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

By

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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.

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(Michelle Deegenaars)

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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 H_2O_2 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

- Deegenaars, M. L. and Watson, K. (1997). Stress proteins and stress tolerance in an Antarctic, psychrophilic yeast, *Candida psychrophila*. *FEMS Lett.* **151,** 191-196.
- Deegenaars, M. L. and Watson, K. (1998). Heat shock response in psychrophilic and psychrotrophic yeast from Antarctica. *Extremophiles* (in press).
- Deegenaars, M. L. and Watson, K. Heat shock and oxidative stress response in the thermophilic yeast, *Arxiozyma telluris*. (submitted).
- Deegenaars, M. L. and Watson, K. Heat shock response in yeast as influenced by growth temperature. (in preparation).
- Deegenaars, M. L. and Watson, K. Oxidative stress response in yeast as influenced by temperature. (in preparation).

Copies of the first two publications are presented in the Appendix.

Parts of this thesis presented at scientific meetings

- Deegenaars, 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.

- Deegenaars, 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 Deegenaars, M. L. (1996). Biochemical adaptations in yeast to stress. *Proc. 2nd Int. Conference on Predictive Microbiology*, Hobart. S2.4.

- Deegenaars, 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 Deegenaars, 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., Deegenaars, 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-(cyclohexlamino)propanesulfonic acid
cDNA:	complementary DNA
cfu ml^{-1}:	colony forming units per ml
cpn:	chaperonin
csp(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

OD_{600}: 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: phenylmethylsulphonylfluoride

psp(s): peroxide shock (inducible) protein(s)

PVDF: polyvinylidene difluoride

RNA: ribonucleic acid

RNase: ribonuclease

ROS: reactive oxygen species

rubisco: ribulose bisphosphate 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

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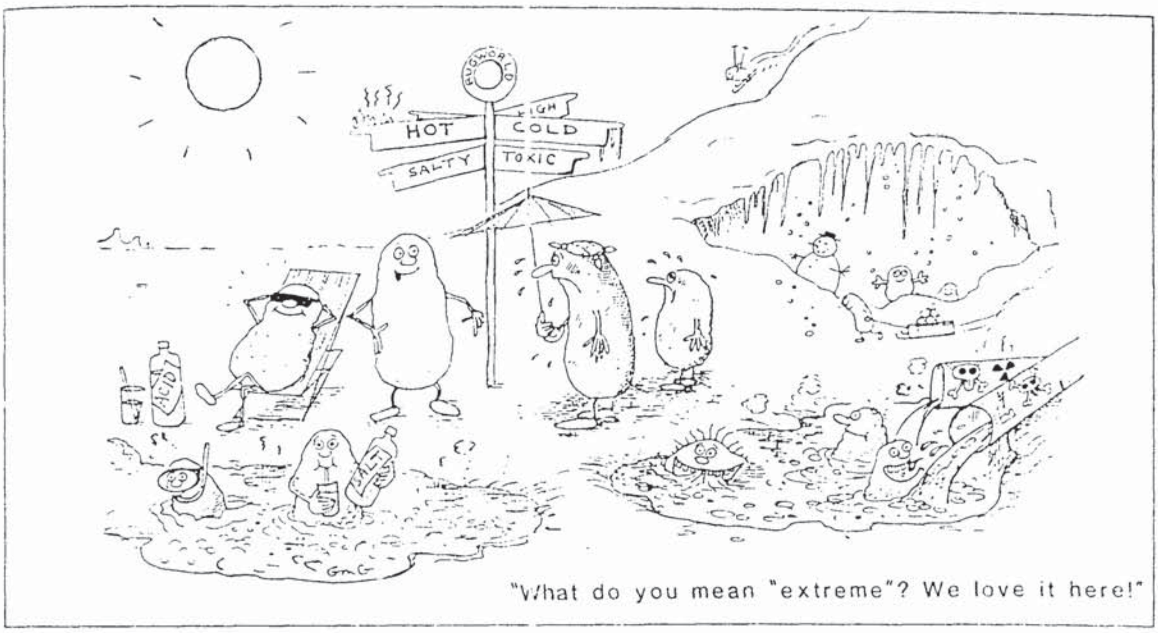
"The role of the infinitely small is infinitely large."

(Louis Pasteur)

(Cited from Aguilar, 1996)



"What do you mean "extreme"? We love it here!"

(From Herbert, 1992)