PERIODICA POLYTECHNICA SER. CHEM. ENG. VOL. 39, NO. 1, PP. 3-12 (1995)

COMPARISON OF STRESSED AND UNSTRESSED YEAST BY DIFFERENTIAL SCANNING CALORIMETRY (DSC) AND SDS-PAGE

Ahmed AMAL*, Anna HALÁSZ*, Anna SIMON** and Ágnes BARÁTH*

*Central Food Research Institute
Herman O. út 15.
H-1022 Budapest, Hungary
**University of Horticulture and Food Industry
Villányi út 29/35.
H-1114 Budapest, Hungary

Received: June 10, 1995

Abstract

Heat shock of *Saccharomyces* yeast strains resulted in the induction of a set of proteins referred to as shock protein (HSPs). SDS-PAGE analysis revealed that at least three induced HSPs were identified in a *Saccharomyces cerevisiae* CBS 1395 and seven in a *Saccharomyces pastorianus* CBS 1503. One of these HSPs with molecular mass of approximately 41 KDa was also identified in two mutant strains of *Saccharomyces cerevisiae*. Differential scanning calorimetry analysis of whole yeast cells revealed that heat shock treatment decreased the enthalpy of denaturation $\Delta(H)$ of total cellular proteins. A direct correlation between the degree of HSP inducibility and protection against extreme temperatures was observed. These results suggest that prior heat shock treatment protects the protein of yeast cells from elevated temperatures.

Keywords: yeast, *Saccharomyces*, heat shock, heat shock proteins, polyacrylamide gel electrophoresis, differential scanning calorimetry.

Introduction

All organisms examined to date, including yeast, are known to respond to temperatures above their normal growth temperature by including the synthesis of a family of specific proteins referred to as heat shock proteins (HSPs) (LI and WERB, 1982; LINDQUIST, 1986; MCALISTER and FINKELSTEIN, 1980; MILLER et al., 1982; YOST et al., 1990; SCHLESINGER et al., 1982). The synthesis of these proteins is also induced in response to a variety of other environmental stresses such as ethanol (LINDQUIST, 1986; MICHEL and STARKA, 1986; PLESSET et al., 1982; SCHLESINGER et al., 1982) anoxia, amino acid analogs, inhibitors of oxidative phosphorylation, heavy metal ions and respiratory poisons (LINDQUIST, 1986; MORIMOTO et al., 1990). The prior induction of HSPs has been reported to confer heat tolerance to extreme temperatures as well as some other kinds of stress. A few known examples of such cross protection are: *Drosophila*

larvae exposed to heat shock were protected against toxic effects of anoxia (VELAZQUEZ and LINDQUIST, 1984) and heat treated yeast cells developed an increased tolerance to ethanol (WATSON et al., 1984; PLESSET et al., 1982; WATSON and CAVICCHIOLI, 1983).

Similarly, prior exposure of cells to ethanol, which induced HSPs, also conferred protection against subsequent lethal temperatures (PLESSET et al., 1982). This, the induction of HSPs either by heat shock or ethanol appears to protect cells against environmental stresses.

Recently, HSPs have been reported to have multiple roles in cell physiology and survival, e.g.,

- their role in signal transduction pathway (PICARD et al., 1990)
- removal of abnormal proteins (ANANTHAN et al., 1986)
- disassembly of hydrophobic protein complexes in an ATP-dependent manner (PELHAM, 1986)
- association with specific proteins (PINHASI-KIMHI et al., 1986), and folding, translocation and assembly of protein–protein complexes (CHIRICO et al., 1988; DESHAIES et al., 1988).

Considering the multiple roles of HSPs, we aimed to study whether the prior heat shock exposure could confer any protection against extreme temperatures, and if significant changes in the protein fine structure could be detected by biochemical and biophysical means.

Materials and Methods

Yeast Strains and Growth Medium

The yeast strains investigated in this study were *S. Pastorianus* CBS 1503; *S. cerevisiae* CBS 1395; *S. cerevisiae* CB 67 and *S. cerevisiae* CB 89. The yeast cells were subcultured in synthetic medium at 30 °C on a shaker prior to use in this study. The synthetic medium consisted of magnesium sulphate, 0.1 g; potassium dihydrogen phosphate, 0.14 g; sodium hydrogen phosphate, 0.8 g; sodium chloride, 0.1 g; ammonium sulphate, 40 g; yeast extract; 5.0 g; glucose, 10.0 g; all dissolved in 1 litre of distilled water, pH 4.8.

