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DNA Microarray Analysis on *Saccharomyces cerevisiae* under High Carbon Dioxide Concentration in Fermentation Process

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Abstract The effect of carbon dioxide on yeast growth was investigated during the cultivation of pH 5.0 and pH 6.8, by replacing the nitrogen part with carbon dioxide under aerobic conditions. The values of the specific growth rate under pH 5.0 and pH 6.8 conditions became 64.0% and 46.9%, respectively, compared to those before the change in gas composition. This suggests that the effect of carbon dioxide was greater pronounced in pH 6.8 than in pH 5.0. The genome-wide transcriptional response to elevated carbon dioxide was examined using a DNA microarray. As for upregulated genes, it was noteworthy that 3 genes were induced upon entry into a stationary phase and 6 genes were involved in stress response. Of 53 downregulated genes, 22 genes were involved in the ribosomal biogenesis and assembly and 5 genes were involved in the lipid metabolism. These facts suggest that carbon dioxide could bring the cell conditions partially to a stationary phase. The *ALD6* gene encoding for cytosolic acetaldehyde dehydrogenase was downregulated, which would lead to a lack of cell components for the growth. The downregulation of *ALD6* was greater in pH 6.8 than in pH 5.0, consistent with physiological response. This suggests that it might be the most effective factor for growth inhibition.

Keywords: carbon dioxide, *Saccharomyces cerevisiae*, transcriptome, growth inhibition, *ALD6*

INTRODUCTION

Saccharomyces cerevisiae has been widely used in fermentation and brewing industries. In fermentation processes, yeast produces carbon dioxide together with ethanol as a result of metabolism. Since carbon dioxide is accumulated in a medium during the yeast fermentation, yeast cells are exposed to high concentrations of carbon dioxide. It has been well-studied that high concentrations of carbon dioxide in the medium affects yeast cellular physiology. The effect of high concentrations of carbon dioxide appears to inhibit growth [1,2] and product formation as well as inhibition of bud formation [3]. The concentration of carbon dioxide may also affect the metabolic reactions in a cell because carbon dioxide could become not only a substrate in carboxylation reactions, but also a product in decarboxylation reactions. Such an effect is problematic, for example, in industrial fermentation and bio ethanol production. Moreover, high concentrations of carbon dioxide could affect flavor and taste in beer and Japanese sake fermentation [4]. Thus, carbon dioxide could be classified as a stress factor such as osmotic and ethanol stresses to which yeast cells are exposed in fermentation processes [1]. Although the relationship between carbon dioxide and the growth of yeast has been argued and the effect of carbon dioxide on yeast cellular physiology is recognized, little is known about the mechanism involved in the effect of carbon dioxide as a stress factor.

Responses of yeast to stresses other than carbon dioxide have been extensively examined [5-8]. Yeast typically responds to stresses through signal transduction pathways which are the first event linked to the transcriptional regulation of the genes involved in the adaptation of stress. However, the cellular response of yeast to carbon dioxide has not been sufficiently studied and the mechanism of the effect of carbon dioxide on cell growth is still unclear.

The genome project of *S. cerevisiae* was completed in 1996 [9]. This led to the development of a DNA microarray technique which enables the measuring of gene expression covering all yeast genes at one time [10]. The DNA microarray seems to be a powerful tool for the analysis of intracellular conditions at the transcriptional

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level. Thus, genome-wide comparative analysis of yeasts with a variety of physiological conditions may provide insight into cellular response to environmental changes. Therefore, a comprehensive analysis using a DNA microarray is expected to be valid as an approach to reveal the mechanism involved in the effect of carbon dioxide. The knowledge obtained from this analysis could be useful to breed yeast strains tolerant of carbon dioxide.

In the present study, we examined the effect of carbon dioxide on yeast growth by changing the composition of the supplied gas from N_2:O_2 = 80:20 to CO_2:O_2 = 80:20 at mid-log phase. The effect of carbon dioxide on yeast growth was evaluated by measuring the extent of the change in the specific growth rate. Comprehensive analyses with DNA microarray were performed using the samples before and after changing the gas composition. Based on the information of DNA microarray analysis, the mechanism involved in the effect of carbon dioxide on inhibition of yeast growth is discussed in this paper.

MATERIALS AND METHODS

Strain and Culture Conditions

S. cerevisiae BY5210 (*MATα his3Δ200 ura3-52 leu2Δ1 lys2Δ202 trp1Δ63*) was obtained from Yeast Genetic Resource Center (YGRC, Osaka University, Japan; http://bio3.tokyo.jst.go.jp/jst/english/), which was previously called *S. cerevisiae* FY834. Cells were grown at 30°C in YPD medium (2% glucose, 1% bacto-yeast extract, and 2% peptone). The growth was monitored by measuring the optical density at wavelength of 660 nm (OD_{660}). The cultivation was carried out using a jar fermentor (Mitsuwa Scientific, Osaka, Japan). The working volume was 2.0 L with agitation of 200 rpm. The pH was automatically kept at 5.0 or 6.8 by the addition of ammonium water. Gas was supplied by an airflow rate of 1.0 vvm. The gas composition was adjusted using a gas control unit (EYELA, Tokyo, Japan) at N_2:O_2=80:20. It was then changed to carbon dioxide-enriched gas with the composition of CO_2:O_2=80:20 when OD_{660} was reached to around unity.

