to stress might be much more important for conferring stress tolerance of yeast cells. The objective of this study was to investigate the phenomena of *S. cerevisiae* during “long-term” ethanol adaptation:

In Chapter 1, the significance of this research was described. General introduction presented the toxicity of ethanol to yeast cells and the ethanol adaptation of yeast cells during fermentation processes. The objective of thesis and an outline of this study were also presented in this chapter.

In Chapter 2, the stepwise adaptation process of *S. cerevisiae* was investigated by carrying out exposure to a stepwise increase in ethanol concentration with repetitive cultivations. When subjecting yeast cells to a stepwise increase in the ethanol concentration of the culture medium, the specific growth rate (SGR) of the ethanol-adapted strain did not change through repetitive cultivation under high ethanol concentration, suggesting that I could successfully obtain a strain, whose SGR is not decreased through repetitive cultivation at high ethanol concentrations. Observations of cell morphology and measurement of fatty acid composition of the cell membrane in the adapted and the non-adapted yeast strains revealed that yeast cells change the intracellular physiological state, such as change in the composition of fatty acid and the increase in the size of yeast, to adapt to ethanol stress.

In Chapter 3, by using DNA microarray, a comprehensive expression analysis of the ethanol-adapted and the control yeast strains was performed and it was revealed that certain genes and functional categories were involved in the ethanol adaptation of yeast to ethanol stress condition. Our results showed that the expressions of ribosomal proteins were specifically up-regulated in the adapted strain under the ethanol stress condition, suggesting an increase in protein synthesis activity in the adapted strain. Furthermore, I found that genes related to ATP synthesis in mitochondria and oxidative stress response were significantly up-regulated in both the adapted and control strains. These results suggested that these functions are involved in growth recovery under the ethanol stress condition. In contrast, I found that genes related to amino acid biosynthesis were up-regulated specifically in the control strain under the ethanol stress condition, indicating that such genes are not involved in the growth recovery under ethanol stress condition.

In Chapter 4, proteomic analysis of the ethanol-adapted strain was performed using 2-D electrophoresis combined with MALDI-TOF MS to identify the differential protein expression of the ethanol adapted and control yeast strains. It was initially shown that the ethanol-adapted strain changes expression level of some proteins. For example, protein abundances of Hsp26p and Eaf5p in the ethanol-adapted strain were significantly reduced than those of the control strain in 10% ethanol. In contrast, increased abundance of five proteins was identified in the adapted yeast. Three of them, Ssb2p, Wtm1p and Egd2p, have main function involved in translation. These proteins might have important impacts on enhancing the activity of protein synthesis and growth recovery of the ethanol-adapted strain during the adaptation to high ethanol concentration.

In conclusion, the stepwise adaptation process of *S. cerevisiae* was investigated in this study by culturing yeast cells to a stepwise increase in ethanol concentration with repetitive cultivations. Observations of cell morphology revealed that yeast cells change the intracellular physiological state, such as a change in the composition of fatty acid and the increase in the size of yeast, to adapt to ethanol stress. Comprehensive analysis of the ethanol-adapted and control yeast strains at transcriptional and translational levels were performed. Certain genes and proteins involved in the "long-term" ethanol adaptation were identified. Results suggested that the recovery of protein synthesis activity plays an important role in the adaptation to high ethanol concentration.

References

1. Hahn-Hägerdal B, Galbe M, Gorwa-Grauslund MF, Lidén G, Zacchi G (2006) Bio-ethanol-the fuel of tomorrow from the residues of today. Trends Biotechnol 24:549–556.

2. Pretorius IS (2000) Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. Yeast 16:675–729.

3. Attfield PV (1997) Stress tolerance: The key to effective strains of industrial baker's yeast. Nat Biotechnol 15: 1351–1357.

4. Gibson BR, Lawrence SJ, Leclaire JP, Powell CD, Smart KA (2007) Yeast responses to stresses associated with industrial brewery handling. FEMS Microbiol Rev 31:535–569.

5. Estruch F (2000) Stress controlled transcription factors, stress-induced genes and stress tolerance in budding yeast. FEMS Microbiol Rev 24:469–486.

6. Casey GP, Ingledew WM (1986) Ethanol tolerance in yeast. CRC Crit Rev Microbiol. 13:219–280.

7. Sajbidor J, Greco J (1992) Fatty acid alterations in *Saccharomyces cerevisiae* exposed to ethanol stress. FEMS Microbiol Lett 72:13–16.

8. Beaven MJ, Chrpentier C, Rose AH (1982) Production and tolerance of ethanol in relation to phospholipids fatty-acyl composition in *Saccharomyces cerevisiae* NCYC 431. J General Microbiol 128:1447–1455.

9. D'amore T, Stewart G (1987) Ethanol tolerance of yeast. Enzyme Microb Technol 9:322–330.

10. Mizoguchi H, Hara S (1997) Ethanol-induced alterations in lipid composition of *Saccharomyces cerevisiae* in the presence of exogenous fatty acid. J Ferment Bioeng 83:12–16.

11. Alexandre H, Ansanay-Galeote V, Dequin S, Blondin B (2001) Global gene expression during short-term ethanol stress in *Saccharomyces cerevisiae*. FEBS Lett 498:98–110.

12. Chandler M, Stanley GA, Rogers P, Chambers P (2004) A genomic approach to defining the ethanol stress response in the yeast *Saccharomyces cerevisiae*. Ann Microbiol 54:427–454.

13. Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, Storz G, Botstein D and Brown PO (2000) Genomic expression programs in the response of yeast cells to environmental changes. Mol Biol Cell 11: 4241–4257.

14. Causton HC, Ren B, Koh SS, Harbison CT, Kanin E, Jennings EG, Lee TI, True HL, Lander ES & Young RA (2001) Remodelling of yeast genome expression in response to environmental changes. Mol Biol Cell 12: 323–337.

15. Takagi H, Takaoka M, Kawaguchi A, Kubo Y (2005) Effect of L-proline on sake brewing and ethanol stress in *Saccharomyces cerevisiae*. Appl Environ Microbiol 71:8656–62.

16. Trabalzini L, Paffetti A, Scaloni A, Talamo F, Ferro E, Coratza G, Bovalini L, Lusini P, Martelli P, Santucchi A (2003) Proteomic response to physiological

fermentation stresses in a wild-type wine strain of *Saccharomyces cerevisiae*. J Biochem 370:35–46.

17. Hirasawa T, Yoshikawa K, Nakakura Y, Nagahisa K, Furusawa C, Katakura Y, Shimizu H, Shioya S (2007) Identification of target genes conferring ethanol stress tolerance to *Saccharomyces cerevisiae* based on DNA microarray data analysis. J Biotechnol 131:34–44.

18. Ismail AA, Ali MM (1971) Selection of high ethanol-yielding *Saccharomyces* I. Ethanol tolerance and the effect of training in *Saccharomycess cerevisiae* Hansen. Folia Microbiol 16:346–369.

19. Lloyd D, Morrell S, Carlsen HN, Degn H, James PE, *et al.* (1993) Effects of growth with ethanol on fermentation and membrane fluidity of *Saccharomyces cerevisiae*. Yeast 9:825–833.

20. Ruis H, Schuller C (1995) Stress signaling in yeast. BioEssays 17:959–965.

21. Martinez-Pastor MT, Marchler G, Schuller C, Marchler-Bauer A, Ruis H, Estruch F (1996) The *Saccharomyces cerevisiae* zinc finger proteins Msn2p and Msn4p are required for transcriptional induction through the stress response element (STRE). EMBO J 15:2227–2235.

22. Schmitt AP, McEntee K (1996) Msn2p, a zinc finger DNA binding protein, is the transcriptional activator of the multistress response in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 93:5777–5782.

23. Treger JM, Schmitt AP, Simon JR, McEntee K (1998) Transcriptional factor mutations reveal regulatory complexities of heat shock and newly identified stress genes in *Saccharomyces cerevisiae*. J Biol Chem 273:26875–9.

24. Bose S, Dutko JA, Zitomer RS (2005) Genetic factors that regulate the attenuation of the stress response in yeast. Genetics 169:1215–1226.

25. Yamamoto A, Mizukami Y, Sakurai H (2005) Identification of a novel class of target genes and a novel type of binding sequence of heat shock transcription factor in *Saccharomyces cerevisiae*. J Biol Chem 280:11911–11919.

26. Hashikawa N, Mizukami Y, Imazu H, Sakurai H (2006) Mutated yeast heat shock transcription factor activates transcription independently of hyperphosphorylation. J Biol Chem 281:3936–42.

27. Eastmond DL, Nelson HC (2006) Genome-wide analysis reveals new roles for the activation domains of the *Saccharomyces cerevisiae* heat shock transcription factor (Hsf1) during the transient heat shock response. J Biol Chem 281:32909–32921.

28. Fernandes L, Corte-Real M, Loureiro V, Loureiro-Dias MC, Leao C (1997) Glucose respiration and fermentation in *Zygosaccharomyces bailii* and *Saccharomyces cerevisiae* express different sensitivity patterns to ethanol and acetic acid. Lett Appl Microbiol 25: 249–253.

29. Canetta E, Adya AK, Walker GM (2006) Atomic force microscopic study of the effects of ethanol on yeast cell surface morphology. FEMS Microbiol Lett 255:308-315

30. Kubota S, Takeo I, Kume K, Kanai M, Shitamukai A, *et al.* (2004) Effect of ethanol on cell growth of budding yeast: genes that are important for cell growth in the presence of ethanol. Biosci Biotechnol Biochem 68:968–972.

31. Alexandre H, Plourde L, Charpentier C, Francois J (1998). Lack of correlation between trehalose accumulation, cell viability, and intracellular acidification as induced by various stresses in *Saccharomyces cerevisiae*. Microbiol 144:1103–1111.

32. Chi Z, Arneborg N (1999) Relationship between lipid composition, frequency of ethanol-induced respiratory deficient mutants, and ethanol tolerance in *Saccharomyces cerevisiae*. J Appl Microbiol 86:1047–1053.

33. Jim'enez J, Longo E, Benítez T (1988) Induction of petite yeast mutants by membrane-active agents. Appl Environ Microbiol 54:3126–3132.

34. Ibeas JI, Jim'enez J (1997) Mitochondrial DNA loss caused by ethanol in Saccharomyces flor yeast. Appl Environ Microbiol 63:7–12.

35. Jim'enez J, Benítez T (1988) Yeast cell viability under conditions of high temperature and ethanol concentrations depends on the mitochondrial genome. Curr Genet 13:461–469.

36. Jones RP, Greenfield PF (1987) Ethanol and the fluidity of the yeast plasma membrane. Yeast 3:223–232.

37. Marza E, Camougrand N, Manon S (2002) Bax expression protects yeast plasma membrane against ethanol-induced permeabilization. FEBS Lett 521:47–52.

38. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P (2007) Molecular Biology of the Cell, Fifth Edition. New York, Garland Science.

39. Chen ECH (1981) Fatty acid profiles of some cultured and wild yeasts in brewing. J Am Soc Brew Chem 39:117–124.

40. Hayashida S, Feng DD, Hongo M (1974) Function of the high alcohol-producing factor. Agr Biol Chem 38:2001–2006.

41. Ingram LO (1976) Adaptation of membrane lipids to alcohols. J Bacteriol 125:670–678.

42. Thomas DS, Hossack AJ, Rose AH (1978) Plasma membrane lipid composition and ethanol tolerance. Arch Microbiol 117:239–245.

43. Mishra P, Prasad R (1989) Relationship between ethanol tolerance and fatty acyl composition of *Saccharomyces cerevisiae.* Appl Microbiol Biotechnol 30:294–298.

44. Alexandre H, Rousseaux I, Charpentier C (1993) Ethanol adaptation mechanisms in *Saccharomyces cerevisiae*. Biotechnol Appl Biochem 20:173–183.

45. Sajbidor J, Ciesarova Z, Smogrovicova (1995) Influence of ethanol on the lipid content and fatty acid composition of *Saccharomyces cerevisiae.* Folia Microbiol 40:508–510.

46. Alexandre H, Rousseaux I, Charpentier C (1994) Relationship between ethanol tolerance, lipid composition and plasma membrane fluidity in *Saccharomyces cerevisiae and Kloeckera apiculata*. FEMS Microbiol. Lett. 124: 17–22.

47. Kajiwara S, Suga K, Sone H, Nakamura K (2000) Improved ethanol tolerance of *Saccharomyces cerevisiae* strain by increases in fatty acid unsaturation via metabolic engineering. Biotechnol Lett 22:1839–1843.

48. You KM, Rosenfield CL, Knipple DC (2003) Ethanol tolerance in the yeast *Saccharomyces cerevisiae* is dependent on cellular oleic acid content. Appl Environ Microbiol 69:1499–1503.

49. Leao C, Van Uden N (1982) Effect of ethanol and other alkanols on the glucose transport system of *Saccharomyces cerevisiae.* Biotechnol. Bioeng. 24:2601–2607.

50. Pascual C, Alonson A, Garcia I, Romay C (1988). Effect of ethanol on glucose transport, key glycolytic enzymes, and proton extrusion in *Saccharomyces cerevisiae*. Biotechnol Bioeng 32:374–378.

51. Rosa MF, Sá-Correia I (1991) In vivo activation by ethanol of plasma membrane ATPase of *Saccharomyces cerevisiae*. Appl Environ Microbiol 57:863–866.

52. Fujita K, Matsuyama A, Kobayashi Y, Iwahashi H (2006) The genome-wide screening of yeast deletion mutants to identify the genes required for tolerance to ethanol and other alcohols. FEMS Yeast Res 6:744–750.

53. Piper PW (1995) The heat shock and ethanol stress responses of yeast exhibit extensive similarity and functional overlap. FEMS Microbiol Lett 134:121–127.

54. Sanchez Y, Taulien J, Borkovich K A, Lindquist S (1992) Hsp104 is required for tolerance to many forms of stress. EMBO J 11: 2357–2364.

55. Glover JR, Lindquist S (1998) Hsp104, Hsp70, and Hsp40 - a novel chaperone system that rescues previously aggregated proteins. Cell 94:73–82.

56. Sales K, Brandt W, Rumbak E, Lindsey G (2000) The LEA-like protein *HSP12* in *Saccharomyces cerevisiae* has a plasma membrane location and protects membranes against desiccation and ethanol-induced stress. Biochimica et Biophysica Acta 1463:267–278.

57. Singer MA, Lindquist S (1998) Thermotolerance in *Saccharomyces cerevisiae*: the Yin and Yang of trehalose. Trends Biotechnol 16:460–468.

58. Londesborough J, Varimo K (1984) Characterization of two trehalases in bakers yeast. Biochem J 219:511–518.

59. Kim J, Alizadeh P, Harding T, Hefner-Gravink A, Klionsky DJ (1996) Disruption of the yeast *ATH1* gene confers better survival after dehydration, freezing, and ethanol shock: potential commercial applications, Appl Environ Microbiol 62:1563–1569.

60. Jung YJ, Park HD (2005) Antisense-mediated inhibition of acid trehalase (*ATH1*) gene expression promotes ethanol fermentation and tolerance in *Saccharomyces cerevisiae*. Biotechnol Lett 27:1855–1859.

61. Alper H, Moxley J, Nevoigt E, Fink GR, Stephanopoulos G (2006) Engineering yeast transcription machinery for improved ethanol tolerance and production. Science 314:1565–1568

62. Goffeau A, Barell BG, Bussey H, Davis RW, Dujon B, Feldmann H, Galibert F, Hoheisel JD, Jacq C, Johnston M, Louis EJ, Mewes HW, Murakami Y, Philippsen P, Tettelin H, Oliver SG (1996) Life with 6,000 genes. Science 274:546–567.

63. van Voorst F, Houghton-Larsen J, Jønson L, Kielland-Brandt MC, Brandt A (2006) Genome-wide identification of genes required for growth of *Saccharomyces cerevisiae* under ethanol stress. Yeast 23:351–359.

64. Yoshikawa K, Tanaka T, Furusawa C, Nagahisa K, Hirasawa T, Shimizu H (2008) Comprehensive phenotypic analysis for identification of genes affecting growth under ethanol stress in *Saccharomyces cereviciae*. FEMS Yeast Res doi:10.1111/j.1567-1364.2008.00456.x

65. Lashkari DA, DeRisi JL, McCusker JH, Namath AF, Gentile C, Hwang SY, Brown PO, Davis RW (1997) Yeast microarrays for genome wide parallel genetic and gene expression analysis. Proc Natl Acad Sci USA 94:13057–13062.

66. Brown PO, Botstein D (1999) Exploring the new world of the genome with DNA microarrays. Nat Genet 21:33–37.

67. Rossignol T, Dulau L, Julien A, Blondin B (2003) Genome-wide monitoring of wine yeast gene expression during alcoholic fermentation. Yeast 20:1369–85

68. Gibson BR, Boulton CA, Box WG, Graham NS, Lawrence SJ, Linforth RS, Smart KA (2008) Carbohydrate utilization and the larger yeast transcriptome during brewery fermentation. Yeast 25:549–562.

69. Zuzuarregui A, Monteoliva L, Gil C, del Olmo M (2006) Transcriptomic and proteomic approach for understanding the molecular basis of adaptation of

Saccharomyces cerevisiae to wine fermentation. Appl Environ Microbiol. 72:836–847.

70. Angelika G, Walter W, Michael J D (2004) Current two-dimensional electrophoresis technology for proteomics. Proteomics 4:3665–3685.

71. Pham TK, Wright PC (2006) Proteomic analysis of calcium alginate-immobilized *Saccharomyces cerevisiae* under high-gravity fermentation conditions. J Proteome Res 7:515–525.

72. Kolkman A, Olsthoorn MM, Heeremans CE, Heck AJ, Slijper M (2005) Comparative proteome analysis of *Saccharomyces cerevisiae* grown in chemostat cultures limited for glucose or ethanol. Mol Cell Proteomics 4:1–11.

73. Villas-Boas SG, Mas S, Akesson M, Smedsgaard J, Nielsen J (2005b) Mass spectrometry in metabolome analysis. Mass Spectro Rev 24:613–646.

74. Jewett MC, Hofmann G, Nielsen J (2006) Fungal metabolite analysis in genomics and phenomics. Curr Opin Biotechnol 17:191–197.

75. Winston F, Dollard C, Ricupero-Hovasse SL (1995) Construction of a set of convenient *Saccharomyces cerevisiae* strains that are isogenic to S288C. Yeast 11:53–55.

76. Walker-Caprioglio HM, Rodriguez RJ, Parks LW (1985) Recovery of *Saccharomyces cerevisiae* from ethanol-induced growth inhibition. Appl Environ Microbiol 50:685–689.

77. Blanch HW, Clark DS (1997) Biochemical engineering. New York: Marcel Dekker Inc.

78. Hama S, Yamaji H, Kaieda M, Oda M, Kondo A, *et al.* (2004) Effect of fatty acid membrane composition on whole-cell biocatalysts for biodiesel-fuel production. Biochem Eng J 21:155–160.

79. María JT, Gemma B, Maite N, Montse P, José MG, *et al.* (2003) Effects of fermentation temperature and *Saccharomyces* species on the cell fatty acid composition and presence of volatile compounds in wine. Int J Food Microbiol 85:127–136.

80. Johnston GC, Pringle JR, Hartwell LH (1977) Coordination of growth with cell division in the yeast *Saccharomyces cerevisiae*. Exp Cell Res 105:79–98.

81. Calvert GR, Dawes IW (1984) Cell size control of development in *Saccharomyces cerevisiae*. Nature 312:61–63.

82. Clotet J, Posas F (2007) Control of cell cycle in response to osmostress: lessons from yeast. Methods Enzymol 428:63–76.

83. Walker-Caprioglio HM, Casey WM, Parks LW (1990) *Saccharomyces cerevisiae* membrane sterol modifications in response to growth in the presence of ethanol. Appl Environ Microbiol 56:2853–2857.

84. Ogawa Y, Nitta A, Uchiyama H, Imamura T, Shimoi H, Ito K (2000) Tolerance mechanism of the ethanol-tolerant mutant of sake yeast. J Biosci Bioeng 90:313–20.

85. Watanabe M, Tamura K, Magbanua JP, Takano K, Kitamoto K, Kitagaki H, Akao T, Shimoi H (2007) Elevated expression of genes under the control of stress response element (STRE) and Msn2p in an ethanol-tolerance sake yeast Kyokai no. 11. J Biosci Bioeng 104:163–170.

86. Pérez-Torrado R, Carrasco P, Aranda A, Gimeno-Alcañiz J, Pérez-Ortín JE, Matallana E, del Olmo ML (2002) Study of the first hours of microvinification by the use of osmotic stress-response genes as probes. Syst Appl Microbiol 25:153–61.

87. Hirasawa T, Nakakura Y, Yoshikawa K, Ashitani K, Nagahisa K, Furusawa C, Katakura Y, Shimizu H, Shioya S (2006) Comparative analysis of transcriptional responses to saline stress in the laboratory and brewing strains of *Saccharomyces cerevisiae* with DNA microarray. Appl Microbiol Biotechnol 70:346–357.

88. Dinh TN, Nagahisa K, Hirasawa T Furusawa C, Shimizu H (2008) Adaptation of *Saccharomyces cerevisiae* cells to high ethanol concentration and changes in fatty acid composition of membrane and cell size. PLoS ONE 3(7):e2623.

89. Nagahisa K, Nakajima T, Yoshikawa K, Hirasawa T, Katakura Y, Furusawa C, Shioya S, Shimizu H (2005) DNA microarray analysis on *Saccharomyces cerevisiae* under high carbon dioxide concentration in fermentation process. Biotechnol Bioprocess Eng 10:451–461.

90. Köhrer K, Domdey H (1991) Preparation of high molecular weight RNA. Methods Enzymol 194:398–405.

91. Yang IV, Chen E, Hasseman JP, Liang W, Frank BC, Wang S, Sharov V, Saeed AI, White J, Li J, Lee NH, Yeatman TJ, Quackenbush J (2002) Within the fold: assessing differential expression measures and reproducibility in microarray assays. Genome Biol 3:research0062.1–0062.12.

92. Ruepp A, Zollner A, Maier D, Albermann K, Hani J, Mokrejs M, Tetko I, Güldener U, Mannhaupt G, Münsterkötter M, Mewes HW (2004) The FunCat, a functional annotation scheme for systematic classification of proteins from whole genomes. Nucleic Acids Res 32:5539–45.

93. Costa V, Amorim MA, Reis E, Quintanilha A, Moradas-Ferreira P (1997) Mitochondrial superoxide dismutase is essential for ethanol tolerance of *Saccharomyces cerevisiae* in the post-diauxic phase. Microbiology 143:1649–1656.

94. Martinez MJ, Roy S, Archuletta AB, Wentzell PD, Anna-Arriola SS, Rodriguez AL, Aragon AD, Quiñones GA, Allen C, Werner-Washburne M (2004) Genomic analysis of stationary-phase and exit in *Saccharomyces cerevisiae*: gene expression and identification of novel essential genes. Mol Biol Cell 15:5295–5305.

95. Herman PK (2002) Stationary phase in yeast. Curr Opin Microbiol 5:602–607.

96. Marks VD, Ho Sui SJ, Erasmus D, van der Merwe GK, Brumm J, Wasserman WW, Bryan J, van Vuuren HJ (2008) Dynamics of the yeast transcriptome during wine fermentation reveals a novel fermentation stress response. FEMS Yeast Res 8:35–52.

97. Powers T, Walter T (1999) Regulation of ribosome biogenesis by the Rapamycinsensitive TOR-signaling pathway in *Saccharomyces cerevisiae.* Mol Biol Cell 10:987–1000

98. Martin DE, Soulard A, Hall MN (2004) TOR regulates ribosomal protein gene expression via PKA and the Forkhead transcription factor FHL1. Cell 119:969–979.

99. Lin Y, Tanaka S (2006) Ethanol fermentation from biomass resources: current state and prospects. Appl Microbiol Biotechnol 69:627–42.

100. Dinh TN, Nagahisa K, Yoshikawa K, Hirasawa T, Furusawa C, Shimizu H (2009) Analysis of adaptation to high ethanol concentration in *Saccharomyces cerevisiae* using DNA microarray. Bioprocess Biosyst Eng doi:10.1007/s00449-008-0292-7.

101. Gygi SP, Rochon Y, Franza BR, Aebersold R (1999) Correlation between protein and mRNA abundance in yeast. Mol Cell Biol 19:1720–1730.

102. Ideker T, Thorsson V, Ranish JA, Christmas R, Buhler J, Eng JK, Bumgarner R, Goodlett DR, Aebersold R, Hood L (2001) Integrated genomic and proteomic analyses of a systematically perturbed metabolic network. Science 292:929–934.

103. Griffin TJ, Gygi SP, Ideker T, Rist B, Eng J, Hood L, Aebersold R (2002) Complementary profiling of gene expression at the transcriptome and proteome levels in *Saccharomyces cerevisiae.* Mol Cell Proteomics 1:323–333.

104. Futcher B, Latter GI, Monardo P, McLaughlin CS, Garrels JI (1999) A sampling of the yeast proteome. Mol Cell Biol 19:7357–68.

105. Laemmli, UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 15:680–685.

106. Bjellqvist B, Ek K, Righetti PG, Gianazza E, Görg A, Westermeier R, Postel W. (1982) Isoelectric focusing in immobilized pH gradients: principle, methodology and some applications. J Biochem Biophys Methods 6:317–39.

107. Yan JX, Harry RA, Spibey C, Dunn MJ (2000) Post-electrophoretic staining of proteins separated by two-dimensional gel electrophoresis using SYPRO dyes. Electrophoresis 21:3657–3665.

108. Tanaka K, Waki H, Ido Y, Akita S, Yoshida Y, Yoshida T (1988) Protein and polymer analysis up to m/z 10000 by laser ionization time-of -flight mass spectrometry. Rapid Communication in Mass Spectrometry 2:151–153.

109. Lahm HW, Langen H (2000) Mass spectrometry: A tool for the identification of proteins separated by gels. Electrophoresis 21:2105–2114.

