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Original Paper

Piezophysiology of genome wide gene expression levels in the yeast *Saccharomyces cerevisiae*

Hitoshi Iwahashi (✉) · Hisayo Shimizu · Mine Odani · Yasuhiko Komatsu

H. Iwahashi
Human Stress Signal Research Center, National Institute of Advanced Industrial Science and Technology, Central-6, Higashi 1-1, Tsukuba 305-8566, Ibaraki, Japan

H. Iwahashi · H. Shimizu · M. Odani · Y. Komatsu
International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology, Central-6, Higashi 1-1, Tsukuba 305-8566, Ibaraki, Japan

✉ H. Iwahashi
Phone: +81-29-8616066
Fax: +81-29-8616066
E-mail: hitoshi.iwahashi@aist.go.jp

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Abstract Hydrostatic pressure is one of the physical factors affecting cellular physiology. Hydrostatic pressure of a few hundred MPa decreases the viability of yeast cells, and pressure of a few tens MPa decreases the growth rate. To understand the effect of hydrostatic pressure, we employed yeast DNA microarrays and analyzed genome-wide gene-expression levels after the pressure treatment with 180 MPa (immediate) at 4°C and recovery incubation for 1 h and 40 MPa (16 h) at 4°C and recovery incubation for 1 h. The transcription of genes involved in energy metabolism, cell defense, and protein metabolism was significantly induced by the pressure treatment. Genome-wide expression profiles suggested that high pressure caused damage to cellular organelles, since the induced gene products were localized in the membrane structure and/or cellular organelles. Hierarchical clustering analysis suggested that the damage caused by the pressure was similar to that caused by detergents, oils, and freezing/thawing. We also estimated

the contribution of induced genes to barotolerance using some strains that have the deletion in the corresponding genes.

Keywords Barotolerance · Gene expression · Hydrostatic pressure · Piezophysiology · *Saccharomyces cerevisiae* · Yeast

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Introduction

Hydrostatic pressure is one of the physical factors affecting cellular physiology (Hayashi 1989). Hydrostatic pressure of a few hundred MPa decreases the viability of yeast cells, and pressure of a few tens MPa decreases the growth rate. New technology for food sterilization is being developed based on these facts, and some food products already undergo hydrostatic pressure sterilization (Hayashi 1989).

As technology develops, effort is being focused on the physiology, growth, and survival of organisms under hydrostatic pressure. In studying the range of pressure that causes growth inhibition, Abe and Horikoshi (1995) found that hydrostatic pressure of 40–60 MPa promoted acidification of the vacuoles in yeast cells; the tryptophan permease gene *TAT2* may be the rate-limiting factor for tryptophan-requiring yeast cells (Abe and Horikoshi 2000). Tamura et al. (1998) showed that expression of the gene encoding yeast heat shock protein was induced in the same range of pressure. In addition, Iwahashi et al. (1991) showed that treatment with a pressure of more than 100 MPa decreased the number of CFU; however, a mild heat-shock treatment of 43°C for 30 min increased barotolerance (resistance to hydrostatic pressure). This was shown to be due to the accumulation of trehalose and Hsp104 (Iwahashi et al. 1997a). They also found that trehalose contributed independently of the temperature to barotolerance and that Hsp104 was dependent on temperature (Iwahashi et al. 1997b). The non-heat-inducible molecular chaperon of Hsc70 was also shown to contribute to barotolerance (Iwahashi et al. 2001). Hamada et al. (1992) observed the induction of tetraploids or homozygous diploids in the industrial yeast strain *Saccharomyces cerevisiae* by hydrostatic pressure (above 100 MPa). In the same range of pressure,

Kobori et al. (1995) also demonstrated, through immunoelectron microscopy using thin frozen sections, that there had been damage to the nuclei of *S. cerevisiae*.

