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Heat Shock Causes Oxidative Stress and Induces a Variety of Cell Rescue Proteins in *Saccharomyces cerevisiae* KNU5377

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In this study, we attempted to characterize the physiological response to oxidative stress by heat shock in *Saccharomyces cerevisiae* KNU5377 (KNU5377) that ferments at a temperature of 40°C. The KNU5377 strain evidenced a very similar growth rate at 40°C as was recorded under normal conditions. Unlike the laboratory strains of *S. cerevisiae*, the cell viability of KNU5377 was affected slightly under 2 hours of heat stress conditions at 43°C. KNU5377 evidenced a time-dependent increase in hydroperoxide levels, carbonyl contents, and malondialdehyde (MDA), which increased in the expression of a variety of cell rescue proteins containing Hsp104p, Ssap, Hsp30p, Sod1p, catalase, glutathione reductase, G6PDH, thioredoxin, thioredoxin peroxidase (Tsa1p), Adhp, Aldp, trehalose and glycogen at high temperature. Pma1/2p, Hsp90p and H^+-ATPase expression levels were reduced as the result of exposure to heat shock. With regard to cellular fatty acid composition, levels of unsaturated fatty acids (USFAs) were increased significantly at high temperatures (43°C), and this was particularly true of oleic acid (C18:1). The results of this study indicated that oxidative stress as the result of heat shock may induce a more profound stimulation of trehalose, antioxidant enzymes, and heat shock proteins, as well as an increase in the USFAs ratios. This might contribute to cellular protective functions for the maintenance of cellular homeostasis, and may also contribute to membrane fluidity.

Keywords: *Saccharomyces cerevisiae* KNU5377, heat shock, oxidative stress, antioxidant enzymes, heat shock proteins, trehalose, fatty acid composition

The thermotolerant strain, *Saccharomyces cerevisiae* KNU5377, which has been isolated from sewage soil, can be fermented at high temperatures, and is considered to constitute a possible alternative energy resource. This strain, which was originally referred to as F38-1 (Kim *et al.*, 1995a), is able to grow and convert glucose to ethanol even at temperatures as high as 40°C, and is also tolerant to high ethanol concentrations (Kim *et al.*, 1995b). This strain, due to these characteristics, appears to harbor some potential for applications in the alcohol fermentation industry.

Under high temperature conditions, yeast can be confronted with certain influences that are deleterious to cell physiology. The general effects of high temperature include the following: (i) reduction of cell viability, including appreciable cell death, (ii) general cell morphological abnormalities (atypical budding, irregular cell growth, and increased cell size), (iii) cell division and growth (inhibition of growth of non-thermotolerant yeasts at temperatures in excess of 40°C and cell cycle arrest in the G1 phase), (iv) plasma membrane structure/function (increased fluidity and reduced permeability to essential nutrients, ergosterol synthesis, reductions in the levels of unsaturated membrane fatty acids, stimulation of ATPase and RAS-adenylate cyclase activity, and declines in intracellular pH), (v) cytoskeletal integrity (extensive disruption of filaments and the microtubular network), (vi) mitochondrial structure/function (reduction in respiratory activity and the induction of respiratory-deficient petite mutants, and aberrant mitochondrial morphology) (Davidson *et al.*, 1996), (vii) intermediary metabolism (inhibition of respiration and fermentation above T_{max}, and immediate increases in cell trehalose and Mn SOD after heat shock) (Elutherio *et al.*, 1995), (viii) protein synthesis (repression of the synthesis of many proteins, specific

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induction of certain heat shock proteins, and mitochondrial protein synthesis), (ix) chromosomal structure/function (increased frequency of mutations during mitotic cross-over and gene conversion, increased mitotic chromosomal non-disjunction, and inefficient repair of heat-damaged DNA) (Walker, 1998; Walker and Dijck, 2006)

Heat shock has been demonstrated to induce oxidative stress (Davidson *et al.*, 1996, 2001a, 2001b; Lushchak *et al.*, 2006a, 2006b). Oxidative stress is not only a common phenomenon inherent to respiratory chain systems for the generation of energy in eukaryotic cells, but is also induced when the cell is exposed to peroxides, glutathione-driving drugs, toxins, radiation, inflammatory cytokines, and heat shock. Oxidative stress has also been observed in a variety of diseases, including cancer, atherosclerosis, rheumatoid arthritis, AIDS, and neurodegenerative disease. In order to protect against or resist such adverse conditions, cells have developed a host of fairly sophisticated mechanisms. These mechanisms include detoxifying enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, vitamins C and E, and thiol-harboring molecules such as glutathione, thioredoxin, and glutaredoxin (Arrigo *et al.*, 2002).

Here, we analyzed the fatty acid composition and H^+-ATPase activity in the plasma membrane, and the specific induction of cell rescue proteins and antioxidant proteins as stress responses against the oxidative stresses induced as the result of high temperatures (43°C).

