

**Physiological and Molecular Analyses of Stress Responses
in Psychrophilic, Psychrotrophic, Mesophilic
and Thermophilic Yeast.**

By

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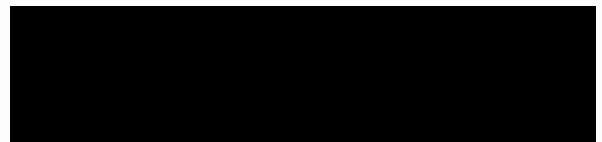
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Declaration

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.



(Michelle Deegenars)

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In loving memory of my Opa (grandfather) who passed away during my candidature.

Abstract

The response to heat and oxidative stress in seven yeast species isolated from Antarctica was examined. The yeast were classified into two groups, one psychrophilic, with a maximum growth temperature of 20°C and the other psychrotrophic, capable of growth at temperatures above 20°C. In addition to species specific heat shock protein (hsp) profiles, a heat shock (15°C to 25°C for 3 h) induced the synthesis of a 110 kDa protein common to the psychrophiles, *Candida psychrophila*, *Mrakia stokesii*, *M. frigida* and *M. gelida*, but not in *Leucosporidium antarcticum*. Preliminary amino acid sequence characterization of hsp 110 revealed similarity to fructose-2,6-bisphosphatase. Immunoblot analyses revealed heat shock inducible proteins corresponding to *Saccharomyces cerevisiae* hsps 70 and 90 in psychrophilic and psychrotrophic yeast (*L. fellii* and *L. scottii*). Interestingly, no protein corresponding to *S. cerevisiae* hsp 104 was observed in any of the psychrophilic species examined, however a hsp 104 homologue was identified in psychrotrophic yeast. In psychrotrophic yeast, as observed in psychrophilic yeast, there was a noticeable absence of a protein corresponding to hsp 60 with the notable exception of a hsp 60 homologue detected in *C. psychrophila*. A 10°C increase in temperature above the growth temperature (15°C) of psychrophiles and psychrotrophs induced thermotolerance. On the other hand in psychrotrophic yeast grown at 25°C, only a 5°C increase in temperature was necessary for heat shock induced thermotolerance. Induced thermotolerance in all psychrophilic and psychrotrophic yeast species was coincident with hsp synthesis and trehalose accumulation.

With respect to intrinsic peroxide stress tolerance, all psychrophilic and psychrotrophic yeast species were relatively intrinsically resistant to a 100 mM H₂O₂ stress and both a heat shock and a peroxide shock (0.2 mM) conferred further tolerance. Species specific peroxide shock proteins were identified in *C. psychrophila*, *M. gelida*, *M. stokesii* and *L. fellii*. However, a peroxide shock did not induce the synthesis of hsps 104, 90, 70 or 60 in *C. psychrophila*.

Heat and oxidative stress tolerance were also examined in a respiratory-competent and respiratory-deficient strain of the thermophilic enteric yeast *Arxiozyma telluris*. Heat shock acquisition of thermotolerance and peroxide stress tolerance was induced by a mild heat shock (35°C to 40°C for 30 min) with concomitant synthesis of hsps 104, 90, 70 and

60. Induction of trehalose synthesis was also stimulated by a mild heat shock but not by a mild peroxide shock (0.2 mM). There were no marked differences in the heat shock response between the respiratory-competent and respiratory-deficient strains, however, a higher sensitivity to peroxide was observed in the respiratory-deficient strain. The heat shock response displayed by the thermophilic yeast strains in many respects paralleled that of the mesophilic yeast, *S. cerevisiae*.

The present studies revealed a relationship between growth temperature and both the heat shock response and oxidative stress response in *S. cerevisiae*. The results indicated that the temperature at which *S. cerevisiae* is grown influences intrinsic thermotolerance, growth rate and constitutive hsp levels but does not greatly influence heat shock induced thermotolerance or trehalose accumulation. The findings suggested that hsp 104 and slow growth contribute to the higher basal tolerance observed in cells grown at 15°C and 35°C. Furthermore, two novel temperature dependent proteins (40 kDa and 80 kDa) were identified which appear to be down-regulated by a heat shock.

In *S. cerevisiae*, results indicated a positive correlation between intrinsic peroxide stress tolerance and lower growth temperatures. However, growth temperature was found not to significantly influence the inducibility of peroxide stress tolerance by a heat or peroxide shock. An interesting finding observed in *S. cerevisiae* and all psychrophilic, psychrotrophic and thermophilic yeast species was that the incubation temperature during a peroxide stress had a profound effect on basal peroxide stress tolerance, with a decrease in incubation temperature corresponding with increased tolerance, regardless of growth temperature.