110. Shevchenko A, Wilm M, Vorm O, Mann M (1996) Mass spectrometric sequencing of proteins from silver-stained polyacrylamide gels. Anal Chem 68:850–858.

111. Amoros M, Estruch F (2001) Hsf1p and Msn2/4p cooperate in the expression of *Saccharomyces cerevisiae* genes *HSP26* and *HSP104* in a gene- and stress type-dependent manner. Mol Microbiol 39:1523–32.

112. Carmelo V, Sá-Correia I (1997) *HySP26* gene transcription is strongly induced during *Saccharomyces cerevisiae* growth at low pH. FEMS Microbiol Lett 149:85–8.

113. Krogan NJ, Baetz K, Keogh MC, Datta N, Sawa C, Kwok TC, Thompson NJ, Davey MG, Pootoolal J, Hughes TR, Emili A, Buratowski S, Hieter P, Greenblatt JF (2004) Regulation of chromosome stability by the histone H2A variant Htz1, the Swr1 chromatin remodeling complex, and the histone acetyltransferase NuA4. Proc Natl Acad Sci U S A 101:13513–8.

114. Werner-Washburne M, Stone DE, Craig EA (1987) Complex interactions among members of an essential subfamily of hsp70 genes in *Saccharomyces cerevisiae.* Mol Cell Biol 7:2568–77.

115. Pemberton LF, Blobel G (1997) Characterization of the Wtm proteins, a novel family of *Saccharomyces cerevisiae* transcriptional modulators with roles in meiotic regulation and silencing. Mol Cell Biol 17:4830–41.

116. Rodríguez A, De La Cera T, Herrero P, Moreno F (2001) The hexokinase 2 protein regulates the expression of the *GLK1, HXK1* and *HXK2* genes of *Saccharomyces cerevisiae.* Biochem J 355:625–31.

117. Querin L, Sanvito R, Magni F, Busti S, Van Dorsselaer A, Alberghina L, Vanoni M (2008) Proteomic Analysis of a Nutritional Shift-up in *Saccharomyces cerevisiae* Identifies Gvp36 as a BAR-containing Protein Involved in Vesicular Traffic and Nutritional Adaptation. J Biol Chem 283:4730–43.

118. George R, Beddoe T, Landl K, Lithgow T (1998) The yeast nascent polypeptide-associated complex initiates protein targeting to mitochondria in vivo. Proc Natl Acad Sci USA 95:2296–301.

Supplemental data

Table I List of up-regulated genes only in the ethanol-adapted strain. The asterisk (*) indicated a main biological process of encoding protein.

Number	Systematic name	Standard name	Biological Process
1	YPL012W	RRP12	processing of 2biological process unknownS pre-rRNA*
2	YCL046W	YCL046W	biological process unknown
3	YLR404W	YLR404W	biological process unknown
4	YDR172W	SUP35	mRNA catabolism, deadenylylation-dependent decay*
5	YLR274W	CDC46	DNA replication initiation*
6	YPL035C	YPL035C	biological process unknown
7	YBR125C	PTC4	biological process unknown
8	YCL002C	YCL002C	biological process unknown
9	YPR027C	YPR027C	biological process unknown
10	YDR237W	MRPL7	protein biosynthesis
11	YPR157W	YPR157W	biological process unknown
12	YPL244C	HUT1	UDP-galactose transport
13	YGL252C	RTG2	intracellular signaling cascade*
14	YJL143W	TIM17	mitochondrial matrix protein import
15	YGL162W	SUT1	regulation of transcription from RNA polymerase II promoter*
16	YDR455C	YDR455C	biological process unknown
17	YDR328C	SKP1	protein complex assembly*
18	YLR372W	SUR4	sphingolipid biosynthesis*
19	YGR263C	YGR263C	biological process unknown
20	YGL041C	YGL041C	biological process unknown
21	YLR374C	YLR374C	biological process unknown
22	YLR316C	TAD3	tRNA modification
23	YLR285W	NNT1	chromatin silencing at ribosomal DNA*
24	YJL114W	YJL114W	biological process unknown
25	YCR047C	BUD23	bud site selection
26	YOR012W	YOR012W	biological process unknown
27	YLR182W	SWI6	meiosis*
28	YJL121C	RPE1	pentose-phosphate shunt
29	YPR147C	YPR147C	biological process unknown
30	YML095C	RAD10	removal of nonhomologous ends*
31	YLR319C	BUD6	actin filament organization*
32	YGL042C	YGL042C	biological process unknown
33	YER060W	FCY21	biological process unknown
34	YPL148C	PPT2	protein-cofactor linkage
35	YOR125C	CAT5	ubiquinone metabolism
36	YPL226W	NEW1	biological process unknown
37	YPL037C	EGD1	nascent polypeptide association
38	YMR158W	MRPS8	protein biosynthesis
39	YLR264W	RPS28B	protein biosynthesis
40	YLR383W	SMC6	DNA repair*
41	YHR131C	YHR131C	biological process unknown
42	YCR015C	YCR015C	biological process unknown

Number	Systematic name	Standard name	Biological Process
43	YPL064C	CWC27	biological process unknown
44	YPR121W	THI22	thiamin biosynthesis
45	YLR393W	ATP10	protein complex assembly
46	YBR070C	ALG14	dolichol-linked oligosaccharide biosynthesis
47	YNL319W	YNL319W	biological process unknown
48	YCR027C	RHB1	L-arginine transport*
49	YDR167W	TAF10	transcription initiation from RNA polymerase II promoter*
50	YFL046W	FMP32	biological process unknown
51	YMR023C	MSS1	protein biosynthesis*
52	YGR274C	TAF1	protein amino acid phosphorylation*
53	YDR162C	NBP2	response to heat*
54	YEL072W	RMD6	biological process unknown
55	YLR361C	DCR2	biological process unknown
56	YOR119C	RIO1	processing of 2biological process unknownS pre-rRNA*
57	YHR192W	YHR192W	biological process unknown
58	YLR379W	YLR379W	biological process unknown
59	YOR166C	YOR166C	biological process unknown
60	YLR265C	NEJ1	DNA repair*
61	YLR323C	CWC24	biological process unknown
62	YDR336W	YDR336W	biological process unknown
63	YLR363C	NMD4	mRNA catabolism, nonsense-mediated decay
64	YPR139C	VPS66	protein-vacuolar targeting
65	YPL080C	YPL080C	biological process unknown
66	YLR273C	PIG1	regulation of glycogen biosynthesis
67	YNL122C	YNL122C	biological process unknown
68	YLR275W	SMD2	nuclear mRNA splicing, via spliceosome
69	YLR312W-A	MRPL15	protein biosynthesis
70	YGL106W	MLC1	endocytosis*
71	YLR033W	RSC58	chromatin remodeling
72	YPL010W	RET3	retrograde transport, Golgi to ER
73	YOR257W	CDC31	microtubule nucleation*
74	YFL032W	YFL032W	biological process unknown
75	YOL038W	PRE6	ubiquitin-dependent protein catabolism
76	YGL166W	CUP2	transcription initiation from RNA polymerase II promoter*
77	YIL069C	RPS24B	protein biosynthesis
78	YPL174C	NIP100	establishment of mitotic spindle orientation
79	YPL215W	CBP3	protein complex assembly
80	YDL081C	RPP1A	translational elongation*
81	YGR135W	PRE9	ubiquitin-dependent protein catabolism*
82	YMR159C	ATG16	autophagy
83	YPL038W	MET31	regulation of transcription*
84	YDR002W	YRB1	ubiquitin-dependent protein catabolism*
85	YFL003C	MSH4	meiotic recombination
86	YDR302W	GPI11	GPI anchor biosynthesis
87	YDR025W	RPS11A	protein biosynthesis*
88	YER071C	YER071C	biological process unknown
89	YLR292C	SEC72	posttranslational protein-membrane targeting
90	YJR063W	RPA12	transcription from RNA polymerase I promoter
91	YLR288C	MEC3	chromatin silencing at telomere*

Number	Systematic name	Standard name	Biological Process
92	YBL021C	HAP3	transcription*
93	YOR224C	RPB8	transcription from RNA polymerase II promoter*
94	YJL055W	YJL055W	biological process unknown
95	YLR320W	MMS22	double-strand break repair
96	YPR146C	YPR146C	biological process unknown
97	YPL094C	SEC62	posttranslational protein-membrane targeting
98	YER002W	NOP16	ribosomal large subunit biogenesis
99	YDL012C	YDL012C	biological process unknown
100	YBR190W	YBR190W	biological process unknown
101	YPR134W	MSS18	Group I intron splicing
102	YCL033C	YCL033C	response to oxidative stress
103	YGR025W	YGR025W	biological process unknown
104	YBR052C	YBR052C	biological process unknown
105	YLR291C	GCD7	translational initiation
106	YGL070C	RPB9	transcription from RNA polymerase II promoter
107	YJR067C	YAE1	biological process unknown
108	YMR123W	PKR1	biological process unknown
109	YHR043C	DOG2	response to stress*
110	YIR009W	MSL1	nuclear mRNA splicing, via spliceosome
111	YKL036C	YKL036C	biological process unknown
112	YPL095C	YPL095C	biological process unknown
113	YPL008W	CHL1	chromosome segregation*
114	YCR087C-A	YCR087C-A	biological process unknown
115	YGR016W	YGR016W	biological process unknown
116	YBR193C	MED8	transcription from RNA polymerase II promoter
117	YPR002W	PDH1	propionate metabolism
118	YLR367W	RPS22B	protein biosynthesis
119	YCL031C	RRP7	35S primary transcript processing*
120	YGR039W	YGR039W	biological process unknown
121	YLR354C	TAL1	pentose-phosphate shunt
122	YLR370C	ARC18	actin filament organization*
123	YDR016C	DAD1	mitotic spindle organization and biogenesis in nucleus*
124	YLR351C	NIT3	biological process unknown
125	YKL154W	SRP102	protein-ER targeting*
126	YNR022C	MRPL50	protein biosynthesis
127	YGR275W	RTT102	biological process unknown
128	YOL039W	RPP2A	translational elongation*
129	YPR086W	SUA7	transcription initiation from RNA polymerase II promoter
130	YLR073C	YLR073C	biological process unknown
131	YGL008C	PMA1	regulation of pH*
132	YLR364W	YLR364W	biological process unknown
133	YCR090C	YCR090C	biological process unknown
134	YFR036W	CDC26	mitotic sister chromatid segregation*
135	YGR148C	RPL24B	protein biosynthesis
136	YLR262C	YPT6	intracellular protein transport*
137	YCR059C	YIH1	regulation of amino acid metabolism
138	YHR044C	DOG1	glucose metabolism
139	YBL090W	MRP21	protein biosynthesis*
140	YMR305C	SCW10	conjugation with cellular fusion
141	YGR260W	TNA1	nicotinamide mononucleotide transport

Number	Systematic name	Standard name	Biological Process
142	YFL017C	GNA1	UDP-N-acetylglucosamine biosynthesis
143	YJL179W	PFD1	protein folding*
144	YCL047C	YCL047C	biological process unknown
145	YIL089W	YIL089W	biological process unknown
146	YPL059W	GRX5	response to osmotic stress*
147	YEL003W	GIM4	tubulin folding
148	YOR258W	HNT3	biological process unknown
149	YPL065W	VPS28	protein-vacuolar targeting*
150	YDR089W	YDR089W	membrane organization and biogenesis
151	YPL100W	ATG21	autophagy*
152	YPL104W	MSD1	protein biosynthesis
153	YLR289W	GUF1	biological process unknown
154	YCL049C	YCL049C	biological process unknown
155	YJL095W	BCK1	protein amino acid phosphorylation*
156	YLR333C	RPS25B	protein biosynthesis
157	YLL015W	BPT1	bilirubin transport*
158	YPL060C-A	YPL060C-A	Ty element transposition
159	YNL217W	YNL217W	biological process unknown
160	YGR216C	GPI1	GPI anchor biosynthesis
161	YPR085C	YPR085C	biological process unknown
162	YHR057C	CPR2	biological process unknown
163	YLR266C	PDR8	response to stress*
164	YPL203W	TPK2	protein amino acid phosphorylation*
165	YLR332W	MID2	cell wall organization and biogenesis*
166	YDR175C	RSM24	protein biosynthesis*
167	YFL044C	YOD1	regulation of transcription
168	YLR016C	YLR016C	biological process unknown
169	YDR319C	YDR319C	biological process unknown
170	YDR041W	RSM10	protein biosynthesis
171	YLR459W	GAB1	attachment of GPI anchor to protein
172	YCR039C	MATALPHA2	regulation of transcription from RNA polymerase II promoter*
173	YER066C-A	YER066C-A	biological process unknown
174	YHR191C	CTF8	mitotic sister chromatid cohesion
175	YGR272C	YGR272C	processing of 2biological process unknownS pre-rRNA
176	YPR131C	NAT3	N-terminal peptidyl-methionine acetylation
177	YDR253C	MET32	sulfur amino acid metabolism
178	YBR217W	ATG12	protein-vacuolar targeting*
179	YHR079C	IRE1	protein amino acid phosphorylation*
180	YFL010C	WWM1	response to dessication
181	YDR201W	SPC19	mitotic spindle organization and biogenesis in nucleus*
182	YPL072W	UBP16	protein deubiquitination
183	YLR360W	VPS38	late endosome to vacuole transport
184	YNL136W	EAF7	regulation of transcription from RNA polymerase II promoter*
185	YLR390W	ECM19	cell wall organization and biogenesis
186	YJL178C	ATG27	vesicle organization and biogenesis
187	YDL121C	YDL121C	biological process unknown
188	YFR041C	ERJ5	biological process unknown
189	YPR101W	SNT309	nuclear mRNA splicing, via spliceosome

Number	Systematic name	Standard name	Biological Process
190	YDL125C	HNT1	nucleotide metabolism
191	YER094C	PUP3	ubiquitin-dependent protein catabolism
192	YOL040C	RPS15	protein biosynthesis*
193	YOR105W	YOR105W	biological process unknown
194	YPL051W	ARL3	intracellular protein transport
195	YDR308C	SRB7	transcription from RNA polymerase II promoter
196	YKL156W	RPS27A	protein biosynthesis
197	YCR003W	MRPL32	protein biosynthesis
198	YHR153C	SPO16	sporulation (sensu Fungi)
199	YDL135C	RDI1	actin filament organization*
200	YGR024C	THG1	tRNA modification
201	YDR337W	MRPS28	protein biosynthesis*
202	YPR143W	RRP15	processing of 27S pre-rRNA
203	YDL015C	TSC13	very-long-chain fatty acid metabolism
204	YGL198W	YIP4	vesicle-mediated transport
205	YLR261C	YLR261C	biological process unknown
206	YBL040C	ERD2	protein-ER retention
207	YGR011W	YGR011W	biological process unknown
208	YPL166W	YPL166W	biological process unknown
209	YCR036W	RBK1	D-ribose metabolism
210	YGL031C	RPL24A	protein biosynthesis
211	YCR075C	ERS1	L-cystine transport
212	YJR091C	JSN1	mRNA catabolism, deadenylylation-dependent decay
213	YPL098C	YPL098C	biological process unknown
214	YFR052W	RPN12	ubiquitin-dependent protein catabolism
215	YER093C-A	YER093C-A	biological process unknown
216	YLR245C	CDD1	cytidine catabolism*
217	YNL310C	ZIM17	protein folding*
218	YNL149C	YNL149C	biological process unknown
219	YPL096W	PNG1	misfolded or incompletely synthesized protein catabolism*
220	YPL036W	PMA2	regulation of pH*
221	YLR066W	SPC3	signal peptide processing
222	YOL012C	HTZ1	regulation of transcription from RNA polymerase II promoter*
223	YPR107C	YTH1	mRNA polyadenylylation*
224	YDR033W	MRH1	biological process unknown
225	YIR019C	MUC1	pseudohyphal growth*
226	YKR074W	YKR074W	biological process unknown
227	YJL004C	SYS1	Golgi to endosome transport*
228	YGL168W	HUR1	DNA replication
229	YPR199C	ARR1	positive regulation of transcription from RNA polymerase II promoter*
230	YNL032W	SIW14	endocytosis*
231	YLR293C	GSP1	rRNA processing*
232	YPR063C	YPR063C	biological process unknown
233	YGL258W	YGL258W	biological process unknown
234	YNL153C	GIM3	tubulin folding
235	YPL268W	PLC1	pseudohyphal growth*
236	YBR066C	NRG2	invasive growth (sensu Saccharomyces)
237	YPR187W	RPO26	transcription from RNA polymerase II promoter*
238	YJR055W	HIT1	biological process unknown

Number	**Systematic name**	**Standard name**	**Biological Process**
239	YDR306C	YDR306C	ubiquitin-dependent protein catabolism
240	YMR119W-A	YMR119W-A	biological process unknown
241	YBL107C	YBL107C	biological process unknown
242	YLR267W	BOP2	biological process unknown
243	YNR009W	YNR009W	biological process unknown
244	YLR362W	STE11	protein amino acid phosphorylation*
245	YDL136W	RPL35B	protein biosynthesis
246	YJL192C	SOP4	ER to Golgi transport
247	YER092W	*IES5*	biological process unknown
248	YBR062C	YBR062C	biological process unknown
249	YDL235C	*YPD1*	response to osmotic stress*
250	YKR051W	YKR051W	biological process unknown
251	YPL218W	*SAR1*	ER to Golgi transport
252	YDL052C	*SLC1*	sphingolipid biosynthesis
253	YPR194C	*OPT2*	oligopeptide transport
254	YBL028C	-	biological process unknown
255	YDR013W	*PSF1*	DNA-dependent DNA replication
256	YPR166C	*MRP2*	protein biosynthesis
257	YLR056W	*ERG3*	ergosterol biosynthesis
258	YDL177C	-	biological process unknown
259	YLR350W	*ORM2*	response to unfolded protein
260	YGR129W	*SYF2*	nuclear mRNA splicing, via spliceosome*
261	YJL032W	_	biological process unknown
262	YGR136W	*LSB1*	biological process unknown
263	YCR020C-A	*MAK31*	N-terminal protein amino acid acetylation
264	YML102C-A	_	biological process unknown
265	YPR007C	*REC8*	meiosis*
266	YFR026C	-	biological process unknown
267	YNR040W	-	biological process unknown
268	YIR022W	*SEC11*	signal peptide processing
269	YIL011W	*TIR3*	biological process unknown
270	YML012W	*ERV25*	ER to Golgi transport
271	YJR056C	-	biological process unknown
272	YPL005W	*AEP3*	mRNA metabolism
273	YBR240C	*THI2*	positive regulation of transcription from RNA polymerase II promoter*
274	YDR469W	*SDC1*	chromatin silencing at telomere*
275	YFL031W	*HAC1*	regulation of transcription from RNA polymerase II promoter*
276	YGR215W	*RSM27*	protein biosynthesis
277	YIR042C	-	biological process unknown
278	YNL277W	*MET2*	methionine biosynthesis*
279	YAR027W	*UIP3*	biological process unknown
280	YLR165C	*PUS5*	rRNA modification*
281	YJL184W	*GON7*	cell wall mannoprotein biosynthesis*
282	YPR049C	*ATG11*	protein-vacuolar targeting*
283	YGR284C	*ERV29*	ER to Golgi transport
284	YHR059W	*FYV4*	biological process unknown
285	YGR020C	*VMA7*	vacuolar acidification
286	YGR139W	_	biological process unknown
287	YBR122C	*MRPL36*	protein biosynthesis
288	YMR171C	-	biological process unknown

Number	Systematic name	Standard name	Biological Process
289	YPL146C	*NOP53*	ribosome biogenesis*
290	YNL157W	-	biological process unknown
291	YDR304C	*CPR5*	biological process unknown
292	YPR167C	*MET16*	methionine metabolism*
293	YER146W	*LSM5*	nuclear mRNA splicing, via spliceosome*
294	YCR091W	*KIN82*	protein amino acid phosphorylation*
295	YPL118W	*MRP51*	protein biosynthesis*
296	YER177W	*BMH1*	sporulation (sensu Fungi)*
297	YIL080W	-	Ty element transposition
298	YCR043C	-	biological process unknown
299	YNR037C	*RSM19*	protein biosynthesis
300	YIL047C	*SYG1*	signal transduction
301	YBR268W	*MRPL37*	protein biosynthesis
302	YPL041C	-	biological process unknown
303	YBL018C	*POP8*	rRNA processing*
304	YOR331C	_	biological process unknown
305	YNL081C	*SWS2*	protein biosynthesis
306	YOR358W	*HAP5*	transcription*
307	YGR188C	*BUB1*	protein amino acid phosphorylation*
308	YPR079W	*MRL1*	vacuolar transport
309	YPL045W	*VPS16*	protein-vacuolar targeting*
310	YBR035C	*PDX3*	fatty acid metabolism
311	YPL165C	*SET6*	biological process unknown
312	YLR346C	-	biological process unknown
313	YOL157C	-	biological process unknown
314	YDR284C	*DPP1*	signal transduction*
315	YDR318W	*MCM21*	chromosome segregation
316	YBL026W	*LSM2*	nuclear mRNA splicing, via spliceosome*
317	YHR193C	*EGD2*	nascent polypeptide association
318	YPL152W	*RRD2*	response to osmotic stress*
319	YGR278W	*CWC22*	nuclear mRNA splicing, via spliceosome
320	YLR298C	*YHC1*	nuclear mRNA splicing, via spliceosome
321	YDR073W	*SNF11*	chromatin remodeling
322	YDR339C	-	biological process unknown
323	YDL246C	*SOR2*	hexose metabolism
324	YBR014C	-	biological process unknown
325	YOR089C	*VPS21*	endocytosis*
326	YPR099C	_	biological process unknown
327	YEL027W	*CUP5*	endocytosis*
328	YPR119W	*CLB2*	G2/M transition of mitotic cell cycle*
329	YLR281C	-	biological process unknown
330	YIL016W	*SNL1*	nuclear pore organization and biogenesis
331	YMR022W	*QRI8*	chromatin assembly or disassembly*
332	YDR210W	-	biological process unknown
333	YOL159C	-	biological process unknown
334	YEL026W	*SNU13*	nuclear mRNA splicing, via spliceosome*
335	YJR058C	*APS2*	vesicle-mediated transport
336	YPL087W	*YDC1*	response to heat*
337	YMR071C	*TVP18*	biological process unknown
338	YLR345W	-	biological process unknown
339	YJL174W	*KRE9*	cell wall organization and biogenesis*

Number	Systematic name	Standard name	Biological Process
340	YML101C	*CUE4*	biological process unknown
341	YPL066W	-	biological process unknown
342	YLL050C	*COF1*	actin filament organization*
343	YGR207C	-	biological process unknown
344	YJL173C	*RFA3*	DNA recombination*
345	YGL029W	*CGR1*	rRNA processing*
346	YOR265W	*RBL2*	microtubule cytoskeleton organization and biogenesis*
347	YMR112C	*MED11*	transcription from RNA polymerase II promoter
348	YHL048W	*COS8*	response to unfolded protein
349	YCR028C-A	*RIM1*	mitochondrial genome maintenance
350	YLR051C	-	biological process unknown
351	YMR193W	*MRPL24*	protein biosynthesis
352	YPL272C	-	biological process unknown
353	YBR201W	*DER1*	ER-associated protein catabolism
354	YBR090C	-	biological process unknown
355	YHR060W	*VMA22*	protein complex assembly*
356	YDR541C	-	biological process unknown
357	YLR074C	*BUD20*	bud site selection
358	YLR203C	*MSS51*	protein biosynthesis*
359	YOR099W	*KTR1*	O-linked glycosylation*
360	YPL052W	*OAZ1*	protein catabolism
361	YLR406C	*RPL31B*	protein biosynthesis
362	YPR005C	*HAL1*	positive regulation of transcription from RNA polymerase II promoter*
363	YPR201W	*ARR3*	arsenite transport
364	YNL131W	*TOM22*	mitochondrial matrix protein import
365	YOR304C-A	-	biological process unknown
366	YDR530C	*APA2*	nucleotide metabolism
367	YPR120C	*CLB5*	G1/S transition of mitotic cell cycle*
368	YBR278W	*DPB3*	chromatin silencing at telomere*
369	YNR036C	-	protein biosynthesis
370	YEL024W	*RIP1*	aerobic respiration*
371	YLR326W	-	biological process unknown
372	YKL160W	*ELF1*	cell growth
373	YMR101C	*SRT1*	protein amino acid glycosylation
374	YPR188C	*MLC2*	cytokinesis, contractile ring contraction
375	YIL098C	*FMC1*	protein complex assembly
376	YDR377W	*ATP17*	ATP synthesis coupled proton transport
377	YBR210W	*ERV15*	axial bud site selection
378	YJL113W	_	biological process unknown
379	YHR049W	*FSH1*	biological process unknown
380	YKL153W	_	biological process unknown
381	YFL038C	*YPT1*	ER to Golgi transport*
382	YNR067C	*DSE4*	cytokinesis, completion of separation
383	YOR307C	*SLY41*	ER to Golgi transport
384	YLR169W	_	biological process unknown
385	YLR349W	_	biological process unknown
386	YJL003W	*COX16*	aerobic respiration*
387	YIR027C	*DAL1*	allantoin catabolism
388	YHR072W-A	*NOP10*	rRNA modification*
389	YKR075C	-	biological process unknown
390	YIL065C	*FIS1*	mitochondrial fission