Total RNA Preparation and Labeling of cDNA with Cy3 and Cy5

Yeast cells were harvested by centrifugation of 50 mL of culture at 4170×*g* for 10 min followed by frozen in liquid nitrogen. The cells were kept at –80°C until use. Total RNA was extracted using the hot phenol method as described [11] and purified with RNeasy kit (Qiagen, Hilden, Germany) according to the instruction manual. The labeled cDNA with Cy3 or Cy5 was synthesized by reverse transcription. A total RNA amount of 25 μg was mixed with 1 μL of 0.5 mg/mL oligo dT primer (Invitrogen, CA, USA) in a volume of 7 μL. Samples were incubated at 70°C for 5 min and incubated at 42°C for 1 to 2 min. Then 4 μL of first strand buffer (Invitrogen, California, USA), 2 μL of dNTP mixture (2 mM of dCTP and 5 mM of dATP, dGTP, and dTTP; Amersham Biosciences, NJ, USA), 2 μL of 100 mM DTT (Invitrogen, CA, USA), 2.5 μL of 40 U RNase inhibitor (Promega, WI, USA), 1 μL of 200 U Superscript II (Invitrogen, CA, USA), and Cy3-labeled dCTP or Cy5 labeled dCTP (Amersham Biosciences, UK) were added. The mixtures were kept at 42°C for 40 min. This was followed by adding 1 μL of 200 U Superscript II and incubation for another 40 min. After the reverse transcription reaction, the reaction solutions were mixed with 20 μL of water, 5 μL of 0.5 M EDTA (pH 8.0), and 10 μL of 1 N NaOH followed by incubating at 65°C for 1 h. The mixtures were neutralized with 25 μL of 1 N Tris-HCl (pH 7.5). Unincorporated nucleotides were removed with a Microcon-30 (Millipore, MA, USA) as follows. The mixtures were concentrated with a Microcon-30 by centrifugation at room temperature. Then 250 μL of TE buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA) was added to the mixtures, and these were concentrated by centrifugation at room temperature. This operation was repeated 6 times. The volume of the mixtures was finally adjusted to 14 μL. The labeling efficiencies with Cy3 and Cy5 were checked by agarose gel electrophoresis, respectively.

Hybridization to DNA Microarray, Washing, and Scanning

Cy3 and Cy5-labeled cDNA were mixed. Then 8.75 μL of 20×SSC (3 M sodium chloride, 0.3 M sodium citrate; pH 7.0) and TE buffer were added, bringing the total volume to 33.25 μL. After heating at 95°C for 1 to 2 min, the sample was left at room temperature for 5 to 10 min. Finally, 1.75 μL of 10% SDS was added. The labeled cDNA solution was dropped on the DNA microarray, Yeast Gene Chip ver. 2 (DNA Chip Research, Kanagawa, Japan). A cover glass was put on the DNA microarray. The DNA microarray was set in a hybridization chamber and left at 65°C for approximately 16 h. After hybridization, the cover glass was removed by shaking in 2 ×SSC, 0.1% SDS. Then the DNA microarray was shaken in 2× SSC, 0.1% SDS at room temperature for 20 min and next in 0.2×SSC, 0.1% SDS at room temperature for 20 min. The DNA microarray was incubated in 0.2×SSC, 0.1% SDS at 55°C for 20 min followed by shaken in 0.2 ×SSC, 0.1% SDS at room temperature for 20 min. The DNA microarray was rinsed in 0.2×SSC at room temperature with shaking and shaken in 0.05×SSC at room temperature. The DNA microarray was scanned using a Genepix 4000A (Molecular Devices, CA, USA) immediately after droplets on the DNA microarray were removed by centrifugation at 1000 rpm for 1 min.

Data Analysis

The signal intensities of Cy3 and Cy5 on DNA microarray were acquired using Genepix pro ver. 3.0 software (Molecular Devices, CA, USA). In comparison between two conditions from the yeast cells, the values of signal intensities were normalized by the locally weighted linear regression (called Lowess) method [12]. During

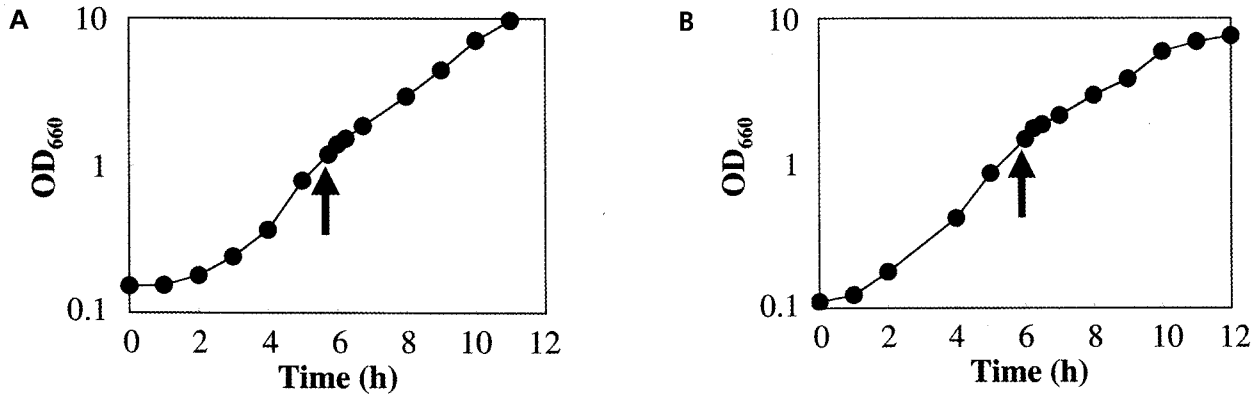

Fig. 1. The growth profiles of yeast under the stress of carbon dioxide. Yeast was grown under the supplement in gas with CO_2:O_2=80:20 following that with N_2:O_2=80:20. The arrow in the figure indicates the time when the gas composition supplied was changed from N_2:O_2=80:20 to CO_2:O_2=80:20. (A) The cultivation under the condition of pH 5.0. (B) The cultivation under the condition of pH 6.8.