Publications arising from this thesis

- Deegenars, M. L. and Watson, K. (1997). Stress proteins and stress tolerance in an Antarctic, psychrophilic yeast, *Candida psychrophila*. *FEMS Lett.* **151**, 191-196.
- Deegenars, M. L. and Watson, K. (1998). Heat shock response in psychrophilic and psychrotrophic yeast from Antarctica. *Extremophiles* (in press).
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Copies of the first two publications are presented in the Appendix.

Parts of this thesis presented at scientific meetings

- Deegenaaars, M. L. and Watson, K. (1994). Crosstolerance between heat and oxidative stress in extremophilic yeast. *Proc. Aust. Soc. Biochem. Mol. Biol.* **26**, Gold Coast. POS-2-30.
- Deegenaaars, M. L. and Watson, K. (1995). The effect of growth temperature on intrinsic and induced thermotolerance. *Proc. 7th FAOBMB Congress* **27**, POS-1-182.
- Watson, K. and Deegenaaars, M. L. (1996). Biochemical adaptations in yeast to stress. *Proc. 2nd Int. Conference on Predictive Microbiology*, Hobart. S2.4.
- Deegenaaars, M. L. and Watson, K. (1996). Comparison of psychrophilic and psychrotrophic stress response systems. *Proc. 9th Int. Symposium on Yeasts*, Sydney. P4-7.
- Watson, K. and Deegenaaars, M. L. (1997). Stress response in Antarctic yeasts. *Conf. Proc. Stress of Life: Stress and Adaptation from Molecules to Man*, Budapest. D3-3.
- Thomas-Hall, S., Deegenaaars, M. L. and Watson, K. (1997). Heat shock proteins in Antarctic yeast. *Proc. Aust. Soc. Biochem. Mol. Biol.*, **29**, Melbourne. B1-58.

Abbreviations

AMPS:	ammonium peroxodisulphate
ATP:	adenosine triphosphate
ATPase:	adenosine triphosphatase
BSA:	bovine serum albumin
cAMP:	cyclic adenosine monophosphate
CAPS:	3-(cyclohexylamino)propanesulfonic acid
cDNA:	complementary DNA
cfu ml⁻¹:	colony forming units per ml
cpn:	chaperonin
esp(s):	cold shock protein(s)
DNA:	deoxyribonucleic acid
ECL:	enhanced chemiluminescence
EDTA:	ethylenediaminetetraacetic acid
ER:	endoplasmic reticulum
f1,6bp:	fructose-1,6-bisphosphate
f1,6bpase:	fructose-1,6-bisphosphatase
f2,6bp:	fructose-2,6-bisphosphate
f2,6bpase:	fructose-2,6-bisphosphatase
f6p:	fructose-6-phosphate
g3pd:	glyceraldehyde-3-phosphate dehydrogenase
g6p:	glucose-6-phosphate
HOG:	high osmolarity glycerol
hsc:	heat shock cognate protein
HSC:	heat shock cognate gene
HSE:	heat shock element
HSF:	heat shock transcription factor
hsp(s):	heat shock protein(s)
HSP(s):	heat shock protein gene(s)
kb:	kilobases
kDa:	kilodaltons

LB:	Luria-Bertani
LM:	Luria medium
M_r:	relative mass
mRNA:	messenger RNA
OD:	optical density
OD₆₀₀:	optical density at 600 nm
PBS:	phosphate buffered saline
PBS-T:	PBS-Tween 20
pfk1:	phosphofructokinase-1
pfk2:	phosphofructokinase-2
pH_i:	intracellular pH
PMSF:	phenylmethylsulphonyl fluoride
psp(s):	peroxide shock (inducible) protein(s)
PVDF:	polyvinylidene difluoride
RNA:	ribonucleic acid
RNase:	ribonuclease
ROS:	reactive oxygen species
rubisco:	ribulose biphosphate carboxylase-oxygenase
SDS:	sodium dodecyl sulphate
SDS-PAGE:	SDS-polyacrylamide gel electrophoresis
SMP:	skim milk powder
SOD:	superoxide dismutase
SSC:	saline sodium citrate
STRE:	stress response element
t6p:	trehalose-6-phosphate
TAE:	Tris acetate EDTA
TCA:	trichloroacetic acid
TE:	Tris EDTA
TEMED:	N,N,N',N'-tetramethylethylenediamine
UV:	ultraviolet
YEP:	yeast extract peptone
YNB:	yeast nitrogen base