Heat Shock Conditions

Exponentially growing yeast cells at 30 °C were harvested by centrifugation, washed twice with distilled water and cell suspensions in 0.6 mol/l KCl buffer (SDS-PAGE analysis) or in distilled water (DSC analysis) were preincubated at 30 °C for 30 min, (Control) or subjected to heat shock at 55 °C

for 10 min, then transferred at 50 °C for 30 min (experimental samples). The cells were cooled to 30 °C and then disruptured by ultrasonication (3 × 3) only for protein analysis. After that the cells were centrifuged (5000 rpm for 15 min 4 °C) and the pelleted cells were used in DSC analysis while the sonicated sample supernatants were used in SDS-PAGE analysis. The pellets and the supernatants were kept frozen until required for analysis.

Protein Analysis

Aliquots of supernatants (7 μl) from control and heat shocked samples were analysed by SDS-PAGE. Electrophoresis was carried out according to (LAEMMLI, 1970). The scanning of electrophoregram was obtained with a Video densitometer MOD H_1-CAM No. 30335008 Biotech-Fisher.

Differential Scanning Calorimetry

The thermal behaviour on the yeast protein was examined with a SETARAM Micro DSC-Hungary. Stainless-steel pan was used for sample and reference. Differential scanning calorimetry (DSC) was performed on whole yeast cells. A 900 ± 10 mg (wet weight) was sealed in stainless-steel pan. The heating rate was 1 °C/min over range of $25 - 95$ °C. The enthalpy of denaturation (ΔH) was expressed as mcal per mg protein and the reference was water. The temperature of denaturation (T_d) was determined as the peak temperature.

Results

1. Detection of HSPs by SDS-PAGE Protein Analysis

Fig. 1 shows the electrophoregram of protein prints for investigated yeast strains separated by SDS-PAGE before and after heat shock treatment at 50 °C for 30 min. The molecular masses and number of heat shock proteins for the investigated yeast strains are summarized in *Table 1 (a,b,c)*.

2. DSC Characterisation of the Investigated Yeast Strains

DSC curves of the investigated whole yeast cells had different shapes. *Fig. 2* shows a broad endothermic peak for *S. pastorianus* CBS 1503 and *S. cerevisiae* CB 89 while a sharp endothermic peak for *S. cerevisiae* CBS 1395. In contrast, *S. cerevisiae* CB 67 shows a sharp multipeak curve, before the