Recent advances in biotechnology enable us to monitor cellular response through almost any level of gene expression. DNA microarray technology is used to monitor almost all levels of gene expression (Eisen et al. 1998). On the yeast DNA microarray, we have 6,000 DNA probes that correspond to almost all of the yeast ORFs. We can estimate the expression level of target yeast RNA transcripts from treated cells by their hybridization to the DNA probes (Momose and Iwahashi 2001).

To understand the effect of hydrostatic pressure, we used yeast DNA microarrays and analyzed 6,000 gene-expression levels. We estimated the contribution of induced genes to barotolerance using some strains that have the deletion in the corresponding genes. The genome-wide expression profiles suggested that high pressure caused damage to cellular organelles and the damage was similar to that caused by detergents, oils, and freezing/thawing.

Materials and methods

Strains and growth conditions

The *S. cerevisiae* strain S288C (*MATα SUC2 mal mel gal2 CUP1*) was grown in YPD medium (2% polypeptone, 1% yeast extract, 2% glucose) at 25°C. This strain was used because the probes on the DNA microarray were produced using S288C as the template for PCR.

Yeast deletion strains were purchased from Research Genetics (USA) (http://www.resgen.com/products/YEASTD.php3). These strains were constructed by the insertion of *KanB* and *KanC* genes into the target genes of strains BY4741 (*MAT a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) and BY4742 (*MATαhis3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) before the mating of these strains. *S. cerevisiae* BY4743 (*MAT a/αhis3Δ1 leu2Δ0 ura3Δ0*), produced through the mating of BY4741 and BY4742, was used as a control strain.

Hydrostatic pressure treatment

To achieve pressure, we used two kinds of equipment; the wp3000 (High Pressure Equipment Co., USA), which has a volume of 50 ml and can be pressurized to 180 MPa in 2 min, and the 30-11HF4 (High Pressure Equipment Co.), which has a volume of 500 ml and can be pressurized to 100 MPa.

The former was used for pressurization of 180 MPa, and the latter for that of 100 and 40 MPa. The temperature was maintained by an ice-water bath.

For the pressure shock treatment, 200 ml of yeast culture, at an exponential growth phase (A660=1.0), was centrifuged and resuspended in 10 ml YPD medium in a 10-ml syringe. These syringes were treated with pressure at 0°C. After decompression, the cells were resuspended in 200 ml fresh YPD medium and incubated for 1 h at 25°C. The yeast cells were then centrifuged and washed in distilled water before storage at −80°C. The same procedure was carried out for the control cells except that the pressure was 0.1 MPa.

To estimate the barotolerance of the deletion strains, they were treated with a pressure of 100 MPa for 16 h; the barotolerance was calculated as follows; (CFU after pressure/CFU before pressure)×100.

DNA microarray analysis

The details of the microarray procedure have been described previously (Momose and Iwahashi 2001). Briefly, DNA microarray analyses were performed using at least three independent culture experiments. Total RNA was isolated by the hot-phenol method (Kohrer and Domdey 1990). Poly(A)+RNA was purified using Oligotex-dT30 mRNA purification kits (Takara, Kyoto, Japan). Fluorescent-labeled cDNA was synthesized by oligo dT-primer polymerization using PowerScript reverse transcriptase (Clontech, CA, USA). cDNA made from the control poly(A)+RNA was fluorescent-labeled with Cy3, and the pressure-treated samples were labeled with Cy5. The reason for this choice of labeling (Cy3 for the control and Cy5 for the pressure treatment) was twofold. The first reason was based on experimental conditions; yeast cells were cultured after pressure shock and they grew slowly during recovery (data not shown). A lower growth rate represses some genes, mainly those related to protein synthesis (Momose and Iwahashi 2001) and the repressed genes do not necessarily reflect the specific effect of the pressure treatment. This means that the information of repressed genes does not help us to understand the specific effect of the pressure treatment. The second reason for the labeling was based on the stability of the dyes during scanning. The scanning laser damages dyes, and this damage is more significant to Cy5; Cy5 breaks more quickly during scanning than Cy3. Furthermore, breakage is significant in spots with low intensities. As a result, control spots gave more induced values when it was labeled with Cy5 than when it was labeled with Cy3. This is important for the actual induced genes, as the intensity of the control should be lower than that of the pressure-treated cells. The mean values of the Cy3-labeled control and the Cy5-labeled control had much greater possibility of error than the values obtained by the