List of Figures

Figure		Page
3.1	Growth curve of <i>C. psychrophila</i> at 15°C with corresponding glucose and ethanol measurements.	51
3.2	Intrinsic and induced thermotolerance to a 35°C heat stress in mid-logarithmic and stationary phase cultures of <i>C. psychrophila</i> grown at 15°C.	53
3.3	Intrinsic and induced thermotolerance to a 35°C heat stress in mid-logarithmic and stationary phase cultures of <i>C. psychrophila</i> grown at 10°C.	53
3.4	The effect of duration and temperature of heat shock on induced thermotolerance to a 35°C heat stress in mid-logarithmic phase cells of <i>C. psychrophila</i> grown at 15°C.	54
3.5	The effect of a 3 hour peroxide shock on induced thermotolerance to a 35°C heat stress in mid-logarithmic and stationary phase cultures of <i>C. psychrophila</i> grown at 15°C.	56
3.6	Intrinsic thermotolerance to a 38°C, 40°C or 42°C heat stress in mid-logarithmic phase cultures of <i>C. psychrophila</i> grown at 15°C.	57
3.7	Intrinsic and induced thermotolerance to a 38°C heat stress in mid-logarithmic phase cultures of <i>C. psychrophila</i> grown at 15°C.	57
3.8	SDS-polyacrylamide gel autoradiogram of ³⁵ S-methionine labelled protein extracts from control and heat shocked cells of <i>C. psychrophila</i> .	59
3.9	Western blot analysis of control and heat shock protein extracts from <i>C. psychrophila</i> .	60
3.10	Trehalose levels in control and heat shock samples of <i>C. psychrophila</i> and <i>S. cerevisiae</i> K7.	62
3.11	Intrinsic ethanol tolerance in mid-logarithmic and late-logarithmic cells of <i>C. psychrophila</i> .	62

3.12	The effect of cold shock (5°C) on growth, trehalose levels and <i>de novo</i> protein synthesis on mid-logarithmic phase cultures of <i>C. psychrophila</i> grown at 15°C.	63
4.1	Growth curves of the Antarctic, psychrophilic yeast <i>M. frigida</i> , <i>M. gelida</i> , <i>M. stokesii</i> and <i>L. antarcticum</i> grown at 15°C.	72
4.2	Growth curves of the Antarctic, psychrotrophic yeast <i>L. fellii</i> and <i>L. scottii</i> grown at 15°C and 25°C.	73
4.3	Intrinsic and induced thermotolerance in mid-logarithmic phase cultures of <i>M. frigida</i> and <i>M. gelida</i> grown at 15°C.	75
4.4	Intrinsic and induced thermotolerance in mid-logarithmic phase cultures of <i>M. stokesii</i> and <i>L. antarcticum</i> grown at 15°C.	76
4.5	Intrinsic and induced thermotolerance in mid-logarithmic phase cultures of <i>L. fellii</i> and <i>L. scottii</i> grown at 15°C.	77
4.6	Intrinsic and induced thermotolerance in mid-logarithmic phase cultures of <i>L. fellii</i> and <i>L. scottii</i> grown at 25°C.	79
4.7	Trehalose levels in control and heat shock samples of the psychrophilic yeast, <i>M. frigida</i> , <i>M. gelida</i> , <i>M. stokesii</i> and <i>L. antarcticum</i> and the psychrotrophic yeast, <i>L. fellii</i> and <i>L. scottii</i> (grown at either 15°C or 25°C).	80
4.8	SDS-polyacrylamide gel autoradiograms of ³⁵ S-methionine labelled protein extracts from control and heat shocked cells of <i>M. frigida</i> , <i>M. gelida</i> , <i>M. stokesii</i> and <i>L. antarcticum</i> .	82
4.9	SDS-polyacrylamide gel autoradiograms of ³⁵ S-methionine labelled protein extracts from control and heat shocked cells of <i>L. fellii</i> and <i>L. scottii</i> grown at 15°C and 25°C.	83
4.10	Western blot analysis of control and heat shocked protein extracts from psychrophilic yeast and psychrotrophic yeast.	85
4.11	SDS-polyacrylamide gel (6%) autoradiograms of ³⁵ S-methionine labelled protein extracts from control and heat shocked cells of <i>M. gelida</i> and <i>M. stokesii</i> to help identify the position of the 110 kDa protein.	87
4.12	BLASTP search of amino acid sequence homology of <i>M. stokesii</i> hsp	88