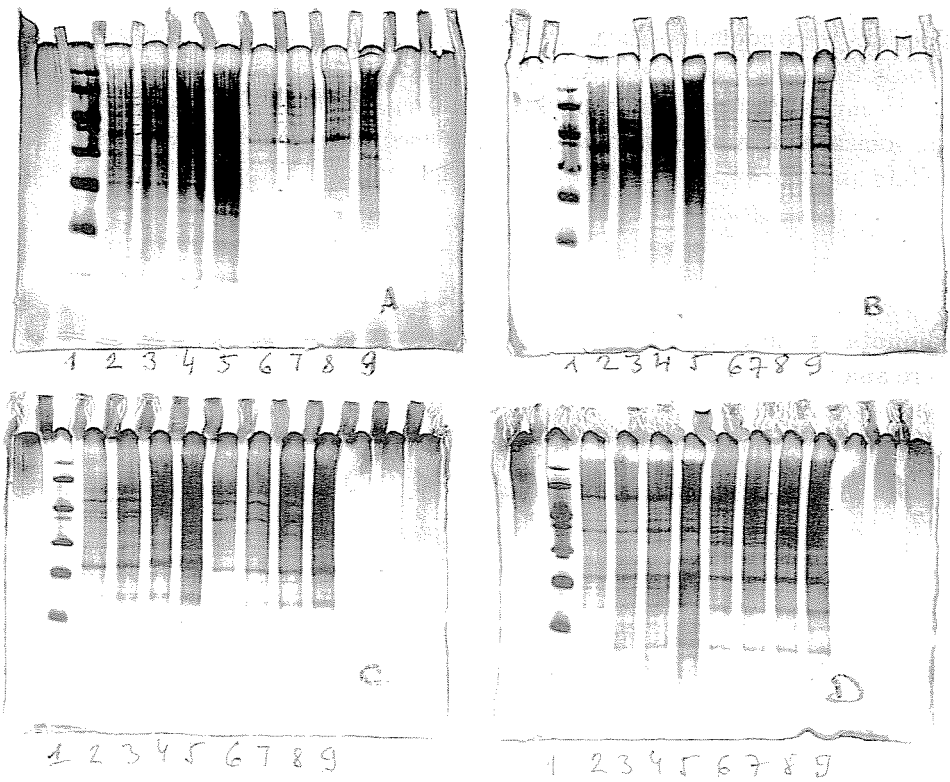

Fig. 1. SDS-PAGE analysis of *Saccharomyces cerevisiae* cells.
A) *S. pastorianus* CBS 1503; B) *S. cerevisiae* CBS 1395; C) *S. cerevisiae* CB67 and D) *S. cerevisiae* CB89. From the left: Lanes 1: Standard; 2,3,4,5: Control (30 °C); 6,7,8,9: Heat shocked cells (55 °C/10 min — 50 °C/30 min)

Table 1a
SDS-PAGE subfractions of heat shocked yeast cells

Yeast strains	No. of subfraction	Approximate molecular mass of HSPs (KDa)
S. pastorianus CBS 1503	7	5,27,34,41,47,62,123
S. cerevisiae CBS 1395	3	30,36,45
S. cerevisiae CB 67	9	16,22,28,33,39,41,49,58,93
S. cerevisiae CB 89	10	11,16,20,28,32,35,37,41,45,54

heat shock treatment. After heat shock treatment the DSC curves of the investigated yeast strains showed two broad endothermic peaks. The temperature of denaturation (T_d) and the enthalpy of denaturation (ΔH) of

Table 1b
SDS-PAGE subfractions of untreated yeast cells

Yeast strains	No. of subfraction	Approximate molecular mass of subfraction (KDa)
S. pastorianus CBS 1503	9	4,8,17,29,36,40,45,52,60
S. cerevisiae CBS 1395	7	27,37,48,54,61,67,100
S. cerevisiae CB 67	7	16,21,30,35,39,49,58,
S. cerevisiae CB 89	10	3,11,17,21,25,33,37,41,58,66

Table 1c
Change in SDS-PAGE after heat shock treatment (KDa)

Yeast strains	New bands	Disappeared bands	More intensive bands
S. pastorianus CBS 1503	27,62,123	17,45,52	62
S. cerevisiae CBS 1395	30,45	27,48,54,61,67	45
S. cerevisiae CB 67	22,28,41,93	30	22
S. cerevisiae CB 89	28,45,54	25,58,66	54

the investigated yeast strains before and after heat treatment are summarized in *Table 2.*

Discussion

In many organisms, induction of heat tolerance to extreme temperatures is directly correlated with induction of a specific set of HSPs (LI, 1983; LI et al., 1980; LINDQUIST, 1986; MITCHELL et al., 1979; WATSON and CAVICCHIOLI, 1983).

These proteins are induced by a variety of stress conditions, most notably heat shock (IIDA and YAHARA, 1984; LINDQUIST and CRAIG, 1988; MICHEL and STARKA, 1986; PLESSET et al., 1982; WATSON and CAVICCHIOLI, 1983) and ethanol stress (BRAZZELL and INGOLIA, 1984; MICHEL and STARKA, 1986; PLESSET et al., 1982). This paper examines the effects of heat shock on the thermal behaviour and the composition of the cell protein of the four investigated yeast strains. Heat shock treatment at 55 °C for 10 min followed by incubation at 50 °C for 30 min resulted in induction of HSPs (*Table 1a*). Several of the HPS bands were identified in the four strains examined, these have also been reported for other *S. cerevisiae* strains (BOSSIER et al., 1989; CAVICCHIOLI and WATSON, 1986; DOWHANICK et al., 1990; MCALISTER et al., 1979; SUSEK and LINDQUIST, 1989).

