Phenotypic & Phylogenetic Analyses of New and Established Antarctic yeast

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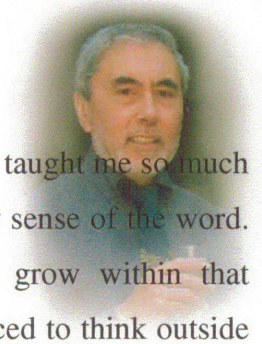
"The smaller the organism, the broader the frontier and the deeper the unmapped terrain."

Edward O Wilson in *The Diversity of Life*

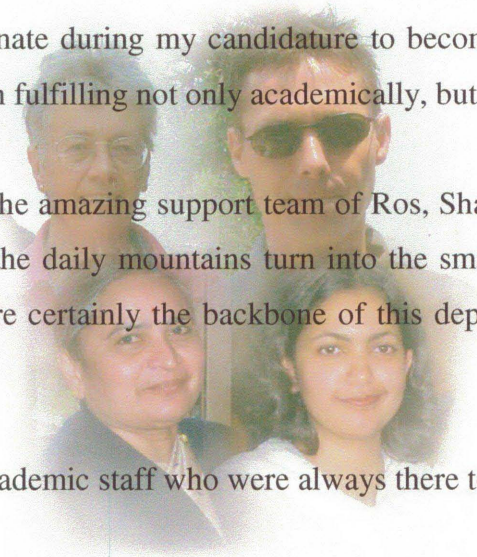
Acknowledgements



The final scene is set, let the credits role…..

Firstly, I would like to thank my supervisor, Prof. Ken Watson. You have taught me so much more than the results show in this thesis. You are truly a teacher in every sense of the word. You provided the framework for the research, then allowed me to grow within that framework developing the skills to analyse and question, often being forced to think outside the square, and finally, to have the confidence to draw conclusions. I'll never be able to thank you enough….

I have been fortunate during my candidature to become part of a family here at UNE. My time here has been fulfilling not only academically, but spiritually.



From the amazing support team of Ros, Sharon, Craig and Ruth, whose ability to make the daily mountains turn into the smallest of molehills are enviable skills. You are certainly the backbone of this department. My deepest gratitude to you all.

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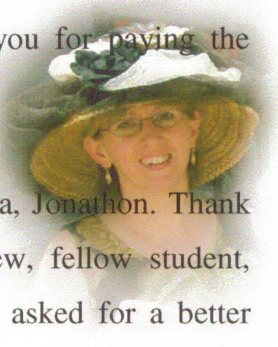

My fellow students, past and present; Skye, Shanchita, Visala, Jonathon. Thank you for making the lab a happy place to be. Linda Agnew, fellow student, colleague, and most importantly, friend. I could never have asked for a better roomy. I'll miss our daily vents but I know that we will be friends forever.