	110 to <i>S. cerevisiae</i> protein sequences.	
5.1	Growth curves of <i>A. telluris</i> 1787 (respiratory-deficient) and <i>A. telluris</i> 2760 (respiratory-competent) grown at 35°C with corresponding glucose and ethanol measurements.	98
5.2	Induced thermotolerance to a 47°C heat stress in mid-logarithmic phase cultures and late-logarithmic cultures of <i>A. telluris</i> 1787 (respiratory-deficient) grown at 37°C.	100
5.3	Intrinsic and induced thermotolerance to a 45°C heat stress in mid-logarithmic phase cultures of <i>A. telluris</i> 1787 (respiratory-deficient) grown at 30°C.	100
5.4	Intrinsic and heat or peroxide shock induced thermotolerance to a 47°C heat stress in mid-logarithmic phase cultures of <i>A. telluris</i> 2760 (respiratory-competent) and <i>A. telluris</i> 1787 (respiratory-deficient) grown at 35°C.	101
5.5	Intrinsic and induced thermotolerance to a 50°C heat stress in mid-logarithmic and late logarithmic phase cultures of <i>A. telluris</i> 2760 (respiratory-competent) grown at 35°C.	103
5.6	Intrinsic and heat or peroxide shock induced thermotolerance to a 47°C heat stress in stationary phase cultures of <i>A. telluris</i> 2760 (respiratory-competent) grown at 35°C.	103
5.7	SDS-polyacrylamide gel autoradiogram of ³⁵ S-methionine labelled protein extracts from control and heat shocked cells from <i>A. telluris</i> 2760 (respiratory-competent) and <i>A. telluris</i> 1787 (respiratory-deficient).	104
5.8	Western blot analysis of control and heat shock protein extracts from <i>A. telluris</i> 2760 (respiratory competent) and <i>A. telluris</i> 1787 (respiratory deficient).	105
5.9	Trehalose levels in control and heat shock samples of <i>A. telluris</i> 2760 (respiratory-competent) and <i>A. telluris</i> 1787 (respiratory-deficient).	107
5.10	The effect of cycloheximide treatment on induced thermotolerance to a 47°C heat stress in mid-logarithmic phase cultures of <i>A. telluris</i> 2760 (respiratory-competent) and <i>A. telluris</i> 1787 (respiratory-	108

	deficient) grown at 35°C.	
5.11	SDS-polyacrylamide gel autoradiogram of ³⁵ S-methionine labelled protein extracts from control, heat shocked and cycloheximide treated cells of <i>A. telluris</i> 2760 (respiratory-competent) and <i>A. telluris</i> 1787 (respiratory-deficient).	109
5.12	The effect of cycloheximide treatment on heat shock induced trehalose accumulation in <i>A. telluris</i> 2760 (respiratory-competent) and <i>A. telluris</i> 1787 (respiratory-deficient) grown at 35°C.	110
5.13	The effect of cold shock (5°C) on growth, trehalose levels and <i>de novo</i> protein synthesis on mid-logarithmic phase cultures of <i>A. telluris</i> grown at 35°C.	111
6.1	Growth curves of <i>S. cerevisiae</i> K7 grown at 15°C, 25°C and 35°C.	120
6.2	Intrinsic and induced thermotolerance to a 35°C heat stress in mid-logarithmic phase cultures of <i>S. cerevisiae</i> K7 and <i>C. psychrophila</i> grown at 15°C.	121
6.3	Intrinsic and induced thermotolerance in mid-logarithmic phase cultures of <i>S. cerevisiae</i> K7 grown at 15°C, 25°C or 35°C.	123
6.4	Trehalose levels in control and heat shock samples of <i>S. cerevisiae</i> K7 grown at 15°C, 25°C or 35°C.	124
6.5	SDS-polyacrylamide gel autoradiogram of ³⁵ S-methionine labelled protein extracts from control and heat shocked cells of <i>S. cerevisiae</i> K7 grown at 15°C, 25°C or 35°C.	126
6.6	SDS-polyacrylamide gel autoradiogram of ³⁵ S-methionine labelled protein extracts from control, heat shocked and cycloheximide treated cells of <i>S. cerevisiae</i> K7 grown at 15°C, 25°C or 35°C.	127
6.7	Western blot analysis of control and heat shock protein extracts from <i>S. cerevisiae</i> K7 grown at 15°C, 25°C or 35°C.	128
7.1	Intrinsic peroxide tolerance in mid-logarithmic and late logarithmic phase cultures of <i>C. psychrophila</i> grown at 15°C.	140
7.2	Intrinsic and induced peroxide tolerance in mid-logarithmic phase cultures of <i>C. psychrophila</i> grown at 15°C.	141
7.3	Intrinsic and heat shock induced peroxide tolerance in mid-	142