Materials and Methods

Yeast strains and culture conditions

S. cerevisiae KNU5377 strain (Kim *et al.*, 1995a, 1995b) and laboratory strains containing *S. cerevisiae* W303, *S. cerevisiae* S288C and *S. cerevisiae* ATCC24858 were grown aerobically in a nutrient-rich YEPD medium (1% yeast extract, 2% peptone, 2% dextrose) at 30°C overnight, with shaking at 160 rpm. Pre-cultured cells were transferred to fresh YPD media and then cultured for 5 h at 30°C with shaking, until log phase was achieved (A_{600}=1.0). In order to elicit heat shock, the mid-log cultured cells were exposed to temperatures of 43°C for 2 h. For PMSF treatment, cells were exposed to heat shock after 30 min of PMSF challenge at 30°C. The growth rate was measured at 600 nm. Cellular viability was evaluated via MTT assay (Teparic *et al.*, 2004).

Hydroperoxide, carbonyl contents and malondialdehyde assay

The intracellular hydroperoxide levels were determined by ferrous ion oxidation in the presence of a ferric ion indicator, xylenol orange (Wolff, 1994). Carbonyl contents were measured via the spectrometric method (Reznick and Packer, 1994). Malondialdehyde (MDA) levels were examined via thiobarbituric acid (TBA) assay (Beuge and Aust, 1978).

Fatty acid analysis

Cells were washed twice in distilled water, then transferred to Teflon-capped tubes. The fatty acids were extracted from 40 mg of wet cells per sample, and fatty acid extractions were conducted in accordance with the Miller method (Miller, 1982). The compositions were determined using the Hewlett-Packard 5890 Series II Gas Chromatograph (Hewlett-Packard Co., USA).

Plasma membrane H^+-ATPase activity

Purified plasma membrane fractions were prepared in accordance with the protocols established by Fernandes (Fernande rosa and Sa-correia, 1991; Fernandes *et al.*, 1998). Plasma membrane H^+-ATPase activity was determined via Serrano's method (Serrano, 1978; Ambesi *et al.*, 2000). Enzyme activity was calculated via linear repression from the slope of released Pi vs. time (up to 10 min).

SDS-PAGE and Western blot analysis

SDS-PAGE was conducted via the method of Laemmli (Laemmli, 1979). 40 μg of denatured proteins were analyzed in 10-12% polyacrylamide gel and electrophoretically transferred to PVDF membranes (Bio-Rad, USA) using trans-buffer (25 mM Tris-base, 192 mM glycine, and 20% methanol). The PVDF membranes were blocked for 60 min at room temperature in TTBS buffer (0.05% Tween-20, 10 mM Tric-HCl: pH 7.6, 150 mM NaCl) containing 5% non-fat skim milk and 0.02% sodium azide. The blotted membranes were then incubated overnight at 4°C with each primary antibody: rabbit anti-glucose-6-phosphate dehydrogenase (Sigma, USA), rabbit anti-Hsp104 and mouse anti-Hsp60 (Stressgen, Canada), rabbit anti-Pma1/2 (Santa Cruz Biotechnology, USA), rabbit anti-alcohol dehydrogenase and rabbit anti-hexokinase (Rockland, USA), rabbit anti-Hsp90, rabbit anti-Ssa and rabbit anti-Ssb antibodies (a gift from E.A. Craig, University of Wisconsin-Madison, USA), rabbit anti-Tsa1p antibody (a gift from J.W. Park, Kyungpook National University, Korea), and rabbit anti-Trr1p, Trx2p, -Trx3p (gifts from K.H. Kim, Chonnam National University, Korea). Rabbit anti-Hsp30 antibody was synthesized. After incubation with the primary antibodies, the membranes were washed four times with TTBS. Secondary antibodies, horseradish peroxidase-conjugated anti-rabbit IgG (H+L) (Promega, USA) or anti-mouse IgG (Amersham, Sweden), were then added and further incubated for

90 min at room temperature. The membranes were then washed 4 times in TTBS. Intensity was developed via enhanced chemiluminescence (ECL kit; Amersham, Sweden).

DNA microarray

Microarray analysis was conducted in accordance with a previously described method (Kim *et al.*, 2005). We utilized the ratios (Cys5 of the stressed cells/Cys3 of the non-stressed cells) of hybridization intensity. Ratios of hybridization intensity (Cys5/Cys3) over 2.0 were determined to have been upregulated, and ratios below 0.5 were regarded as downregulated.