Number	Systematic name	Standard name	Biological Process
391	YDR030C	*RAD28*	DNA repair
392	YGR230W	*BNS1*	meiosis
393	YOR086C	*TCB1*	biological process unknown
394	YDR139C	*RUB1*	protein deneddylation*
395	YMR297W	*PRC1*	vacuolar protein catabolism
396	YOR016C	*ERP4*	secretory pathway
397	YGR033C	*TIM21*	mitochondrial matrix protein import
398	YDL016C	_	biological process unknown
399	YIR024C	-	biological process unknown
400	YJL110C	*GZF3*	negative regulation of transcription from RNA polymerase II promoter*
401	YLR250W	*SSP120*	protein secretion
402	YHR125W	_	biological process unknown
403	YPR182W	*SMX3*	nuclear mRNA splicing, via spliceosome
404	YEL004W	*YEA4*	cell wall chitin biosynthesis*
405	YCR010C	*ADY2*	transport*
406	YDR373W	*FRQ1*	regulation of signal transduction
407	YHR198C	*FMP22*	biological process unknown
408	YJR044C	*VPS55*	late endosome to vacuole transport
409	YMR081C	*ISF1*	aerobic respiration
410	YIL070C	*MAM33*	aerobic respiration
411	YNL031C	*HHT2*	chromatin assembly or disassembly
412	YBR203W	*COS111*	signal transduction
413	YLR376C	*PSY3*	error-free DNA repair
414	YPR008W	*HAA1*	transcription initiation from RNA polymerase II promoter
415	YOR103C	*OST2*	N-linked glycosylation
416	YOL030W	*GAS5*	biological process unknown
417	YJL078C	*PRY3*	biological process unknown
418	YLR300W	*EXG1*	cell wall organization and biogenesis*
419	YDR315C	*IPK1*	myo-inositol metabolism
420	YBL071C	_	biological process unknown
421	YLR280C	_	biological process unknown
422	YPL183W-A	-	protein biosynthesis
423	YER087C-A	_	biological process unknown
424	YLR290C	-	biological process unknown
425	YNL255C	*GIS2*	intracellular signaling cascade
426	YDR063W	-	biological process unknown
427	YLR388W	*RPS29A*	protein biosynthesis
428	YOL026C	*MIM1*	protein complex assembly*
429	YGL127C	*SOH1*	DNA repair*
430	YDR177W	*UBC1*	endocytosis*
431	YDR286C	-	biological process unknown
432	YOL005C	*RPB11*	transcription from RNA polymerase II promoter
433	YJR029W	_	biological process unknown
434	YBR173C	*UMP1*	ubiquitin-dependent protein catabolism*
435	YGL028C	*SCW11*	cytokinesis, completion of separation
436	YCR020C	*PET18*	mitochondrion organization and biogenesis*
437	YOR252W	*RBF17*	biological process unknown
438	YNL147W	*LSM7*	nuclear mRNA splicing, via spliceosome*
439	YBR055C	*PRP6*	nuclear mRNA splicing, via spliceosome
440	YDR298C	*ATP5*	ATP synthesis coupled proton transport

Number	Systematic name	Standard name	Biological Process
441	YPL123C	*RNY1*	cellular morphogenesis
442	YMR008C	*PLB1*	glycerophospholipid metabolism
443	YBR077C	*SLM4*	signal transduction
444	YJL185C	-	biological process unknown
445	YAL049C	-	biological process unknown
446	YPL071C	-	biological process unknown
447	YJL140W	*RPB4*	mRNA-nucleus export*
448	YCR007C	-	biological process unknown
449	YGL219C	*MDM34*	mitochondrion organization and biogenesis
450	YCR060W	*TAH1*	protein folding
451	YKR048C	*NAP1*	budding cell bud growth*
452	YCL007C	_	biological process unknown
453	YNL214W	*PEX17*	peroxisome organization and biogenesis
454	YML002W	-	biological process unknown
455	YDR511W	*ACN9*	gluconeogenesis*
456	YGL035C	*MIG1*	regulation of transcription from RNA polymerase II promoter*
457	YLR218C	-	biological process unknown
458	YNL030W	*HHF2*	chromatin assembly or disassembly
459	YGR105W	*VMA21*	protein complex assembly
460	YBR058C-A	*TSC3*	sphingolipid biosynthesis*
461	YLR286C	*CTS1*	cytokinesis, completion of separation*
462	YDL018C	*ERP3*	secretory pathway
463	YOR264W	*DSE3*	biological process unknown
464	YNL336W	*COS1*	biological process unknown
465	YDR115W	-	protein biosynthesis*
466	YML058W	*SML1*	mitochondrion organization and biogenesis*
467	YNL156C	*NSG2*	biological process unknown
468	YOL162W	-	transport
469	YCR020W-B	*HTL1*	regulation of cell cycle*
470	YNL338W	_	biological process unknown
471	YGR030C	*POP6*	rRNA processing*
472	YKR106W	-	transport
473	YOR031W	*CRS5*	response to metal ion

Table II List of up-regulated genes in both the control and the ethanol-adapted strains. The asterisk (*) indicated a main biological process of encoding protein.

Number	Systematic name	Standard name	Biological Process
1	YDL127W	*PCL2*	cell cycle
2	YPR200C	*ARR2*	response to arsenic
3	YDR100W	*TVP15*	biological process unknown
4	YLR283W	-	biological process unknown
5	YJR082C	*EAF6*	biological process unknown
6	YOR245C	*DGA1*	triacylglycerol biosynthesis*
7	YER131W	*RPS26B*	protein biosynthesis
8	YFL017W-A	*SMX2*	nuclear mRNA splicing, via spliceosome
9	YIL086C	_	biological process unknown
10	YGR053C	-	biological process unknown
11	YJR161C	*COS5*	biological process unknown
12	YDR512C	*EMI1*	sporulation (sensu Fungi)
13	YNL327W	*EGT2*	cytokinesis
14	YPR082C	*DIB1*	nuclear mRNA splicing, via spliceosome
15	YBL005W-B	_	biological process unknown
16	YGR133W	*PEX4*	peroxisome organization and biogenesis*
17	YLR282C	_	biological process unknown
18	YBR010W	*HHT1*	chromatin assembly or disassembly
19	YJL023C	*PET130*	biological process unknown
20	YMR178W	-	biological process unknown
21	YOR107W	*RGS2*	G-protein signaling, coupled to cAMP nucleotide second messenger
22	YDL098C	*SNU23*	nuclear mRNA splicing, via spliceosome
23	YPR052C	*NHP6A*	regulation of transcription from RNA polymerase II promoter*
24	YLR200W	*YKE2*	protein folding*
25	YHL035C	*VMR1*	transport
26	YPR078C	-	meiosis*
27	YHR195W	*NVJ1*	microautophagy
28	YLR254C	*NDL1*	biological process unknown
29	YLR456W	-	biological process unknown
30	YKL067W	*YNK1*	purine nucleotide biosynthesis*
31	YLR093C	*NYV1*	vesicle fusion
32	YBL107W-A	_	biological process unknown
33	YGR181W	*TIM13*	mitochondrial inner membrane protein import
34	YPL020C	*ULP1*	G2/M transition of mitotic cell cycle*
35	YER012W	*PRE1*	response to stress*
36	YGL010W	-	biological process unknown
37	YLR375W	*STP3*	biological process unknown
38	YMR244W	-	biological process unknown
39	YER124C	*DSE1*	cell wall organization and biogenesis
40	YLR325C	*RPL38*	protein biosynthesis
41	YML110C	*COQ5*	aerobic respiration*
42	YKL141W	*SDH3*	tricarboxylic acid cycle*
43	YJR115W	-	biological process unknown
44	YPR061C	*JID1*	biological process unknown
45	YPL234C	*TFP3*	vacuolar acidification
46	YDL137W	*ARF2*	ER to Golgi transport*

Number	**Systematic name**	**Standard name**	**Biological Process**
47	YER058W	*PET117*	cytochrome c oxidase complex assembly
48	YOL129W	*VPS68*	protein-vacuolar targeting
49	YBR253W	*SRB6*	transcription from RNA polymerase II promoter
50	YER087C-B	*SBH1*	SRP-dependent cotranslational protein-membrane targeting, translocation*
51	YOL165C	*AAD15*	aldehyde metabolism
52	YJL151C	*SNA3*	biological process unknown
53	YLR010C	*TEN1*	telomere capping
54	YBR162W-A	*YSY6*	protein secretion
55	YCL056C	-	biological process unknown
56	YDR183W	*PLP1*	beta-tubulin folding*
57	YNR032C-A	*HUB1*	cellular morphogenesis during conjugation with cellular fusion*
58	YPL265W	*DIP5*	amino acid transport
59	YJL171C	-	biological process unknown
60	YLR343W	*GAS2*	biological process unknown
61	YMR298W	*LIP1*	ceramide biosynthesis
62	YHR092C	*HXT4*	hexose transport
63	YML116W	*ATR1*	multidrug transport
64	YGR041W	*BUD9*	bud site selection
65	YDR079W	*PET100*	aerobic respiration*
66	YOR360C	*PDE2*	cAMP-mediated signaling
67	YPL078C	*ATP4*	ATP synthesis coupled proton transport
68	YLR118C	-	biological process unknown
69	YPR159W	*KRE6*	cell wall organization and biogenesis*
70	YIR032C	*DAL3*	allantoin catabolism
71	YHR162W	-	biological process unknown
72	YJL189W	*RPL39*	protein biosynthesis
73	YDL115C	*IWR1*	meiosis
74	YBR040W	*FIG1*	cellular morphogenesis during conjugation with cellular fusion*
75	YDR022C	*CIS1*	regulation of cyclin dependent protein kinase activity
76	YKL013C	*ARC19*	mitochondrion inheritance*
77	YLR271W	-	biological process unknown
78	YJR034W	*PET191*	cytochrome c oxidase complex assembly
79	YKR071C	*DRE2*	biological process unknown
80	YJL152W	_	biological process unknown
81	YLR034C	*SMF3*	intracellular sequestering of iron ion
82	YMR046W-A	_	biological process unknown
83	YLR262C-A	*RBF7*	biological process unknown
84	YGL006W	*PMC1*	calcium ion homeostasis*
85	YNL208W	-	biological process unknown
86	YGR108W	*CLB1*	G2/M transition of mitotic cell cycle*
87	YER163C	-	biological process unknown
88	YJL160C	-	biological process unknown
89	YPL057C	*SUR1*	sphingolipid biosynthesis*
90	YDR326C	-	biological process unknown
91	YDR032C	*PST2*	biological process unknown
92	YPR156C	*TPO3*	polyamine transport
93	YML131W	-	biological process unknown
94	YIL040W	*APQ12*	mRNA-nucleus export

Number	Systematic name	Standard name	Biological Process
95	YGL187C	*COX4*	aerobic respiration*
96	YEL070W	*DSF1*	biological process unknown
97	YML066C	*SMA2*	spore wall assembly (sensu Fungi)
98	YDR031W	-	biological process unknown
99	YOR032C	*HMS1*	pseudohyphal growth
100	YEL012W	*UBC8*	protein monoubiquitination*
101	YPR094W	*RDS3*	nuclear mRNA splicing, via spliceosome*
102	YDR156W	*RPA14*	transcription from RNA polymerase I promoter
103	YPL221W	*BOP1*	biological process unknown
104	YEL068C	_	biological process unknown
105	YGR066C	-	biological process unknown
106	YPL135W	*ISU1*	iron ion homeostasis*
107	YML055W	*SPC2*	signal peptide processing
108	YER039C	*HVG1*	biological process unknown
109	YPL264C	-	biological process unknown
110	YJL142C	_	biological process unknown
111	YPL185W	_	biological process unknown
112	YJR061W	-	biological process unknown
113	YML078W	*CPR3*	protein folding
114	YLR348C	*DIC1*	dicarboxylic acid transport
115	YBR009C	*HHF1*	chromatin assembly or disassembly
116	YDR157W	_	biological process unknown
117	YDL183C	-	biological process unknown
118	YNL244C	*SUI1*	translational initiation
119	YER185W	-	biological process unknown
120	YDR474C	_	biological process unknown
121	YOR161C	*PNS1*	biological process unknown
122	YLR378C	*SEC61*	protein-ER targeting*
123	YMR244C-A	-	biological process unknown
124	YLR392C	-	biological process unknown
125	YOR357C	*GRD19*	protein localization
126	YBR071W	-	biological process unknown
127	YIR039C	*YPS6*	biological process unknown
128	YDR467C	_	biological process unknown
129	YLR294C	_	biological process unknown
130	YER044C	*ERG28*	ergosterol biosynthesis
131	YIR043C	-	biological process unknown
132	YDR322C-A	*TIM11*	ATP synthesis coupled proton transport
133	YMR141C	_	biological process unknown
134	YCR046C	*IMG1*	protein biosynthesis
135	YFR053C	*HXK1*	glucose metabolism*
136	YOL048C	-	biological process unknown
137	YOR210W	*RPB10*	transcription from RNA polymerase II promoter*
138	YML129C	*COX14*	aerobic respiration*
139	YGL072C	_	biological process unknown
140	YGR258C	*RAD2*	nucleotide-excision repair, DNA incision, 3'-to lesion
141	YIR044C	-	biological process unknown
142	YDL045W-A	*MRP10*	protein biosynthesis
143	YML072C	*TCB3*	biological process unknown
144	YDR275W	*BSC2*	biological process unknown
145	YER063W	*THO1*	transcription, DNA-dependent

Number	Systematic name	Standard name	Biological Process
146	YOR045W	*TOM6*	mitochondrial matrix protein import
147	YPL277C	-	biological process unknown
148	YLR119W	*SRN2*	protein-vacuolar targeting*
149	YPR020W	*ATP20*	ATP synthesis coupled proton transport
150	YPL054W	*LEE1*	biological process unknown
151	YER085C	-	biological process unknown
152	YDR396W	_	biological process unknown
153	YOR122C	*PFY1*	response to osmotic stress*
154	YOR003W	*YSP3*	protein catabolism
155	YCL058C	_	biological process unknown
156	YDR133C	_	biological process unknown
157	YLR423C	*ATG17*	autophagy
158	YGR209C	*TRX2*	response to oxidative stress*
159	YMR286W	*MRPL33*	protein biosynthesis
160	YER057C	*HMF1*	biological process unknown
161	YNL281W	*HCH1*	response to stress*
162	YFL062W	*COS4*	biological process unknown
163	YML009C	*MRPL39*	protein biosynthesis
164	YAL030W	*SNC1*	endocytosis*
165	YGR244C	*LSC2*	tricarboxylic acid cycle*
166	YMR291W	-	biological process unknown
167	YNL259C	*ATX1*	response to oxidative stress*
168	YPR030W	*CSR2*	cell wall organization and biogenesis*
169	YPL225W	-	biological process unknown
170	YLR407W	-	biological process unknown
171	YLR204W	*QRI5*	biological process unknown
172	YJR085C	-	biological process unknown
173	YER050C	*RSM18*	protein biosynthesis
174	YJL131C	-	biological process unknown
175	YER142C	*MAG1*	DNA dealkylation
176	YPR100W	*MRPL51*	protein biosynthesis*
177	YER019C-A	*SBH2*	cotranslational protein-membrane targeting
178	YHR210C	-	biological process unknown
179	YDR179C	*CSN9*	adaptation to pheromone during conjugation with cellular fusion*
180	YBR139W	-	biological process unknown
181	YOL133W	*HRT1*	G1/S transition of mitotic cell cycle*
182	YLL049W	-	biological process unknown
183	YIL077C	-	biological process unknown
184	YFL006W	_	biological process unknown
185	YBR262C	*FMP51*	biological process unknown
186	YCL038C	*ATG22*	protein-vacuolar targeting*
187	YOR015W	_	biological process unknown
188	YLR107W	*REX3*	RNA processing
189	YMR272C	*SCS7*	fatty acid metabolism
190	YGL259W	*YPS5*	biological process unknown
191	YLR395C	*COX8*	aerobic respiration*
192	YMR252C	-	biological process unknown
193	YPL017C	-	biological process unknown
194	YEL073C	-	biological process unknown
195	YMR319C	*FET4*	intracellular copper ion transport*

Number	**Systematic name**	**Standard name**	**Biological Process**
196	YBR082C	*UBC4*	response to stress*
197	YBL002W	*HTB2*	chromatin assembly or disassembly
198	YDR086C	*SSS1*	protein secretion*
199	YLR054C	*OSW2*	spore wall assembly (sensu Fungi)
200	YPR158W	-	biological process unknown
201	YBL001C	*ECM15*	cell wall organization and biogenesis
202	YBR039W	*ATP3*	ATP synthesis coupled proton transport
203	YEL059C-A	*SOM1*	proteolysis and peptidolysis
204	YDR342C	*HXT7*	hexose transport
205	YGL146C	-	biological process unknown
206	YHR180W	_	biological process unknown
207	YPL186C	*UIP4*	biological process unknown
208	YER067W	-	biological process unknown
209	YPR138C	*MEP3*	ammonium transport
210	YKL167C	*MRP49*	protein biosynthesis
211	YGR138C	*TPO2*	polyamine transport
212	YJL166W	*QCR8*	aerobic respiration*
213	YGL080W	*FMP37*	biological process unknown
214	YNL306W	*MRPS18*	protein biosynthesis
215	YJR135W-A	*TIM8*	mitochondrial inner membrane protein import
216	YGL045W	*RIM8*	meiosis*
217	YDR134C	-	biological process unknown
218	YIR017C	*MET28*	regulation of transcription from RNA polymerase II promoter*
219	YDR510W	*SMT3*	protein sumoylation
220	YML030W	-	biological process unknown
221	YPL154C	*PEP4*	sporulation*
222	YEL049W	*PAU2*	biological process unknown
223	YNL333W	*SNZ2*	thiamin biosynthesis*
224	YGL226C-A	*OST5*	N-linked glycosylation
225	YHR051W	*COX6*	aerobic respiration*
226	YPR026W	*ATH1*	response to stress*
227	YDL123W	*SNA4*	biological process unknown
228	YHR175W	*CTR2*	intracellular copper ion transport*
229	YDR545W	*YRF1-1*	telomerase-independent telomere maintenance
230	YOR285W	-	biological process unknown
231	YFR033C	*QCR6*	aerobic respiration*
232	YGL133W	*ITC1*	chromatin remodeling*
233	YMR199W	*CLN1*	regulation of cyclin dependent protein kinase activity
234	YGR042W	-	biological process unknown
235	YGR143W	*SKN1*	cell wall organization and biogenesis*
236	YKL192C	*ACP1*	fatty acid biosynthesis
237	YDR042C	-	biological process unknown
238	YJR074W	*MOG1*	protein-nucleus import
239	YHR039C-A	*VMA10*	vacuolar acidification*
240	YOR327C	*SNC2*	endocytosis*
241	YFR003C	*YPI1*	protein amino acid dephosphorylation*
242	YDL243C	*AAD4*	aldehyde metabolism
243	YPL024W	*RMI1*	response to DNA damage stimulus
244	YJL161W	*FMP33*	biological process unknown
245	YDR224C	*HTB1*	chromatin assembly or disassembly*

Number	Systematic name	Standard name	Biological Process
246	YDR357C	-	biological process unknown
247	YDR363W-A	*SEM1*	ubiquitin-dependent protein catabolism*
248	YDR078C	*SHU2*	error-free DNA repair
249	YKL224C	-	biological process unknown
250	YLL064C	-	biological process unknown
251	YEL005C	*VAB2*	biological process unknown
252	YDL232W	*OST4*	N-linked glycosylation
253	YJR119C	-	biological process unknown
254	YGR282C	*BGL2*	cell wall organization and biogenesis
255	YLR295C	*ATP14*	ATP synthesis coupled proton transport
256	YGL188C	_	biological process unknown
257	YKL065C	*YET1*	biological process unknown
258	YDL024C	*DIA3*	pseudohyphal growth*
259	YHR143W	*DSE2*	cell wall organization and biogenesis*
260	YBR089C-A	*NHP6B*	regulation of transcription from RNA polymerase II promoter*
261	YMR040W	*YET2*	biological process unknown
262	YER106W	*MAM1*	meiotic chromosome segregation
263	YDR277C	*MTH1*	signal transduction*
264	YOR020C	*HSP10*	protein folding
265	YOR052C	-	biological process unknown
266	YOL035C	_	biological process unknown
267	YKR076W	*ECM4*	cell wall organization and biogenesis
268	YDL010W	-	biological process unknown
269	YIL087C	-	biological process unknown
270	YDR276C	*PMP3*	cation transport
271	YNL070W	*TOM7*	mitochondrial matrix protein import*
272	YOR303W	*CPA1*	arginine biosynthesis
273	YIR037W	*HYR1*	response to oxidative stress
274	YLR356W	-	biological process unknown
275	YDR343C	*HXT6*	hexose transport
276	YIL060W	-	biological process unknown
277	YGL191W	*COX13*	aerobic respiration
278	YLL025W	-	biological process unknown
279	YDR154C	_	biological process unknown
280	YGL248W	*PDE1*	cAMP-mediated signaling
281	YBR026C	*ETR1*	aerobic respiration*
282	YBR157C	*ICS2*	biological process unknown
283	YLR037C	*DAN2*	biological process unknown
284	YHR199C	*FMP34*	biological process unknown
285	YOR389W	-	biological process unknown
286	YOR100C	*CRC1*	fatty acid metabolism
287	YDR513W	*TTR1*	response to oxidative stress*
288	YPL013C	*MRPS16*	protein biosynthesis
289	YNL200C	-	biological process unknown
290	YJL048C	*UBX6*	ubiquitin-dependent protein catabolism*
291	YLR110C	*CCW12*	cell wall organization and biogenesis*
292	YGR044C	*RME1*	meiosis*
293	YLR390W-A	*CCW14*	cell wall organization and biogenesis
294	YPL278C	-	biological process unknown
295	YNL274C	-	metabolism

Number	Systematic name	Standard name	Biological Process
296	YKL016C	*ATP7*	protein complex assembly*
297	YDL124W	-	metabolism
298	YGL218W	_	biological process unknown
299	YLR297W	-	biological process unknown
300	YLR149C	-	biological process unknown
301	YDL182W	*LYS20*	lysine biosynthesis, aminoadipic pathway
302	YCL039W	*GID7*	negative regulation of gluconeogenesis
303	YLL039C	*UBI4*	response to stress*
304	YNL332W	*THI12*	thiamin biosynthesis
305	YIR041W	-	biological process unknown
306	YOR028C	*CIN5*	regulation of transcription from RNA polymerase II promoter*
307	YPL026C	*SKS1*	protein amino acid phosphorylation*
308	YLR251W	*SYM1*	ethanol metabolism
309	YGL261C	-	biological process unknown
310	YBL112C	-	biological process unknown
311	YIL015C-A	_	biological process unknown
312	YOR044W	-	biological process unknown
313	YNL322C	*KRE1*	cell wall organization and biogenesis
314	YDL004W	*ATP16*	ATP synthesis coupled proton transport
315	YER096W	*SHC1*	sporulation (sensu Fungi)*
316	YLR312C	-	biological process unknown
317	YNL234W	-	response to stress
318	YLL060C	*GTT2*	glutathione metabolism
319	YPR204W	-	biological process unknown
320	YAL061W	-	biological process unknown
321	YHR145C	_	biological process unknown
322	YFL068W	-	biological process unknown
323	YKL142W	*MRP8*	protein biosynthesis
324	YOR062C	-	biological process unknown
325	YBR006W	*UGA2*	response to oxidative stress*
326	YLR461W	*PAU4*	biological process unknown
327	YFL020C	*PAU5*	biological process unknown
328	YPL230W	-	biological process unknown
329	YNR076W	*PAU6*	biological process unknown
330	YER158C	-	biological process unknown
331	YKR049C	*FMP46*	biological process unknown
332	YLL009C	*COX17*	cytochrome c oxidase complex assembly*
333	YLR270W	*DCS1*	deadenylylation-dependent decapping
334	YOL161C	-	biological process unknown
335	YCL042W	-	biological process unknown
336	YDL067C	*COX9*	aerobic respiration*
337	YJL223C	*PAU1*	biological process unknown
338	YOL083W	-	biological process unknown
339	YDL244W	*THI13*	thiamin biosynthesis
340	YDR059C	*UBC5*	endocytosis*
341	YOR215C	-	biological process unknown
342	YCR082W	*AHC2*	biological process unknown
343	YGR243W	*FMP43*	biological process unknown
344	YBR012C	_	biological process unknown
345	YGL037C	*PNC1*	chromatin silencing at telomere*

Number	Systematic name	Standard name	Biological Process
346	YDR178W	*SDH4*	tricarboxylic acid cycle*
347	YGR122C-A	_	biological process unknown
348	YKL185W	*ASH1*	pseudohyphal growth*
349	YLL020C	_	biological process unknown
350	YJL205C-A	_	biological process unknown
351	YNR073C	-	biological process unknown
352	YKR042W	*UTH1*	mitochondrion organization and biogenesis*
353	YER138W-A	-	biological process unknown
354	YJR008W	-	biological process unknown
355	YBR067C	*TIP1*	cell wall organization and biogenesis
356	YMR158C-B	_	biological process unknown
357	YDR340W	_	biological process unknown
358	YJL144W	-	response to dessication
359	YGR279C	*SCW4*	conjugation with cellular fusion
360	YEL039C	*CYC7*	electron transport
361	YCL035C	*GRX1*	response to oxidative stress*
362	YDL020C	*RPN4*	ubiquitin-dependent protein catabolism*
363	YMR181C	-	biological process unknown
364	YNL334C	*SNO2*	thiamin biosynthesis*
365	YGR102C	-	biological process unknown
366	YML117W-A	_	biological process unknown
367	YNL055C	*POR1*	aerobic respiration*
368	YOR338W	-	biological process unknown
369	YER035W	*EDC2*	deadenylylation-dependent decapping
370	YCL025C	*AGP1*	amino acid transport
371	YDR218C	*SPR28*	cell wall organization and biogenesis*
372	YPR002C-A	_	biological process unknown
373	YHR033W	-	biological process unknown
374	YLR252W	_	biological process unknown
375	YFL030W	*AGX1*	glycine biosynthesis
376	YHR056C	*RSC30*	regulation of transcription, DNA-dependent
377	YBR105C	*VID24*	vesicle-mediated transport*
378	YGR183C	*QCR9*	aerobic respiration*
379	YGR035C	-	biological process unknown
380	YER188W	_	biological process unknown
381	YKL096W	*CWP1*	cell wall organization and biogenesis
382	YJR127C	*ZMS1*	biological process unknown
383	YOL119C	*MCH4*	transport
384	YDR529C	*QCR7*	aerobic respiration*
385	YMR122W-A	-	biological process unknown
386	YFR011C	-	biological process unknown
387	YGR146C	-	biological process unknown
388	YPL092W	*SSU1*	sulfite transport
389	YNL305C	-	biological process unknown
390	YCR005C	*CIT2*	glutamate biosynthesis*
391	YDR055W	*PST1*	cell wall organization and biogenesis
392	YKL063C	-	biological process unknown
393	YPR150W	_	biological process unknown
394	YKR105C	-	biological process unknown
395	YER053C	*PIC2*	phosphate transport
396	YCR071C	*IMG2*	protein biosynthesis