control labeled with Cy3 and the sample labeled with Cy5 (data not shown). The possibility of error using Cy3 and Cy5 can be summed up by the following; (Cy3/Cy5) > average between (Cy3/Cy5) and (Cy5/Cy3)>(Cy5/Cy3).

On the microarray, ORFs of 200–8,000 bp DNA in TE buffer (0.1–0.5 ng), prepared by a PCR primer for ORFs (Invitrogen Corporation, CA, USA), were spotted so that 5,880 genes could be analyzed under our conditions. Two to four micrograms of poly(A)+RNA was used for each labeling, and the same amount of each poly(A)+RNA was used on each slide. The two labeled cDNA pools were mixed and hybridized with a yeast DNA chip (DNA Chip Research, Yokohama, Japan) for 24–36 h at 65°C in a plastic bag. After hybridization, the labeled microarrays were washed, dried, and scanned using a confocal laser ScanArray 4000 system (GSI Luminics, MA, USA). The resulting image data were quantified using Quantarray (GSI Luminics, MA, USA). The background around the spot was subtracted from the fluorescence intensity of each spot and any signal arising from TE buffer wassubtracted using the value obtained from a spot of TE only in each block of 16 spots. The ratios of intensity (Cy5/Cy3) were calculated and normalized with a median value using GeneSpring (Silicon Genetics, CA, USA).

Hierarchical clustering

Hierarchical clustering was carried out using GeneSpring (Ohmine et al. 2001). The settings for the calculations were as follows: similarity was measured by standard correlation, the separation ratio was 1.0, and the minimum distance was 0.001 (Ohmine et al. 2001; Murata et al. 2002). Genes were selected if they had ever showed expression levels with above-average intensity (not induction) under at least one condition. This selection was made to ignore low-intensity genes, since low intensity means a high possibility of erroneous results. In total, 3,874 genes were used in the calculation, the data for which can be obtained from our database (http://kasumi.nibh.jp/~egenomix/).

Results

Conditions of the pressure treatment

Hydrostatic pressure affects the cellular physiology of yeast cells. This effect is apparently observed as cellular death or growth inhibition. Generally, cellular death is observed at more than 150 MPa at 4°C for a few minutes and at 30–100 MPa at 4°C for a few days. Thus, cellular death is dependent

on both the degree of pressure and the period of pressure treatment. We tried to study the responses under the high-pressure and short-period condition and the low-pressure and long-period condition. For the high-pressure and short-period condition, we chose 180-MPa (4°C) treatments as these matched the upper limit of our equipment. Figure 1 shows the time-dependent viability using the 180-MPa treatment. The time required for pressurization of the syringe was 2 min and even after immediate release from pressure, yeast cells were damaged. Thus, we selected the 0 min (instant) treatment. Cell viability was approximately 60% compared with the control under these conditions (Fig. 1). For lower pressure conditions, we chose a 16 h period at 10–100 MPa. Yeast cells may be treated for 1 week or 1 year at this pressure range, but we considered 16 h to be significantly different from the instant treatment at 180 MPa. Figure 2 shows the pressure-dependent viability of the cells during the pressure treatment for 16 h at 4°C. With the 40-MPa pressure treatment, yeast cells showed a viability of approximately 60% compared with the control; thus, we selected conditions of 40 MPa at 4°C. After the yeast cells had been damaged by pressure treatment, they were incubated for 1 h at 25°C in order for them to recover. In a previous experiment of damage caused by freezing/thawing, we found that yeast cells need 1 h to recover before RNA transcripts could be isolated for analysis by DNA microarray (see http://kasumi.nibh.jp/~egenomix/). For the control cells, yeast cells were treated under the same conditions except that the pressure was 0.1 MPa (without pressure).