Table 1. Effect of carbon dioxide on yeast growth

pH in the medium	Specific growth rate (h^{-1})		
	Before change in gas composition	After change in gas composition	Ratio of change in specific growth rate
5.0	0.602	0.385	0.640
6.8	0.625	0.293	0.469

our research, the thresholds for significant change were determined to be twice and half from the experiment of comparison between the same samples. The data analysis accompanied by gene ontology was done using the program GO Slim Mapper of the *Saccharomyces* genome database (SGD) [13; http://www.yeastgenome.org/]. This helped categorize genes involved in the transcriptional responses to high concentrations of carbon dioxide.

RESULTS AND DISCUSSION

Effect of Carbon Dioxide on Yeast Growth

In order to investigate the effect of carbon dioxide on yeast growth, it is important to cultivate yeast under the optimal condition for the obvious growth inhibition by carbon dioxide. Aerobic cultivation has been reported to show a strong negative effect of carbon dioxide on yeast growth [2]. In other research, carbon dioxide poorly affected the yeast growth under anaerobic conditions [14]. In this study, yeast was grown in a jar fermentor under aerobic conditions with an airflow rate of 1 vvm. The effect of carbon dioxide was examined by replacing the nitrogen part in the supplied gas with carbon dioxide, while the ratio of oxygen in the gas supplied was maintained at 20%. Two different conditions of pH 5.0 and pH 6.8 were performed.

Growth profiles of yeast under two different conditions are shown in Fig. 1, exhibiting the decrease of growth rate after the change to carbon dioxide-enriched gas both in pH 5.0 and 6.8. These results suggest that increased concentrations of carbon dioxide under aerobic cultivation lead to the growth inhibition. The extent of the effect of carbon dioxide under the two cultivation conditions was evaluated using the ratio of change in the specific growth rate between before and after changes in gas composition were supplied. The values of the specific growth rate and the ratio of the change in the specific growth rate under two conditions are summarized in Table 1. The specific growth rate under aerobic cultivation in pH 6.8 decreased from 0.625 to 0.293 after the change in gas composition to high concentrations of carbon dioxide, showing the inhibition of yeast growth with a ratio of 0.469. On the other hand, the specific growth rate under aerobic cultivation in pH 5.0 decreased from 0.602 to 0.385 after changes in gas composition to higher concentrations of carbon dioxide, showing the inhibition of yeast growth with the ratio of 0.640. These results indicate that the inhibitory effect of carbon dioxide appeared more in pH 6.8 than in pH 5.0. The inhibitory effect of carbon dioxide on yeast growth might increase with a raise in pH in the medium.

The concentration of dissolved carbon dioxide is determined by temperature and the partial pressure of carbon dioxide in the gas phase. Dissolved carbon dioxide occurs in four forms in the liquid phase, CO_2, H_2CO_3, HCO_3^-, and CO_3^{2-}. Each form has an equilibrium relationship with each other. The concentration of CO_3^{2-} can be negligible in the range of pH 5.0~7.0. The ratio of HCO_3^- to CO_2 in the liquid phase is increased with increased pH, while the ratio of H_2CO_3 to CO_2 in the liquid phase is