Number	Systematic name	Standard name	Biological Process
397	YDL181W	*INH1*	ATP synthesis coupled proton transport
398	YOR306C	*MCH5*	transport
399	YDR525W-A	*SNA2*	biological process unknown
400	YPL088W	-	aldehyde metabolism
401	YPR149W	*NCE102*	protein secretion
402	YIL111W	*COX5B*	mitochondrial electron transport, cytochrome c to oxygen*
403	YBR047W	*FMP23*	biological process unknown
404	YBL048W	_	biological process unknown
405	YBR230C	-	biological process unknown
406	YOR247W	*SRL1*	nucleobase, nucleoside, nucleotide and nucleic acid metabolism
407	YMR103C	_	biological process unknown
408	YPL250C	*ICY2*	biological process unknown
409	YPR127W	-	biological process unknown
410	YCR083W	*TRX3*	response to oxidative stress
411	YLR109W	*AHP1*	regulation of cell redox homeostasis*
412	YOR010C	*TIR2*	response to stress
413	YGL117W	-	biological process unknown
414	YER011W	*TIR1*	response to stress
415	YPR098C	-	biological process unknown
416	YJL159W	*HSP150*	cell wall organization and biogenesis
417	YPR184W	*GDB1*	glycogen catabolism
418	YLR205C	*HMX1*	iron ion homeostasis*
419	YMR271C	*URA10*	pyrimidine base biosynthesis
420	YLR038C	*COX12*	cytochrome c oxidase complex assembly
421	YOR289W	-	biological process unknown
422	YMR195W	*ICY1*	biological process unknown
423	YNL036W	*NCE103*	response to oxidative stress
424	YPL027W	*SMA1*	spore wall assembly (sensu Fungi)
425	YBL064C	*PRX1*	regulation of cell redox homeostasis
426	YKL062W	*MSN4*	response to stress*
427	YMR174C	*PAI3*	vacuolar protein catabolism
428	YGR008C	*STF2*	ATP synthesis coupled proton transport*
429	YCL040W	*GLK1*	carbohydrate metabolism*
430	YPL271W	*ATP15*	ATP synthesis coupled proton transport
431	YOR248W	_	biological process unknown
432	YIL101C	*XBP1*	response to stress
433	YGR052W	*FMP48*	biological process unknown
434	YHL040C	*ARN1*	iron-siderophore transport
435	YGL158W	*RCK1*	protein amino acid phosphorylation*
436	YOL084W	*PHM7*	biological process unknown
437	YDR001C	*NTH1*	response to stress*
438	YER121W	_	biological process unknown
439	YDL110C	-	biological process unknown
440	YFL058W	*THI5*	thiamin biosynthesis
441	YMR322C	*SNO4*	pyridoxine metabolism
442	YBR285W	-	biological process unknown
443	YKL026C	*GPX1*	response to oxidative stress
444	YLL028W	*TPO1*	polyamine transport
445	YGL156W	*AMS1*	carbohydrate metabolism

Number	Systematic name	Standard name	Biological Process
446	YKL103C	*LAP4*	vacuolar protein catabolism
447	YBR054W	*YRO2*	biological process unknown
448	YKL096W-A	*CWP2*	cell wall organization and biogenesis*
449	YBR241C	-	biological process unknown
450	YOL077W-A	*ATP19*	ATP synthesis coupled proton transport
451	YKL086W	*SRX1*	response to oxidative stress
452	YDL049C	*KNH1*	beta-1,6 glucan biosynthesis
453	YHR054C	-	biological process unknown
454	YMR105C	*PGM2*	glucose 1-phosphate utilization*
455	YIL136W	*OM45*	biological process unknown
456	YKR046C	*PET10*	aerobic respiration
457	YHR008C	*SOD2*	replicative cell aging*
458	YDR453C	*TSA2*	regulation of cell redox homeostasis
459	YDR534C	*FIT1*	siderophore transport
460	YCR098C	*GIT1*	glycerophosphodiester transport
461	YOR134W	*BAG7*	small GTPase mediated signal transduction
462	YCL069W	*VBA3*	basic amino acid transport
463	YDL130W-A	*STF1*	ATP synthesis coupled proton transport
464	YLR136C	*TIS11*	mRNA catabolism*
465	YNR014W	-	biological process unknown
466	YGR248W	*SOL4*	biological process unknown
467	YER037W	*PHM8*	biological process unknown
468	YBR085C-A	-	biological process unknown
469	YKR093W	*PTR2*	peptide transport
470	YGR161C	*RTS3*	protein amino acid dephosphorylation
471	YJL052W	*TDH1*	glycolysis*
472	YGR144W	*THI4*	mitochondrial genome maintenance*
473	YOL058W	*ARG1*	arginine biosynthesis*
474	YDR077W	*SED1*	cell wall organization and biogenesis*
475	YBL078C	*ATG8*	protein-vacuolar targeting*
476	YML128C	*MSC1*	meiotic recombination
477	YJR073C	*OPI3*	phosphatidylcholine biosynthesis
478	YML081C-A	*ATP18*	ATP synthesis coupled proton transport
479	YEL011W	*GLC3*	glycogen metabolism
480	YCR021C	*HSP30*	response to stress
481	YHR071W	*PCL5*	cell cycle
482	YDR258C	*HSP78*	response to stress*
483	YBR147W	-	biological process unknown
484	YMR090W	-	biological process unknown
485	YLR178C	*TFS1*	regulation of proteolysis and peptidolysis
486	YDL021W	*GPM2*	biological process unknown
487	YHR053C	*CUP1-1*	response to copper ion
488	YHR055C	*CUP1-2*	response to copper ion
489	YDR043C	*NRG1*	regulation of transcription from RNA polymerase II promoter*
490	YGR043C	-	biological process unknown
491	YML100W	*TSL1*	response to stress*
492	YOR173W	*DCS2*	biological process unknown
493	YFR032C	-	biological process unknown
494	YPR160W	*GPH1*	glycogen catabolism
495	YDR533C	*HSP31*	biological process unknown

Number	Systematic name	Standard name	Biological Process
496	YPL223C	*GRE1*	response to stress*
497	YNL160W	*YGP1*	response to stress*
498	YJL079C	*PRY1*	biological process unknown
499	YLR327C	*RBF9*	biological process unknown
500	YOR374W	*ALD4*	ethanol metabolism
501	YJL116C	*NCA3*	mitochondrion organization and biogenesis
502	YGL121C	*GPG1*	signal transduction
503	YOR383C	*FIT3*	siderophore transport
504	YMR251W-A	*HOR7*	response to stress
505	YOR391C	*HSP33*	biological process unknown
506	YER150W	*SPI1*	biological process unknown
507	YOL047C	-	biological process unknown
508	YNL015W	*PBI2*	vacuole fusion, non-autophagic*
509	YOR382W	*FIT2*	siderophore transport
510	YOL052C-A	*DDR2*	response to stress
511	YBR072W	*HSP26*	response to stress*
512	YFL014W	*HSP12*	response to oxidative stress*

Table III List of up-regulated genes only in the control strain. The asterisk (*) indicated a main biological process of encoding protein.

Number	Systematic name	Standard name	Biological Process
1	YGL193C	_	biological process unknown
2	YIR029W	*DAL2*	allantoin catabolism
3	YIL023C	-	biological process unknown
4	YMR097C	*MTG1*	protein biosynthesis*
5	YJL129C	*TRK1*	potassium ion homeostasis
6	YPL222W	*FMP40*	biological process unknown
7	YLR168C	-	biological process unknown
8	YDL075W	*RPL31A*	protein biosynthesis
9	YKL034W	*TUL1*	protein ubiquitination*
10	YGR151C	_	biological process unknown
11	YKR033C	_	biological process unknown
12	YDL209C	*CWC2*	nuclear mRNA splicing, via spliceosome
13	YDL027C	-	biological process unknown
14	YER137C	-	biological process unknown
15	YEL017C-A	*PMP2*	cation transport
16	YKL138C	*MRPL31*	protein biosynthesis
17	YOR309C	_	biological process unknown
18	YNL125C	*ESBP6*	transport
19	YMR134W	-	iron ion homeostasis
20	YLL024C	*SSA2*	protein folding
21	YPL177C	*CUP9*	transcription initiation from RNA polymerase II promoter*
22	YKR014C	*YPT52*	endocytosis*
23	YKR100C	*SKG1*	cell wall organization and biogenesis
24	YMR135C	*GID8*	negative regulation of gluconeogenesis
25	YMR031C	-	biological process unknown
26	YHR157W	*REC104*	meiotic recombination*
27	YOR036W	*PEP12*	Golgi to vacuole transport
28	YML070W	*DAK1*	response to stress*
29	YPL275W	*FDH2*	formate catabolism
30	YOL071W	*EMI5*	sporulation (sensu Fungi)
31	YEL020C	-	biological process unknown
32	YGL054C	*ERV14*	ER to Golgi transport*
33	YOR085W	*OST3*	protein complex assembly*
34	YCL009C	*ILV6*	branched chain family amino acid biosynthesis
35	YHR140W	-	biological process unknown
36	YPR154W	*PIN3*	actin cytoskeleton organization and biogenesis
37	YLR402W	_	biological process unknown
38	YLR213C	*CRR1*	biological process unknown
39	YML108W	-	biological process unknown
40	YCR061W	-	biological process unknown
41	YAR035W	*YAT1*	alcohol metabolism*
42	YOR108W	*LEU9*	leucine biosynthesis
43	YER020W	*GPA2*	sporulation (sensu Fungi)*
44	YAL044C	*GCV3*	one-carbon compound metabolism*
45	YKL023W	-	biological process unknown
46	YML034W	*SRC1*	mitotic sister chromatid segregation
47	YLL029W	-	biological process unknown

Number	Systematic name	Standard name	Biological Process
48	YMR052C-A	_	biological process unknown
49	YBL030C	*PET9*	aerobic respiration*
50	YDR497C	*ITR1*	myo-inositol transport
51	YGR206W	-	biological process unknown
52	YHR194W	*MDM31*	mitochondrion organization and biogenesis*
53	YIL153W	*RRD1*	DNA repair*
54	YOL152W	*FRE7*	biological process unknown
55	YER048W-A	-	biological process unknown
56	YBR001C	*NTH2*	response to stress*
57	YOR182C	*RPS30B*	protein biosynthesis
58	YGR222W	*PET54*	protein biosynthesis*
59	YDR003W	*RCR2*	biological process unknown
60	YOR121C	_	biological process unknown
61	YOL082W	*ATG19*	protein-vacuolar targeting
62	YMR114C	-	biological process unknown
63	YNL293W	*MSB3*	actin filament organization*
64	YDL139C	*SCM3*	biological process unknown
65	YDR282C	-	biological process unknown
66	YLR145W	*RMP1*	rRNA processing*
67	YML087C	-	biological process unknown
68	YOR337W	*TEA1*	transcription
69	YFR047C	*BNA6*	NAD biosynthesis
70	YGR023W	*MTL1*	cell wall organization and biogenesis
71	YEL041W	-	biological process unknown
72	YJR052W	*RAD7*	nucleotide-excision repair, DNA damage recognition
73	YIL097W	*FYV10*	negative regulation of gluconeogenesis
74	YDR034C	*LYS14*	lysine biosynthesis, aminoadipic pathway
75	YIL012W	_	biological process unknown
76	YKL018W	*SWD2*	histone methylation*
77	YHR037W	*PUT2*	glutamate biosynthesis*
78	YKR011C	-	biological process unknown
79	YDL197C	*ASF2*	chromatin silencing at silent mating-type cassette
80	YFL013C	*IES1*	chromatin remodeling
81	YHR217C	_	biological process unknown
82	YOL153C	-	biological process unknown
83	YLL041C	*SDH2*	tricarboxylic acid cycle*
84	YOR302W	-	regulation of protein biosynthesis
85	YOR305W	-	biological process unknown
86	YOR170W	_	biological process unknown
87	YDL053C	*PBP4*	biological process unknown
88	YOR300W	_	biological process unknown
89	YOR226C	*ISU2*	iron ion homeostasis*
90	YJR079W	-	biological process unknown
91	YDR314C	-	biological process unknown
92	YOR027W	*STI1*	protein folding
93	YER054C	*GIP2*	protein amino acid dephosphorylation
94	YNL283C	*WSC2*	cell wall organization and biogenesis*
95	YKL107W	-	biological process unknown
96	YHR018C	*ARG4*	arginine biosynthesis
97	YFL059W	*SNZ3*	thiamin biosynthesis*
98	YHR219W	-	biological process unknown

Number	Systematic name	Standard name	Biological Process
99	YLR202C	_	biological process unknown
100	YFR014C	*CMK1*	protein amino acid phosphorylation*
101	YKL190W	*CNB1*	cell wall organization and biogenesis*
102	YIL008W	*URM1*	protein modification
103	YIL154C	*IMP2'*	DNA repair*
104	YOR298W	*MUM3*	spore wall assembly (sensu Fungi)*
105	YJL089W	*SIP4*	regulation of transcription from RNA polymerase II promoter*
106	YDR094W	_	biological process unknown
107	YBR119W	*MUD1*	nuclear mRNA splicing, via spliceosome
108	YLL066C	-	biological process unknown
109	YDR259C	*YAP6*	positive regulation of transcription from RNA polymerase II promoter
110	YER042W	*MXR1*	response to oxidative stress
111	YGR255C	*COQ6*	ubiquinone metabolism
112	YCL018W	*LEU2*	leucine biosynthesis
113	YNL285W	_	biological process unknown
114	YGL038C	*OCH1*	N-linked glycosylation
115	YLL005C	*SPO75*	sporulation (sensu Fungi)*
116	YJR159W	*SOR1*	fructose metabolism*
117	YDL199C	-	biological process unknown
118	YKL133C	-	biological process unknown
119	YOL060C	*MAM3*	mitochondrion organization and biogenesis*
120	YDR320C	*SWA2*	ER organization and biogenesis
121	YMR251W	*GTO3*	biological process unknown
122	YOL027C	*MDM38*	mitochondrion organization and biogenesis*
123	YNL103W	*MET4*	positive regulation of transcription from RNA polymerase II promoter*
124	YOL123W	*HRP1*	mRNA polyadenylylation*
125	YDR272W	*GLO2*	carbohydrate metabolism
126	YCL019W	_	biological process unknown
127	YGL058W	*RAD6*	ubiquitin-dependent protein catabolism*
128	YOL085C	_	biological process unknown
129	YLR260W	*LCB5*	response to heat*
130	YNR004W	-	biological process unknown
131	YAL069W	_	biological process unknown
132	YOR071C	-	transport
133	YOR058C	*ASE1*	mitotic spindle organization and biogenesis in nucleus*
134	YEL035C	*UTR5*	biological process unknown
135	YKL137W	-	biological process unknown
136	YMR094W	*CTF13*	centromere and kinetochore complex maturation
137	YNL331C	*AAD14*	aldehyde metabolism
138	YIL050W	*PCL7*	regulation of glycogen biosynthesis*
139	YNL211C	-	biological process unknown
140	YOR152C	-	biological process unknown
141	YHR021W-A	*ECM12*	cell wall organization and biogenesis
142	YBR114W	*RAD16*	nucleotide-excision repair, DNA damage recognition
143	YOL140W	*ARG8*	arginine biosynthesis*
144	YGL011C	*SCL1*	ubiquitin-dependent protein catabolism
145	YEL074W	_	biological process unknown
146	YIL007C	*NAS2*	ubiquitin-dependent protein catabolism
147	YER073W	*ALD5*	electron transport*
148	YIL108W	-	proteolysis and peptidolysis

Number	Systematic name	Standard name	Biological Process
149	YMR254C	_	biological process unknown
150	YDR293C	*SSD1*	cell wall organization and biogenesis
151	YMR189W	*GCV2*	one-carbon compound metabolism*
152	YCR107W	*AAD3*	aldehyde metabolism
153	YER017C	*AFG3*	protein complex assembly*
154	YDR405W	*MRP20*	protein biosynthesis
155	YLL065W	_	biological process unknown
156	YDR397C	*NCB2*	negative regulation of transcription from RNA polymerase II promoter
157	YNR051C	*BRE5*	protein deubiquitination
158	YOR324C	*FRT1*	response to stress
159	YOL013W-A	-	biological process unknown
160	YKL129C	*MYO3*	cell wall organization and biogenesis*
161	YDL009C	_	biological process unknown
162	YHL005C	_	biological process unknown
163	YDR080W	*VPS41*	vacuole organization and biogenesis*
164	YBL111C	-	biological process unknown
165	YIL152W	-	biological process unknown
166	YOR137C	*SIA1*	proton transport
167	YPR191W	*QCR2*	aerobic respiration*
168	YER091C	*MET6*	methionine biosynthesis
169	YLR046C	-	biological process unknown
170	YAL039C	*CYC3*	cytochrome c-heme linkage
171	YJR048W	*CYC1*	electron transport
172	YHR030C	*SLT2*	cell wall organization and biogenesis*
173	YEL077C	-	biological process unknown
174	YOL106W	_	biological process unknown
175	YLL046C	*RNP1*	ribosome biogenesis and assembly
176	YGL005C	*COG7*	intra-Golgi transport
177	YGR295C	*COS6*	biological process unknown
178	YGL033W	*HOP2*	synapsis
179	YJR109C	*CPA2*	arginine biosynthesis
180	YMR137C	*PSO2*	DNA repair*
181	YMR188C	*MRPS17*	protein biosynthesis*
182	YDR452W	*PPN1*	polyphosphate metabolism
183	YDL215C	*GDH2*	nitrogen compound metabolism
184	YDR230W	_	biological process unknown
185	YFL067W	-	biological process unknown
186	YBR222C	*PCS60*	biological process unknown
187	YPL061W	*ALD6*	acetate biosynthesis
188	YLR223C	*IFH1*	rRNA processing*
189	YGL059W	-	biological process unknown
190	YOR227W	-	biological process unknown
191	YLR444C	_	biological process unknown
192	YOR148C	*SPP2*	nuclear mRNA splicing, via spliceosome
193	YAL004W	_	biological process unknown
194	YDL042C	*SIR2*	chromatin silencing at telomere*
195	YIL144W	*TID3*	chromosome segregation*
196	YLR030W	-	biological process unknown
197	YHR005C-A	*MRS11*	mitochondrial inner membrane protein import
198	YIL063C	*YRB2*	mRNA-nucleus export*
199	YNL155W	-	biological process unknown

Number	Systematic name	Standard name	Biological Process
200	YIR034C	*LYS1*	lysine biosynthesis, aminoadipic pathway
201	YBR086C	*IST2*	response to osmotic stress
202	YCR013C	_	biological process unknown
203	YDR403W	*DIT1*	spore wall assembly (sensu Fungi)
204	YLR194C	-	biological process unknown
205	YPL276W	-	formate catabolism
206	YOR355W	*GDS1*	aerobic respiration
207	YLR225C	-	biological process unknown
208	YHR080C	-	biological process unknown
209	YOL036W	-	biological process unknown
210	YJR077C	*MIR1*	phosphate transport
211	YJL141C	*YAK1*	protein amino acid phosphorylation
212	YNL213C	-	mitochondrion organization and biogenesis
213	YHR176W	*FMO1*	protein folding
214	YNL133C	*FYV6*	double-strand break repair via nonhomologous end-joining
215	YJL164C	*TPK1*	protein amino acid phosphorylation*
216	YHR182W	-	biological process unknown
217	YDR313C	*PIB1*	protein ubiquitination
218	YAL034C	*FUN19*	biological process unknown
219	YKL005C	*BYE1*	negative regulation of transcription from RNA polymerase II promoter
220	YNR077C	_	biological process unknown
221	YLL056C	-	biological process unknown
222	YDR199W	_	biological process unknown
223	YNL117W	*MLS1*	glyoxylate cycle
224	YKR101W	*SIR1*	chromatin silencing at silent mating-type cassette*
225	YOR139C	_	biological process unknown
226	YDL106C	*PHO2*	transcription*
227	YLR170C	*APS1*	vesicle-mediated transport
228	YJR086W	*STE18*	signal transduction during conjugation with cellular fusion
229	YLR237W	*THI7*	thiamin transport
230	YKL199C	_	biological process unknown
231	YKR097W	*PCK1*	gluconeogenesis
232	YOR007C	*SGT2*	biological process unknown
233	YLR152C	-	biological process unknown
234	YBR024W	*SCO2*	copper ion transport
235	YDL086W	-	biological process unknown
236	YFL056C	*AAD6*	aldehyde metabolism
237	YLR089C	*ALT1*	biological process unknown
238	YDL022W	*GPD1*	intracellular accumulation of glycerol
239	YDR019C	*GCV1*	one-carbon compound metabolism*
240	YKL053C-A	*MDM35*	mitochondrion organization and biogenesis
241	YJR120W	-	mitochondrion organization and biogenesis*
242	YER175C	*TMT1*	biological process unknown
243	YPR075C	*OPY2*	cell cycle arrest in response to pheromone
244	YML013W	*SEL1*	protein secretion*
245	YDR273W	*DON1*	meiosis*
246	YPL046C	*ELC1*	RNA elongation from RNA polymerase II promoter*
247	YKR096W	-	biological process unknown
248	YIL113W	*SDP1*	MAPKKK cascade during cell wall biogenesis
249	YER103W	*SSA4*	response to stress*
250	YFL063W	_	biological process unknown

Number	Systematic name	Standard name	Biological Process
251	YBR218C	*PYC2*	gluconeogenesis*
252	YNL111C	*CYB5*	sterol biosynthesis
253	YJL017W	_	biological process unknown
254	YDL061C	*RPS29B*	protein biosynthesis
255	YLR299W	*ECM38*	glutathione catabolism
256	YGL182C	_	biological process unknown
257	YBR078W	*ECM33*	cell wall organization and biogenesis
258	YMR204C	*INP1*	peroxisome inheritance
259	YKR058W	*GLG1*	glycogen biosynthesis
260	YIL042C	-	biological process unknown
261	YGR149W	-	biological process unknown
262	YIL051C	*MMF1*	mitochondrial genome maintenance*
263	YOR009W	*TIR4*	biological process unknown
264	YDR058C	*TGL2*	lipid metabolism
265	YNR001C	*CIT1*	tricarboxylic acid cycle*
266	YHR110W	*ERP5*	secretory pathway
267	YDR034C-A	-	biological process unknown
268	YMR191W	*SPG5*	biological process unknown
269	YHR209W	-	biological process unknown
270	YDR270W	*CCC2*	intracellular copper ion transport*
271	YBL099W	*ATP1*	ATP synthesis coupled proton transport
272	YNL143C	_	biological process unknown
273	YKL093W	*MBR1*	aerobic respiration
274	YLL058W	-	sulfur metabolism
275	YOR350C	*MNE1*	biological process unknown
276	YBR162C	*TOS1*	biological process unknown
277	YGL180W	*ATG1*	autophagy
278	YEL020W-A	*TIM9*	mitochondrial inner membrane protein import
279	YNL179C	_	biological process unknown
280	YDR056C	-	biological process unknown
281	YNL134C	-	biological process unknown
282	YGR086C	*PIL1*	response to heat
283	YJL149W	-	biological process unknown
284	YMR072W	*ABF2*	mitochondrion inheritance*
285	YDR216W	*ADR1*	transcription*
286	YKR067W	*GPT2*	phospholipid biosynthesis
287	YLR162W	-	biological process unknown
288	YHL046C	-	biological process unknown
289	YPL282C	-	biological process unknown
290	YJL163C	-	biological process unknown
291	YKR013W	*PRY2*	biological process unknown
292	YPR140W	*TAZ1*	phospholipid biosynthesis*
293	YFL016C	*MDJ1*	protein folding*
294	YOR284W	*HUA2*	actin cortical patch assembly
295	YHR104W	*GRE3*	response to stress*
296	YER030W	-	biological process unknown
297	YJL042W	*MHP1*	cell wall organization and biogenesis*
298	YGL116W	*CDC20*	mitotic sister chromatid segregation*
299	YNR068C	-	biological process unknown
300	YKR069W	*MET1*	methionine metabolism*
301	YDR374C	-	biological process unknown