Fig. 1. Viability of yeast cells under 180-MPa pressure conditions. Yeast cells in the logarithmic phase were pressurized to 180 MPa in 2 min and depressed for 2 min in ice-water conditions. The incubation time is the period at 180 MPa. The viability is shown as the percentage viability (CFU) to the cells not pressurized (*NP*). All data from three independent experiments are in the range of the *closed boxes*

Fig. 2. Viability of yeast cells under various pressure conditions for 16 h. Yeast cells in the logarithmic phase were pressurized at various MPa levels for 16 h in ice-water conditions. The viability is shown as the percentage viability (CFU) to the 0.1-MPa treatment. All data from three independent experiments are in the range of the *closed boxes*

Genome-wide gene responses after an instant treatment at 180 MPa and 4°C and recovery at 0.1 MPa and 25°C for 60 min

After the instant treatment at 180 MPa and 4°C (increasing the pressure to 180 MPa and decreasing it to 0.1 MPa for 4 min), the viability of the yeast cells decreased to approximately 60% of the control, and some genes were induced. The top 40 genes induced are listed in Table 1. The most highly induced gene was *HSP26*, followed by molecular chaperons of heat-shock proteins. Out of 5,880 genes, 286 were induced greater than two times. We characterized these genes according to the functional categories of the Munich Information Center for Protein Sequences (MIPS) (http://www.mips.biochem.mpg.de/) (Fig. 3). The proportion of induced genes (induced genes/number of genes in the category) was high in the categories of "energy," "cell rescue," and "protein destination." More than 100 induced genes fell into the category of "cellular organization." Heat-shock proteins such as *HSP26*, *SSA4*, and *HSP78* were in the category of "cell rescue." The molecular chaperons *SSA4, HSP78*, and *HSP104* were in the protein destination category. These genes were shown to be induced by heat shock treatment (Piper 1993). It was also shown that many genes related to energy metabolism including ATP production were induced by heat-shock treatment (Piper 1993). *CYC7*, *HXK1*, and *HSP30* (Table 1) are factors that contribute to energy metabolism and are induced by heat-shock treatment.

[Table 1. will appear here. See end of document.]

Fig. 3. Functional characterization of induced genes (180 MPa) according to the MIPS categories. The genes selected to be induced were grouped according to the functional categories of MIPS using GeneSpring. The percentage is calculated as follows: (number of genes induced by pressure)/(number of genes in the categories)×100

Genome-wide gene responses after the pressure treatment at 40 MPa, 4°C for 16 h, followed by recovery at 0.1 MPa, 25°C for 60 min

After the 40 MPa treatment at 4°C for 16 h and recovery at 0.1 MPa and 25°C for 60 min, the viability of the yeast cells decreased to approximately 60% compared with the control. The top 40 genes induced are listed in Table 2. The most highly induced gene was *SSA4*, followed by *TIP1*,

HSP12, and an unknown gene. In all, 218 genes out of a total 5,880 genes, were induced greater than two times. These genes were characterized according to the functional categories of MIPS (Fig. 4). The proportion of induced genes was high in the categories of "cell rescue," "protein destination," and "energy." The highest number of genes was in the category of cellular organization. This was very similar to the genes induced by the 180-MPa pressure treatment except that the standard deviation (SD) values were smaller than those seen after the 180-MPa treatment. The large SD values of the 180-MPa treatment may suggest uneven stress over the yeast cells due to rapidly increasing pressure conditions.

[Table 2. will appear here. See end of document.]