Transcriptional analysis via RT-PCR

Cells were harvested by centrifugation. Total RNA was isolated using a QIAGEN® RNA/DNA Mini Kit (Qiagen, Germany), and was employed in the synthesis of cDNA using AccuPower® RT PreMix (Bioneer, Korea). PCR reactions were conducted using AccuPower® PCR PreMix (Bioneer, Korea). After an initial denaturation period at 94°C for 4 min, the reaction mixture was cycled 35 times. The PCR conditions used were as follows: 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min. After 35 cycles, a final extension step was run for 10 min at 72°C. After PCR, the PCR products were electrophoresed, stained with ethidium bromide, visualized, and photographed. PCR amplicon of *PDA1* was employed as a reference control (Wenzel *et al.*, 1995). The oligonucleotides utilized were as follows; PDA-F: 5'-CTTCATTCAAACGCCAACCA-3' and PDA-R: 5'-GAGGCAAAACCTTGCTTTTTG-3'; HSP30-F: 5'-ACATGGCCTGGATA TGCACAT-3' and HSP30-R: 5'-TTCCACATCGTCCGTAGCAT-3'; PMA1-F: 5'-AG CCAACTCAAGAAAAGCCTG-3' and PMA1-R: 5'-T TCTGGAAGCAGCCAAACAA-3'; PMA2-F: 5'-TCTT CCACT GAAGCAAAGCA-3' and PMA2-R: 5'-TCA

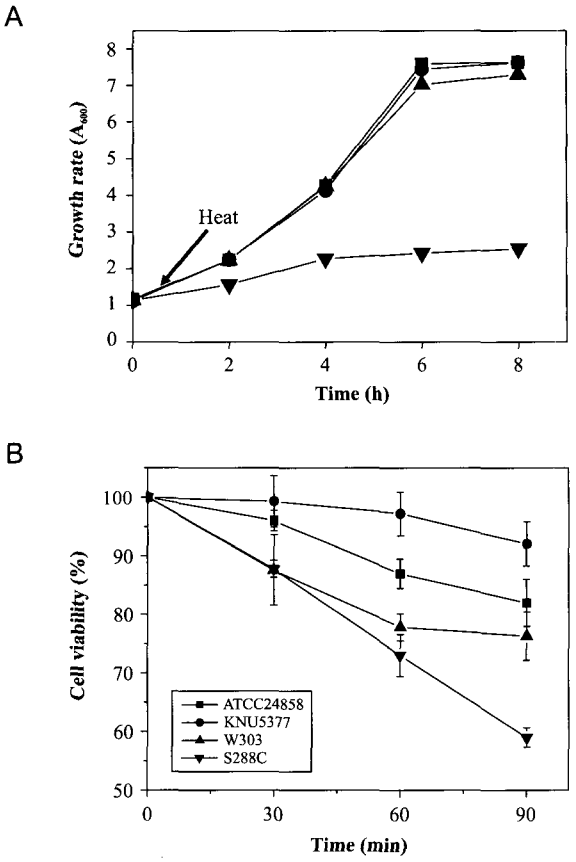

Fig. 1. Cellular growth rate and viability. (A) Growth rate at 30°C (■), 37°C (●), 40°C (▲) and 43°C (▼) on the acquisition of increased thermotolerance in *S. cerevisiae* KNU5377. Absorbance value at 600 nm represents the increase in cell numbers when cells were subjected to different temperatures. (B) Cell viability at 43°C for 2 hours at different times between KNU5377 and the laboratory strains (W303, S288C and ATCC24858). Survival was determined as the relative percentages of the normal cells (100%) without heat exposure.

Table 1. Strains used in this study

W303	*MATa/MATα, leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*	KCCM
ATCC24858	*MATa/MATα, trp1, his3, leu2, ura3*	KCCM
BY4741 (S288C)	*MATa, his3Δ1, leu Δ0, met15 Δ0, ura Δ0*	EUROSCARF
KNU5377	wild type	This study

KCCM: Korean Culture Center of Microorganisms
EUROSCARF: European *Saccharomyces cerevisiae* Archives for Functional Analysis

Table 2. Level of hydroperoxide, carbonyl content and malondialdehyde (MDA) during heat shock. Each unit is presented as μmol/mg protein in hydroperoxide, nmol/mg protein in carbonyl content, and nmol/mg protein in MDA.

	0	15	30	60	120
Hydroperoxide	0.2 ± 0.005	0.8 ± 0.081	1.8 ± 0.113	4.4 ± 0.111	9.8 ± 0.156
Carbonyl content	0.4 ± 0.021	0.55 ± 0.026	0.72 ± 0.054	0.93 ± 0.045	1.25 ± 0.067
MDA	0.34 ± 0.004	0.48 ± 0.022	0.52 ± 0.043	0.6 ± 0.012	0.66 ± 0.035

CAGTGGATGAGGAGA ACA-3'. Each primer was designed using the *Saccharomyces* Genome Database (SGD) (http://seq. yeastgenome.org/cgi-bin/web-primer). The expected size of the PCR products was approximately 1 kb.