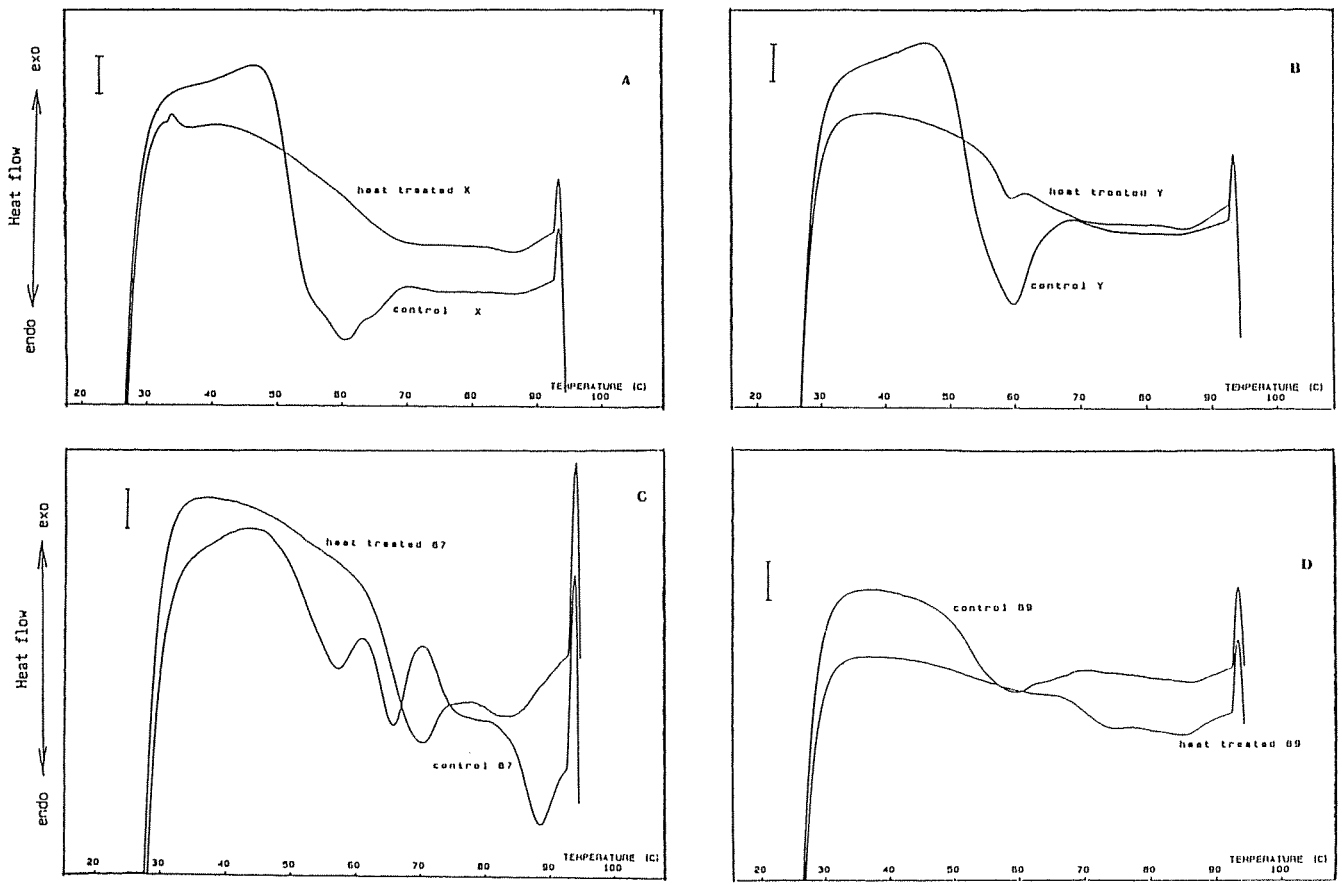

Fig. 2. DSC scans of whole *Saccharomyces* yeast cells before and after heat shock treatment. A) *S. pastorianus* CBS 1503; B) *S. cerevisiae* CBS 1395; C) *S. cerevisiae* CB 67 and D) *S. cerevisiae* CB 89

Table 2
Temperature of denaturation (T_d) and enthalpy contents (ΔH) of the endotherms of the investigated yeast strains (whole cells) before and after heat shock treatment

Yeast strains	Endotherm			
	Control (30 °C)		Heat shock treatment	
	T_d (°C)	ΔH (mcalmg^{-1})	T_d (°C)	ΔH (mcalmg^{-1})
S. pastorianus CBS 1503 (X)	60.4	255.33	67.8	19.80
			86.6	15.88
S. cerevisiae CBS 1395 (Y)	59.3	228.04	58.9	5.71
			86.2	18.24
S. cerevisiae CB 67	56.4	19.67	60.95	10.22
	65.7	16.96	83.6	2.87
	88.2	14.73		
S. cerevisiae CB89	58.7	72.66	73.2	1.39
			85.1	16.58

S. cerevisiae CB 89, the most heat resistance strain produced the highest number of HSPs as compared to the other strains (*Table 1a, 1c*). Perhaps some of these HSPs are synthesized constitutively in higher amount in the themotolerant strains and these proteins may account for their thermotolerance.