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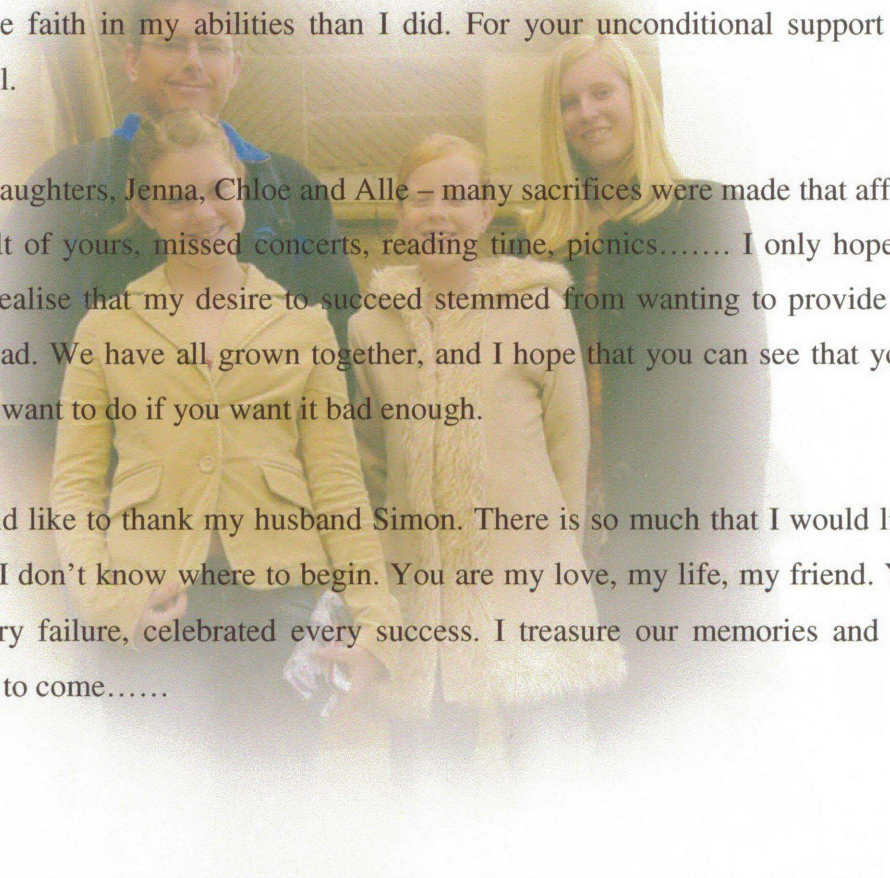

My beautiful daughters, Jenna, Chloe and Alle – many sacrifices were made that affected you though no fault of yours, missed concerts, reading time, picnics..... I only hope that you will come to realise that my desire to succeed stemmed from wanting to provide you with what I never had. We have all grown together, and I hope that you can see that you can do what-ever you want to do if you want it bad enough.

Finally, I would like to thank my husband Simon. There is so much that I would like to say about you but I don't know where to begin. You are my love, my life, my friend. Your love cushioned every failure, celebrated every success. I treasure our memories and await the adventures yet to come.....

Far better it is to dare mighty things, to win glorious triumphs even though checkered by failure, than to rank with those poor spirits who neither enjoy nor suffer much because they live in the gray twilight that knows neither victory nor defeat.

Theodore Roosevelt (1858 - 1919)

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.

30/8/05

Sharon Patricia Guffogg

Abstract

Yeasts isolated from the extreme environment of Antarctica, offer a unique opportunity to exploit characteristics not found in other microorganisms. The focus of this study was sixty yeast samples originating from the Vestfold Hills area located near the Australian Davis Base, Antarctica. Functional studies of these extremophiles using a combination of classical and molecular techniques provided a correlation analysis to be achieved verifying the placement of new species against positions of existing species. Isolates were screened using one-dimensional-sodium dodecyl sulphate polyacrylamide gel electrophoresis (1D-SDS-PAGE) of whole cell proteins and yeasts grouped together according to their protein profiles. This analysis reduced the study group to 39. Phylogenetic analysis of the sequences of the D1/D2 region and the internal transcribed spacers of the 26S ribosomal DNA determined relatedness among known yeast isolates. Sequence data revealed 2 unique species and a further 3 species that were closely related, but not identical, to established yeast species. Two isolates, shown to be indistinguishable through rDNA sequencing and protein analysis by 1D-SDS-PAGE, were described as *Cryptococcus watticus* sp. nov. [type culture = CBS 9496^{T}, NRRL Y-27556]. Sequence analyses of the D1/D2 region [26S] ribosomal DNA placed *Cr. watticus* in the Hymenomycetous yeasts in a cluster with *Holtermannia corniformis* and *Cr. nyarrowii*. This species has been allocated to the genus *Cryptococcus* on the basis of physiological and morphological characteristics. Sequence analyses placed five psychrophilic isolates (UNE116c designated type strain) in the Archiascomycete clade with *Leuconeurospora pulcherrima* and *Oosporidium margaritiferum*. Preliminary sequencing results using the 18S (small ribosomal subunit) region indicated that strain UNE116c was 99.8% similar with a black, Aureobasidium-like strain. This was highly unusual as UNE116c was orange in colour and did not produce any black meristematic cells at any stage. There were also 29 isolates that were identified as previously established basidiomycetous yeast species (*Cr. victoriae, Cr. nyarrowii, Cr. gilvescens, Cr. gastricus, Cr. sp. KCTC 17063, Leucosporidium antarcticum, Rhodotorula mucilaginosa, R. laryngis, R. minuta*) and ascomycetous yeast species (*Candida norvegica, C. parapsilosis, Debaryomyces hansenii*). Some of these yeasts have not previously been