Results

S. cerevisiae KNU5377 strain evidences normal growth pattern at high temperature (40°C)

Fig. 1 shows cell growth results at different temperatures and cell viability at high temperatures (43°C). The *S. cerevisiae* KNU5377 strain evidenced a growth pattern at 40°C identical to that observed under normal conditions (30°C) (Fig. 1A). On the basis of these results, we selected 43°C as an appropriate temperature for the elicitation of heat stress. Fig. 1B shows cell viability results at different times under high temperature between the KNU5377 and laboratory strains (W303, S288C, and ATCC24858). Cell survival characteristics in laboratory strains decreased in a time-dependent manner. The rate of reduction

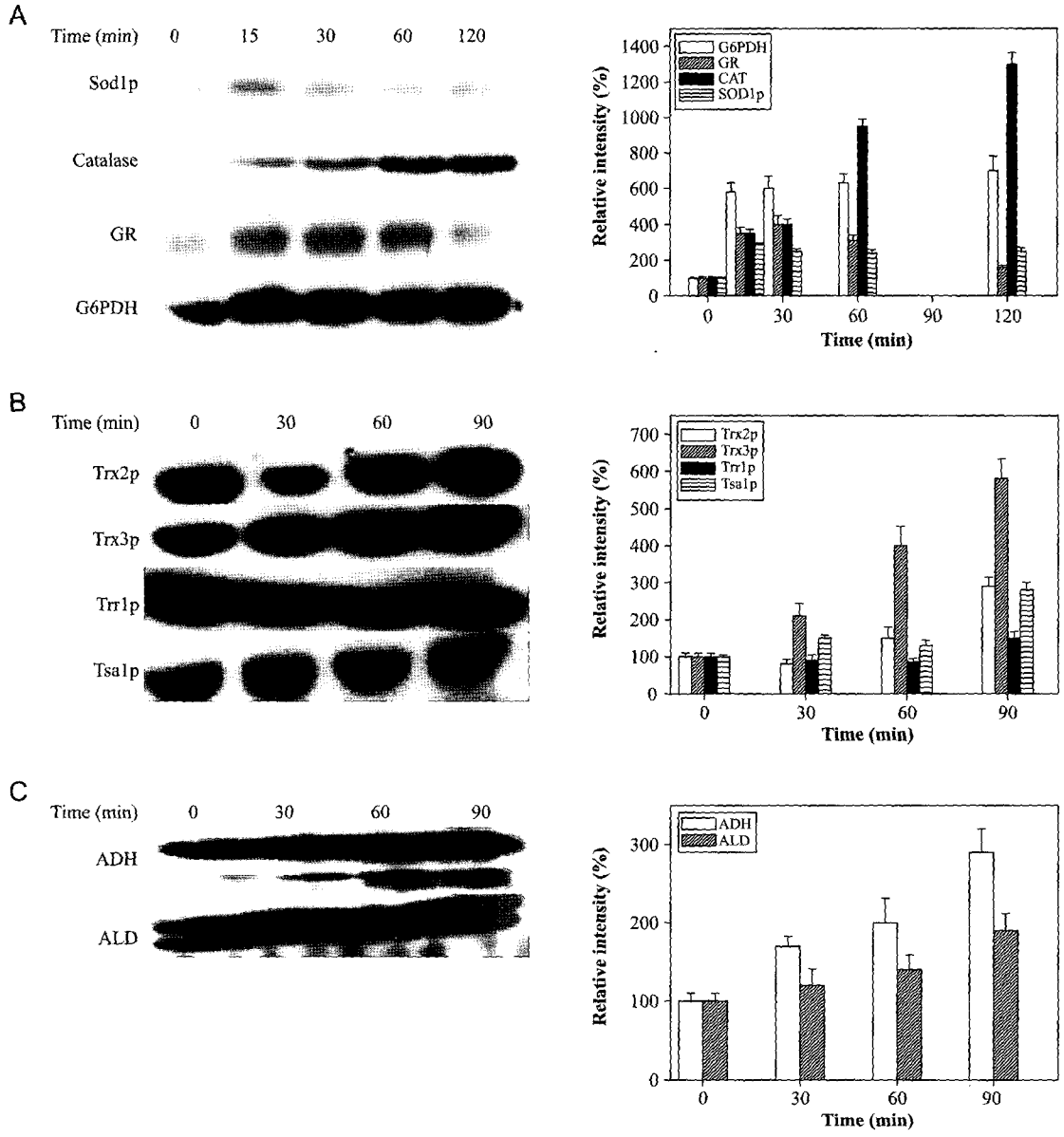

Fig. 2. Expression changes of proteins involved in antioxidant enzymes and -molecules. Total cellular proteins from log-phase cells exposed to heat shock (43°C) for the indicated times were separated via SDS-PAGE (12 or 17%), transferred to PVDF membranes, and reacted with each first and second antibody. The intensity of Sod1p (Cu/Zn superoxide dismutase), catalase, GR (glutathione reductase) and G6PDH (glucose-6-phosphate dehydrogenase) (A), Trx2p (thioredoxin 2), Trx3p (thioredoxin 3), Trr1p (thioredoxin reductase) and Tsa1p (thioredoxin peroxidase 1) (B), and ADH (alcohol dehydrogenase) and ALD (aldehyde dehydrogenase) (C) was processed by ECL Plus

reached a maximal level of 40% under the conditions mandated in the experiment. Unlike the laboratory strains, the sensitivity of the KNU5377 strain remained unaffected under high temperature conditions (43°C).

These findings indicate that the KNU5377 strain was more tolerant than the laboratory strains at high temperatures.