Protein unfolding of denaturation is accompanied by enthalpy changes (PRIVALOV and KHECHINASHVITY, 1974) which can be monitored by thermoanalytical techniques such as differential scanning calorimetry (DSC). The enthalpy changes are measured as differential heat flow between sample and reference and recorded as a peak by DSC. The peak analysis enables determination of temperature of transition and enthalpy of denaturation from peak temperature and area of the peak, respectively. The sharpness of the peak also indicates the cooperative nature of the transition from native to denatured state. If rupture of intramolecular bonds occurs within a very narrow range of temperature (very sharp peak), the transition is considered highly cooperative. The broader the peak the less cooperative the transition (WRIGHT et al., 1977). DSC has been used to study thermal denaturation of some food proteins such as muscle proteins (WRIGHT et al., 1977), egg albumin (DONOVAN et al., 1975), soybean proteins (HERMANSSON 1978 and 1979), whey proteins (DE WIT and KLARENBEEK, 1984), thermal behaviour of whole *Saccharomyces cerevisiae* (KAUL et al., 1992), and fababean proteins (ARNTFIELD and MURRAY, 1981). DSC is a simple technique since samples can be analysed directly and the size of sample required is small.

DSC scans of heat shocked yeast cells exhibited decreased (92.2, 93.7; 97.4, 92.0; 48.0, 83.1 and 98.1, 77.2 %) enthalpy (ΔH) values of the endotherms (*Fig. 2*, A,B,C and D) with respect to the control of the investigated yeast strains, respectively. These are indication of heat shock induced stability of the system. LEPOCK et al. (1990) in their model for protein denaturation, $N \underset{K-1}{\overset{K}{=}} D_R \overset{K_1}{\rightarrow} D_I$ where N and D are native and denatured states, respectively, K is the rate constant for killing and K_{-1} is the rate constant for refolding, have suggested that protein denaturation is made irreversible at higher concentrations such as those occurring in the cell by aggregation which follows actual denaturation. Heat shock proteins could either prevent such aggregation as has been reported by PELHAM, (1986) or alternatively HSPs themselves could be heat resistant so as to enable the whole complex mixture of cellular proteins to attain significantly lower ΔH values on DSC scans. In *S. cerevisiae* CB 98 which produced the highest number of HSPs, the DSC scans of this strain of this strain showed a higher decrease in ΔH value (98.1 %)as compared with other strains. This reflects that the heat shock effects, most if not all, are mediated by proteins as shown by altered values of ΔH for the main endotherm. Since the main component of the endotherm in this state is protein denaturation it can be said that HSPs impart the stabilization to the system as determined by the reduced ΔH values of the investigated yeast strains.

References

1. ANANTHAN, J. – GOLDBERG, M. L. – VOELLMY, R. (1986): Abnormal Protein Serve as Eukaryotic Stress Signals and Trigger the Activation of Heat-Shock Genes. *Science*, Vol. 232, pp. 252–254.

2. ARNTFIELD, S. D. – MURRAY, E. D. (1981): The Influence of Processing Parameters on Food Protein Functionality. I. Differential Scanning Calorimetry as an Indicator of Protein Denaturation. *Can. Inst. Food. Sci. Technol. J.*, Vol. 14, p. 289.

3. BOSSIER, P. – FITCH, I. T. – BOUCHERIE, H. – TUITE, M. F. (1989): Structure and Expression of a Yeast Gene Encoding the Small Heat Shock Protein HSP26. *Gene*, Vol. 78, pp. 323–330.

4. BRAZZELL, C. – INGOLIA, T. D. (1986): Stimuli that Induce a Yeast Heat Shock Gene Fused to β-Galactosidase. *Mol. Cell. Biol.*, Vol. 4, pp. 2573–2579.