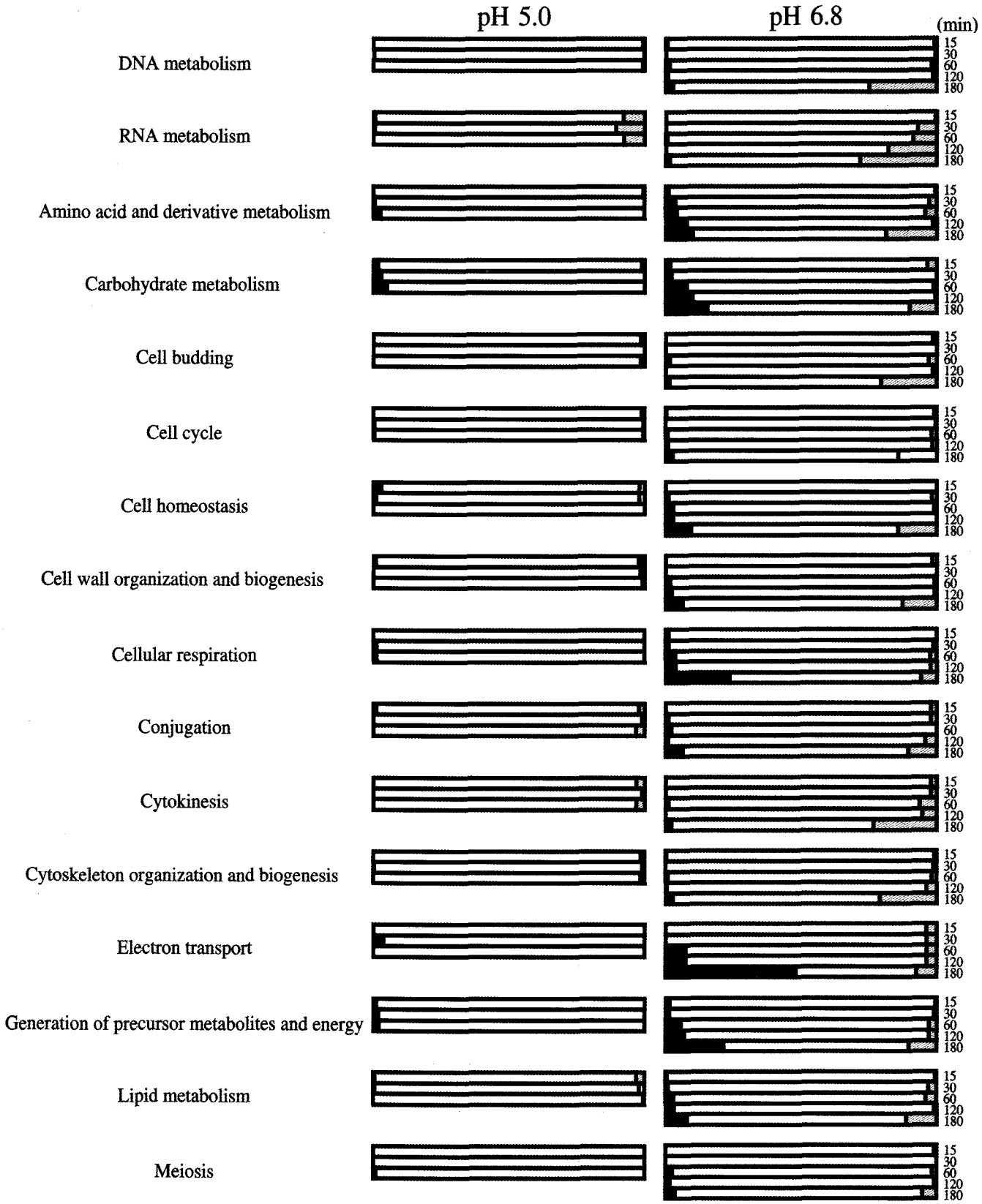

Fig. 2. Transcriptional response to carbon dioxide with the categorization of genes. Black bars, shaded bars, and open bars indicate the ratios of significantly upregulated genes, downregulated genes, and unchanged genes, respectively.

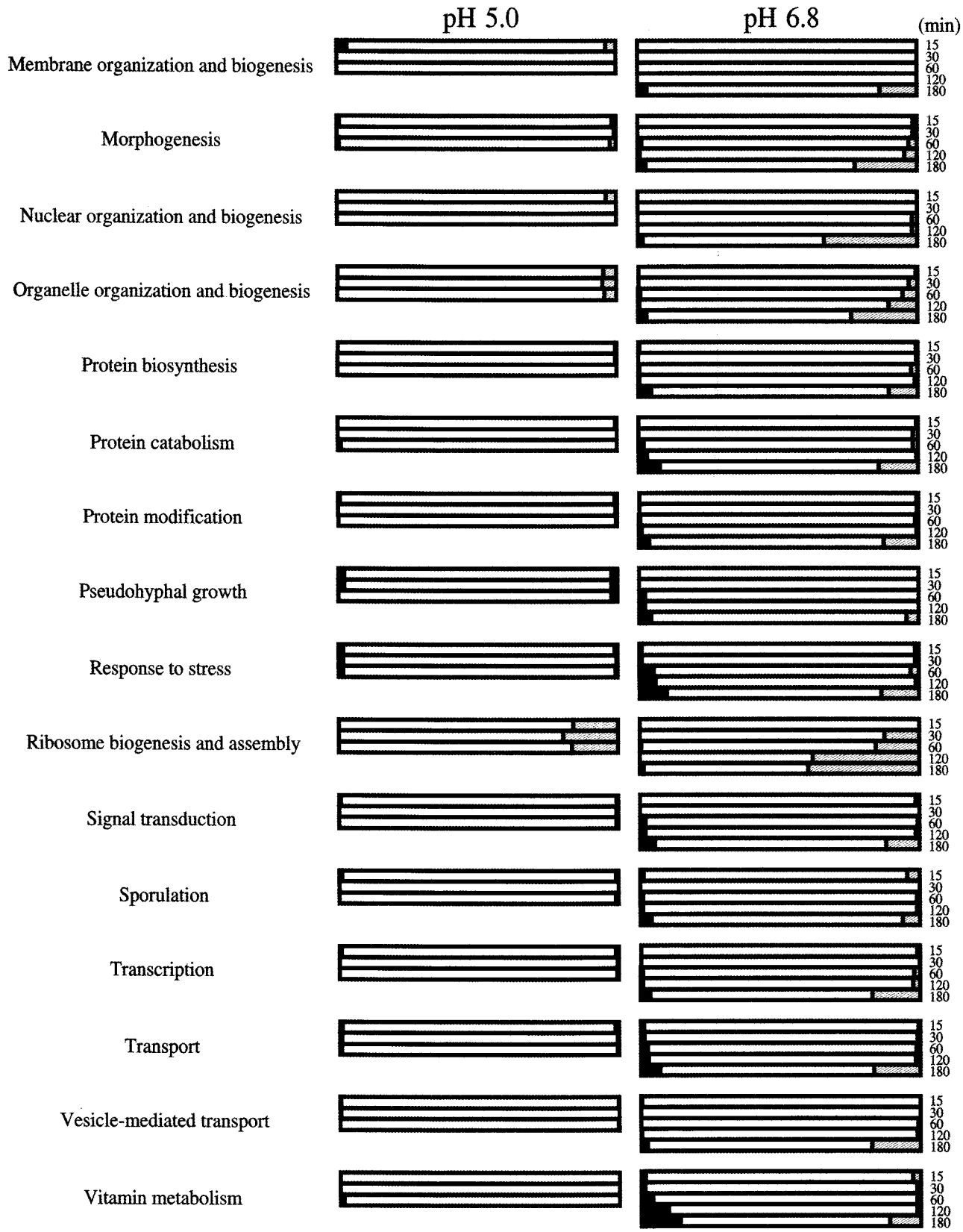

Fig. 2. Continued.