Number	**Systematic name**	**Standard name**	**Biological Process**
302	YPR172W	-	biological process unknown
303	YDR085C	*AFR1*	signal transduction during conjugation with cellular fusion*
304	YBR256C	*RIB5*	riboflavin biosynthesis
305	YLR043C	*TRX1*	response to oxidative stress*
306	YIL045W	*PIG2*	biological process unknown
307	YGR189C	*CRH1*	biological process unknown
308	YIR016W	-	biological process unknown
309	YML133C	-	biological process unknown
310	YMR197C	*VTI1*	vesicle fusion*
311	YGL062W	*PYC1*	gluconeogenesis*
312	YML130C	*ERO1*	protein folding*
313	YLR217W	_	biological process unknown
314	YPL025C	_	biological process unknown
315	Q0160	*SCEI*	intron homing
316	YBR301W	*DAN3*	biological process unknown
317	YMR104C	*YPK2*	protein amino acid phosphorylation
318	YBR016W	-	response to dessication
319	YLR164W	-	biological process unknown
320	YJL066C	*MPM1*	biological process unknown
321	YPL130W	*SPO19*	meiosis
322	YLR216C	*CPR6*	protein folding
323	YJR095W	*SFC1*	fumarate transport*
324	YKR091W	*SRL3*	nucleobase, nucleoside, nucleotide and nucleic acid metabolism
325	YAL062W	*GDH3*	glutamate biosynthesis
326	YGL087C	*MMS2*	ubiquitin-dependent protein catabolism*
327	YML058C-A	_	biological process unknown
328	YDL072C	*YET3*	biological process unknown
329	YDR436W	*PPZ2*	sodium ion homeostasis
330	YMR304C-A	_	biological process unknown
331	YGR203W	-	biological process unknown
332	YER038C	*KRE29*	biological process unknown
333	YER004W	*FMP52*	biological process unknown
334	Q0275	*COX3*	aerobic respiration*
335	YOR178C	*GAC1*	meiosis*
336	YFL012W	-	biological process unknown
337	YNL289W	*PCL1*	cell cycle
338	YJL146W	*IDS2*	meiosis
339	YNL241C	*ZWF1*	pentose-phosphate shunt
340	YBR297W	*MAL33*	regulation of transcription, DNA-dependent*
341	YGL183C	*MND1*	meiotic recombination
342	YOR267C	*HRK1*	cell ion homeostasis
343	YAR069C	_	biological process unknown
344	YDL114W	-	biological process unknown
345	YDR262W	-	biological process unknown
346	YDL046W	*NPC2*	biological process unknown
347	YDR542W	-	biological process unknown
348	YOL011W	*PLB3*	phosphatidylserine catabolism*
349	YJR104C	*SOD1*	zinc ion homeostasis*
350	YFL054C	-	water transport
351	YMR041C	-	biological process unknown
352	YJR152W	*DAL5*	allantoate transport

Number	Systematic name	Standard name	Biological Process
353	YIL024C	-	biological process unknown
354	YJR059W	*PTK2*	G1/S transition of mitotic cell cycle*
355	YFL057C	*AAD16*	aldehyde metabolism
356	YKL035W	*UGP1*	protein amino acid glycosylation*
357	YJL015C	_	biological process unknown
358	YNL013C	_	biological process unknown
359	YDR223W	*CRF1*	negative regulation of transcription
360	YJR155W	*AAD10*	aldehyde metabolism
361	YBR132C	*AGP2*	response to osmotic stress*
362	YHL050C	-	biological process unknown
363	YDL066W	*IDP1*	glutamate biosynthesis*
364	YLR311C	_	biological process unknown
365	YGR273C	-	biological process unknown
366	YOR185C	*GSP2*	nuclear organization and biogenesis
367	YJL108C	*PRM10*	conjugation with cellular fusion
368	YOR023C	*AHC1*	histone acetylation
369	YGL081W	-	biological process unknown
370	YML054C	*CYB2*	electron transport
371	YMR056C	*AAC1*	aerobic respiration*
372	YAL054C	*ACS1*	acetyl-CoA biosynthesis*
373	YOL132W	*GAS4*	biological process unknown
374	YOR394W	-	biological process unknown
375	YDR523C	*SPS1*	protein amino acid phosphorylation*
376	YDL223C	*HBT1*	cellular morphogenesis during conjugation with cellular fusion
377	YGL215W	*CLG1*	cell cycle
378	YGR201C	-	biological process unknown
379	YJL153C	*INO1*	myo-inositol metabolism
380	YMR173W	*DDR48*	DNA repair
381	YER190W	*YRF1-2*	telomerase-independent telomere maintenance
382	YOR214C	-	biological process unknown
383	YOR273C	*TPO4*	polyamine transport
384	YNL142W	*MEP2*	pseudohyphal growth*
385	YBL015W	*ACH1*	acetate metabolism*
386	YER187W-A	_	biological process unknown
387	YER159C	*BUR6*	negative regulation of transcription from RNA polymerase II promoter
388	YNL300W	-	biological process unknown
389	YBR292C	_	biological process unknown
390	YLL018C	*DPS1*	protein biosynthesis
391	YNL180C	*RHO5*	Rho protein signal transduction
392	YIL119C	*RPI1*	thiamin biosynthesis*
393	YOR255W	*OSW1*	spore wall assembly (sensu Fungi)
394	YMR017W	*SPO20*	spore wall assembly (sensu Fungi)*
395	YKL085W	*MDH1*	tricarboxylic acid cycle*
396	YBR269C	*FMP21*	biological process unknown
397	YOR120W	*GCY1*	response to salt stress*
398	YER069W	*ARG5,6*	arginine biosynthesis*
399	YOR208W	*PTP2*	protein amino acid dephosphorylation*
400	YNL335W	-	biological process unknown
401	YLL023C	-	biological process unknown
402	YGR121C	*MEP1*	ammonium transport
403	YMR180C	*CTL1*	RNA processing

Number	Systematic name	Standard name	Biological Process
404	YDL131W	*LYS21*	lysine biosynthesis, aminoadipic pathway
405	YDR393W	*SHE9*	mitochondrion organization and biogenesis*
406	YNL093W	*YPT53*	endocytosis*
407	YIL146C	*ECM37*	cell wall organization and biogenesis
408	YJR078W	*BNA2*	NAD biosynthesis
409	YDR231C	*COX20*	aerobic respiration*
410	YFR049W	*YMR31*	protein biosynthesis
411	YFR016C	-	biological process unknown
412	YDR074W	*TPS2*	response to stress*
413	YDR476C	-	biological process unknown
414	YFL053W	*DAK2*	response to stress*
415	YGR142W	*BTN2*	intracellular protein transport*
416	YLR307W	*CDA1*	spore wall assembly (sensu Fungi)
417	YBR126C	*TPS1*	response to stress*
418	YNL104C	*LEU4*	leucine biosynthesis
419	Q0130	*OLI1*	protein complex assembly*
420	YGR236C	*SPG1*	biological process unknown
421	YKL006C-A	*SFT1*	intra-Golgi transport
422	YLR438W	*CAR2*	arginine catabolism
423	YMR030W	*RSF1*	aerobic respiration*
424	YER088C	*DOT6*	regulation of transcription from RNA polymerase II promoter*
425	YIR038C	*GTT1*	glutathione metabolism
426	YLR031W	-	biological process unknown
427	YMR250W	*GAD1*	response to oxidative stress*
428	YDR504C	*SPG3*	biological process unknown
429	YKL150W	*MCR1*	response to oxidative stress*
430	YLL055W	-	biological process unknown
431	YOR298C-A	*MBF1*	positive regulation of transcription from RNA polymerase II promoter
432	YER084W	_	biological process unknown
433	YOR065W	*CYT1*	mitochondrial electron transport, ubiquinol to cytochrome c*
434	YIL176C	-	biological process unknown
435	YER079W	-	biological process unknown
436	YGR182C	_	biological process unknown
437	YKL151C	-	biological process unknown
438	YJL158C	*CIS3*	cell wall organization and biogenesis
439	YGL184C	*STR3*	methionine biosynthesis
440	YLR121C	*YPS3*	protein metabolism
441	YKR039W	*GAP1*	amino acid transport*
442	YBL075C	*SSA3*	response to stress*
443	YJL038C	-	biological process unknown
444	YJL037W	-	biological process unknown
445	YDR271C	_	biological process unknown
446	YKL124W	*SSH4*	biological process unknown
447	YAR053W	_	biological process unknown
448	YKL148C	*SDH1*	tricarboxylic acid cycle*
449	YBR076W	*ECM8*	cell wall organization and biogenesis
450	YML042W	*CAT2*	carnitine metabolism
451	YMR170C	*ALD2*	aldehyde metabolism*
452	YPL171C	*OYE3*	biological process unknown
453	YER119C	*AVT6*	amino acid transport
454	YLR142W	*PUT1*	glutamate biosynthesis*

Number	Systematic name	Standard name	Biological Process
455	YIL155C	*GUT2*	carbohydrate metabolism*
456	YJR025C	*BNA1*	NAD biosynthesis
457	YMR169C	*ALD3*	response to stress*
458	YDL218W	-	biological process unknown
459	YGR087C	*PDC6*	ethanol metabolism
460	YDL186W	-	biological process unknown
461	YHR138C	-	vacuole fusion, non-autophagic
462	YLL026W	*HSP104*	response to stress*
463	YMR095C	*SNO1*	pyridoxine metabolism*
464	YJL088W	*ARG3*	arginine biosynthesis*
465	YDR171W	*HSP42*	response to stress*
466	YJR150C	*DAN1*	sterol transport
467	YNL195C	-	biological process unknown
468	YBR117C	*TKL2*	pentose-phosphate shunt
469	YML058W-A	*HUG1*	response to DNA damage stimulus*
470	YKL163W	*PIR3*	cell wall organization and biogenesis
471	YMR107W	*SPG4*	biological process unknown
472	YHR139C	*SPS100*	spore wall assembly (sensu Fungi)
473	YOR348C	*PUT4*	proline catabolism*
474	YLR337C	*VRP1*	endocytosis*
475	YJR162C	_	biological process unknown
476	YJR156C	*THI11*	thiamin biosynthesis
477	YPR151C	*SUE1*	protein catabolism
478	YIL099W	*SGA1*	sporulation (sensu Fungi)*
479	YIL107C	*PFK26*	fructose 2,6-bisphosphate metabolism*
480	YBR169C	*SSE2*	protein folding
481	YKL221W	*MCH2*	transport
482	YPL280W	*HSP32*	biological process unknown
483	YDL222C	*FMP45*	cell wall organization and biogenesis
484	YGR088W	*CTT1*	response to stress
485	YDL204W	*RTN2*	biological process unknown
486	YMR096W	*SNZ1*	pyridoxine metabolism*
487	YFR017C	-	biological process unknown
488	YBR116C	_	biological process unknown
489	YFL021W	*GAT1*	transcription initiation from RNA polymerase II promoter*
490	YDR034W-B	-	biological process unknown
491	YNR034W-A	-	biological process unknown
492	YHR087W	-	RNA metabolism
493	YMR175W	*SIP18*	response to osmotic stress*
494	YHR126C	-	Biological process unknown

Table IV List of down-regulated genes only in the ethanol-adapted strain. The asterisk (*) indicated a main biological process of encoding protein.

Number	Systematic name	Standard name	Biological Process
1	YHR213W	-	biological process unknown
2	YDR502C	*SAM2*	methionine metabolism
3	YKL147C	_	biological process unknown
4	YPR181C	*SEC23*	ER to Golgi transport*
5	YGR012W	-	biological process unknown
6	YGR119C	*NUP57*	mRNA-nucleus export*
7	YMR221C	*FMP42*	biological process unknown
8	YMR241W	*YHM2*	tricarboxylic acid transport
9	YGL175C	*SAE2*	meiotic DNA double-strand break processing
10	YNR015W	*SMM1*	tRNA modification*
11	YOR156C	*NFI1*	protein sumoylation*
12	YGL229C	*SAP4*	G1/S transition of mitotic cell cycle
13	YHL030W	*ECM29*	protein catabolism
14	YAR014C	*BUD14*	cellular morphogenesis during vegetative growth
15	YDR346C	*SVF1*	biological process unknown
16	YNR060W	*FRE4*	iron-siderophore transport
17	YKR003W	*OSH6*	steroid biosynthesis
18	YCR106W	*RDS1*	response to xenobiotic stimulus
19	YGL137W	*SEC27*	ER to Golgi transport*
20	YPR045C	-	biological process unknown
21	YMR227C	*TAF7*	transcription initiation from RNA polymerase II promoter*
22	YBR133C	*HSL7*	regulation of cell cycle*
23	YAR062W	-	biological process unknown
24	YPR016C	*TIF6*	ribosomal large subunit biogenesis*
25	YHR084W	*STE12*	pseudohyphal growth*
26	YDL138W	*RGT2*	signal transduction*
27	YGL199C	_	biological process unknown
28	YDL225W	*SHS1*	establishment of cell polarity (sensu Fungi)*
29	YOR048C	*RAT1*	35S primary transcript processing*
30	YNL210W	*MER1*	meiosis*
31	YOR184W	*SER1*	purine base biosynthesis*
32	YBR096W	-	biological process unknown
33	YDL205C	*HEM3*	heme biosynthesis
34	YDR365C	*ESF1*	rRNA processing
35	YLR175W	*CBF5*	rRNA modification*
36	YLR446W	-	biological process unknown
37	YOR197W	*MCA1*	apoptosis
38	YOR164C	-	biological process unknown
39	YMR287C	*MSU1*	RNA catabolism
40	YIR020C	_	biological process unknown
41	YOL056W	*GPM3*	biological process unknown
42	YJL196C	*ELO1*	fatty acid elongation, unsaturated fatty acid
43	YNR074C	*AIF1*	response to singlet oxygen*
44	YNL199C	*GCR2*	positive regulation of transcription from RNA polymerase II promoter*
45	YNL020C	*ARK1*	protein amino acid phosphorylation*
46	YLR428C	_	biological process unknown

Number	Systematic name	Standard name	Biological Process
47	YOR112W	-	biological process unknown
48	YGL235W	-	biological process unknown
49	YKR001C	*VPS1*	protein-vacuolar targeting*
50	YDR472W	*TRS31*	ER to Golgi transport
51	YOL067C	*RTG1*	transcription initiation from RNA polymerase II promoter*
52	YKR082W	*NUP133*	mRNA-nucleus export*
53	YBR134W	_	biological process unknown
54	YMR049C	*ERB1*	rRNA processing
55	YJL009W	_	biological process unknown
56	YNR063W	-	biological process unknown
57	YAL043C-A	_	biological process unknown
58	YGR193C	*PDX1*	acetyl-CoA biosynthesis from pyruvate
59	YNR019W	*ARE2*	sterol metabolism
60	YOR386W	*PHR1*	photoreactive repair
61	YKL106W	*AAT1*	aspartate biosynthesis*
62	YNL193W	-	biological process unknown
63	YOR160W	*MTR10*	protein-nucleus import*
64	YLR146C	*SPE4*	pantothenate biosynthesis*
65	YOR219C	*STE13*	peptide pheromone maturation
66	YJR137C	*ECM17*	cell wall organization and biogenesis*
67	YMR265C	-	biological process unknown
68	YMR047C	*NUP116*	mRNA-nucleus export*
69	YLR412W	-	biological process unknown
70	YOR377W	*ATF1*	fatty acid metabolism*
71	YMR120C	*ADE17*	purine nucleotide biosynthesis*
72	YJR117W	*STE24*	peptide pheromone maturation
73	YGR242W	_	biological process unknown
74	YDL220C	*CDC13*	telomere maintenance*
75	YKL078W	*DHR2*	ribosome biogenesis
76	YBR172C	*SMY2*	cytoskeleton organization and biogenesis
77	YGR266W	-	biological process unknown
78	YKL120W	*OAC1*	sulfate transport*
79	YNL073W	*MSK1*	lysyl-tRNA aminoacylation
80	YBL097W	*BRN1*	mitotic sister chromatid segregation*
81	YGR061C	*ADE6*	purine nucleotide biosynthesis
82	YLR232W	_	biological process unknown
83	YER170W	*ADK2*	nucleotide metabolism
84	YDL094C	_	biological process unknown
85	YDL146W	-	biological process unknown
86	YBR178W	_	biological process unknown
87	YLR042C	-	biological process unknown
88	YOR345C	_	biological process unknown
89	YDL043C	*PRP11*	spliceosome assembly
90	YMR208W	*ERG12*	ergosterol biosynthesis
91	YGL111W	*NSA1*	ribosomal large subunit biogenesis
92	YFL011W	*HXT10*	hexose transport
93	YNL172W	*APC1*	mitotic sister chromatid segregation*
94	YKR092C	*SRP40*	nucleocytoplasmic transport
95	YPL128C	*TBF1*	loss of chromatin silencing
96	YBR242W	-	biological process unknown
97	YMR122C	_	biological process unknown

Number	Systematic name	Standard name	Biological Process
98	YBR294W	*SUL1*	sulfate transport
99	YHR114W	*BZZ1*	actin filament organization*
100	YGL082W	-	biological process unknown
101	YBR089W	_	biological process unknown
102	YBL069W	*AST1*	protein-membrane targeting
103	YHR027C	*RPN1*	ubiquitin-dependent protein catabolism
104	YFL025C	*BST1*	ER to Golgi transport*
105	YLR116W	*MSL5*	nuclear mRNA splicing, via spliceosome
106	YJR007W	*SUI2*	translational initiation
107	YIL031W	*ULP2*	mitotic spindle checkpoint*
108	YBL079W	*NUP170*	mRNA-nucleus export*
109	YLR096W	*KIN2*	exocytosis
110	YGR103W	*NOP7*	processing of 2biological process unknownS pre-rRNA*
111	YCR105W	*ADH7*	alcohol metabolism
112	YNL267W	*PIK1*	sporulation (sensu Fungi)*
113	YDL164C	*CDC9*	DNA recombination*
114	YLR197W	*SIK1*	rRNA modification*
115	YFL048C	*EMP47*	ER to Golgi transport
116	YDL141W	*BPL1*	protein amino acid biotinylation
117	YLR418C	*CDC73*	RNA elongation from RNA polymerase II promoter*
118	YJL215C	_	biological process unknown
119	YBL070C	_	biological process unknown
120	YDR170C	*SEC7*	ER to Golgi transport*
121	YNL298W	*CLA4*	protein amino acid phosphorylation*
122	YAL019W	*FUN30*	chromosome organization and biogenesis (sensu Eukaryota)
123	YNL123W	-	biological process unknown
124	YOR325W	_	biological process unknown
125	YHR074W	*QNS1*	biological process unknown
126	YER105C	*NUP157*	mRNA-nucleus export*
127	YOL098C	-	proteolysis and peptidolysis
128	YBL023C	*MCM2*	DNA replication initiation*
129	YDL172C	_	biological process unknown
130	YNL204C	*SPS18*	sporulation
131	YMR277W	*FCP1*	transcription*
132	YER019W	*ISC1*	response to salt stress*
133	YKL211C	*TRP3*	tryptophan biosynthesis
134	YMR214W	*SCJ1*	protein folding*
135	YGL124C	*MON1*	protein-vacuolar targeting*
136	YHL017W	-	biological process unknown
137	YJR018W	_	biological process unknown
138	YBR223C	*TDP1*	DNA repair
139	YHR025W	*THR1*	methionine metabolism*
140	YBR124W	_	biological process unknown
141	YOL020W	*TAT2*	aromatic amino acid transport
142	YBR275C	*RIF1*	chromatin silencing at telomere*
143	YKL052C	*ASK1*	mitotic spindle organization and biogenesis in nucleus*
144	YJR128W	_	biological process unknown
145	YOL148C	*SPT20*	histone acetylation*
146	YGL120C	*PRP43*	U2-type spliceosome dissembly
147	YPL139C	*UME1*	regulation of meiosis
148	YDR508C	*GNP1*	amino acid transport

Number	Systematic name	Standard name	Biological Process
149	YEL036C	*ANP1*	N-linked glycosylation
150	YGR092W	*DBF2*	protein amino acid phosphorylation*
151	YBR208C	*DUR1,2*	allantoin catabolism*
152	YJL106W	*IME2*	protein amino acid phosphorylation*
153	YLR144C	*ACF2*	actin cytoskeleton organization and biogenesis
154	YPR152C	-	biological process unknown
155	YAR061W	-	biological process unknown
156	YJL120W	_	biological process unknown
157	YGL249W	*ZIP2*	synaptonemal complex formation
158	YNL014W	*HEF3*	translational elongation
159	YBR225W	-	biological process unknown
160	YOR329C	*SCD5*	endocytosis*
161	YBR248C	*HIS7*	histidine biosynthesis*
162	YKL114C	*APN1*	DNA repair*
163	YDL156W	-	biological process unknown
164	YBL067C	*UBP13*	biological process unknown
165	YDR299W	*BFR2*	ER to Golgi transport
166	YGR126W	-	biological process unknown
167	YML109W	*ZDS2*	establishment of cell polarity (sensu Fungi)*
168	YML008C	*ERG6*	ergosterol biosynthesis
169	YDR120C	*TRM1*	tRNA methylation
170	YIL141W	_	biological process unknown
171	YNL181W	-	biological process unknown
172	YAL043C	*PTA1*	mRNA polyadenylylation*
173	YBR113W	_	biological process unknown
174	YDR256C	*CTA1*	oxygen and reactive oxygen species metabolism
175	YNL196C	-	biological process unknown
176	YNL317W	*PFS2*	mRNA polyadenylylation*
177	YGL214W	_	biological process unknown
178	YJL084C	-	biological process unknown
179	YER128W	-	biological process unknown
180	YDL147W	*RPN5*	ubiquitin-dependent protein catabolism
181	YFR030W	*MET10*	sulfate assimilation
182	YBR012W-A	_	biological process unknown
183	YNL169C	*PSD1*	phosphatidylcholine biosynthesis
184	YLR256W-A	_	biological process unknown
185	YMR226C	-	metabolism
186	YIR021W	*MRS1*	Group I intron splicing
187	YEL056W	*HAT2*	chromatin silencing at telomere*
188	YML057W	*CMP2*	cell ion homeostasis*
189	YOL107W	-	biological process unknown
190	YGR211W	*ZPR1*	regulation of cell cycle
191	YHR014W	*SPO13*	positive regulation of sister chromatid cohesion
192	YEL046C	*GLY1*	threonine catabolism*
193	YKR015C	-	biological process unknown
194	YJR016C	*ILV3*	branched chain family amino acid biosynthesis
195	YBR233W	*PBP2*	biological process unknown
196	YOR310C	*NOP58*	rRNA modification*
197	YHR086W	*NAM8*	nuclear mRNA splicing, via spliceosome*
198	YOR098C	*NUP1*	mRNA-nucleus export*
199	YHL009C	*YAP3*	regulation of transcription from RNA polymerase II

Number	Systematic name	Standard name	Biological Process
			promoter
200	YHR135C	*YCK1*	protein amino acid phosphorylation*
201	YBL022C	*PIM1*	proteolysis and peptidolysis*
202	YEL066W	*HPA3*	histone acetylation
203	YLR244C	*MAP1*	proteolysis and peptidolysis
204	YIL133C	*RPL16A*	protein biosynthesis
205	YOR233W	*KIN4*	biological process unknown
206	YFR024C-A	*LSB3*	actin filament organization
207	YBL042C	*FUI1*	uridine transport
208	YBL011W	*SCT1*	phospholipid biosynthesis
209	YDR352W	-	biological process unknown
210	YJL056C	*ZAP1*	regulation of transcription from RNA polymerase II promoter*
211	YDR011W	*SNQ2*	response to drug*
212	YGL122C	*NAB2*	mRNA polyadenylylation*
213	YOR271C	-	biological process unknown
214	YEL030W	*ECM10*	protein-mitochondrial targeting*
215	YML018C	-	biological process unknown
216	YMR084W	-	biological process unknown
217	YML083C	-	biological process unknown
218	YJL102W	*MEF2*	translational elongation
219	YMR301C	*ATM1*	iron ion homeostasis
220	YKR057W	*RPS21A*	protein biosynthesis
221	YGR038W	*ORM1*	response to unfolded protein
222	YMR259C	-	biological process unknown
223	YGR168C	-	biological process unknown
224	YDL221W	_	biological process unknown
225	YNL164C	*IBD2*	mitotic spindle checkpoint
226	YMR258C	-	biological process unknown
227	YMR102C	-	biological process unknown
228	YMR186W	*HSC82*	response to stress*
229	YDR501W	*PLM2*	G1/S-specific transcription in mitotic cell cycle
230	YDR106W	*ARP10*	biological process unknown
231	YOR349W	*CIN1*	post-chaperonin tubulin folding pathway*
232	YHL045W	_	biological process unknown
233	YKL113C	*RAD27*	DNA repair*
234	YDR432W	*NPL3*	mRNA-nucleus export
235	YNL184C	_	biological process unknown
236	YJL019W	*MPS3*	mitotic sister chromatid cohesion*
237	YOR083W	*WHI5*	G1/S transition of mitotic cell cycle*
238	YLL018C-A	*COX19*	cytochrome c oxidase complex assembly*
239	YLR138W	*NHA1*	monovalent inorganic cation homeostasis
240	YDR126W	*SWF1*	spore wall assembly (sensu Fungi)
241	YER027C	*GAL83*	protein amino acid phosphorylation*
242	YMR133W	*REC114*	meiotic recombination*
243	YOR381W	*FRE3*	iron ion homeostasis*
244	YPR148C	-	biological process unknown
245	YIL036W	*CST6*	transcription initiation from RNA polymerase II promoter*
246	YKL197C	*PEX1*	peroxisome organization and biogenesis
247	YIL072W	*HOP1*	meiosis*
248	YJL212C	*OPT1*	sulfur metabolism

Number	**Systematic name**	**Standard name**	**Biological Process**
249	YKL102C	_	biological process unknown
250	YER086W	*ILV1*	branched chain family amino acid biosynthesis
251	YER122C	*GLO3*	ER to Golgi transport*
252	YLR139C	*SLS1*	protein biosynthesis*
253	YOR138C	*RUP1*	biological process unknown
254	YMR192W	*GYL1*	ER to Golgi transport*
255	YLR150W	*STM1*	telomere maintenance*
256	YLL057C	*JLP1*	sulfur metabolism
257	YER065C	*ICL1*	glyoxylate cycle
258	YGL013C	*PDR1*	regulation of transcription from RNA polymerase II promoter*
259	YNR034W	*SOL1*	tRNA-nucleus export*
260	YBR080C	*SEC18*	ER to Golgi transport*
261	YJL041W	*NSP1*	mRNA-nucleus export*
262	YER048C	*CAJ1*	biological process unknown
263	YDL187C	_	biological process unknown
264	YDL227C	*HO*	mating type switching and recombination*
265	YIL109C	*SEC24*	ER to Golgi transport*
266	YML099C	*ARG81*	arginine metabolism*
267	YGL196W	-	biological process unknown
268	YNL290W	*RFC3*	mismatch repair*
269	YCR052W	*RSC6*	chromatin remodeling
270	YKR055W	*RHO4*	actin filament organization*
271	YNL042W	*BOP3*	response to mercury ion
272	YDR142C	*PEX7*	peroxisome organization and biogenesis*
273	YDL214C	*PRR2*	MAPKKK cascade
274	YOL080C	*REX4*	rRNA processing*
275	YOR030W	*DFG16*	invasive growth (sensu Saccharomyces)*
276	YDR294C	*DPL1*	sphingolipid metabolism*
277	YHR094C	*HXT1*	hexose transport
278	YPR145W	*ASN1*	asparagine biosynthesis
279	YER137C-A	_	biological process unknown
280	YOR113W	*AZF1*	regulation of transcription, DNA-dependent*
281	YBR213W	*MET8*	sulfate assimilation*
282	YJL024C	*APS3*	vesicle-mediated transport*
283	YNL088W	*TOP2*	meiotic recombination*
284	YBR179C	*FZO1*	mitochondrion organization and biogenesis*
285	YFL034W	-	biological process unknown
286	YKL051W	*SFK1*	actin cytoskeleton organization and biogenesis*
287	YIL137C	*RBF108*	proteolysis and peptidolysis
288	YER189W	-	biological process unknown
289	YMR037C	*MSN2*	response to stress*
290	YDL054C	*MCH1*	transport
291	YHR106W	*TRR2*	response to oxidative stress
292	YBL091C	*MAP2*	proteolysis and peptidolysis
293	YIL140W	*AXL2*	bud site selection*
294	YML005W	*TRM12*	biological process unknown
295	YGR097W	*ASK10*	response to oxidative stress*
296	YBL066C	*SEF1*	biological process unknown
297	YIL022W	*TIM44*	mitochondrial matrix protein import
298	YGL130W	*CEG1*	positive regulation of transcription from RNA polymerase II