Heat shock elicits oxidative stress and induces cell rescue proteins

As oxidative stress markers, the levels of hydroperoxide, carbonyl contents, and MDA were evaluated. These factors all increased in a time-dependent manner for 120 min at 43°C (Table 2). These results indicate that heat shock elicits oxidative stress. Under heat stress conditions, the KNU5377 cells expressed a host of proteins and molecules to protect against or resist the deleterious effects of heat stress. Fig. 2 shows the antioxidant proteins induced by heat shock. The proteins that were elevated are as follows: Cu/Zn superoxide dismutase (Sod1p), CAT (catalase), glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PDH), thioredoxin 2 (Trx2p), and thioredoxin 3 (Trx3p). Interestingly, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALD) were also found to be upregulated as the result of heat shock. In addition, KNU5377 cells maintained high trehalose and glycogen levels under stress conditions. For the indicated times, the levels of trehalose were 4-fold higher than those of the basal level. Glycogen levels were highest (2 fold) for 30 min, and then decreased gradually (Fig. 3).

As is shown in Fig. 4 and Fig. 5B, the expression levels of Hsp104, Ssa, Hsp60, and Hsp30 protein

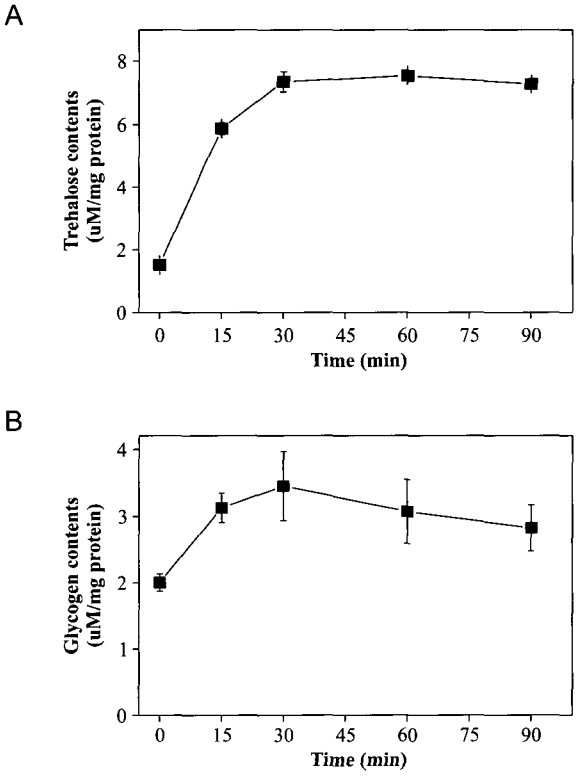

Fig. 3. Trehalose and glycogen assay. KNU5377 cells were aerobically grown in nutrient rich YEPD medium at 30°C with shaking at 160 rpm. Once a mid-log phase to OD_{600} of 1.0 was achieved, the cells were challenged for 120 min at 43°C. (A) trehalose content (B) glycogen content. The data are expressed as the mean values ± SD from three independent experiments.

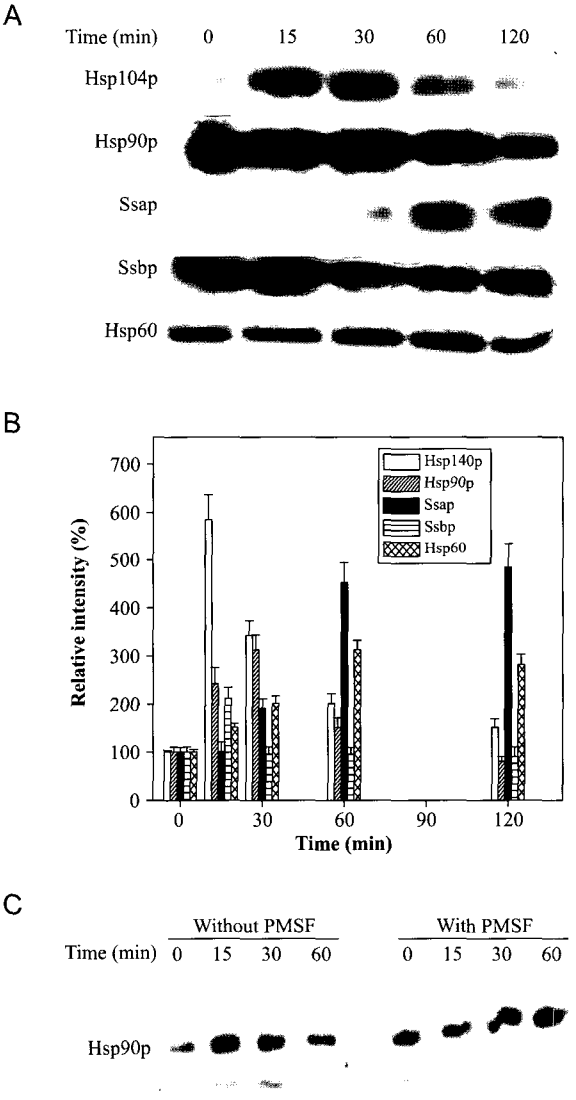

Fig. 4. Expression changes of heat shock proteins during heat shock. Total cellular proteins from log-phase cells exposed to heat shock (43°C) for the indicated durations were separated via SDS-PAGE (10 or 12%), transferred to PVDF membranes, and reacted with each first and second antibody. The intensities of Hsp104p, Hsp90p, Ssap, Ssbp, and Hsp60p were determined by ECL Plus (A, B). (C) Expression analysis of Hsp90 under heat shock conditions after PMSF treatment.