Localization of proteins encoded by genes induced by pressure

In both pressure treatments, many genes that belong to the category of "cellular organization" were induced. This category includes the genes whose localizations were characterized. Thus, we analyzed the localization of proteins encoded by genes induced greater than two times according to the MIPS (Table 3). After the 180-MPa treatment, many genes whose products were localized in the ER, mitochondria, nucleus, and cytoplasm were highly induced (Table 3). The gene products localized in the cytoplasm came from Hsps or energy metabolism genes (Fig. 2). By the 40-MPa treatment, many gene products induced were localized in the endoplasmic reticulum (ER), nucleus, and cytoplasm (Table 3). This suggests that pressure damages or activates the ER, mitochondria, and nucleus.

[Table 3. will appear here. See end of document.]

Cluster analysis of induced genes

We have accumulated genome-wide expression profiles of genes induced by environmental stress (http://kasumi.nibh.jp/~egenomix/). We compared the expression profiles of 3,875 ORFs after pressure shock and other environmental stresses, using hierarchical cluster analysis (Murata et al. 2002). The selected ORFs were those that had shown previous high intensity (greater than average) under at least one stress condition. This selection was carried out because ORFs of low intensity are likely to yield erroneous results. In Fig. 5, the 180-MPa and 40-MPa treatments are located at

the same cluster and are close to the clusters made by LAS, SDS, and Capsaicin. The LAS and SDS treatment caused damage to cellular organelles, a result which is in keeping with the localization of proteins encoded by genes induced by pressure treatment (Table 3). It is also interesting that pressure stress is similar to freezing/thawing stress.

Fig. 5. The hierarchical clustering analysis of genome-wide expression profiles by pressure treatment. Hierarchical clustering was carried out using GeneSpring. The setting for the calculation was as follows: The similarity was measured by standard correlation, the separation ratio was 1.0 and the minimum distance was 0.001 (Murata et al. 2002). The 3,874 genes used in the calculation were selected on the basis that they had been induced with an above-average intensity under at least one condition. All the data and the experimental conditions can be obtained from the database (http://kasumi.nibh.jp/~egenomix/)

Barotolerance of the deletion strains

The highly induced genes may contribute to barotolerance. To estimate the contribution of these genes (Tables 1 and 2) to barotolerance, we examined barotolerance (100 MPa for 16 h at 4°C) of the strain whose corresponding genes were deleted. We examined 23 strains obtained from the *Saccharomyces* deletion project through Research Genetics (Table 4). The lowest barotolerance was observed in the strain lacking *UBI4* (13.8%). YGR142 W, *CPR6*, YLR327C, *HSP104*, and *RPN7* minus strains showed less than 50% relative barotolerance. It was shown that *HSP104*, *SSB*, and *NTH1* contributed to barotolerance (Iwahashi et al. 1997a), and we confirmed the contribution of *HSP104* to barotolerance in this report. It is interesting that the genes whose functions are not understood showed lower barotolerance, and this suggests that high-pressure conditions can be used for studying functions of these unknown genes.

[Table 4. will appear here. See end of document.]

Discussion

We studied barotolerance by focusing on each biological factor and have demonstrated the contribution of *HSP104* (Iwahashi et al. 1997a), trehalose (Iwahashi et al. 1997a), *NTH1* (Iwahashi et al. 2000), and *SSBs* (Iwahashi et al. 2001) to barotolerance. In this study, we expanded the factors to make them genome-wide, and we found the factors contributing to “energy,” “cell rescue,” and “protein destination” were dominantly induced by both of the pressure treatments.

We had previously confirmed the contribution of *HSP104* (Iwahashi et al. 1997a), *SSBs* (Iwahashi et al. 2001), and *NTH1* (Iwahashi et al. 2000). *HSP104* and *SSBs* were categorized into "cell rescue" and "protein destination" and *NTH1* into "energy". However, it seems that these factors are not always dominant in barotolerance. We found evidence suggesting that *UBI4*, YGR142 W, *CPR6*, YLR327C, and *RPN7* also contribute to barotolerance. For example, *HSP104* may be merely one of the factors, like *UBI4* and *RPN7*, contributing the rescue of cells after pressure shock treatments.