Number	Systematic name	Standard name	Biological Process
			promoter*
299	YBR115C	*LYS2*	lysine biosynthesis, aminoadipic pathway
300	YDR486C	*VPS60*	late endosome to vacuole transport*
301	YMR016C	*SOK2*	pseudohyphal growth
302	YIL117C	*PRM5*	conjugation with cellular fusion
303	YOR039W	*CKB2*	protein amino acid phosphorylation*
304	YOL115W	*TRF4*	mitotic sister chromatid cohesion*
305	YMR106C	*YKU80*	chromatin assembly or disassembly*
306	YOR378W	-	biological process unknown
307	YNL228W	_	biological process unknown
308	YLR095C	*IOC2*	chromatin remodeling
309	YLR198C	_	biological process unknown
310	YGR083C	*GCD2*	translational initiation
311	YLR460C	-	biological process unknown
312	YNL243W	*SLA2*	cell wall organization and biogenesis*
313	YPR096C	_	biological process unknown
314	YNL061W	*NOP2*	rRNA processing
315	YMR203W	*TOM40*	mitochondrial matrix protein import
316	YBL082C	*ALG3*	protein amino acid glycosylation*
317	YER176W	*ECM32*	regulation of translational termination
318	YLR228C	*ECM22*	sterol biosynthesis
319	YBR198C	*TAF5*	transcription initiation from RNA polymerase II promoter*
320	YBR263W	*SHM1*	one-carbon compound metabolism
321	YGR205W	-	biological process unknown
322	YMR183C	*SSO2*	vesicle fusion*
323	YGL203C	*KEX1*	protein processing
324	YNL166C	*BNI5*	cytokinesis*
325	YLR172C	*DPH5*	peptidyl-diphthamide biosynthesis from peptidyl-histidine
326	YOR198C	*BFR1*	meiosis*
327	YDL023C	_	biological process unknown
328	YKR066C	*CCP1*	response to oxidative stress
329	YAR015W	*ADE1*	purine nucleotide biosynthesis*
330	YOR218C	_	biological process unknown
331	YNR006W	*VPS27*	protein-Golgi retention*
332	YIL055C	-	biological process unknown
333	YOR365C	-	biological process unknown
334	YLR338W	_	biological process unknown
335	YJL020C	*BBC1*	actin cytoskeleton organization and biogenesis
336	YEL076C	-	biological process unknown
337	YML010W-A	_	biological process unknown
338	YEL042W	*GDA1*	protein amino acid glycosylation
339	YMR018W	-	biological process unknown
340	YFL065C	-	biological process unknown
341	YAL014C	*SYN8*	transport
342	YNL176C	-	biological process unknown
343	YLR188W	*MDL1*	oligopeptide transport
344	YBL007C	*SLA1*	cell wall organization and biogenesis*
345	YGR009C	*SEC9*	vesicle fusion*
346	YDR289C	*RTT103*	negative regulation of DNA transposition
347	YDR351W	*SBE2*	cell wall organization and biogenesis
348	YDR480W	*DIG2*	invasive growth (sensu Saccharomyces)

Number	Systematic name	Standard name	Biological Process
349	YBR083W	*TEC1*	pseudohyphal growth*
350	YBL081W	-	biological process unknown
351	YHR146W	*CRP1*	biological process unknown
352	YGL023C	*PIB2*	vesicle-mediated transport
353	YJL083W	*TAX4*	cell wall organization and biogenesis*
354	YER089C	*PTC2*	G1/S transition of mitotic cell cycle*
355	YIR015W	*RPR2*	tRNA processing
356	YMR210W	-	biological process unknown
357	YMR044W	*IOC4*	chromatin remodeling
358	YMR213W	*CEF1*	nuclear mRNA splicing, via spliceosome
359	YEL076W-C	_	biological process unknown
360	YNL230C	*ELA1*	RNA elongation from RNA polymerase II promoter
361	YHR006W	*STP2*	positive regulation of transcription from RNA polymerase II promoter
362	YBR280C	-	biological process unknown
363	YDR485C	*VPS72*	protein-vacuolar targeting*
364	YOL059W	*GPD2*	glycerol metabolism
365	YHL006C	*SHU1*	error-free DNA repair
366	YKL029C	*MAE1*	pyruvate metabolism*
367	YJR092W	*BUD4*	bud site selection*
368	YKL187C	-	biological process unknown
369	YAR044W	_	biological process unknown
370	YEL075C	-	biological process unknown
371	YNL092W	-	biological process unknown
372	YOL126C	*MDH2*	gluconeogenesis*
373	YMR300C	*ADE4*	purine nucleotide biosynthesis*
374	YMR323W	*ERR3*	biological process unknown
375	YDR490C	*PKH1*	protein amino acid phosphorylation*
376	YFR018C	-	biological process unknown
377	YGR204W	*ADE3*	purine base biosynthesis*
378	YLR134W	*PDC5*	pyruvate metabolism*
379	YKR045C	-	biological process unknown
380	YEL065W	*SIT1*	iron ion homeostasis*
381	YKR043C	-	biological process unknown
382	YOR290C	*SNF2*	chromatin remodeling
383	YDR384C	*ATO3*	transport*
384	YFL055W	*AGP3*	amino acid transport
385	YBR145W	*ADH5*	alcohol metabolism
386	YLR234W	*TOP3*	meiotic recombination*
387	YBR212W	*NGR1*	mitochondrion organization and biogenesis*
388	YBR015C	*MNN2*	protein amino acid glycosylation
389	YFR010W	*UBP6*	protein deubiquitination
390	YHR100C	-	biological process unknown
391	YHL004W	*MRP4*	protein biosynthesis
392	YHR208W	*BAT1*	branched chain family amino acid biosynthesis*
393	YJL107C	-	biological process unknown
394	YGL255W	*ZRT1*	high-affinity zinc ion transport
395	YLL013C	*PUF3*	mRNA catabolism, deadenylylation-dependent decay
396	YBL061C	*SKT5*	response to osmotic stress*
397	YLR248W	*RCK2*	protein amino acid phosphorylation*
398	YLR207W	*HRD3*	ER-associated protein catabolism

Number	Systematic name	Standard name	Biological Process
399	YGL125W	*MET13*	methionine metabolism*
400	YML074C	*FPR3*	biological process unknown
401	YBL077W	_	biological process unknown
402	YKL025C	*PAN3*	DNA repair*
403	YJL162C	*JJJ2*	biological process unknown
404	YDL224C	*WHI4*	regulation of cell size
405	YDR522C	*SPS2*	sporulation (sensu Fungi)*
406	YPL281C	*ERR2*	biological process unknown
407	YGR213C	*RTA1*	biological process unknown
408	YGL157W	-	biological process unknown
409	YGR013W	*SNU71*	nuclear mRNA splicing, via spliceosome
410	YPR203W	-	biological process unknown
411	YDR207C	*UME6*	sporulation (sensu Fungi)*
412	YDR169C	*STB3*	biological process unknown
413	YKR035C	_	biological process unknown
414	YIL095W	*PRK1*	protein amino acid phosphorylation*
415	YDR464W	*SPP41*	negative regulation of transcription from RNA polymerase II promoter
416	YLR058C	*SHM2*	one-carbon compound metabolism
417	YHL008C	-	biological process unknown
418	YPL162C	-	biological process unknown
419	YOR181W	*LAS17*	endocytosis*
420	YHR218W	-	biological process unknown
421	YDR448W	*ADA2*	histone acetylation*
422	YNL190W	-	response to dessication
423	YDL184C	*RPL41A*	protein biosynthesis
424	YIL160C	*POT1*	fatty acid beta-oxidation
425	YMR108W	*ILV2*	branched chain family amino acid biosynthesis
426	YJL172W	*CPS1*	proteolysis and peptidolysis*
427	YDL233W	-	biological process unknown
428	YDL085W	*NDE2*	ethanol fermentation*
429	YKL050C	-	biological process unknown
430	YOR042W	*CUE5*	biological process unknown
431	YGR256W	*GND2*	glucose metabolism
432	YNL007C	*SIS1*	protein folding*
433	YCR004C	*YCP4*	biological process unknown
434	YIR014W	-	biological process unknown
435	YOL019W	-	biological process unknown
436	YJR045C	*SSC1*	protein folding*
437	YLR417W	*VPS36*	protein-vacuolar targeting*
438	YDR054C	*CDC34*	G1/S transition of mitotic cell cycle*
439	YAL058C-A	_	biological process unknown
440	YHR096C	*HXT5*	hexose transport
441	YGL204C	_	biological process unknown
442	YJL018W	_	biological process unknown
443	YNL057W	_	biological process unknown
444	YFR021W	*ATG18*	protein-vacuolar targeting*
445	YAR035W	*YAT1*	alcohol metabolism*
446	YBL030C	*PET9*	aerobic respiration*
447	YOL152W	*FRE7*	biological process unknown
448	YDR282C	-	biological process unknown

Number	Systematic name	Standard name	Biological Process
449	YOR027W	*STI1*	protein folding
450	YOL027C	*MDM38*	mitochondrion organization and biogenesis*
451	YMR189W	*GCV2*	one-carbon compound metabolism*
452	YOR324C	*FRT1*	response to stress
453	YFL067W	-	biological process unknown
454	YDL042C	*SIR2*	chromatin silencing at telomere*
455	YDL106C	*PHO2*	transcription*
456	YJR120W	-	mitochondrion organization and biogenesis*
457	YER103W	*SSA4*	response to stress*
458	YBL099W	*ATP1*	ATP synthesis coupled proton transport
459	YML058C-A	_	biological process unknown
460	YDL223C	*HBT1*	cellular morphogenesis during conjugation with cellular fusion
461	YDR187C	_	biological process unknown
462	YKL184W	*SPE1*	pantothenate biosynthesis*

Table V List of down-regulated genes both in the ethanol-adapted and the control strains. The asterisk (*) indicated a main biological process of encoding protein.

Number	Systematic name	Standard name	Biological Process
1	YDL037C	*BSC1*	biological process unknown
2	YGL089C	*MF(ALPHA)2*	response to pheromone during conjugation with cellular fusion
3	YHR215W	*PHO12*	biological process unknown
4	YJR004C	*SAG1*	agglutination during conjugation with cellular fusion
5	YAR071W	*PHO11*	phosphate metabolism
6	YKL216W	*URA1*	pyrimidine base biosynthesis
7	YLR432W	*IMD3*	GTP biosynthesis
8	YHR136C	*SPL2*	response to temperature
9	YOL014W	-	biological process unknown
10	YDL039C	*PRM7*	conjugation with cellular fusion
11	YBR093C	*PHO5*	phosphate metabolism*
12	YDR144C	*MKC7*	proteolysis and peptidolysis
13	YPR118W	-	methionine salvage
14	YKL177W	_	biological process unknown
15	YGL263W	*COS12*	biological process unknown
16	YFR055W	-	sulfur metabolism*
17	YEL040W	*UTR2*	cell wall organization and biogenesis
18	YPL263C	*KEL3*	biological process unknown
19	YBR238C	-	biological process unknown
20	YNL040W	-	biological process unknown
21	YKL178C	*STE3*	signal transduction during conjugation with cellular fusion
22	YAR073W	*IMD1*	biological process unknown
23	YPR116W	-	biological process unknown
24	YJL218W	-	biological process unknown
25	YNR003C	*RPC34*	transcription from RNA polymerase III promoter
26	YBR092C	*PHO3*	phosphate metabolism*
27	YPL126W	*NAN1*	processing of 2biological process unknownS pre-rRNA
28	YJL014W	*CCT3*	protein folding*
29	YNR075W	*COS10*	endocytosis
30	YMR205C	*PFK2*	glycolysis
31	YHR214W	-	biological process unknown
32	YLR359W	*ADE13*	purine nucleotide biosynthesis*
33	YGR200C	*ELP2*	regulation of transcription from RNA polymerase II promoter
34	YDR091C	*RLI1*	translational initiation*
35	YMR217W	*GUA1*	GMP metabolism
36	YBR031W	*RPL4A*	protein biosynthesis
37	YOR243C	*PUS7*	tRNA modification*
38	YBR196C	*PGI1*	glycolysis*
39	YBR220C	-	biological process unknown
40	YNL209W	*SSB2*	protein biosynthesis
41	YKL021C	*MAK11*	ribosomal large subunit biogenesis
42	YHR216W	*IMD2*	GTP biosynthesis
43	YBR296C	*PHO89*	phosphate transport
44	YOL141W	*PPM2*	biological process unknown
45	YNL049C	*SFB2*	ER to Golgi transport
46	YML075C	*HMG1*	ergosterol biosynthesis

Number	Systematic name	Standard name	Biological Process
47	YPL028W	*ERG10*	ergosterol biosynthesis
48	YKL143W	*LTV1*	response to osmotic stress*
49	YJR024C	-	biological process unknown
50	YER156C	-	biological process unknown
51	YJL012C	*VTC4*	vacuole fusion, non-autophagic
52	YGR177C	*ATF2*	steroid metabolism
53	YDR012W	*RPL4B*	protein biosynthesis
54	YOL105C	*WSC3*	cell wall organization and biogenesis*
55	YMR229C	*RRP5*	rRNA processing*
56	YLR304C	*ACO1*	mitochondrial genome maintenance*
57	YJL080C	*SCP160*	chromosome segregation*
58	YPR033C	*HTS1*	histidyl-tRNA aminoacylation
59	YER110C	*KAP123*	protein-nucleus import
60	YAR068W	-	biological process unknown
61	YFR056C	_	biological process unknown
62	YJR002W	*MPP10*	rRNA modification*
63	YLR060W	*FRS1*	phenylalanyl-tRNA aminoacylation
64	YDL241W	-	biological process unknown
65	YKL215C	-	biological process unknown
66	YML127W	*RSC9*	regulation of transcription from RNA polymerase II promoter*
67	YOR341W	*RPA190*	transcription from RNA polymerase I promoter
68	YGL009C	*LEU1*	leucine biosynthesis
69	YDL150W	*RPC53*	transcription from RNA polymerase III promoter
70	YDL084W	*SUB2*	mRNA-nucleus export*
71	YKR026C	*GCN3*	translational initiation
72	YHR197W	*RIX1*	35S primary transcript processing*
73	YDR361C	*BCP1*	biological process unknown
74	YLL002W	*RTT109*	negative regulation of DNA transposition
75	YJL157C	*FAR1*	signal transduction during conjugation with cellular fusion*
76	YDL154W	*MSH5*	meiotic recombination
77	YHR170W	*NMD3*	ribosomal large subunit assembly and maintenance*
78	YDR324C	*UTP4*	processing of 2biological process unknownS pre-rRNA
79	YDL151C	_	biological process unknown
80	YJR153W	*PGU1*	pseudohyphal growth*
81	YGR159C	*NSR1*	rRNA processing*
82	YKL127W	*PGM1*	glucose 1-phosphate utilization*
83	YIL121W	*QDR2*	multidrug transport
84	YJR105W	*ADO1*	purine base metabolism
85	YDL036C	-	biological process unknown
86	YBR281C	-	biological process unknown
87	YDR341C	-	protein biosynthesis
88	YOR153W	*PDR5*	response to drug*
89	YLR040C	-	biological process unknown
90	YML123C	*PHO84*	phosphate transport*
91	YDR376W	*ARH1*	iron ion homeostasis*
92	YMR211W	*DML1*	mitochondrial genome maintenance*
93	YDL062W	_	biological process unknown
94	YCL029C	*BIK1*	mitotic spindle organization and biogenesis in nucleus*
95	YKL181W	*PRS1*	histidine biosynthesis*
96	YEL045C	_	biological process unknown

Number	Systematic name	Standard name	Biological Process
97	YOR146W	_	biological process unknown
98	YLR143W	-	biological process unknown
99	YBR298C	*MAL31*	alpha-glucoside transport
100	YFR009W	*GCN20*	regulation of translational elongation
101	YLR041W	_	biological process unknown
102	YMR093W	*UTP15*	processing of 2biological process unknownS pre-rRNA
103	YPL145C	*KES1*	vesicle-mediated transport*
104	YJL198W	*PHO90*	phosphate transport
105	YGL149W	_	biological process unknown
106	YHR064C	*SSZ1*	protein biosynthesis
107	YLR129W	*DIP2*	processing of 2biological process unknownS pre-rRNA
108	YDR165W	*TRM82*	tRNA methylation
109	YDR537C	_	biological process unknown
110	YDL090C	*RAM1*	protein amino acid farnesylation
111	YNL174W	_	biological process unknown
112	YNL132W	*KRE33*	biological process unknown
113	YJL069C	*UTP18*	35S primary transcript processing
114	YHR005C	*GPA1*	signal transduction during conjugation with cellular fusion
115	YNL148C	*ALF1*	post-chaperonin tubulin folding pathway*
116	YLR181C	*VTA1*	late endosome to vacuole transport
117	YDL096C	_	biological process unknown
118	YIR026C	*YVH1*	sporulation (sensu Fungi)*
119	YGR162W	*TIF4631*	translational initiation
120	YJR042W	*NUP85*	mRNA-nucleus export*
121	YMR038C	*CCS1*	intracellular copper ion transport
122	YBL012C	_	biological process unknown
123	YLR413W	-	biological process unknown
124	YBR219C	-	biological process unknown
125	YOL155C	-	cell wall organization and biogenesis
126	YIL078W	*THS1*	protein biosynthesis
127	YLR100W	*ERG27*	ergosterol biosynthesis
128	YCL030C	*HIS4*	histidine biosynthesis
129	YDR044W	*HEM13*	heme biosynthesis
130	YDR321W	*ASP1*	asparagine catabolism
131	YBR079C	*RPG1*	translational initiation
132	YAR042W	*SWH1*	steroid biosynthesis
133	YJL182C	_	biological process unknown
134	YBL076C	*ILS1*	protein biosynthesis
135	YBR085W	*AAC3*	anaerobic respiration
136	YGR190C	_	biological process unknown
137	YMR212C	*EFR3*	cellular morphogenesis
138	YNL307C	*MCK1*	protein amino acid phosphorylation*
139	YFL002C	*SPB4*	35S primary transcript processing*
140	YBR186W	*PCH2*	regulation of meiosis
141	YBL009W	-	meiosis
142	YOL104C	*NDJ1*	synapsis*
143	YGR249W	*MGA1*	filamentous growth
144	YOR301W	*RAX1*	bud site selection
145	YKL191W	*DPH2*	peptidyl-diphthamide biosynthesis from peptidyl-histidine
146	YER044C-A	*MEI4*	sporulation (sensu Fungi)*

Number	Systematic name	Standard name	Biological Process
147	YDR292C	*SRP101*	protein-ER targeting*
148	YMR012W	*CLU1*	translational initiation*
149	YGR115C	_	biological process unknown
150	YLR180W	*SAM1*	methionine metabolism
151	YOR254C	*SEC63*	posttranslational protein-membrane targeting*
152	YJR146W	_	biological process unknown
153	YMR290W-A	_	biological process unknown
154	YJL008C	*CCT8*	protein folding*
155	YER025W	*GCD11*	translational initiation
156	YFR028C	*CDC14*	protein amino acid dephosphorylation*
157	YGL239C	_	biological process unknown
158	YDR023W	*SES1*	seryl-tRNA aminoacylation
159	YEL055C	*POL5*	rRNA transcription
160	YHR183W	*GND1*	response to oxidative stress*
161	YHR070W	*TRM5*	tRNA methylation
162	YDL033C	*SLM3*	protein biosynthesis*
163	YDR344C	_	biological process unknown
164	YKR060W	*UTP30*	35S primary transcript processing
165	YBR034C	*HMT1*	mRNA-nucleus export*
166	YNR043W	*MVD1*	ergosterol biosynthesis*
167	YGR068C	-	biological process unknown
168	YBL013W	*FMT1*	translational initiation*
169	YJR129C	-	biological process unknown
170	YCR011C	*ADP1*	transport
171	YJR015W	-	biological process unknown
172	YBL016W	*FUS3*	protein amino acid phosphorylation*
173	YLR196W	*PWP1*	rRNA processing
174	YCL020W	_	biological process unknown
175	YGL069C	_	biological process unknown
176	YPL082C	*MOT1*	regulation of transcription from RNA polymerase II promoter
177	YJL219W	*HXT9*	hexose transport
178	YML037C	-	biological process unknown
179	YAL025C	*MAK16*	ribosomal large subunit biogenesis
180	YDR112W	_	biological process unknown
181	YDR060W	*MAK21*	ribosomal large subunit assembly and maintenance
182	YBL104C	-	biological process unknown
183	YOR315W	-	biological process unknown
184	YOR361C	*PRT1*	translational initiation
185	YDL148C	*NOP14*	processing of 2biological process unknownS pre-rRNA
186	YBL014C	*RRN6*	transcription from RNA polymerase I promoter
187	YOL022C	-	biological process unknown
188	YOR272W	*YTM1*	ribosomal large subunit biogenesis*
189	YHR120W	*MSH1*	DNA repair
190	YPR197C	_	biological process unknown
191	YER118C	*SHO1*	pseudohyphal growth*
192	YOL021C	*DIS3*	35S primary transcript processing*
193	YBR084W	*MIS1*	nucleobase, nucleoside, nucleotide and nucleic acid metabolism*
194	YMR233W	-	biological process unknown
195	YLR029C	*RPL15A*	protein biosynthesis
196	YBR266C	_	biological process unknown

Number	**Systematic name**	**Standard name**	**Biological Process**
197	YDR192C	*NUP42*	mRNA-nucleus export*
198	YPR176C	*BET2*	protein amino acid geranylgeranylation
199	YLR114C	*AVL9*	biological process unknown
200	YGL097W	*SRM1*	rRNA-nucleus export*
201	YIL134W	*FLX1*	FAD transport
202	YHL039W	-	biological process unknown
203	YJR147W	*HMS2*	pseudohyphal growth
204	YIR012W	*SQT1*	ribosomal large subunit assembly and maintenance
205	YBL032W	*HEK2*	telomerase-dependent telomere maintenance*
206	YJL216C	-	biological process unknown
207	YJL186W	*MNN5*	protein amino acid glycosylation
208	YJR053W	*BFA1*	conjugation with cellular fusion*
209	YKL201C	*MNN4*	response to stress*
210	YML126C	*ERG13*	ergosterol biosynthesis
211	YHR019C	*DED81*	asparaginyl-tRNA aminoacylation
212	YAL048C	*GEM1*	vesicle-mediated transport
213	YDL143W	*CCT4*	protein folding*
214	YBR104W	*YMC2*	transport
215	YGL234W	*ADE5,7*	purine nucleotide biosynthesis*
216	YER055C	*HIS1*	histidine biosynthesis
217	YLR449W	*FPR4*	biological process unknown
218	YHR124W	*NDT80*	meiosis*
219	YJR132W	*NMD5*	protein-nucleus import
220	YNL019C	-	biological process unknown
221	YHL011C	*PRS3*	histidine biosynthesis*
222	YLR339C	_	biological process unknown
223	YDR437W	-	biological process unknown
224	YDR390C	*UBA2*	protein sumoylation
225	YOR393W	*ERR1*	biological process unknown
226	YOR168W	*GLN4*	glutaminyl-tRNA aminoacylation
227	YCL066W	*HMLALPHA1*	regulation of transcription from RNA polymerase II promoter*
228	YBR244W	*GPX2*	response to oxidative stress
229	YNL033W	-	biological process unknown
230	YMR224C	*MRE11*	DNA repair*
231	YER107C	*GLE2*	mRNA-nucleus export*
232	YMR209C	-	biological process unknown
233	YDR037W	*KRS1*	lysyl-tRNA aminoacylation
234	YNL288W	*CAF40*	regulation of transcription from RNA polymerase II promoter
235	YGL170C	*SPO74*	sporulation (sensu Fungi)
236	YGL201C	*MCM6*	DNA replication initiation*
237	YHR089C	*GAR1*	rRNA modification*
238	YFL004W	*VTC2*	vacuole fusion, non-autophagic*
239	YHR177W	-	biological process unknown
240	YGL207W	*SPT16*	chromatin remodeling*
241	YGR145W	*ENP2*	rRNA processing
242	YBL106C	*SRO77*	exocytosis*
243	YDL129W	-	biological process unknown
244	YJL195C	_	biological process unknown
245	YAR060C	_	biological process unknown
246	YDR395W	*SXM1*	mRNA-nucleus export*