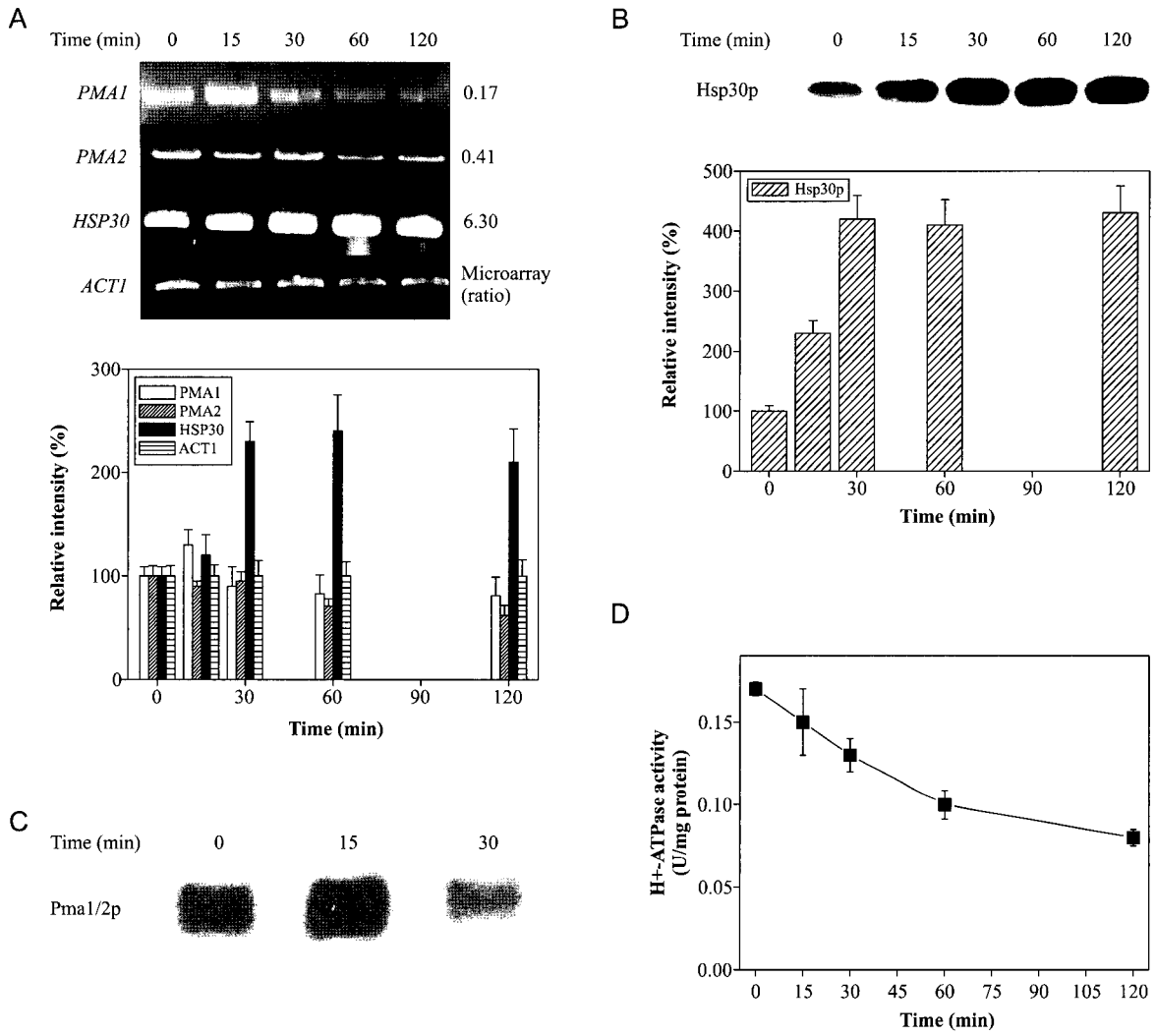

Fig. 5. Transcriptional and translational analysis of the genes involved in plasma membrane H+-ATPase and Hsp30. (A) Transcriptional analysis of *PMA1*, *PMA2*, and *HSP30* using RT-PCR and microarray. *ACT1* was utilized as a standard control for normalization. Immunoblotting analysis of Hsp30 (B) and Pma1/2 (C). (D) Enzyme activity of plasma membrane H+-ATPase. Specific activity was represented as units per mg of protein.