It is also interesting that the pressure-shock response is similar to that caused by detergents, oils, and freezing/thawing (Fig. 5). It is easy to speculate that these kinds of stress cause damage to the membrane structure and/or cellular organelles. This speculation is supported by the localization of gene products induced by pressure shock to the ER, mitochondria, and nucleus. On the other hand, we cannot exclude the activation of ER and mitochondria. The ER is usually an essential organelle for the assembly of the membrane protein complex in cellular organelles (Kaiser et al. 1997). The damage done by the pressure treatment to the organelle require the repair or the synthesis of the damaged organelle. The similarity of expression profiles by the pressure treatment to those of detergents, oils, and freezing/thawing does not conflict with the activation of ER synthesis. Mitochondria are important for the synthesis of energy.

In conclusion, we would like to suggest the following: pressure treatment causes damage to cellular organelles, possibly to the nucleus, mitochondria, and/or ER; and during the repair or synthesis process, factors for protein metabolism, such as the organelle of ER and genes of *HSP104*, *UBI4*, and *RPN7*, are activated for the repair of cellular organelles, or else they degrade the damaged proteins from organelles. For these types of metabolism, cells require ATP for energy.

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Table 1. Genes induced by 180 MPa pressure shock

Gene code	Mean value	SD value	Common name	Function
YBR072W	17.2	16.5	HSP26	Heat shock protein 26
YGR142W	16.2	16.7	BTN2	Gene/protein whose expression is elevated by *btn1*
YER103W	14.5	7.8	SSA4	Member of 70 kDa heat shock protein family
YLR162W	8.7	12.5		Hypothetical protein
YLL026W	8.6	1.8	HSP104	Heat shock protein 104//heat shock protein
YDR516C	8.4	6.1		Strong similarity to glucokinase
YDR258C	8.4	6.6	HSP78	Mitochondrial heat shock protein 78 kDa
YDR445C	6.5	4.8		Questionable ORF
YDL172C	6.4	5.2		Questionable ORF
YBL078C	6.3	4.2		Essential for autophagy
YGL228W	6.2	2.9	SHE10	Causes lethality when overexpressed
YGR180C	6.0	4.0	RNR4	Ribonucleotide reductase
YLR216C	5.9	3.3	CPR6	Member of the cyclophilin family
YBR284W	5.7	4.2		Similarity to AMP deaminase
YDL173W	5.7	4.4		Hypothetical protein
YFR053C	5.5	3.2	HXK1	Hexokinase I (PI) (also called hexokinase A)
YOL123W	5.4	3.6	HRP1	SCCXV106 K SCYOL123 W SCU38535 SC35737
YLR217W	5.1	4.9		Questionable ORF
YKR011C	5.1	2.7		Hypothetical protein
YNL212W	5.1	4.0	VID27	Regulation of fructose-1,6-bisphosphatase import
YHR054C	5.1	1.1		Weak similarity to YOR262w
YER069W	5.1	3.9	ARG5,6	N-AGGP reductase and acetylglutamate kinase