Number	Systematic name	Standard name	Biological Process
247	YGR031W	-	biological process unknown
248	YNL313C	-	karyogamy
249	YDR281C	*PHM6*	biological process unknown
250	YBR245C	*ISW1*	chromatin remodeling
251	YGR124W	*ASN2*	asparagine biosynthesis
252	YOL061W	*PRS5*	histidine biosynthesis*
253	YER172C	*BRR2*	U2-type spliceosome conformational change to release U4 and U1
254	YOR217W	*RFC1*	DNA repair*
255	YDR353W	*TRR1*	regulation of cell redox homeostasis
256	YNL035C	-	biological process unknown
257	YOR212W	*STE4*	signal transduction during conjugation with cellular fusion
258	YIL131C	*FKH1*	pseudohyphal growth*
259	YDR398W	*UTP5*	processing of 2biological process unknownS pre-rRNA
260	YIL039W	-	biological process unknown
261	YCL017C	*NFS1*	iron ion homeostasis*
262	YDL226C	*GCS1*	ER to Golgi transport*
263	YDR132C	-	biological process unknown
264	YMR290C	*HAS1*	rRNA processing
265	YNL220W	*ADE12*	purine nucleotide biosynthesis*
266	YNL105W	_	biological process unknown
267	YOR127W	*RGA1*	actin filament organization*
268	YNR062C	-	biological process unknown
269	YNL279W	*PRM1*	plasma membrane fusion
270	YMR266W	*RSN1*	biological process unknown
271	YMR246W	*FAA4*	lipid metabolism*
272	YDR521W	_	biological process unknown
273	YJL050W	*MTR4*	35S primary transcript processing*
274	YIL003W	*CFD1*	iron-sulfur cluster assembly
275	YGL060W	*YBP2*	biological process unknown
276	YMR128W	*ECM16*	processing of 2biological process unknownS pre-rRNA*
277	YLR115W	*CFT2*	mRNA polyadenylylation*
278	YGL251C	*HFM1*	meiosis*
279	YNL175C	*NOP13*	biological process unknown
280	YGR185C	*TYS1*	tyrosyl-tRNA aminoacylation
281	YAR066W	-	biological process unknown
282	YLL016W	*SDC25*	Ras protein signal transduction
283	YOR206W	*NOC2*	ribosome assembly*
284	YEL037C	*RAD23*	ER-associated protein catabolism*
285	YDL102W	*CDC2*	nucleotide-excision repair*
286	YJL175W	_	biological process unknown
287	YMR235C	*RNA1*	rRNA-nucleus export*
288	YMR220W	*ERG8*	ergosterol biosynthesis*
289	YDR380W	*ARO10*	leucine catabolism*
290	YGL076C	*RPL7A*	protein biosynthesis
291	YBR017C	*KAP104*	protein-nucleus import*
292	YDR535C	_	biological process unknown
293	YGL148W	*ARO2*	aromatic amino acid family biosynthesis
294	YMR092C	*AIP1*	response to osmotic stress*
295	YGR233C	*PHO81*	phosphate metabolism

Number	**Systematic name**	**Standard name**	**Biological Process**
296	YOR241W	*MET7*	one-carbon compound metabolism
297	YBR288C	*APM3*	vesicle-mediated transport*
298	YLR083C	*EMP70*	transport
299	YHR214W-A	_	biological process unknown
300	YDR443C	*SSN2*	negative regulation of transcription from RNA polymerase II promoter
301	YPL190C	*NAB3*	regulation of transcription from RNA polymerase II promoter
302	YOR141C	*ARP8*	biological process unknown
303	YOL046C	_	biological process unknown
304	YNL095C	-	biological process unknown
305	YLR032W	*RAD5*	DNA repair
306	YML082W	-	sulfur metabolism
307	YHR137W	*ARO9*	aromatic amino acid family metabolism
308	YJR060W	*CBF1*	chromatin assembly or disassembly*
309	YNL233W	*BNI4*	chitin biosynthesis*
310	YDR004W	*RAD57*	telomerase-independent telomere maintenance*
311	YER003C	*PMI40*	protein amino acid glycosylation*
312	YLR085C	*ARP6*	protein-vacuolar targeting*
313	YLR410W-A	_	biological process unknown
314	YOL077C	*BRX1*	ribosomal large subunit assembly and maintenance
315	YJL105W	*SET4*	biological process unknown

Table VI List of down-regulated genes only in the control strain. The asterisk (*) indicated a main biological process of encoding protein.

Number	Systematic name	Standard name	Biological Process
1	YGL253W	*HXK2*	replicative cell aging*
2	YBL039C	*URA7*	phospholipid biosynthesis*
3	YML056C	*IMD4*	biological process unknown
4	YLR249W	*YEF3*	translational elongation
5	YLR385C	*SWC7*	chromatin remodeling
6	YML093W	*UTP14*	processing of 2biological process unknownS pre-rRNA
7	YPL019C	*VTC3*	vacuole fusion, non-autophagic
8	YKR059W	*TIF1*	translational initiation
9	YJR071W	_	biological process unknown
10	YDL167C	*NRP1*	biological process unknown
11	YNL024C	-	biological process unknown
12	YNL141W	*AAH1*	adenine catabolism
13	YPL266W	*DIM1*	rRNA modification*
14	YPL012W	*RRP12*	processing of 2biological process unknownS pre-rRNA*
15	YOL097C	*WRS1*	tryptophanyl-tRNA aminoacylation
16	YLR303W	*MET17*	methionine metabolism*
17	YNL182C	*IPI3*	35S primary transcript processing*
18	YGR265W	_	biological process unknown
19	YLR409C	*UTP21*	35S primary transcript processing
20	YNR042W	_	biological process unknown
21	YPL207W	-	biological process unknown
22	YKR027W	*FMP50*	biological process unknown
23	YAL022C	*FUN26*	nucleoside transport
24	YAR075W	-	biological process unknown
25	YPR163C	*TIF3*	translational initiation
26	YNL198C	_	biological process unknown
27	YMR116C	*ASC1*	biological process unknown
28	YPL044C	_	biological process unknown
29	YLR433C	*CNA1*	cell wall organization and biogenesis*
30	YCR072C	-	biological process unknown
31	YPR041W	*TIF5*	regulation of translational initiation*
32	YMR131C	*RRB1*	ribosome biogenesis
33	YJR070C	*LIA1*	microtubule cytoskeleton organization and biogenesis
34	YCL059C	*KRR1*	rRNA processing*
35	YCR057C	*PWP2*	35S primary transcript processing*
36	YDR020C	-	biological process unknown
37	YPR009W	*SUT2*	regulation of transcription from RNA polymerase II promoter*
38	YPR048W	*TAH18*	biological process unknown
39	YDR496C	*PUF6*	regulation of transcription, mating-type specific
40	YNL026W	*SAM50*	protein complex assembly*
41	YCL046W	_	biological process unknown
42	YDR434W	*GPI17*	attachment of GPI anchor to protein
43	YCL054W	*SPB1*	processing of 27S pre-rRNA
44	YGL001C	*ERG26*	ergosterol biosynthesis
45	YGR175C	*ERG1*	ergosterol biosynthesis
46	YNL178W	*RPS3*	protein biosynthesis*
47	YBR189W	*RPS9B*	protein biosynthesis*

Number	Systematic name	Standard name	Biological Process
48	YPR198W	*SGE1*	response to drug*
49	YNR053C	*NOG2*	ribosome assembly*
50	YHR109W	*CTM1*	protein modification
51	YLR420W	*URA4*	pyrimidine nucleotide biosynthesis
52	YLR452C	*SST2*	signal transduction*
53	YLR222C	*UTP13*	processing of 2biological process unknownS pre-rRNA
54	YNL296W	_	biological process unknown
55	YIL009W	*FAA3*	lipid metabolism*
56	YPL208W	-	biological process unknown
57	YPL131W	*RPL5*	protein biosynthesis*
58	YPR010C	*RPA135*	transcription from RNA polymerase I promoter
59	YPR144C	*NOC4*	processing of 2biological process unknownS pre-rRNA*
60	YDL080C	*THI3*	thiamin biosynthesis
61	YGL012W	*ERG4*	ergosterol biosynthesis
62	YPL273W	*SAM4*	sulfur amino acid metabolism
63	YDR190C	*RVB1*	regulation of transcription from RNA polymerase II promoter*
64	YDR428C	-	biological process unknown
65	YOR385W	-	biological process unknown
66	YDL178W	*DLD2*	biological process unknown*
67	YGL034C	_	biological process unknown
68	YDL014W	*NOP1*	rRNA modification*
69	YPR019W	*CDC54*	DNA replication initiation*
70	YOR192C-A	_	biological process unknown
71	YNL324W	_	biological process unknown
72	YER007W	*PAC2*	post-chaperonin tubulin folding pathway*
73	YHR149C	*SKG6*	biological process unknown
74	YDR465C	*RMT2*	peptidyl-arginine methylation
75	YGR091W	*PRP31*	nuclear mRNA splicing, via spliceosome
76	YGL123W	*RPS2*	protein biosynthesis*
77	YJR003C	-	biological process unknown
78	YLR212C	*TUB4*	mitotic spindle organization and biogenesis in nucleus*
79	YHR072W	*ERG7*	ergosterol biosynthesis
80	YDR268W	*MSW1*	tryptophanyl-tRNA aminoacylation
81	YJL025W	*RRN7*	transcription from RNA polymerase I promoter
82	YDL104C	*QRI7*	proteolysis and peptidolysis
83	YML060W	*OGG1*	DNA repair*
84	YER181C	_	biological process unknown
85	YBR174C	_	biological process unknown
86	YJL183W	*MNN11*	protein amino acid glycosylation
87	YGR245C	*SDA1*	actin cytoskeleton organization and biogenesis*
88	YER005W	*YND1*	protein amino acid glycosylation
89	YMR243C	*ZRC1*	zinc ion homeostasis*
90	YDL103C	*QRI1*	UDP-N-acetylglucosamine biosynthesis
91	YOL052C	*SPE2*	pantothenate biosynthesis*
92	YPL086C	*ELP3*	regulation of transcription from RNA polymerase II promoter
93	YDL112W	*TRM3*	tRNA methylation
94	YMR011W	*HXT2*	hexose transport
95	YDR149C	_	biological process unknown
96	YJL058C	*BIT61*	biological process unknown
97	YGL047W	*ALG13*	dolichol-linked oligosaccharide biosynthesis

Number	Systematic name	Standard name	Biological Process
98	YNL248C	*RPA49*	transcription from RNA polymerase I promoter
99	YLL031C	*GPI13*	GPI anchor biosynthesis
100	YCL055W	*KAR4*	meiosis*
101	YOL093W	*TRM10*	tRNA methylation
102	YPR136C	_	biological process unknown
103	YDR327W	_	biological process unknown
104	YJL010C	-	rRNA processing
105	YJL109C	*UTP10*	processing of 2biological process unknownS pre-rRNA
106	YPR054W	*SMK1*	protein amino acid phosphorylation*
107	YGR264C	*MES1*	methionyl-tRNA aminoacylation
108	YHR169W	*DBP8*	35S primary transcript processing
109	YLL061W	*MMP1*	S-methylmethionine transport
110	YJR139C	*HOM6*	methionine metabolism*
111	YGR160W	_	biological process unknown
112	YDR385W	*EFT2*	translational elongation
113	YLL062C	*MHT1*	sulfur amino acid metabolism
114	YKL080W	*VMA5*	vacuolar acidification
115	YCL014W	*BUD3*	cytokinesis*
116	YPR060C	*ARO7*	aromatic amino acid family biosynthesis
117	YOR087W	*YVC1*	cation homeostasis
118	YBR074W	-	proteolysis and peptidolysis
119	YCR067C	*SED4*	ER to Golgi transport
120	YLR373C	*VID22*	vacuolar protein catabolism
121	YDR245W	*MNN10*	actin filament organization*
122	YJR001W	*AVT1*	neutral amino acid transport
123	YLR384C	*IKI3*	regulation of transcription from RNA polymerase II promoter
124	YIL132C	*CSM2*	meiotic chromosome segregation*
125	YHR085W	*IPI1*	rRNA processing*
126	YJR041C	*URB2*	ribosome biogenesis*
127	YJL098W	*SAP185*	G1/S transition of mitotic cell cycle
128	YJL064W	_	biological process unknown
129	YPR006C	*ICL2*	propionate metabolism*
130	YKL009W	*MRT4*	rRNA processing*
131	YPR189W	*SKI3*	mRNA catabolism*
132	YDL063C	-	biological process unknown
133	YCR045C	-	biological process unknown
134	YBR252W	*DUT1*	pyrimidine deoxyribonucleoside triphosphate catabolism
135	YCR034W	*FEN1*	vesicle-mediated transport*
136	YAR018C	*KIN3*	chromosome segregation
137	YGL143C	*MRF1*	protein biosynthesis*
138	YLR183C	*TOS4*	G1/S-specific transcription in mitotic cell cycle
139	YBR160W	*CDC28*	protein amino acid phosphorylation*
140	YDR137W	*RGP1*	intracellular protein transport
141	YGL169W	*SUA5*	aerobic respiration
142	YLL038C	*ENT4*	endocytosis*
143	YHR129C	*ARP1*	establishment of mitotic spindle orientation*
144	YLR028C	*ADE16*	aerobic respiration*
145	YPL183C	-	biological process unknown
146	YNL207W	*RIO2*	processing of 2biological process unknownS pre-rRNA
147	YIL103W	*DPH1*	peptidyl-diphthamide biosynthesis from peptidyl-histidine
148	YOL101C	*IZH4*	lipid metabolism*

Number	Systematic name	Standard name	Biological Process
149	YER117W	*RPL23B*	protein biosynthesis
150	YML046W	*PRP39*	nuclear mRNA splicing, via spliceosome
151	YBL054W	-	biological process unknown
152	YKL073W	*LHS1*	response to unfolded protein*
153	YKL014C	*URB1*	rRNA processing*
154	YDR111C	*ALT2*	biological process unknown
155	YMR202W	*ERG2*	ergosterol biosynthesis
156	YJL111W	*CCT7*	protein folding*
157	YKL209C	*STE6*	peptide pheromone export
158	YFL001W	*DEG1*	RNA processing
159	YLR328W	*NMA1*	NAD metabolism
160	YPR066W	*UBA3*	protein neddylation
161	YBR175W	*SWD3*	chromatin silencing at telomere*
162	YOL130W	*ALR1*	magnesium ion transport*
163	YPL235W	*RVB2*	regulation of transcription from RNA polymerase II promoter*
164	YPL248C	*GAL4*	regulation of transcription, DNA-dependent*
165	YGR198W	-	MAPKKK cascade
166	YCR017C	*CWH43*	cell wall organization and biogenesis*
167	YGR099W	*TEL2*	telomerase-dependent telomere maintenance
168	YJL138C	*TIF2*	translational initiation*
169	YNL023C	*FAP1*	biological process unknown
170	YDR399W	*HPT1*	purine nucleotide biosynthesis
171	YIL019W	*FAF1*	processing of 2biological process unknownS pre-rRNA
172	YDR101C	*ARX1*	ribosomal large subunit biogenesis
173	YGR169C	*PUS6*	tRNA modification*
174	YDR429C	*TIF35*	translational initiation
175	YGR214W	*RPS0A*	protein biosynthesis*
176	YNL139C	*RLR1*	mRNA-nucleus export*
177	YHL033C	*RPL8A*	protein biosynthesis
178	YLR063W	-	biological process unknown
179	YDR267C	-	biological process unknown
180	YPR175W	*DPB2*	nucleotide-excision repair*
181	YAR008W	*SEN34*	tRNA splicing
182	YLL007C	-	biological process unknown
183	YML080W	*DUS1*	tRNA modification
184	YLL008W	*DRS1*	35S primary transcript processing*
185	YML022W	*APT1*	AMP biosynthesis*
186	YPL143W	*RPL33A*	protein biosynthesis
187	YGR128C	*UTP8*	processing of 2biological process unknownS pre-rRNA
188	YOR095C	*RKI1*	pentose-phosphate shunt
189	YJR124C	-	biological process unknown
190	YER049W	-	biological process unknown
191	YOR063W	*RPL3*	protein biosynthesis*
192	YPL217C	*BMS1*	rRNA processing*
193	YLR397C	*AFG2*	response to drug
194	YMR289W	*ABZ2*	biological process unknown
195	YOL158C	*ENB1*	ferric-enterobactin transport
196	YCR101C	-	biological process unknown
197	YJL002C	*OST1*	N-linked glycosylation*
198	YMR288W	*HSH155*	spliceosome assembly
199	YPL101W	*ELP4*	regulation of transcription from RNA polymerase II

Number	Systematic name	Standard name	Biological Process
			promoter
200	YCR081W	*SRB8*	negative regulation of transcription from RNA polymerase II promoter
201	YDL040C	*NAT1*	protein amino acid acetylation
202	YLR441C	*RPS1A*	protein biosynthesis
203	YOR060C	-	biological process unknown
204	YJL039C	*NUP192*	nuclear pore organization and biogenesis
205	YBR247C	*ENP1*	rRNA processing*
206	YGR073C	_	biological process unknown
207	YLL033W	-	sporulation (sensu Fungi)
208	YMR006C	*PLB2*	glycerophospholipid metabolism
209	YJR114W	*SRF2*	biological process unknown
210	YNL330C	*RPD3*	chromatin silencing at telomere*
211	YDR047W	*HEM12*	heme biosynthesis
212	YNL299W	*TRF5*	sister chromatid cohesion*
213	YBR180W	*DTR1*	spore wall assembly (sensu Fungi)*
214	YNL328C	*MDJ2*	protein folding
215	YGL084C	*GUP1*	glycerol transport*
216	YDL051W	*LHP1*	tRNA processing
217	YOR175C	-	biological process unknown
218	YLR167W	*RPS31*	protein biosynthesis*
219	YDL058W	*USO1*	ER to Golgi transport*
220	YDR280W	*RRP45*	35S primary transcript processing*
221	YLR243W	-	biological process unknown
222	YCR032W	*BPH1*	cell wall organization and biogenesis*
223	YML021C	*UNG1*	DNA repair
224	YBR065C	*ECM2*	cell wall organization and biogenesis*
225	YDR431W	_	biological process unknown
226	YAR064W	-	biological process unknown
227	YBR061C	*TRM7*	protein biosynthesis*
228	YER127W	*LCP5*	rRNA modification*
229	YDL060W	*TSR1*	rRNA processing*
230	YIL018W	*RPL2B*	protein biosynthesis
231	YKL172W	*EBP2*	rRNA processing
232	YPL181W	*CTI6*	positive regulation of transcription
233	YPR110C	*RPC40*	transcription from RNA polymerase I promoter*
234	YJL061W	*NUP82*	mRNA-nucleus export*
235	YNL120C	_	biological process unknown
236	YLR153C	*ACS2*	acetyl-CoA biosynthesis
237	YDL152W	_	biological process unknown
238	YGL223C	*COG1*	intra-Golgi transport*
239	YPL200W	*CSM4*	meiotic chromosome segregation
240	YPL245W	-	biological process unknown
241	YDL118W	_	biological process unknown
242	YLR404W	-	biological process unknown
243	YOL145C	*CTR9*	transcription from RNA polymerase II promoter*
244	YDR172W	*SUP35*	mRNA catabolism, deadenylylation-dependent decay*
245	YKL075C	-	biological process unknown
246	YPL043W	*NOP4*	rRNA processing
247	YKL033W	-	biological process unknown
248	YDR538W	*PAD1*	aromatic compound catabolism
249	YPL113C	-	metabolism

Number	**Systematic name**	**Standard name**	**Biological Process**
250	YJR031C	*GEA1*	ER to Golgi transport*
251	YNR066C	-	biological process unknown
252	YAL033W	*POP5*	rRNA processing*
253	YGR074W	*SMD1*	nuclear mRNA splicing, via spliceosome
254	YDR518W	*EUG1*	protein folding
255	YAL058W	*CNE1*	protein folding*
256	YCR006C	_	biological process unknown
257	YNL089C	_	biological process unknown
258	YGL245W	*GUS1*	glutamyl-tRNA aminoacylation
259	YKL183W	*LOT5*	biological process unknown
260	YDR098C-A	_	biological process unknown
261	YEL053C	*MAK10*	N-terminal protein amino acid acetylation
262	YIR033W	*MGA2*	positive regulation of transcription from RNA polymerase II promoter*
263	YDR257C	*SET7*	biological process unknown
264	YJL145W	*SFH5*	phospholipid transport
265	YLL035W	*GRC3*	rRNA processing
266	YGR114C	_	biological process unknown
267	YDR446W	*ECM11*	cell wall organization and biogenesis
268	YDR416W	*SYF1*	nuclear mRNA splicing, via spliceosome*
269	YCL052C	*PBN1*	GPI anchor biosynthesis*
270	YLR075W	*RPL10*	protein biosynthesis*
271	YKL056C	*RBF18*	biological process unknown
272	YPL150W	-	biological process unknown
273	YDR015C	_	biological process unknown
274	YDL145C	*COP1*	ER to Golgi transport*
275	YJR143C	*PMT4*	O-linked glycosylation
276	YBR044C	*TCM62*	protein complex assembly
277	YKL055C	*OAR1*	aerobic respiration*
278	YDL074C	*BRE1*	chromatin silencing at telomere*
279	YER006W	*NUG1*	rRNA processing
280	YLR084C	*RAX2*	bud site selection
281	YNL022C	-	biological process unknown
282	YCR054C	*CTR86*	biological process unknown
283	YGR173W	*RBG2*	biological process unknown
284	YLR130C	*ZRT2*	low-affinity zinc ion transport
285	YOR026W	*BUB3*	mitotic spindle checkpoint
286	YLL054C	-	biological process unknown
287	Q0250	*COX2*	aerobic respiration*
288	YPL157W	*TGS1*	ribosome biogenesis*
289	YPL243W	*SRP68*	protein-ER targeting*
290	YNL256W	*FOL1*	folic acid and derivative biosynthesis
291	YDR312W	*SSF2*	ribosomal large subunit assembly and maintenance*
292	YML125C	-	biological process unknown
293	YPL112C	*PEX25*	peroxisome organization and biogenesis*
294	YMR218C	*TRS130*	ER to Golgi transport
295	YLR302C	_	biological process unknown
296	YIL020C	*HIS6*	histidine biosynthesis
297	YGR187C	*HGH1*	biological process unknown
298	YLR015W	*BRE2*	chromatin silencing at telomere*
299	YBL008W	*HIR1*	regulation of transcription from RNA polymerase II promoter

Number	Systematic name	Standard name	Biological Process
300	YHR031C	*RRM3*	DNA replication
301	YPR029C	*APL4*	vesicle-mediated transport
302	YIR008C	*PRI1*	DNA replication initiation*
303	YNL075W	*IMP4*	rRNA modification*
304	YOL076W	*MDM20*	mitochondrion inheritance*
305	YOR213C	*SAS5*	chromatin silencing at telomere
306	YLR274W	*CDC46*	DNA replication initiation*
307	YCL024W	*KCC4*	protein amino acid phosphorylation*
308	YBL096C	_	biological process unknown
309	YMR117C	*SPC24*	chromosome segregation*
310	YDR161W	-	biological process unknown
311	YDR212W	*TCP1*	protein folding*
312	YJR140C	*HIR3*	G1/S-specific transcription in mitotic cell cycle
313	YMR262W	-	biological process unknown
314	YFR001W	*LOC1*	ribosomal large subunit biogenesis*
315	YIL145C	*PAN6*	pantothenate biosynthesis
316	YLR401C	*DUS3*	tRNA modification
317	YBR159W	-	fatty acid elongation
318	YDR348C	-	biological process unknown
319	YGR261C	*APL6*	vesicle-mediated transport*
320	YDL087C	*LUC7*	mRNA splice site selection
321	YBR267W	*REI1*	budding cell bud growth*
322	YDL100C	*ARR4*	response to heat*
323	YLR186W	*EMG1*	35S primary transcript processing*
324	YLR154C	*RNH203*	DNA replication
325	YKL099C	*UTP11*	processing of 2biological process unknownS pre-rRNA
326	YNR021W	-	biological process unknown
327	YOR135C	_	biological process unknown
328	YBR181C	*RPS6B*	protein biosynthesis
329	YBL052C	*SAS3*	chromatin silencing at telomere*
330	YGL094C	*PAN2*	postreplication repair*
331	YGL211W	*NCS6*	biological process unknown
332	YPR112C	*MRD1*	35S primary transcript processing
333	YPL035C	_	biological process unknown
334	YPL160W	*CDC60*	leucyl-tRNA aminoacylation
335	YER171W	*RAD3*	transcription initiation from RNA polymerase II promoter*
336	YLR276C	*DBP9*	35S primary transcript processing*
337	YML014W	*TRM9*	response to stress*
338	YNL021W	*HDA1*	regulation of transcription, DNA-dependent*
339	YBR259W	-	biological process unknown
340	YDR354W	*TRP4*	tryptophan biosynthesis
341	YJR145C	*RPS4A*	protein biosynthesis*
342	YPL161C	*BEM4*	establishment of cell polarity (sensu Fungi)*
343	YDL050C	_	biological process unknown
344	YBR143C	*SUP45*	cytokinesis*
345	YDR488C	*PAC11*	nuclear migration, microtubule-mediated*
346	YGL172W	*NUP49*	mRNA-nucleus export*
347	YNL216W	*RAP1*	chromatin silencing at telomere*
348	YPL030W	-	biological process unknown
349	YLR101C	_	biological process unknown
350	YPR153W	-	biological process unknown

Number	**Systematic name**	**Standard name**	**Biological Process**
351	YIL104C	*SHQ1*	snoRNA metabolism
352	YGR234W	*YHB1*	response to stress
353	YBR164C	*ARL1*	protein-vacuolar targeting*
354	YLR341W	*SPO77*	sporulation (sensu Fungi)*
355	YHR045W	-	biological process unknown
356	YDR300C	*PRO1*	proline biosynthesis
357	YML069W	*POB3*	chromatin remodeling*
358	YNR061C	-	biological process unknown
359	YER015W	*FAA2*	lipid metabolism*
360	YLR419W	-	biological process unknown
361	YDR191W	*HST4*	chromatin silencing at telomere*
362	YNR012W	*URK1*	pyrimidine salvage
363	YOR312C	*RPL20B*	protein biosynthesis
364	YLR230W	_	biological process unknown
365	YLR405W	*DUS4*	tRNA modification
366	YPR105C	*COG4*	intra-Golgi transport*
367	YJR069C	*HAM1*	DNA repair
368	YBR205W	*KTR3*	cell wall organization and biogenesis*
369	YLR002C	*NOC3*	rRNA processing*
370	YOL139C	*CDC33*	translational initiation*
371	YDR274C	_	biological process unknown
372	YGL221C	*NIF3*	biological process unknown
373	YBR125C	*PTC4*	biological process unknown

Table VII List of 494 genes whose deletion results growth defect under ethanol stress condition specifically.