	logarithmic and stationary phase cultures of <i>C. psychrophila</i> grown at 15°C.	
7.4	Intrinsic and induced peroxide tolerance in mid-logarithmic phase cultures of <i>M. frigida</i> , <i>M. gelida</i> , <i>M. stokesii</i> and <i>L. antarcticum</i> grown at 15°C.	143
7.5	Intrinsic and induced peroxide tolerance in mid-logarithmic phase cultures of <i>L. fellii</i> and <i>L. scottii</i> grown at 15°C and 25°C.	145
7.6	Intrinsic peroxide tolerance in mid-logarithmic and late logarithmic phase cultures of <i>A. telluris</i> 2760 (respiratory-competent) grown at 35°C.	146
7.7	Intrinsic and induced peroxide tolerance to a 2 mM H ₂ O ₂ stress in mid-logarithmic phase cultures of <i>A. telluris</i> 2760 (respiratory-competent) and <i>A. telluris</i> 1787 (respiratory-deficient) grown at 35°C.	147
7.8	Intrinsic and induced peroxide tolerance to a 5 or 10 mM H ₂ O ₂ stress in mid-logarithmic phase cultures of <i>A. telluris</i> 2760 (respiratory-competent) grown at 35°C.	148
7.9	Intrinsic and induced peroxide tolerance to a 10 mM H ₂ O ₂ stress in stationary phase cultures of <i>A. telluris</i> 2760 (respiratory-competent) grown at 35°C.	150
7.10	Trehalose levels in control, heat shock and peroxide shock samples of <i>C. psychrophila</i> , <i>A. telluris</i> 2760 (respiratory-competent) and <i>A. telluris</i> 1787 (respiratory-deficient).	150
7.11	Western blot analysis of control, heat shock and peroxide shock extracts from <i>C. psychrophila</i> , <i>A. telluris</i> 2760 (respiratory-competent) and <i>A. telluris</i> 1787 (respiratory-deficient).	151
7.12	SDS-polyacrylamide gel autoradiogram of ³⁵ S-methionine labelled protein extracts from control, heat shocked and peroxide shocked cells of <i>C. psychrophila</i> .	152
7.13	SDS-polyacrylamide gel autoradiograms of ³⁵ S-methionine labelled protein extracts from control and peroxide shocked cells of <i>M. frigida</i> , <i>M. gelida</i> , <i>M. stokesii</i> and <i>L. antarcticum</i> .	153

7.14	SDS-polyacrylamide gel autoradiogram of ³⁵ S-methionine labelled protein extracts from control and peroxide shocked cells of <i>L. fellii</i> and <i>L. scottii</i> grown at 15°C and 25°C.	154
7.15	SDS-polyacrylamide gel autoradiogram of ³⁵ S-methionine labelled protein extracts from control, heat shocked and peroxide shocked cells of <i>A. telluris</i> 2760 (respiratory-competent) and <i>A. telluris</i> 1787 (respiratory-deficient).	155
8.1	Intrinsic peroxide tolerance in mid-logarithmic phase cultures of <i>S. cerevisiae</i> grown at 15°C, 25°C and 35°C.	164
8.2	Intrinsic and induced peroxide tolerance in mid-logarithmic phase cultures of <i>S. cerevisiae</i> K7 grown at 15°C, 25°C and 35°C.	165
8.3	SDS-polyacrylamide gel autoradiogram of ³⁵ S-methionine labelled protein extracts from control and peroxide shocked cells of <i>S. cerevisiae</i> K7 grown at 15°C, 25°C or 35°C.	166
8.4	Intrinsic peroxide tolerance in mid-logarithmic phase cultures of <i>S. cerevisiae</i> K7 grown at 15°C, 25°C and 35°C and peroxide stressed at different temperatures.	168
8.5	Intrinsic peroxide tolerance in mid-logarithmic cultures of <i>C. psychrophila</i> , <i>M. frigida</i> , <i>M. gelida</i> , <i>M. stokesii</i> and <i>L. antarcticum</i> grown at 15°C and peroxide stressed at different temperatures.	169
8.6	Intrinsic peroxide tolerance in mid-logarithmic cultures of <i>L. fellii</i> and <i>L. scottii</i> grown at 15°C or 25°C and peroxide stressed at different temperatures.	171
8.7	Intrinsic peroxide tolerance in mid-logarithmic cultures of <i>A. telluris</i> 2760 (respiratory-competent) grown at 35°C and peroxide stressed at different temperatures.	173
8.8	Intrinsic peroxide tolerance in mid-logarithmic cultures of <i>A. telluris</i> 1787 (respiratory-deficient) grown at 35°C and peroxide stressed at different temperatures.	174