isolated from Antarctica. The number of yeasts isolated from the Vestfold Hills thus far represented the most comprehensive biodiversity data available for Antarctic yeasts.

R. mucilaginosa, a highly pigmented red yeast, was shown to be extremely UVA-resistant and exhibited the classic heat shock response of acquired thermotolerance. Increased expression of hsp70 or hsp90 was not observed following a heat shock as determined by 1D-SDS-PAGE, however, increased expression of a smaller hsp at ~ 30 kDa was observed following UVA exposure. The coenzyme Q_8 ratio ($CoQH_2/CoQH_2$ + CoQ), a sensitive measure of cellular redox potential and oxidative stress, was measured during exposure of *R. mucilaginosa* cells to UVA. Preliminary results indicated a reductive response in cellular CoQ balance with increasing $CoQH_2$/total CoQ ratio on exposure to UVA radiation. The regulation of these processes to maintain high levels of the reduced form of coenzyme Q was a novel cellular response to UVA photooxidative stress not observed in any other Antarctic or mesophilic yeasts. As far as one was aware, this was the first report in eukaryotes of high levels of reduced CoQ in response to UVA-radiation.

The positive effects of coenzyme Q_{10} (CoQ_{10}) in compromised individuals with various forms of cardiovascular disease has been documented. The aim of the present study was to evaluate the effect of oral administration of CoQ_{10} (100 mg/day for fourteen days) in twelve healthy individuals (34 to 61 years) by measuring the expression of heat shock protein 70 (hsp70) in lymphocytes, and a number of oxidative stress parameters in plasma and erythrocytes. Expression of hsp70 in lymphocytes and total plasma antioxidant status did not alter following supplementation. Malondialdehyde (MDA), as a measure of lipid peroxidation, decreased following supplementation as did protein oxidation, as measured by protein carbonyl formation. Supplementation resulted in an increase in the rate of erythrocyte haemolysis when challenged with the free radical generator AAPH (2,2'-azobis-(2-amidinopropane) dihydrochloride). The results from this small-scale clinical trial indicated an improvement in plasma antioxidant defences in healthy individuals but a prooxidative effect against free radical induced erythrocyte haemolysis following oral supplementation with CoQ_{10}.

Publications arising from this thesis

S. Guffogg, S. Thomas-Hall, P. Holloway & K. Watson (2004). **A novel psychrotolerant member of the hymenomycetous yeasts from Antarctica: *Cryptococcus watticus* sp. nov.** *International Journal of Systematic and Evolutionary Microbiology* 54: 275-277

Tsimako M, Guffogg S, Thomas-Hall S, Watson K. (2002). **Resistance to UVB radiation in Antarctic yeasts.** *Redox Report* 7(5): 312-4

Guffogg, S. Khan. S, Bowman. J, Dunlap, W & Watson, K (2005). ***Rhodotorula mucilaginosa* isolated from Antarctica is highly resistant to UVA-inducible redox response**. In preparation.

Guffogg, S. Thomas-Hall, S & Watson, K (2005). **The biodiversity of Antarctic yeast**. In preparation.

Guffogg, S. Agnew, L. Oliver, C & Watson, K (2005). **Dietary intake of ubiquinone (CoQ10) does not protect erythrocytes from oxidative damage in *in vivo* studies**. In preparation.