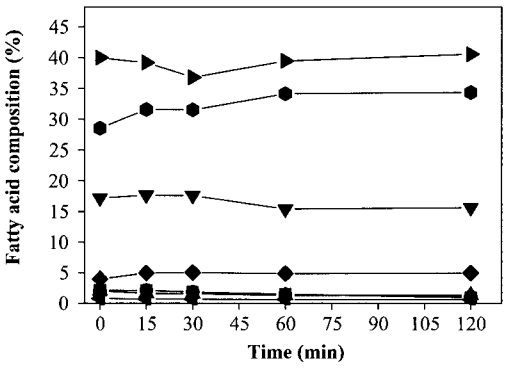

Fig. 6. The percentage of individual fatty acids present in cells grown at 43°C under the indicated time. (A) C16:0 (▼), C16:1 (●) and C18:1 (▶). (B) C10:0 (■), C12:0 (○), C14:0 (▲), C14:1 (▽) and C18:0 (◆).

were increased profoundly under heat shock conditions, whereas the levels of Hsp90p and Ssbp decreased. In addition to Hsp30, the expression level of PMA1 and PMA2 encoding for membrane H^+-ATPase was found to have been downregulated (Fig. 5A, 5C). The downregulation of PMA1/2 also was shown to affect the enzyme activity of H^+-ATPase (Fig. 5D).

Heat shock maintains a steady ratio of saturated to unsaturated fatty acids

Fig. 6 shows the result of fatty acid composition as an indicator of cell physiology under high temperature conditions. As is shown in Fig. 6, the ratio of saturated to unsaturated fatty acids was maintained under high temperature conditions (43°C). The level of unsaturated fatty acids increased significantly with

time. The highest contents were observed primarily with C18:1 (oleic acid) and, to a lesser extent, C16:1 (palmitoleic acid). In addition, the proportion of these unsaturated fatty acids was more than 70%.

Discussion

The common optimal temperature of *S. cerevisiae* is within a range of 25 to 35°C. In the range of 35 to 37°C, yeast cells are moderately stressed but continue to grow, developing a protective tolerance against higher levels of lethal shock (Davidson *et al.*, 2001a, 2001b). Species evidencing T_{opt} values in excess of 40°C are referred to as thermotolerant yeasts (Walker, 1998). Here, we first refer to *S. cerevisiae* KNU5377 as a thermotolerant yeast, as KNU5377 evidences normal growth at 40°C (Fig. 1).

S. cerevisiae has long been studied as a model for research into the stress response. A major stress facing yeast cells during aerobic growth is oxidative stress, which induces the generation of reactive oxygen species (ROSs) including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH^-) (Christian *et al.*, 1998; Hohnman and Mager, 2003). These ROSs can be generated via a variety of extra-environmental conditions including high temperature, or during the fermentation process. The generated ROS inflicts cellular damage on a variety of cellular components, including DNA fragmentation, protein or enzyme inactivation, the modification of carbohydrate compounds, and changes in membrane fluidity via lipid peroxidation (Elisa *et al.*, 2000). As is shown in Table 2, the observed increases in hydroperoxide, carbonyl content, and MDA levels at high temperatures show that heat shock induces oxidative stress. Recently, the body of information available regarding oxidative stress in the context of heat shock has grown considerably. When cells are subjected to abnormal conditions, the cells must carry out a rapid and effective genomic expression program, in order to adapt, proliferate, or survive in new environments (Patrica *et al.*, 2004). Under the oxidative stress conditions elicited by heat shock, KNU5377 produced a variety of antioxidant enzymes, including Sod1p, catalase, GR, G6PDH, Trx2p, Trx3p, and Tsa1p. The previous studies indicated that mutants lacking the antioxidant genes encoding for catalase, superoxide dismutase, and cytochrome c peroxidase (Davidson *et al.*, 1996), and thioredoxin peroxidase (Lee and Park, 1998) is more sensitive to the lethal effects of heat than are isogenic wild-type cells against oxidative stress. However, the overexpression of catalase and superoxide dismutase genes (Davidson *et al.*, 1996), as well as thioredoxin peroxidase (*TSA1* and *AHP1*) and cytochrome c peroxidase (*CCP1*) (Davidson *et al.*, 2001a, 2001b) induces an increase in thermotolerance in *S. cerevisiae*. Thioredoxin overexpression has been remained to occur under heat shock conditions, but not oxidative stress. In addition, alcohol dehydrogenase (ADH) and aldehyde dehydrogensae (ALD) were induced profoundly under heat shock conditions. The cellular functions of these proteins under stress conditions in yeast are gradually being elucidated. In *E. coli,* cells lacking the *adhE* gene are extremely sensitive to oxidative stress, which results in levels of internal peroxides and increased protein carbonyl content are higher than in wild-type cells (Echave *et al.*, 2003). Among the genes encoding for ALD, *ALD3* is induced via a variety of stresses, including osmotic shock, heat shock, glucose exhaustion, oxidative stress, and drugs, whereas *ALD2* is induced only by osmotic stress and glucose exhaustion (Navarro-Avino *et al.*, 1999). However, the results of microarray analyses revealed that KNU5377 cells induced *ALD2* gene expression under conditions of menadione-induced oxidative stress (data not shown). The KNU5377 cells accumulated trehalose and glycogen as a protective response to heat shock. Trehalose has been shown to play a role as a stress-protective molecule under adverse environments including osmotic pressure, heat shock, oxidative stress, and chemical drugs in *S. cerevisiae* (Majara *et al.*, 1996; Ogawa *et al.*, 2000; Benaroudj *et al.*, 2001; Pereira *et al.*, 2003; Voit, 2003; Herdeiro *et al.*, 2006) and in *Aspergillus nidulans* (Fillinger *et al.*, 2001). The role of glycogen under stressful conditions is, however, thus far poorly documented.