constant independent on pH. For example, the ratios of HCO_3^- and H_2CO_3 to CO_2 in the liquid phase are 0.03 and 0.001 in pH 5.0, respectively, while those are 2.5 and 0.001 in pH 7.0, respectively [1]. In this study, the concentration of dissolved carbon dioxide was the same under both conditions of pH 5.0 and pH 6.8, since temperature and partial pressure of carbon dioxide were same. Therefore, it was suggested that the concentration of HCO_3^- in the medium could affect the growth inhibition of yeast from the result that inhibitory effect on the growth was increased with an increase in pH.

Transcriptional Analysis of Yeast under High Concentrations of Carbon Dioxide

A DNA microarray analysis was performed to examine the transcriptional response to increased carbon dioxide concentration. In order to apply the DNA microarray, yeast cells in the cultivation of pH 5.0 were harvested before and 15 min, 30 min, and 60 min after the change in gas composition, respectively, while those of pH 6.8 were harvested before and 15 min, 30 min, 60 min, 120 min, and 180 min after the change in gas composition, respectively. RNA was purified from each sample and used as a template for the synthesis of cDNA labeled with Cy3 or Cy5 through reverse transcription. The mixture of Cy3-labeled and Cy5-labeled cDNA was applied to the DNA microarray. The expression level ratios in each sample after the change in gas composition to that before the change in gas composition were calculated based on the data of DNA microarray analysis.

In the cultivation of pH 5.0, 4.5% of genes out of 6,059 genes used for the analysis of the transcriptional response showed a significant change in at least one time point after the change in gas composition. In the cultivation of pH 6.8, 27% of genes out of 6,114 genes used for analysis of the transcriptional response, showed significant changes in at least one time point after the change in gas composition, while 7.2% of genes showed the significant change in at least one time point of 15 min, 30 min, and 60 min after the change in gas composition. A larger number of significant transcriptional responses were observed in the cultivation of pH 6.8 than with pH 5.0 were inconsistent with the physiological response to carbon dioxide.

Subsequently, genome-wide transcriptional response to high concentrations of carbon dioxide was analyzed dividing the categorization of genes into 32 biological processes. Fractions of upregulated, downregulated, and unchanged genes in each category and each time point under each condition are exhibited as the ratio in Fig. 2. The ratio of genes with the significant change by elevated carbon dioxide was low in almost all categories except for the time point of 180 min. Conversely, the ratio of genes with a significant change was observed to some extent in case of the investigation of osmotic response [15]. Moreover, the change in gene expression in a category of "response to stress" was significantly small. These results suggest that high concentrations of carbon dioxide are not as stressful to yeast. In contrast, the ratio of the expression change in genes classified into "ribosome biogenesis and assembly" was noteworthy when compared to genes in other categories. More than 12% of the genes were significantly downregulated under both conditions of pH 5.0 and 6.8. This indicates that yeast cells would stop the synthesis of translation instruments involved in the protein synthesis.

Comparative Analysis of the Transcriptional Response between the Cultivation of pH 5.0 and pH 6.8

A comparative analysis between the conditions of pH 5.0 and pH 6.8 was performed to identify the genes with specific responses to elevated carbon dioxide at transcriptional levels. The genes which showed significant change a each time point and under both conditions of pH 5.0 and pH 6.8 are listed in Table 2. The number of genes with common responses to carbon dioxide was a total of 75, of which 21 genes were upregulated, 53 genes were downregulated, and one gene was downregulated and then upregulated in response to elevated carbon dioxide concentration.

Of the 21 upregulated genes, 4 genes (*GPH1*, *GSY1*, *PGM2*, and *GLK1*) were involved in carbohydrate metabolism. *GSY1* encodes glycogen synthase [16], while *GPH1* encodes glycogen phosphorylase, which is not essential for cell viability based on the deletion analysis [17]. The upregulated expression of *GSY1* and *GPH1* genes suggests that the cycle of synthesis and degradation of glycogen might be activated. *GLK1* encoding glucokinase catalyzes the phosphorylation of glucose at C6 [18]. *PGM2* encodes phosphoglucomutase [19]. These upregulated genes suggest that carbohydrate metabolism is enhanced. In upregulated genes, it was remarkable that 3 genes, *YGP1*, *HSP26*, and *GSY1* were identified. These are the genes induced upon entry into a stationary phase [20-22], while the genes which are also induced in a stationary phase such as *HSP30*, *HSP78*, and *UBI4* were not significantly altered [20]. It has been reported that the acquisition of some a stationary phase property might not only be due to nutrient limitation but also due to other factors [23]. These facts suggest that accumulated carbon dioxide might bring the condition of yeast cells partially to a stationary phase. The expression of *YGP1* encoding cell wall-related secretary glycoprotein is regulated in response to nutrient deprivation such as glucose concentrations below 1% and depletion of nitrogen and phosphate [24]. In this study, it was shown that the expression of *YGP1* responds also to high concentrations of carbon dioxide. Attention should be paid to 6 genes (*YGP1*, *HSP26*, *HOR7*, *GAD1*, *WSC4*, and *GPX2*) which were involved in the stress response in 21 upregulated genes. Of these 6 stress response genes, 2 genes, *GAD1* and *GPX2*, respond to oxidative stress [25,26], *HOR7* whose name was derived from HyperOsmolarity-Responsive gene is involved in response to osmotic pressure [27], and *HSP26* is a general stress response gene [28].