Gene code	Mean value	SD value	Common name	Function
YHR146W	5.0	1.5		Similarity to pheromone-response G-protein Mdg1p
YJL026W	4.7	2.3	RNR2	Small subunit of ribonucleotide reductase
YML128C	4.7	2.8	GIN3	Similarity to subtelomerically-encoded proteins
YDL021W	4.6	3.1	GPM2	Phosphoglycerate mutase
YAL005C	4.6	2.4	SSA1	Heat shock protein of HSP70 family, cytosolic
YEL039C	4.5	3.3	CYC7	Iso-2-cytochrome c
YHR179W	4.5	3.0	OYE2	NAPDH dehydrogenase (old yellow enzyme)
YEL012W	4.5	4.0	UBC8	Ubiquitin-conjugating enzyme
YGR286C	4.5	4.6	BIO2	Biotin synthase
YHL021C	4.5	1.1		Similarity to pseudomonas butyrobetaine hydroxylase
YDL020C	4.4	1.8	RPN4	Involved in ubiquitin degradation pathway
YHR138C	4.4	3.8		Hypothetical protein
YNL009W	4.4	3.2	IDP3	NADP-dependent isocitrate dehydrogenase
YKL142W	4.4	0.9	MRP8	Mitochondrial ribosomal protein
YPR150W	4.3	4.2		Questionable ORF
YFL031W	4.3	3.1	HAC1	bZIP (basic-leucine zipper) protein
YPL240C	4.3	3.9	HSP82	Heat shock protein
YLL039C	4.2	2.2	UBI4	Ubiquitin//ubiquitin

Table 2. Genes induced by 40 MPa pressure shock

Gene code	Mean value	SD value	Common name	Function
YER103W	6.4	3.4	SSA4	Member of 70 kDa heat shock protein family
YFL014W	3.8	1.3	HSP12	12 kDa heat shock protein
YGR286C	3.7	2.7	BIO2	Biotin synthase//biotin synthetase
YER042W	3.5	3.4	MXR1	Responsible for the reduction of methionine sulfoxide
YGR142W	3.2	1.7	BTN2	Gene/protein whose expression is elevated in a *btn1*
YBR067C	3.2	2.2	TIP1	Cell wall mannoprotein
YLR216C	3.1	1.2	CPR6	Member of the cyclophilin family
YBR173C	3.0	2.1	UMP1	20S proteasome maturation factor
YNL274C	3.0	1.1		Similarity to glycerate- and formate-dehydrogenases
YLL039C	2.8	1.8	UBI4	Ubiquitin
YHR138C	2.8	1.1		Hypothetical protein
YDL007W	2.5	0.7	RPT2	26S proteasome regulatory subunit
YJL116C	2.5	0.6	NCA3	Regulation of synthesis of Atp6p and Atp8p
YBL041W	2.4	0.8	PRE7	Proteasome subunit
YML092C	2.4	0.8	PRE8	Proteasome component Y7
YLR303W	2.4	1.3	MET17	*o*-Acetylhomoserine sulfhydrylase
YOR117W	2.4	0.9	RPT5	26S proteasome regulatory subunit
YML125C	2.3	0.6		Mitochondrial membrane protein
YGR048W	2.3	0.6	UFD1	Ubiquitin fusion degradation protein
YPR108W	2.3	0.4	RPN7	Subunit of the regulatory particle of the proteasome
YER012W	2.3	0.4	PRE1	22.6 k Da proteasome subunit
YER004W	2.3	0.6		Similarity to hypothetical *E. coli* proteins
YNL155W	2.3	0.2		Hypothetical protein

Gene code	Mean value	SD value	Common name	Function
YFR024C	2.3	0.8		
YAL005C	2.2	1.0	SSA1	Heat shock protein of HSP70 family, cytosolic
YNL036W	2.2	0.7	NCE103	Involved in secretion of proteins
YBR072W	2.2	1.8	HSP26	Heat shock protein 26
YDL126C	2.2	0.8	CDC48	Microsomal protein of CDC48
YLR387C	2.2	0.5		Similarity to YBR267w
YBL078C	2.2	0.3		Essential for autophagy
YML004C	2.2	0.5	GLO1	Lactoylglutathione lyase (glyoxalase I)
YBR284W	2.1	1.4		Similarity to AMP deaminase
YLL026W	2.1	0.7	HSP104	Heat shock protein 104
YOR362C	2.1	0.5	PRE10	20S proteasome subunit C1 (alpha7)
YNL312W	2.1	0.5	RFA2	Subunit 2 of replication factor RF-A
YGR180C	2.1	0.4	RNR4	Ribonucleotide reductase
YFR004W	2.1	0.8	RPN11	Similar to *S. pombe* PAD1 gene product
YFR050C	2.1	0.2	PRE4	Proteasome subunit
YLR327C	2.1	1.1		Strong similarity to Stf2p
YEL037C	2.0	0.3	RAD23	Ubiquitin-like protein