Number	Systematic Name	Standard Name	From references
1	YAL009W	SPO7	iv
2	YAL022C	FUN26	iv
3	YAL027W	SAW1	iv
4	YAL037W	YAL037W	i
5	YAL039C	CYC3	i, iv
6	YAL040C	CLN3	i
7	YAL044C	GCV3	iv
8	YAL049C	YAL049C	iv
9	YAL055W	PEX22	iv
10	YAR015W	ADE1	iv
11	YBL022C	PIM1	iv
12	YBL027W	RPL19B	iv
13	YBL051C	PIN4	iv
14	YBL053W	YBL053W	iv
15	YBL062W	YBL062W	iv
16	YBL072C	RPS8A	iv
17	YBL083C	YBL083C	iv
18	YBL094C	YBL094C	i
19	YBL103C	RTG3	iv
20	YBR003W	COQ1	iv
21	YBR021W	FUR4	i, iv
22	YBR026C	ETR1	iv
23	YBR036C	CSG2	i, ii, iv
24	YBR044C	TCM62	i
25	YBR072W	HSP26	iv
26	YBR077C	SLM4	i, iv
27	YBR078W	ECM33	iv
28	YBR095C	RXT2	iv
29	YBR112C	CYC8	i
30	YBR126C	TPS1	i, iii, iv
31	YBR131W	CCZ1	i
32	YBR173C	UMP1	ii
33	YBR231C	SWC5	iv
34	YBR251W	MRPS5	iv
35	YBR268W	MRPL37	iv
36	YBR282W	MRPL27	iv
37	YBR283C	SSH1	iv
38	YBR291C	CTP1	iv
39	YBR292C	YBR292C	iv
40	YBR296C	PHO89	iv
41	YCL007C	YCL007C	i, ii
42	YCL008C	STP22	i, ii, iv
43	YCL010C	SGF29	iv
44	YCR002C	CDC10	iv
45	YCR003W	MRPL32	iv
46	YCR009C	RVS161	i
47	YCR024C	SLM5	iv

Number	Systematic Name	Standard Name	From references
48	YCR025C	YCR025C	iv
49	YCR036W	RBK1	iv
50	YCR045C	YCR045C	iv
51	YCR046C	IMG1	iii, iv
52	YCR049C	YCR049C	iv
53	YCR050C	YCR050C	iv
54	YCR059C	YIH1	iv
55	YCR061W	YCR061W	iv
56	YCR066W	RAD18	iv
57	YCR067C	SED4	iv
58	YCR068W	ATG15	iv
59	YCR071C	IMG2	iv
60	YCR079W	PTC6	iv
61	YCR087C-A	YCR087C-A	iv
62	YCR087W	YCR087W	iv
63	YDL001W	RMD1	iv
64	YDL012C	YDL012C	iv
65	YDL013W	HEX3	ii
66	YDL040C	NAT1	ii
67	YDL044C	MTF2	iii, iv
68	YDL056W	MBP1	iv
69	YDL065C	PEX19	iv
70	YDL066W	IDP1	ii
71	YDL067C	COX9	iv
72	YDL068W	YDL068W	iv
73	YDL081C	RPP1A	i
74	YDL088C	ASM4	iv
75	YDL090C	RAM1	iv
76	YDL091C	UBX3	iv
77	YDL096C	OPI6	iv
78	YDL100C	ARR4	iv
79	YDL107W	MSS2	iv
80	YDL114W	YDL114W	iii, iv
81	YDL118W	YDL118W	iv
82	YDL191W	RPL35A	iv
83	YDL198C	GGC1	iv
84	YDL225W	SHS1	ii
85	YDL226C	GCS1	i, iv
86	YDR010C	YDR010C	iv
87	YDR035W	ARO3	iv
88	YDR049W	YDR049W	i
89	YDR065W	YDR065W	iv
90	YDR074W	TPS2	i
91	YDR079W	PET100	iv
92	YDR108W	GSG1	iv
93	YDR121W	DPB4	iv
94	YDR126W	SWF1	iv
95	YDR149C	YDR149C	ii
96	YDR162C	NBP2	iv
97	YDR175C	RSM24	iv
98	YDR192C	NUP42	iv
99	YDR194C	MSS116	iv

Number	Systematic Name	Standard Name	From references
100	YDR197W	CBS2	iv
101	YDR202C	RAV2	iv
102	YDR204W	COQ4	iv
103	YDR219C	MFB1	iv
104	YDR230W	YDR230W	iv
105	YDR237W	MRPL7	i, iv
106	YDR245W	MNN10	i
107	YDR255C	RMD5	iv
108	YDR260C	SWM1	i, iv
109	YDR261C	EXG2	iv
110	YDR265W	PEX10	iv
111	YDR274C	YDR274C	iv
112	YDR283C	GCN2	iv
113	YDR293C	SSD1	i, ii, iv
114	YDR300C	PRO1	i, iv
115	YDR317W	HIM1	iv
116	YDR332W	IRC3	iv
117	YDR337W	MRPS28	iv
118	YDR347W	MRP1	iv
119	YDR354W	TRP4	i, ii, iv
120	YDR372C	VPS74	iv
121	YDR375C	BCS1	iv
122	YDR393W	SHE9	iv
123	YDR435C	PPM1	ii, iv
124	YDR484W	VPS52	i
125	YDR485C	VPS72	i
126	YDR486C	VPS60	iv
127	YDR488C	PAC11	iv
128	YDR491C	YDR491C	iv
129	YDR493W	FMP36	iv
130	YDR523C	SPS1	iv
131	YDR529C	QCR7	iv
132	YEL009C	GCN4	iv
133	YEL024W	RIP1	i, iv
134	YEL042W	GDA1	iv
135	YEL046C	GLY1	iv
136	YER005W	YND1	ii
137	YER050C	RSM18	iv
138	YER058W	PET117	iv
139	YER087W	YER087W	iv
140	YER090W	TRP2	i, iv
141	YER110C	KAP123	iv
142	YER111C	SWI4	i, iii, iv
143	YER114C	BOI2	iv
144	YER122C	GLO3	i
145	YER131W	RPS26B	iv
146	YER145C	FTR1	iv
147	YER177W	BMH1	iv
148	YFL028C	CAF16	ii
149	YFL031W	HAC1	iv
150	YFR011C	YFR011C	i
151	YFR033C	QCR6	iv

Number	Systematic Name	Standard Name	From references
152	YFR048W	RMD8	iii
153	YGL007W	BRP1	iv
154	YGL019W	CKB1	iv
155	YGL020C	MDM39	iv
156	YGL023C	PIB2	iv
157	YGL026C	TRP5	iv
158	YGL042C	YGL042C	iv
159	YGL043W	DST1	i, iv
160	YGL054C	ERV14	iv
161	YGL105W	ARC1	i
162	YGL117W	YGL117W	iv
163	YGL124C	MON1	iv
164	YGL129C	RSM23	iv
165	YGL136C	MRM2	iv
166	YGL143C	MRF1	iv
167	YGL148W	ARO2	iv
168	YGL152C	YGL152C	iv
169	YGL153W	PEX14	iv
170	YGL197W	MDS3	iv
171	YGL212W	VAM7	i, iv
172	YGL219C	MDM34	i
173	YGL227W	VID30	iv
174	YGL237C	HAP2	iv
175	YGL242C	YGL242C	iv
176	YGL252C	RTG2	i, iv
177	YGR027C	RPS25A	iv
178	YGR056W	RSC1	i, iv
179	YGR062C	COX18	iv
180	YGR064W	YGR064W	iv
181	YGR076C	MRPL25	iv
182	YGR077C	PEX8	iv
183	YGR078C	PAC10	i, ii, iv
184	YGR081C	SLX9	iv
185	YGR085C	RPL11B	iv
186	YGR092W	DBF2	iv
187	YGR112W	SHY1	iv
188	YGR133W	PEX4	iv
189	YGR150C	YGR150C	iv
190	YGR166W	KRE11	iv
191	YGR171C	MSM1	i, iv
192	YGR174C	CBP4	iv
193	YGR181W	TIM13	i
194	YGR183C	QCR9	iv
195	YGR196C	FYV8	ii
196	YGR206W	MVB12	iv
197	YGR229C	SMI1	i, ii, iii, iv
198	YGR270W	YTA7	ii, iv
199	YGR284C	ERV29	iv
200	YHL009C	YAP3	ii
201	YHL029C	OCA5	iv
202	YHL038C	CBP2	iv
203	YHR004C	NEM1	iv

Number	Systematic Name	Standard Name	From references
204	YHR006W	STP2	iv
205	YHR008C	SOD2	i, ii, iv
206	YHR009C	YHR009C	iv
207	YHR011W	DIA4	i, iv
208	YHR012W	VPS29	iii
209	YHR029C	YHI9	iv
210	YHR030C	SLT2	ii, iii
211	YHR038W	RRF1	iv
212	YHR067W	HTD2	iv
213	YHR086W	NAM8	iv
214	YHR116W	COX23	iv
215	YHR129C	ARP1	iv
216	YHR147C	MRPL6	iv
217	YHR189W	PTH1	iv
218	YIL017C	VID28	iv
219	YIL029C	YIL029C	iv
220	YIL034C	CAP2	iv
221	YIL036W	CST6	ii
222	YIL039W	TED1	iv
223	YIL052C	RPL34B	iv
224	YIL053W	RHR2	iv
225	YIL124W	AYR1	iv
226	YIL125W	KGD1	iv
227	YIL157C	FMP35	iv
228	YJL003W	COX16	iv
229	YJL006C	CTK2	i
230	YJL024C	APS3	iv
231	YJL046W	YJL046W	iv
232	YJL080C	SCP160	iv
233	YJL095W	BCK1	ii
234	YJL096W	MRPL49	iv
235	YJL101C	GSH1	iv
236	YJL102W	MEF2	iv
237	YJL131C	YJL131C	iv
238	YJL154C	VPS35	iv
239	YJL164C	TPK1	iv
240	YJL175W	YJL175W	iv
241	YJL179W	PFD1	i, ii, iv
242	YJL187C	SWE1	iv
243	YJL208C	NUC1	iv
244	YJL209W	CBP1	iv
245	YJL211C	YJL211C	iv
246	YJL212C	OPT1	iv
247	YJR011C	YJR011C	i
248	YJR018W	YJR018W	i
249	YJR025C	BNA1	i
250	YJR033C	RAV1	i, iv
251	YJR043C	POL32	i, ii
252	YJR059W	PTK2	iv
253	YJR074W	MOG1	iv
254	YJR075W	HOC1	ii, iv
255	YJR090C	GRR1	i, ii

Number	Systematic Name	Standard Name	From references
256	YJR102C	VPS25	i, iv
257	YJR120W	YJR120W	iv
258	YJR145C	RPS4A	iv
259	YJR152W	DAL5	i
260	YKL002W	DID4	i, iv
261	YKL005C	BYE1	iv
262	YKL016C	ATP7	iv
263	YKL037W	YKL037W	i, ii, iv
264	YKL041W	VPS24	iii, iv
265	YKL048C	ELM1	ii
266	YKL053W	YKL053W	iv
267	YKL055C	OAR1	iv
268	YKL068W	NUP100	iv
269	YKL073W	LHS1	ii, iv
270	YKL087C	CYT2	iv
271	YKL109W	HAP4	iv
272	YKL118W	YKL118W	i, ii
273	YKL134C	OCT1	iv
274	YKL155C	RSM22	iv
275	YKL167C	MRP49	iv
276	YKL169C	YKL169C	iv
277	YKL170W	MRPL38	iv
278	YKL178C	STE3	iv
279	YKL197C	PEX1	iv
280	YKL206C	ADD66	iv
281	YKL211C	TRP3	i, iv
282	YKR006C	MRPL13	iv
283	YKR007W	MEH1	i, ii, iv
284	YKR026C	GCN3	iv
285	YKR028W	SAP190	iv
286	YKR035C	OPI8	i, iv
287	YKR035W-A	DID2	iv
288	YKR055W	RHO4	i
289	YKR084C	HBS1	iv
290	YKR085C	MRPL20	iv
291	YKR093W	PTR2	iv
292	YLL007C	YLL007C	iv
293	YLL018C-A	COX19	iv
294	YLL027W	ISA1	i, iv
295	YLL042C	ATG10	iv
296	YLL043W	FPS1	ii
297	YLR021W	IRC25	iv
298	YLR025W	SNF7	ii, iv
299	YLR027C	AAT2	iv
300	YLR038C	COX12	iv
301	YLR039C	RIC1	i, iv
302	YLR056W	ERG3	iv
303	YLR065C	YLR065C	iv
304	YLR070C	XYL2	iv
305	YLR077W	FMP25	iv
306	YLR119W	SRN2	iv
307	YLR125W	YLR125W	iv

Number	Systematic Name	Standard Name	From references
308	YLR138W	NHA1	i
309	YLR144C	ACF2	iv
310	YLR201C	FMP53	iv
311	YLR203C	MSS51	iv
312	YLR239C	LIP2	iv
313	YLR260W	LCB5	iv
314	YLR288C	MEC3	
315	YLR289W	GUF1	iv
316	YLR304C	ACO1	iv
317	YLR319C	BUD6	iv
318	YLR331C	JIP3	ii
319	YLR332W	MID2	ii
320	YLR360W	VPS38	iv
321	YLR386W	VAC14	ii, iii, iv
322	YLR393W	ATP10	i
323	YLR396C	VPS33	i
324	YLR414C	YLR414C	i
325	YLR417W	VPS36	i, ii, iii, iv
326	YLR426W	YLR426W	iv
327	YLR447C	VMA6	i, ii, iv
328	YML001W	YPT7	iv
329	YML008C	ERG6	iii, iv
330	YML010W-A	YML010W-A	iv
331	YML014W	TRM9	iv
332	YML034W	SRC1	iv
333	YML041C	VPS71	i
334	YML078W	CPR3	iv
335	YML087C	YML087C	iv
336	YML110C	COQ5	iv
337	YML129C	COX14	iv
338	YMR003W	YMR003W	ii
339	YMR004W	MVP1	iv
340	YMR015C	ERG5	iv
341	YMR016C	SOK2	iv
342	YMR021C	MAC1	iv
343	YMR026C	PEX12	iv
344	YMR035W	IMP2	iv
345	YMR038C	CCS1	iv
346	YMR063W	RIM9	i, iv
347	YMR064W	AEP1	iv
348	YMR072W	ABF2	i, iv
349	YMR089C	YTA12	iv
350	YMR092C	AIP1	iv
351	YMR098C	ATP25	iv
352	YMR135C	GID8	iv
353	YMR140W	SIP5	i
354	YMR144W	YMR144W	iv
355	YMR150C	IMP1	iv
356	YMR151W	YIM2	iv
357	YMR157C	FMP39	i
358	YMR158W	MRPS8	iv
359	YMR166C	YMR166C	iv

Number	Systematic Name	Standard Name	From references
360	YMR186W	HSC82	i
361	YMR188C	MRPS17	i, iv
362	YMR202W	ERG2	i, iv
363	YMR216C	SKY1	iv
364	YMR228W	MTF1	iv
365	YMR257C	PET111	iv
366	YMR267W	PPA2	iv
367	YMR282C	AEP2	iv
368	YMR286W	MRPL33	iv
369	YMR287C	MSU1	iv
370	YMR293C	HER2	iv
371	YMR300C	ADE4	iv
372	YMR311C	GLC8	iv
373	YMR326C	YMR326C	i
374	YNL001W	DOM34	iv
375	YNL021W	HDA1	iv
376	YNL045W	YNL045W	iv
377	YNL051W	COG5	iv
378	YNL056W	OCA2	i
379	YNL070W	TOM7	i
380	YNL073W	MSK1	iii
381	YNL076W	MKS1	i, iv
382	YNL079C	TPM1	i, ii, iv
383	YNL081C	SWS2	iv
384	YNL098C	RAS2	i, ii, iv
385	YNL107W	YAF9	i, iv
386	YNL121C	TOM70	i
387	YNL136W	EAF7	iv
388	YNL140C	YNL140C	iv
389	YNL147W	LSM7	iv
390	YNL167C	SKO1	iv
391	YNL177C	MRPL22	iv
392	YNL187W	YNL187W	iv
393	YNL197C	WHI3	iv
394	YNL214W	PEX17	iv
395	YNL220W	ADE12	iv
396	YNL224C	SQS1	iv
397	YNL233W	BNI4	iv
398	YNL235C	YNL235C	iv
399	YNL288W	CAF40	iv
400	YNL298W	CLA4	iv
401	YNL315C	ATP11	i
402	YNL335W	DDI3	iv
403	YNR004W	YNR004W	iv
404	YNR005C	YNR005C	iv
405	YNR020C	ATP23	iv
406	YNR025C	YNR025C	i
407	YNR041C	COQ2	iv
408	YNR045W	PET494	iv
409	YNR051C	BRE5	i, iv
410	YOL008W	COQ10	iv
411	YOL023W	IFM1	iv

Number	Systematic Name	Standard Name	From references
412	YOL033W	MSE1	iv
413	YOL044W	PEX15	iv
414	YOL061W	PRS5	i
415	YOL067C	RTG1	i, iv
416	YOL083W	YOL083W	iv
417	YOL095C	HMI1	iii, iv
418	YOL105C	WSC3	iv
419	YOL111C	MDY2	iv
420	YOL115W	TRF4	iv
421	YOL129W	VPS68	iv
422	YOR002W	ALG6	i, ii, iv
423	YOR008C	SLG1	ii, iii, iv
424	YOR014W	RTS1	iv
425	YOR039W	CKB2	iv
426	YOR067C	ALG8	iv
427	YOR069W	VPS5	iii
428	YOR070C	GYP1	iv
429	YOR106W	VAM3	iv
430	YOR115C	TRS33	i, ii, iv
431	YOR123C	LEO1	iv
432	YOR127W	RGA1	iv
433	YOR128C	ADE2	iv
434	YOR155C	ISN1	iv
435	YOR158W	PET123	iv
436	YOR187W	TUF1	iv
437	YOR196C	LIP5	iv
438	YOR198C	BFR1	ii
439	YOR216C	RUD3	iv
440	YOR258W	HNT3	ii, iv
441	YOR265W	RBL2	iv
442	YOR270C	VPH1	i, iii, iv
443	YOR275C	RIM20	iv
444	YOR298C-A	MBF1	iv
445	YOR305W	YOR305W	iv
446	YOR312C	RPL20B	iv
447	YOR318C	YOR318C	iv
448	YOR322C	LDB19	iv
449	YOR323C	PRO2	iv
450	YOR331C	YOR331C	i, iv
451	YOR349W	CIN1	i
452	YOR350C	MNE1	iv
453	YOR358W	HAP5	iv
454	YOR360C	PDE2	iv
455	YOR375C	GDH1	iv
456	YPL002C	SNF8	i, ii, iv
457	YPL061W	ALD6	iv
458	YPL062W	YPL062W	iv
459	YPL065W	VPS28	i, ii, iv
460	YPL069C	BTS1	iv
461	YPL077C	YPL077C	iv
462	YPL084W	BRO1	i, ii, iv
463	YPL100W	ATG21	iv

Number	Systematic Name	Standard Name	From references
464	YPL104W	MSD1	iv
465	YPL114W	YPL114W	iv
466	YPL120W	VPS30	iii, iv
467	YPL132W	COX11	iv
468	YPL150W	YPL150W	iv
469	YPL155C	KIP2	i
470	YPL165C	SET6	i
471	YPL172C	COX10	iv
472	YPL173W	MRPL40	iv
473	YPL174C	NIP100	iv
474	YPL180W	TCO89	ii, iv
475	YPL181W	CTI6	iv
476	YPL212C	PUS1	iv
477	YPL227C	ALG5	iv
478	YPL241C	CIN2	i
479	YPL260W	YPL260W	i
480	YPR023C	EAF3	iv
481	YPR060C	ARO7	i, iv
482	YPR074C	TKL1	iv
483	YPR099C	YPR099C	iv
484	YPR111W	DBF20	iv
485	YPR116W	YPR116W	iv
486	YPR122W	AXL1	iv
487	YPR123C	YPR123C	iv
488	YPR124W	CTR1	i, iv
489	YPR134W	MSS18	iv
490	YPR157W	YPR157W	iv
491	YPR160W	GPH1	i
492	YPR173C	VPS4	i, iv
493	YPR191W	QCR2	iv
494	YPR194C	OPT2	i

References

i. Kubota S, Takeo I, Kume K, Kanai M, Shitamukai A, Mizunuma M, Miyakawa T, Shimoi H, Iefuji H, Hirata D (2004) Effect of ethanol on cell growth of budding yeast: genes that are important for cell growth in the presence of ethanol. Biosci Biotechnol Biochem 68:968–972

ii. Fujita K, Matsuyama A, Kobayashi Y, Iwahashi H (2006) The genome-wide screening of yeast deletion mutants to identify the genes required for tolerance to ethanol and other alcohols. FEMS Yeast Res 6:744–50

iii. van Voorst F, Houghton-Larsen J, Jønson L, Kielland-Brandt MC, Brandt A (2006) Genome-wide identification of genes required for growth of *Saccharomyces cerevisiae* under ethanol stress. Yeast, 23:351–359

iv. Yoshikawa K, Tanaka T, Furusawa C, Nagahisa K, Hirasawa T, Shimizu H (2008) Comprehensive phenotypic analysis for identification of genes affecting growth under ethanol stress in *Saccharomyces ceneviciae*. FEMS Yeast Res doi: 10.1111/j.1567-1364.2008.00456.x

List of publications

Publications related to this present study

1. Thai Nho Dinh, Keisuke Nagahisa, Takashi Hirasawa, Chikara Furusawa, Hiroshi Shimizu (2008) Adaptation of *Saccharomyces cerevisiae* cells to high ethanol concentration and changes in fatty acid composition of membrane and cell size. PLoS ONE 3(7):e2623
2. Thai Nho Dinh, Keisuke Nagahisa, Katsunori Yoshikawa, Takashi Hirasawa, Chikara Furusawa and Hiroshi Shimizu (2009) Analysis of adaptation to high ethanol concentration in *Saccharomyces cerevisiae* using DNA microarray. Bioprocess and Biosystems Engineering DOI:10.1007/s00449-008-0292-7

Other publication

1. Takashi Hirasawa, Kazuyuki Yamada, Keisuke Nagahisa, Thai Nho Dinh, Chikara Furusawa, Yoshio Katakura, Suteaki Shioya, and Hiroshi Shimizu (2009) Proteomic analysis of responses to osmotic stress in laboratory and sake-brewing strains of *Saccharomyces cerevisiae*. Process Biochemistry DOI:10.1016/j.procbio.2009.02.004

Acknowledgements

I would like to express my deepest gratitude and appreciation to Professor Dr. Hiroshi Shimizu, Metabolic Engineering Laboratory, Department of Bioinformatic Engineering, Graduate School of Information Science and Technology, Osaka University, for giving me the opportunity to study in his laboratory and for all of his enthusiastic support, careful guidance and continuous encouragement for my research and daily life in Osaka, Japan.

I am very grateful to the members of my committee, Professor Dr. Satoshi Harashima and Professor Dr. Eiichiro Fukusaki, Department of Biotechnology, Graduate School of Engineering, Osaka University, for their helpful comments and constructive suggestions. I would like to express my sincere thank to Professor Dr. Suteaki Shioya, Department of Applied Life Science, Faculty of Biotechnology and Life Science, Sojo University, for all his kind support.

I wish to specially thank to Dr. Keisuke Nagahisa, Niigata Research Laboratory, Mitsubishi Gas Chemical Company Inc., for his wise advices, encouragement, help and discussion in this study. I would like to gratefully acknowledge to Associate Professor Dr. Chikara Furusawa, Assistant Professor Dr. Takashi Hirasawa and Associate Professor Dr. Naoaki Ono, Department of Bioinformatic Engineering, Graduate School of Information Science and Technology, Osaka University, for their insightful suggestions and valuable comments throughout my research and thesis writing period.

I want to express my thankfulness to Associate Professor Dr. Yoshinobu Kaneko, Department of Biotechnology, Graduate School of Engineering, Osaka University, for providing FY834 yeast strain. I would like to thank Associate Professor Dr. Yoshio Katakura, Department of Biotechnology, Graduate School of Engineering, Osaka University, for his kind help with the analysis of the fatty acid composition.

I would like to gratefully acknowledge the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan for the Japanese government (Monbukagakusho) scholarship. I am really grateful to Professor Dr. Masanori Fujita, Department of Environmental Engineering, Graduate School of Engineering, Osaka University, Professor Dr. Pham Hung Viet, Associate Professor Dr. Trinh Dinh Dat, Associate Professor Dr. Nguyen Xuan Quynh and Dr. Pham Thi Kim Trang, Vietnam National University, Hanoi, for giving me the chance to apply for this scholarship.

I am grateful to Mr. Yasuyuki Masuda, Mr. Kazuyuki Yamada, Dr. Katsunori Yoshikawa and Mr. Siraje Arif Mahmud for their generous technical help. My thanks to all present and ex-members of Metabolic Engineering Lab (Prof. Shimizu's Lab) and Bioprocess Systems Engineering Lab (Prof. Shioya's Lab) for their kindness and friendship.

I wish to thank my classmates from the Special program of Biotechnology and friends at Osaka University, for sharing their time and friendship.

Finally, my particular acknowledgement goes to my family and close friends for their support, love and continuous encouragement.

Thai Nho Dinh