In addition to *HOR7*, *GLK1* were also previously cloned as a HyperOsmolarity-Responsive gene [27]. These genes were upregulated in response to elevated carbon dioxide,

Table 2. Genes with significant transcroptional change in both pH 5.0 and pH 6.8

Gene/ORF	Description	Fold change				
		15 min	30 min	60 min	120 min	180 min
Upregulated						
YGP1	Cell wall-related secretory glycoprotein	3.18 1.57	3.04 2.66	2.04 2.97	4.89	12.5
HSP26	Small heat shock protein	1.49 1.17	2.27 1.53	3.65 3.73	3.05	12.5
GPH1	Non-essential glycogen phosphorylase	-1.42 	2.21 -1.03	4.06 3.93	6.93	6.16
GSY1	Glycogen synthase	1.23 -1.60	2.76 2.27	3.03 3.55	6.16	3.90
YKL162C-A	Similar to PIR1, PIR2 and PIR3 proteins	1.69 1.21	1.31 1.90	2.49 2.54	3.55	7.11
HOR7	Protein of unknown function	1.57 -1.09	1.60 1.61	2.43 2.78	4.09	6.32
YRO2	Putative plasma membrane protein	1.89 2.88	2.19 3.14	1.40 2.48	2.45	4.54
OPT2	Oligopeptide transporter	1.45 1.03	2.20 2.59	2.54 1.74	4.20	4.26
GAD1	Glutamate decarboxylase	1.44 -1.06	1.95 1.98	2.01 2.10	5.03	4.00
TFS1	Carboxypeptidase Y inhibitor	1.01 1.48	1.22 1.71	2.10 2.01	3.12	6.33
PGM2	Phosphoglucomutase	1.29 -1.49	2.51 1.32	2.25 2.80	3.63	4.46
YPC1	Alkaline ceramidase	1.78 1.20	2.02 2.46	1.62 2.50	3.02	3.96
WSC4	ER membrane protein	2.57 1.55	2.26 2.25	2.70 1.88	3.00	1.75
GLK1	Glucokinase	1.85 -2.11	2.23 1.61	2.98 2.35	4.34	1.90
LYS1	Saccharopine dehydrogenase	1.70 1.66	1.48 2.00	2.05 2.10	3.05	3.02
TIS11	mRNA-binding protein expressed during iron starvation	2.70 1.76	2.30 2.25	1.74 2.12	1.00	1.34
YCL042W	Hypothetical protein	1.80 -1.61	2.25 2.02	2.50 3.55	2.26	-12.1
SPL2	Protein with similarity to cyclin-dependent kinase inhibitors	2.01 2.04	1.54 2.11	1.33 1.59	1.35	1.57
GPX2	Phospholipid hydroperoxide glutathione peroxidase	3.18 2.35	1.87 1.81	1.26 -1.01	-1.09	1.02
YOR225W	Hypothetical protein	2.22 2.55	1.00 1.58	1.46 1.23	1.27	1.70
PHO12	Repressible acid phosphatases	2.45 2.18	1.28 2.62	1.62 1.22	-1.16	-1.97
Downregulated						
DBP2	Essential ATP-dependent RNA helicase	-3.32 -1.40	-4.26 -3.31	-2.84 -2.21	-5.01	-18.2
BUD23	Protein involved in bud-site selection	-2.96 -1.57	-2.28 -2.10	-2.42 -2.66	-3.88	-4.32
YBL054W	Hypothetical protein	-3.98 -1.49	-3.50 -2.72	-3.17 -1.44	-3.37	-3.04
SDA1	Nuclear protein	-2.38 -1.50	-2.59 -2.42	-2.29 -2.29	-3.25	-4.45