Table 3. Localization of proteins encoded by genes induced by the pressure treatment

Localization to	Number (%) of genes induced by		Total number of localized genes
	40 MPa	180 MPa	
Cell wall[a]	1 (2.6)	2 (5.3)	38
Cytoplasm	22 (3.8)	44 (7.7)	571
Cytoskeleton	3 (2.6)	3 (2.6)	112
Endosome	0 (0)	1 (9.1)	11
ER	16 (11)	18 (13)	143
Extracellular	0 (0)	0 (0)	19
Golgi	2 (2.4)	1 (1.2)	82
Lipid particle	0 (0)	0 (0)	4
Microsomes	0 (0)	0 (0)	3
Mitochondria	7 (1.8)	27 (7.1)	381
Nucleus	45 (5.6)	33 (4.2)	791
Peroxisome	0 (0)	1 (2.5)	40
Plasma membrane	3 (1.9)	3 (1.9)	156
Transport vesicle	1 (2.0)	1 (2.0)	49
Vacuole	0 (0)	1 (1.9)	54

[a]Localization is based on MIPS

Table 4. Barotolerance of the deletion strains

Deleted gene	Barotolerance			Common name	Function
	Mean value[a]	SD value	Relative[b]		
Wild type	0.0158	0.0036	100.0		
YGR142W	0.0075	0.0046	46.7		Expression is elevated in a *btn1* minus
YLR216C	0.0067	0.0008	41.9	CPR6	Member of the cyclophilin family
YHR138C	0.0087	0.0011	54.6		Hypothetical protein
YNL274C	0.0102	0.0039	63.9		Similar to dehydrogenases
YLR303W	0.0092	0.0065	57.8	MET17	*o*-Acetylhomoserine sulfhydralase
YDR059C	0.0109	0.0052	67.9	UBC5	Homologue of the yeast CDC3/10/11/12
YLR327C	0.0076	0.0034	47.5		Strong similarity to Stf2p
YER004W	0.0119	0.0075	74.1		Similar to *E. coli* and *C. elegans* proteins
YML092C	0.0097	0.0048	60.4	PRE8	Proteasome component Y7
YLL026W	0.0071	0.0041	44.7	HSP104	Heat shock protein 104
YOR117W	0.0220	0.0237	137.2	RPT5	26S proteasome regulatory subunit
YGR286C	0.0111	0.0082	69.7	BIO2	Biotin synthase/biotin synthetase
YDL100C	0.0095	0.0080	59.4		Similar to *E. coli* ATPase
YPR108W	0.0074	0.0073	46.5	RPN7	Regulatory particle of the proteasome
YML125C	0.0113	0.0035	70.6		Hypothetical ORF
YFR004W	0.0212	0.0282	132.5	RPN11	Similar to *S. pombe PAD1* gene product
YLL039C	0.0022	0.0015	13.8	UBI4	Ubiquitin
YMR002W	0.0113	0.0019	70.8		Similar to *S. pombe* and *C. elegans* proteins
YOR007C	0.0211	0.0109	131.6	SGT2	Essential for autophagocytosis
YOR285W	0.0191	0.0094	119.1		Similar to *D. melanogaster HSP 67B2*

Deleted gene	Barotolerance			Common name	Function
	Mean value[a]	SD value	Relative[b]		
YFR024C	0.0089	0.0031	55.5		Unknown
YNL155W	0.0099	0.0052	61.6		Hypothetical protein

[a]Mean value of barotolerance (CFU after pressure/CFU before pressure) was calculated from three of independent experiments
[b]Relative barotolerance was calculated as that of wild type=100%