List of Tables

Table		Page
2.1	Experimental yeast strains	31
2.2	Restriction endonucleases required to produce appropriately sized HSP gene probe for Southern hybridization.	32
3.1	Southern hybridization analysis with heat shock genes in <i>C. psychrophila</i> .	60
4.1	Culture doubling times for psychrophilic and psychrotrophic yeast.	74
5.1	Southern hybridization analysis with heat shock genes in <i>A. telluris</i> .	105
8.1	Experimental conditions used to examine the influence of growth temperature and incubation temperature (during peroxide stress) on intrinsic peroxide stress tolerance.	162

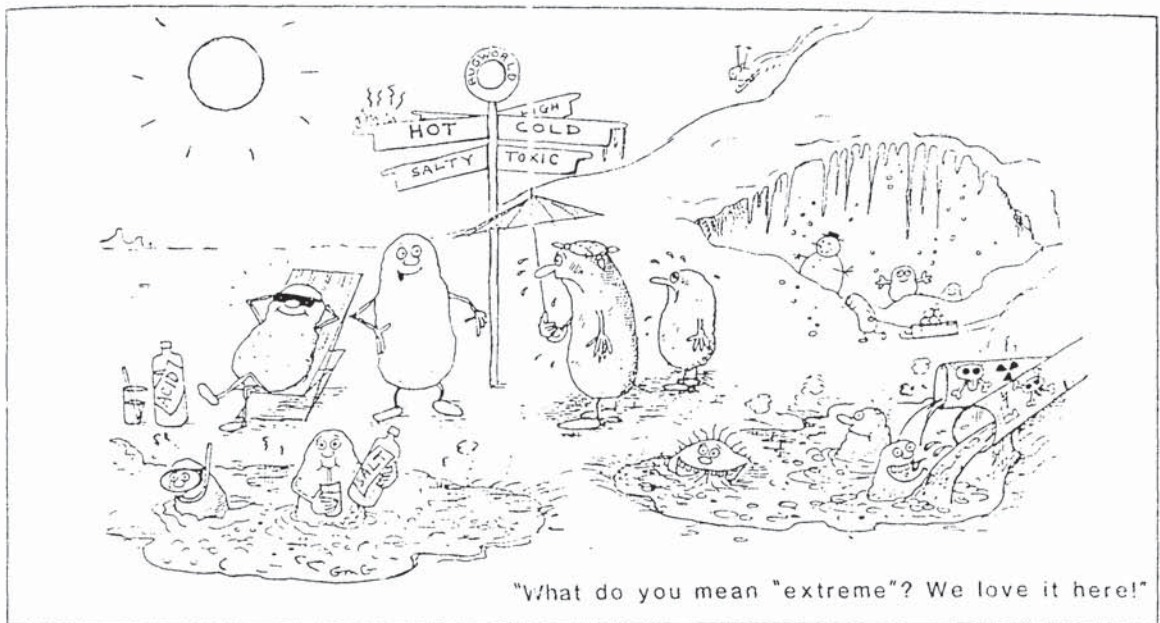
Contents

Declaration	i
Acknowledgements	ii
Abstract	iii
Publications arising from this thesis	v
Parts of this thesis presented at scientific meetings	vi
Abbreviations	vii
List of Figures	ix
List of Tables	xv
CHAPTER 1: Introduction	1
1.1 The heat shock response	1
1.2 Cellular events during heat shock	1
1.3 History of the stress response	2
1.4 Heat shock protein families	2
1.5 Heat shock genes in <i>Saccharomyces cerevisiae</i>	3
<i>1.5.1 HSP 104</i>	3
<i>1.5.2 HSP 90 gene family</i>	4
<i>1.5.3 HSP 70 gene family</i>	5
<i>1.5.3.1 Subgroup SSA</i>	6
<i>1.5.3.2 Subgroup SSB</i>	7
<i>1.5.3.3 Subgroup SSC</i>	8
<i>1.5.3.4 Subgroup SSD</i>	8
<i>1.5.3.5 Subgroup SSE</i>	8
<i>1.5.4 HSP 60</i>	9
<i>1.5.5 HSP 30</i>	10

"The role of the infinitely small is infinitely large."