Table 2. Continued

Gene/ORF	Description	Fold change				
		15 min	30 min	60 min	120 min	180 min
HXT2	High-affinity glucose transporter	-8.22 -7.05	-2.66 -4.10	-3.39 1.21	-1.63	-3.37
NOP4	Nucleolar protein	-2.00 -1.42	-3.06 -2.35	-2.13 -2.65	-2.64	-4.82
YOR004W	Protein required for cell viability	-2.66 -1.42	-2.46 -2.36	-2.50 -2.02	-4.02	-2.89
FAS2	Alpha subunit of fatty acid synthetase	-2.10 -1.76	-1.69 -1.90	-4.34 -2.54	-1.81	-14.8
DBP10	Putative ATP-dependent RNA helicase	-2.18 -1.43	-2.67 -2.29	-2.67 -2.16	-3.00	-3.70
SAS10	Component of the small (ribosomal) subunit (SSU) processosome	-1.98 -1.56	-2.55 -2.35	-2.74 -2.12	-3.28	-2.80
MRD1	Essential conserved protein that associates with 35S precursor rRNA	-3.50 -1.45	-2.73 -2.19	-2.97 -1.45	-2.69	-3.48
YDL063c	Hypothetical protein	-2.57 -1.22	-2.28 -2.20	-2.60 -2.59	-3.15	-3.27
UTP14	Nucleolar protein	-1.96 -1.54	-2.58 -2.25	-2.04 -2.50	-2.48	-4.77
DHR2	Predominantly nucleolar DEAH-box RNA helicase	-4.27 1.01	-2.38 -2.40	-2.82 -1.88	-3.54	-3.94
NUG1	GTPase that associates with nuclear 60S pre-ribosomes	-2.24 -1.52	-2.79 -2.30	-2.44 -1.66	-3.00	-3.80
HCA4	Putative nucleolar DEAD box RNA helicase	-3.57 -1.38	-2.13 -2.14	-2.94 -1.55	-3.02	-3.27
ECM16	Essential DEAH-box ATP-dependent RNA helicase	-1.87 -1.22	-2.35 -2.18	-2.28 -2.58	-2.64	-7.01
LTV1	Protein required for growth at low temperature	-2.86 -1.14	-2.94 -2.20	-2.67 -2.21	-2.70	-2.41
RRP5	Protein required for the synthesis of both 18S and 5.8S rRNA	-1.41 -1.23	-2.94 -2.26	-1.85 -2.86	-2.36	-19.25
PPM2	Putative carboxyl methyl transferase	-3.24 -1.10	-2.38 -2.06	-2.38 -1.71	-2.13	-6.88
STP4	Protein involved in pre-tRNA splicing and in uptake of branched-chain amino acids	-1.05 -2.06	-2.65 -2.89	-3.10 -2.29	-1.82	-3.06
FAF1	Protein required for pre-rRNA processing and 40S ribosomal subunit assembly	-2.20 -1.37	-3.36 -2.32	-1.62 -2.41	-2.37	-2.30
ALD6	Cytosolic aldehyde dehydrogenase	-6.78 -8.89	-1.78 -2.56	-1.59 -1.50	-1.35	-1.56
ENP2	Essential nucleolar protein of unknown function	-2.87 -1.18	-2.67 -2.32	-2.82 -1.23	-2.69	-2.81
IPI3	Protein required for cell viability	-1.96 -1.26	-2.65 -2.26	-1.69 -2.37	-2.50	-2.69
TRM1	tRNA methyltransferase	-1.63 -1.40	-3.38 -2.40	-1.75 -3.03	-1.88	-2.28
FPR4	Nuclear protein, putative peptidyl-prolyl cis-trans isomerase (PPIase)	-1.10 -1.23	-2.54 -2.00	-2.08 -3.16	-2.66	-7.08
YNL174W	Hypothetical protein	 -1.05	-2.74 -2.58	-1.82 -2.44	-2.06	-3.05
MAK5	Essential nucleolar protein	-2.12 -1.34	-2.33 -2.19	-1.94 -2.07	-2.37	-2.16
OLE1	Fatty acid desaturase	-4.65 -1.03	-6.42 -6.33	-1.56 -3.25	-1.40	-1.14

Table 2. Continued

Gene/ORF	Description	Fold change				
		15 min	30 min	60 min	120 min	180 min
GFD2	Protein of unknown function	-2.14	-2.17	-1.47		
		-1.43	-2.67	-2.13	-2.37	-1.99
IPI1	Protein of unknown function	-1.46	-2.48	-1.50		
		-1.50	-2.32	-2.42	-2.00	-2.19
NOP7	Nucleolar protein involved in rRNA processing and 60S ribosomal subunit biogenesis	-2.49	-2.56	-2.62		
		1.34	-2.41	-1.59	-3.75	-2.19
NOP13	Protein of unknown function	-1.42	-2.31	-2.10		
		-1.15	-1.77	-2.28	-2.30	-2.72
KRE33	Essential protein of unknown function	-1.57	-2.27	-2.02		
		1.00	-1.70	-2.18	-1.95	-4.43
RPF1	Nucleolar protein involved in the assembly of the large ribosomal subunit	-1.43	-2.63	-1.97		
		-1.06	-2.06	-2.34	-1.98	-1.98
IZH4	Membrane protein involved in zinc metabolism	-4.26	-2.32	-1.40		
		-1.93	-4.42	-2.96	-1.15	1.21
YDL152w	Hypothetical protein	-1.36	-2.27	-1.28		
		-1.19	-2.29	-2.38	-1.63	-3.56
YLR413W	Protein of unknown function	-3.20	-2.53	-1.07		
		-2.18	-3.61	-1.64	1.17	-1.91
BSC1	Protein of unconfirmed function	1.42	-2.22	-3.02		
		1.39	-2.50	-1.71	-6.28	-31.88
MRT4	Protein involved in mRNA turnover and ribosome assembly	-1.01	-2.35	-1.54		
		-1.16	-2.39	-2.45	-2.09	-1.84
PRP43	RNA helicase in the DEAH-box family	-1.05	-2.23	-1.27		
		-1.18	-2.21	-1.87	-1.76	-3.66
FAA4	Long chain fatty acyl-CoA synthetase	-2.23	-2.23	1.02		
		-1.00	-2.73	-1.83	-1.31	-3.23
KRR1	Essential nucleolar protein	1.17	-2.92	-1.79		
		1.03	-2.39	-2.91	-1.93	-1.89
PRM7	Pheromone-regulated protein	1.26	-2.19	-2.40		
		1.53	-3.11	-1.37	-4.34	-7.66
IZH1	Membrane protein involved in zinc metabolism	-2.44	-2.37	-1.04		
		-1.60	-2.41	-1.49	-1.02	-1.05
YML125C	Protein required for cell viability	1.05	-2.63	-1.22		
		-1.13	-2.01	-1.92	-1.33	-1.41
YDR491C	Hypothetical protein	-1.87	-2.19	1.18		
		-1.36	-2.04	-1.29	-1.05	-1.34
CWP1	Cell wall mannoprotein	-3.41	-1.61	1.17		
		-3.18	-1.95	-1.25	2.00	1.55
ADR1	Carbon source-responsive zinc-finger transcription factor	-2.02	1.35	1.24		
		-2.20	-1.77	2.07	1.46	-3.18
HXT3	Low affinity glucose transporter	-2.53	-1.08	1.14		
		-2.37	-1.74	3.69	2.78	2.16
HXT4	High-affinity glucose transporter	-2.11	1.37	1.58		
		-2.27	-1.74	3.01	3.23	2.72
HXT6	High-affinity glucose transporter	-2.61	1.15	1.68		
		-2.57	-1.65	5.85	7.20	6.30
HXK1	Hexokinase isoenzyme 1	-2.08	2.01	3.05		
		-2.25	-1.31	3.29	6.33	4.09