5. CAVICCHIOLI, R. – WATSON, K. (1986): Loss of Heat Shock Acquisition of Thermotolerance in Yeast is not Correlated with Loss of Heat Shock Proteins. *FEBS Lett.*, Vol. 207, pp. 149–152.

6. CHIRICO, W. J. – GERARD WATERS, M. – BLOHEL, G. (1988): 70K Heat Shock Related Proteins Stimulate Protein Translocation into Microsomes. *Nature*, Vol. 332, pp. 805–810.

7. DESHAIES, R. J. – KOCH, B. D. – WERNER-WASHBURNE, M. – CRAIG, E. A. – SCHEKMAN, R. (1988): A Subfamily of Stress Facilitates Translocation of Secretory and Mitochondrial Precursor Polypeptides. *Nature*, Vol. 332, pp. 800–805.
8. DE WIT, J. N. – KLARENBEEK, G. (1984): Effects of Various Heat Treatments on Structure and Solubility of Whey Proteins. *J. Dairy Sci.* Vol. 67, p. 2701.
9. DONOVAN, J. W. – MAXES, C. J. – DAVIS, J. G. – GARBALDI, J. A. (1975): A Differential Scanning Calorimetric Study of Eggwhite to Heat Denaturation. *J. Sci. Fd. Agric.* Vol. 26, p. 73.
10. DOWHANICK, T. – SOLCZAK, J. – RUSSELL, I. – STEWART, G. G. (1990): The Rapid Identification by Protein Fingerprinting of Yeast and Bacterial Brewery Contaminants. *J. Am. Soc. Brew. Chem.* Vol. 48, pp. 75–79.
11. HERMANSSON, A. M. (1978): Physico-Chemical Aspects of Soy Protein Structure Formation. *J. Texture Studies*, Vol. 9, p. 33.
12. HERMANSSON, A. M. (1979): Methods of Studying Functional Characteristics of Vegetable Proteins. *J. Am. Oil Chem. Soc.*, Vol. 56, p. 272.
13. IIDA, H. – YAHARA, I. (1984): Heat Shock Resistant Mutant of *Saccharomyces cerevisiae* Shows Constitutive Synthesis of Two Heat Shock Proteins and Altered Growth. *J. Cell. Biol.* Vol. 99, pp. 1441–1450.
14. KAUL, S. C. – OBUCHI, K. – IWAHASHI, H. – KOMATSU, Y. (1992): Cryoprotection Provided by Heat Shock Treatment in *Saccharomyces Cerevisiae. Cell. Mol. Biol.*, Vol. 38(2), pp. 135–143.
15. LAEMMLI, U. K. (1970): Cleavage of Structural Proteins During the Assembly of the Heat of Bacteriophage T_4. *Nature*, Vol. 227, pp. 680–685.
16. LEPOCK, J. R. – FREY, H. E. – HEYNEN, M. P. – NISHIO, J. – WATERS, B. – RITCHIE, K. P. – KRUUV, J. (1990): Increased Thermostability of Thermotolerant CHLV79 Cells as Determined by Differential Scanning Calorimetry. *J. Cell. Physiol.*, Vol. 142, pp. 628–634.
17. LI, G. C. – SHIU, E. C. – HAHN, G. M. (1980): Similarities in Cellular Inactivation by Hyperthermia or Ethanol. *Rad. Res.* Vol. 82, pp. 257–268.
18. LI, G. C. – WERB, Z.: (1982): Correlation between Synthesis of Heat Shock Proteins and Development of Thermotolerance in Chinese Hamster Fibroblasts. *Proc. Nath. Acad. Sci. USA*, Vol. 79, pp. 3218–3222.
19. LI, G. C. (1983): Induction of Thermotolerance and Enhanced Heat Shock Protein Synthesis in Chinese Hamster Fibroblasts by Sodium Arsenite and by Ethanol. *J. Cell. Physiol.*, Vol. 115, pp. 116–122.
20. LINDQUIST, S. (1986): The Heat Shock Response. *Annu. Rev. Biochem.*, Vol. 55, pp. 1151–1191.
21. LINDQUIST, S. – CRAIG, E. A. (1988): The Heat Shock Proteins. *Annu. Rev. Genet.*, Vol. 22, pp. 631–677.
22. MCALISTER, L. – STRAUSBERG, S. – KULAGA, A. – FINKELSTEIN, D.B.: (1979): Altered Patterns of Protein Synthesis Induced by Heat Shock of Yeast. *Curr. Genet.*, Vol. 1, pp. 63–74.
23. MCALISTER, L. – FINKELSTEIN, D. B.: (1980): Heat Shock Proteins and Thermal Resistance in Yeast. *Biochem. Biophys. Res. Commun.*, Vol. 93, pp. 819–824.
24. MICHEL, G. P. F. – STARKA, J. (1986): Effects of Ethanol and Heat Stresses on the Protein Pattern of *Zymomonas mobilis. J. Bacteriol.*, Vol. 165, pp. 1040–1042.
25. MILLER, M.J. – XUONG, N. H. – GEIDUSCHEK, E.P. (1982): Quantitative Analysis of the Heat Shock Response of *Saccharomyces cerevisiae. J. Bacteriol.*, Vol. 151, pp. 311–327.