(Louis Pasteur)

(Cited from Aguilar, 1996)



(From Herbert, 1992)

1.5.6	<i>Small HSPs</i>	10
1.5.6.1	<i>HSP 26</i>	11
1.5.6.2	<i>HSP 12</i>	11
1.5.7	<i>Other S. cerevisiae heat shock proteins</i>	12
1.6	Functions of heat shock proteins	13
1.6.1	<i>Molecular chaperones</i>	13
1.6.2	<i>Thermotolerance</i>	14
1.6.3	<i>Immune system</i>	15
1.7	Heat shock gene regulation	16
1.7.1	<i>Heat shock element</i>	16
1.7.2	<i>Stress response element</i>	16
1.8	Trehalose	17
1.9	Other factors involved in the heat shock response	19
1.10	Stress induced proteolysis	19
1.11	Oxidative stress response	20
1.11.1	<i>Non-enzymatic antioxidant defences</i>	21
1.11.2	<i>Enzymatic antioxidant defences</i>	22
1.11.3	<i>Transcriptional regulation of the oxidative stress response</i>	22
1.12	Cold shock response	23
1.13	Yeast from relatively extreme temperature environments	25
1.13.1	<i>Psychrophiles and psychrotrophs</i>	26
1.13.2	<i>Thermophiles</i>	27
1.14	Thesis aims	28
 CHAPTER 2: Materials and Methods		29
2.1	Materials	29
2.1.1	<i>Chemicals</i>	29
2.1.2	<i>Strains</i>	30
2.1.3	<i>Heat shock protein DNA probes</i>	30

2.1.4	<i>Anti-hsp antibodies</i>	32
2.2	Methods	33
2.2.1	General	33
2.2.2	Maintenance of cultures	33
2.2.2.1	<i>Liquid and solid media</i>	33
2.2.2.2	<i>Glycerol stocks</i>	34
2.2.2.3	<i>Starter cultures</i>	34
2.2.3	Growth curves	34
2.2.4	Glucose determination	35
2.2.5	Ethanol determination	35
2.2.6	Shock / stress conditions	35
2.2.6.1	<i>Heat shock / heat stress</i>	35
2.2.6.2	<i>Peroxide shock / peroxide stress</i>	36
2.2.6.3	<i>Crosstolerance</i>	36
2.2.6.4	<i>Ethanol stress</i>	36
2.2.6.5	<i>Cold shock</i>	37
2.2.6.6	<i>Viable plate counts - % Survivors</i>	37
2.2.6.7	<i>Reproducibility of experiments</i>	37
2.2.7	Southern hybridization	38
2.2.7.1	<i>Bacterial transformation</i>	38
2.2.7.2	<i>Selection of transformed host cells</i>	38
2.2.7.3	<i>Plasmid DNA preparations</i>	38
2.2.7.4	<i>Genomic DNA preparations</i>	39
2.2.7.5	<i>DNA quantification</i>	39
2.2.7.6	<i>Restriction endonuclease digestion</i>	39
2.2.7.7	<i>Agarose gel electrophoresis</i>	40
2.2.7.8	<i>DNA purification (GeneClean, Bresaclean)</i>	40
2.2.7.9	<i>Oligo-labelling of DNA probe</i>	41
2.2.7.10	<i>Alkaline Southern blotting</i>	41
2.2.7.11	<i>Hybridization, stringency washes and autoradiography</i>	41
2.2.8	Sodium dodecyl sulphate – polyacrylamide gel electrophoresis	42
2.2.8.1	<i>³⁵S-methionine labelling of proteins</i>	42
2.2.8.2	<i>Protein extraction</i>	42