The values of fold change in upper side and lower side of each line were obtained from the sample under the conditions of pH 5.0 and pH 6.8, respectively.

Positive and negative values represent the upregulated or downregulated changes, respectively.

Fold change data are shown at 15, 30, 60 min in pH 5.0 and at 15, 30, 60, 120, 180 min in pH 6.8, respectively.

suggesting that yeast cells might be subjected to osmotic stress as well as oxidative stress under increased carbon dioxide concentration conditions. *WSC4* is the homolog of WSC (cell Wall integrity and Stress response Component) and plays a role in stress responses [29]. The gene expression was increased about *HOR7* and *YGP1* other than *WSC4*, which are cell wall-related genes, suggesting that yeast cells would intend to maintain the cell wall integrity against the stress of carbon dioxide.

Of 53 downregulated genes, 22 genes were related to the ribosome biogenesis and assembly which are necessary for translational instruments, suggesting that the stop of translation process might be caused by increased carbon dioxide concentration. As for the rest, 5 genes (*FAS2*, *OLE1*, *IZH4*, *FAA4*, and *IZH1*) were involved in the lipid metabolism. *IZH2* and *IZH4* are membrane proteins involved in zinc metabolism for zinc homeostasis [30]. The expressions of these genes were induced by fatty acids, indicating that these genes play a role in lipid metabolism [31]. *FAA4* encodes long chain acyl-CoA synthetase [32]. *OLE1* and *FAS2* are related to cellular lipid metabolism. *OLE1* encodes Δ-9 fatty acid desaturase, which catalyzes double bond formations between carbon 9 and 10 of palmitoyl and stearoyl coenzyme A for the formation of unsaturated fatty acids [33]. *FAS2* encodes an alpha subunit of fatty acid synthetase which is responsible for β-ketoacyl reductase and β-ketoacyl synthase activities [34]. The downregulation of these genes suggests that carbon dioxide hinders the synthesis of membrane components required for cell proliferation.

The downregulation of the *ALD6* gene was also observed in common to the cultivation of pH 5.0 and pH 6.8. *ALD6* was responsible for the conversion of acetaldehyde to acetate to synthesize the acetyl-CoA in the cytosol [35]. The deletion of *ALD6* gene has been reported to lead to a one-third decrease in specific growth rates [35]. These facts indicate that a downregulation of *ALD6* gene expression would cause a lack of acetyl-CoA in the cytosol, which leads to a lack of cell components for growth. The extent of change in *ALD6* gene expression was greater in pH 6.8 than in pH 5.0 was inconsistent with physiological response. This suggests that the downregulation of *ALD6* might be the most effective factor for inhibition of growth.

One of the genes which significantly changed only during the cultivation of pH 6.8 was *CIT2* [36]. *CIT2*, which is one of catalysts that worked in the TCA cycle, was strongly upregulated. It is speculated that the TCA cycle was activated to provide energy for the growth under high concentrations of carbon dioxide.

CONCLUSION

The inhibitory effect of carbon dioxide on yeast growth under aerobic conditions was observed under both pH 5.0 and pH 6.8 conditions by replacing the nitrogen part in gas supplied with carbon dioxide. The values of the specific growth rate in the cultivation of pH 5.0 and pH 6.8 became 62.5% and 29.3% compared to those before the change in gas supplied, respectively. This result suggests that the inhibitory effect of carbon dioxide appeared more in pH 6.8 than in pH 5.0, which might be due to the concentration of HCO_3^- in the medium.

The genome-wide transcriptional response to elevated carbon dioxide concentration was investigated using DNA microarray. The number of genes with significant changes in expression in each time point and cultivation was a total of 75 (of which 21 genes were upregulated, 53 genes were downregulated). One gene was downregulated soon after changes in gas composition and then upregulated. Three upregulated genes were involved in entry into a stationary phase. Six upregulated genes were involved in stress response. Twenty-two genes out of 53 downregulated genes were categorized into the ribosome biogenesis and assembly. These facts indicate that accumulated carbon dioxide might bring the condition of yeast cells partially to a stationary phase. The *ALD6* gene encoding a cytosolic acetaldehyde dehydrogenase was downregulated greater in pH 6.8 than in pH 5.0 was consistent with physiological response. This suggests that the downregulation of *ALD6* might be the most effective factor for growth inhibition.

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