26. MITCHELL, H. K. – MULLER, G. – PETERSON, N. S. – LIPPS-SARMIENTO, L. (1979): Specific Protection from Phenocopy Induction by Heat Shock. *Dev. Genet.*, Vol. 1, pp. 181–192.
27. MORIMOTO, R. I. – TISSIERES, A. – GEORGOPOULOS, C. (1990): Stress Proteins in Biology and Medicine. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. p. 442.
28. PELHAM, H. R. B. (1986): Speculation on the Functions of the Major Heat Shock and Glucose-Regulated Proteins. *Cell*, Vol. 46, pp. 959–961.
29. PICARD, D. – KHURSHEED, B. – GARABEDIAN, M. J. – FORTIN, M. G. – LINDQUIST, S. – YAMAMOTO, K. R. (1990): Reduced Levels os hsp90 Compromise Steroid Receptor Action in Vivo. *Nature*, Vol. 348, pp. 166–168.
30. PINHASI-KIMHI, O. – MICHALOVITZ, G. – BENZEER, A – OREN, M. (1986): Specific Interactions between the p53 Cellular Tumor Antigen and Major Heat-Shock Proteins. *Nature*, Vol. 330, pp. 182–185.
31. PLESSET, J. – PALM, C. – MCLAUGHIN, C. S. (1982): Induction of Heat Shock Proteins and Thermotolerance by Ethanol. *Biochem. Biophys. Res. Commu.*, Vol. 108, pp. 1340–1345.
32. PRIVALOV, P. L. – KHECHINASHVILI, N. N. (1974): A Thermodynamic Approach to the Problem of Stabilization of Globular Proteins: A Calorimetric Approach. *J. Mol. Biol.*, Vol. 86, p. 665.
33. SCHLESINGER, M. J. – ASHBURNER, M. – TISSIERES, A. (1982): Heat Shock from Bacteria to Man, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. p. 440.
34. SUSEK, R. E. – LINDQUIST, S. L. (1989): HSP26 of *Saccharomyces cerevisiae* is Related to the Super Family of Small Heat Shock Proteins But is without a Demonstrable Function. *Mol. Cell. Biol.* Vol. 9, pp. 5265–5271.
35. VELAZQUEZ, J. M. – LINDQUIST, S. (1984): Hsp70: Nuclear Concentration During Environmental Stress and Cytoplasmic Storage During Recovery. *Cell*, Vol. 36, pp. 655–662.
36. WATSON, K. – CAVICCHIOLI, R. (1983): Acquisition of Ethanol Tolerance in Yeast Cells by Heat Shock. *Biotechnol. Lett.*, Vol. 5, pp. 683–688.
37. WATSON, K. – DUNLOP, G. – CAVICCHIOLI, R. (1984): Mitochondrial and Cytoplasmic Protein Synthesis are not Required for Heat Shock Acquisition of Ethanol and Thermotolerance in Yeast. *FEBS Lett.*, Vol. 172, pp. 299–302.
38. WRIGHT, D. J. – LEACH, J. B. – WILDING, P. (1977): Differential Scanning Calorimetric Studies of Muscle and its Constituent Proteins. *J. Sci. Fd. Agric.*, Vol. 28, p. 557.
39. YOST, H. J. – PETERSON, R. B. – LINDQUIST, S. (1990): In: Stress Proteins in Biology and Medicine. Cold Spring Harbor Laboratory Press, N.Y. pp. 379–409.