In general, Hsps are molecular chaperones, which are critical to survival under various stresses (Brosnan *et al.*, 2000). Heat shock proteins are induced when yeasts are exposed to stresses other than heat shock. Levels of Hsp104, the Hsp70 family, and Hsp60 were shown to have increased (Fig. 4). Hsp104 acts synergistically with trehalose to confer thermoprotection on *S. cerevisiae* (Estruch, 2000). The mechanism inherent to the cellular functions of Hsps, with the exception of Hsp104, is quite limited. Interestingly, heat-inducible Hsp90p (Borkovich *et al.*, 1989) was downregulated under heat stress conditions in KNU5377. The reduction of Hsp90 is questionable in KNU5377, which did not affect the total activity of the molecular chaperone (data not shown). Hsp30, a small Hsp, was also significantly upregulated in *S. cerevisiae* KNU5377 under heat shock conditions, as well as under high concentrations of ethanol, oxidative stress, and sulfuric acid stress (data not shown). Hsp30 is known to be induced by a variety of stresses (Panaretou and Piper, 1992; Piper *et al.*, 1994; Piper *et al.*, 1997; Seymour and Piper, 1999). Hsp30p, a negative regulator of the H^+-ATPase Pma1p, enables the cells to cope with rapid transitions in energy requirements, via its function as

a stress-inducible regulator of plasma membrane H^+-ATPase activity (Carmelo *et al.*, 1996; Piper *et al.*, 1997). The resistance to lethal heat of a mutant strain with reduced membrane ATPase expression was significantly less than that of the wild-type parent (Coote *et al.*, 1994). This phenomenon is associated with ethanol tolerance in different strains of industrial wine yeast (Aguilera *et al.*, 2006). In addition to yeast Hsp30, human Hsp27 or *Drosophila* Hsp27 reduced basal intracellular ROS levels under oxidative stress conditions, which may modulate the intracellular redox state (Mehlen *et al.*, 1995; Preville *et al.*, 1999; Rogalla *et al.*, 1999; Cashikar *et al.*, 2005). Taken together, the induction of Hsps harboring small Hsp may contribute to the stress tolerance capacity of yeasts, by acting as molecular chaperones or redox regulators.

With regard to fatty acid analysis, the levels of unsaturated fatty acids increased considerably with time. High contents were observed primarily with C18:1 (oleic acid) and, to a lesser extent, C16:1 (palmitoleic acid) under heat shock conditions, whereas C16:1 evidenced the highest proportions to oxidative stress response (data not shown). In addition, the proportion of these unsaturated fatty acids was measured to be in excess of 70%. These results demonstrate the elastic regulation of fatty acid compositions against various stresses in the KNU5377 strain. In *S. cerevisiae*, sensitivity to heat stress depends on membrane lipid composition. Aerobic cells with membranes enriched in palmitoleic (C16:1) and oleic acids (C18:1) evidenced the highest degrees of resistance to heat stress (52°C, 5 min) and oxidative stress (20 mM H_2O_2, 15 min) (Steels *et al.*, 1994; Swan and Watson, 1997). Ethanol-tolerant yeast harbored a higher percentage of unsaturated fatty acids, primarily acyl-chained fatty acids (Chen, 1981; Beaven *et al.*, 1982; Lentini *et al.*, 1998; Walker, 1998). The presence of these higher levels of unsaturated fatty acids results in an increase in the fluidity of the cell membrane in response to the physical effects of adverse conditions. Cellular lipid composition also influences the stress activation of the yeast general stress response element (STRE), which binds to transcription factors via two homologous and functionally redundant genes, *MSN2* and *MSN4*. The activation of these genes by Msn2/4p involves the acquisition of stress tolerance (Chatterjee *et al.*, 2000). The strategic flexibility of cellular fatty acid composition may function in the regulation of membrane fluidity and gene expression in KNU5377.

Recently, the amount of available information regarding oxidative stress under heat shock conditions has been advanced considerably. However, the majority of information regarding the manner in which cell rescue proteins containing antioxidant enzymes and Hsps function in yeasts has been determined in studies of laboratory strains. Little information is currently available regarding the induction of the stress response under alternative physiological conditions, in particular the basic stress responses of wild-type and industrial yeast strains. Hence, the results of our study indicate that the KNU5377 strain may differ, to some degree, from the responses that have been characterized in the laboratory strains.

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