2.2.8.3	<i>Protein determination</i>	43
2.2.8.4	<i>Polyacrylamide gel electrophoresis</i>	43
2.2.8.5	<i>Silver staining of polyacrylamide gels</i>	44
2.2.8.6	<i>Gel drying and autoradiography</i>	44
2.2.9	<i>Western immunoblot analysis</i>	44
2.2.9.1	<i>Transfer of proteins to nitrocellulose</i>	44
2.2.9.2	<i>Enhance chemiluminescence detection of bound antibody</i>	45
2.2.9.3	<i>Antibody homology to <i>S. cerevisiae</i> hsp90</i>	45
2.2.10	<i>Protein isolation</i>	46
2.2.10.1	<i>SDS – PAGE</i>	46
2.2.10.2	<i>Transfer of protein to PVDF membrane</i>	46
2.2.10.3	<i>Coomassie brilliant blue staining and protein band excision</i>	46
2.2.10.4	<i>Amino acid sequencing</i>	47
2.2.11	<i>Trehalose assay</i>	47
2.2.11.1	<i>Sample preparation</i>	47
2.2.11.2	<i>Anthrone assay</i>	47
 CHAPTER 3: Heat shock response in the Antarctic, <i>Psychrophilic</i>		48
Yeast, <i>Candida psychrophila</i>.		
3.1	Introduction	48
3.2	Results	50
3.2.1	<i>Growth curves</i>	50
3.2.2	<i>Thermotolerance</i>	50
3.2.3	<i>Southern hybridization analysis</i>	58
3.2.4	<i>Heat shock proteins</i>	58
3.2.5	<i>Trehalose</i>	61
3.2.6	<i>Ethanol tolerance</i>	61
3.2.7	<i>Cold shock</i>	61
3.3	Discussion	64
3.3.1	<i>Heat shock response</i>	64
3.3.2	<i>Ethanol tolerance</i>	67

3.3.3	<i>Cold shock response</i>	68
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**CHAPTER 4: Heat shock response in Antarctic Psychrophilic
and Psychrotrophic Yeast.** 70

4.1	Introduction	70
4.2	Experimental outline	70
4.3	Results	71
4.3.1	<i>Growth curves</i>	71
4.3.2	<i>Thermotolerance</i>	74
4.3.3	<i>Trehalose</i>	78
4.3.4	<i>Heat shock proteins</i>	81
4.3.5	<i>Isolation and preliminary characterization of the 110 kDa hsp</i>	89
4.4	Discussion	89
4.4.1	<i>Thermotolerance</i>	89
4.4.2	<i>Preliminary characterization of hsp 110</i>	95

**CHAPTER 5: Heat Shock Response in the Thermophilic Enteric
Yeast, *Arxiozyma telluris*.** 96

5.1	Introduction	96
5.2	Results	97
5.2.1	<i>Growth curves</i>	97
5.2.2	<i>Thermotolerance</i>	99
5.2.3	<i>Southern hybridization analysis</i>	102
5.2.4	<i>Heat shock proteins</i>	102
5.2.5	<i>Trehalose</i>	106
5.2.6	<i>Cycloheximide treatment</i>	106
5.2.7	<i>Cold shock</i>	106
5.3	Discussion	106

CHAPTER 6: Influence of Growth Temperature on the Heat Shock Response in <i>Saccharomyces cerevisiae</i>.	117
6.1 Introduction	117
6.2 Experimental outline	118
6.3 Results	119
6.3.1 <i>Growth curves</i>	119
6.3.2 <i>Thermotolerance</i>	119
6.3.3 <i>Trehalose</i>	122
6.3.4 <i>Heat shock proteins</i>	125
6.4 Discussion	129
CHAPTER 7: Oxidative Stress Tolerance.	137
7.1 Introduction	137
7.2 Experimental outline	138
7.3 Results	139
7.3.1 <i>Peroxide stress tolerance</i>	139
7.3.1.1 <i>Antarctic yeast</i>	139
7.3.1.2 <i>Thermophilic yeast</i>	144
7.3.2 <i>Trehalose</i>	149
7.3.3 <i>Stress proteins</i>	149
7.3.3.1 <i>Antarctic yeast</i>	149
7.3.3.2 <i>Thermophilic yeast</i>	156
7.4 Discussion	156
CHAPTER 8: Oxidative Stress Response as Influenced by Temperature.	161
8.1 Introduction	161
8.2 Experimental outline	161

8.3	Results	163
8.3.1	<i>Peroxide stress tolerance in S. cerevisiae</i>	163
8.3.2	<i>Peroxide stress tolerance in psychrophilic and psychrotrophic yeast</i>	167
8.3.3	<i>Peroxide stress tolerance in thermophilic yeast</i>	172
8.4	Discussion	175
8.4.1	<i>Intrinsic peroxide stress tolerance</i>	175
8.4.2	<i>Induced peroxide stress tolerance</i>	179
	References	182
	Appendix	