Khan, S. Guffogg, S & Watson, K (2005). **Yeasts isolated from Antarctica exhibit high resistance to UVA, UVB & UVC radiation**. In preparation.

Copy of papers appear in appendix III

Aspects of thesis presented at conference proceedings

Guffogg, S. Khan, S. Dunlap, W. and Watson, K. Antarctic yeast: How to stay cool when the heat is on. Presented at the *Canadian Society for Microbiology Annual Scientific Meeting*, Edmonton Alberta, Canada, 20 – 23 June 2004. Poster.

Guffogg, S. Khan, S. Dunlap, W. and Watson, K. Stress response in Novel Antarctic Yeasts. Presented at the *Yeasts: Products and Discovery Conference*, Barossa Valley SA, 4 – 6 April 2004. Poster and presentation.

Guffogg, S. Thomas-Hall, S. Tsimago, M. Holloway, P and Watson, K. Antarctic yeasts: phenotypic and phylogenetic analysis. Presented at the *Australian Society for Microbiology Annual Scientific Meeting*, Melbourne, 29 September – 3 October 2002. Poster.

Khan, S. Guffogg, S. Tsimako, M. and Watson, K. UVB sensitivity of mesophilic yeasts and psychrophilic Antarctic yeasts at different stages of growth: effects of pigmentation and glutathione. Presented at the *Yeast: Products and Discovery Conference*, Melbourne, 27 – 29 November 2002. Poster.

Tsimako, M. Guffogg, S. Thomas-Hall, S. and Watson, K. Resistance to UVB-irradiation in Antarctic yeasts. Presented at the *9th Annual Meeting of the Society for Free Radical Research [Australia & Japan], Redox Processes in Chemistry, Biology & Medicine*. Sydney, 30 November – 4 December 2001. poster.

Thomas-Hall, S. Guffogg, S. Fell, J. and Watson, K. A comprehensive molecular study of the basidiomycetes incorporating new Antarctic yeasts. Presented at the *Yeast: Products and Discovery Conference,* South Stradbroke Island, 29 June – 1 July 2000. Poster.

Copy of posters and abstracts appear in appendix II

Abbreviations

1-D	One dimensional
AAPH	2,2'-azobis-(2-amidinopropane) dihydrochloride
AMPS	Ammonium peroxidisulphate
bisacrylamide	N,N'-methylenebisacrylamide
bp	Base pairs
CBS	Centraalbureau voor Schimmelcultures
CHD	Chronic heart disease
CoQ	Coenzyme Q
CRC	Cooperative Research Centre
DBB	Diazonium Blue B
DNA	Deoxyribonucleic acid
DNPH	2,4-dinitrophenylhydrazine
DTT	Dithiothreitol
EDTA	Ethylene diamine tetraacetic acid
hsp	Heat shock protein
kDa	Kilodaltons
IGS	Intragenic spacer
ITS	Internal transcribed spacer
MAAs	Micosporine-like amino acids
MDA	Malondialdehyde
NQR	NAD(P)H: quinone oxidoreductase
nt	Nucleotide
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PBS-T	PBS-Tween 20
PCR	Polymerase chain reaction
PMSF	Phenyl methyl sulphonyl floride
PUFA	Polyunsaturated fatty acids
rDNA	Ribosomal deoxyribonucleic acid

RAPD	Randomly amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SDS	Sodium dodecyl sulphate
TBARS	Thiobarbituric acid reactive substances
TEMED	N,N,N',N'-tetramethylene diamine
TCA	Trichloroacetic acid
Tris	Tris-[hydroxymethyl] aminomethane
UV	Ultraviolet
UVA	Ultraviolet A
UVB	Ultraviolet B
UVC	Ultraviolet C
UVR	Ultraviolet radiation
YEP	Yeast extract peptone
YNB	Yeast nitrogen base

Table of Contents

List of Figures

List of Tables