

# Phenotypic & Phylogenetic Analyses of New and Established Antarctic yeast

*Sharon Patricia Guffogg*

BSc (Biomed. Sci.),  
BSc 1<sup>st</sup> Class Honours (Microbiology)  
University of New England  
Armidale, Australia

*A thesis submitted for the degree of Doctor of Philosophy from the University of  
New England, Armidale, Australia*

2005

*“The smaller the organism, the broader the frontier and the deeper the unmapped terrain.”*

Edward O Wilson in *The Diversity of Life*

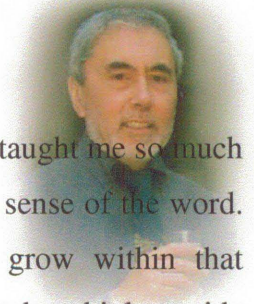
---

# 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.

The academic staff who were always there to listen and offer advice, thank you.

The administration staff, Annette, Frank and Chris, thank you for paying the bills...

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.



This work would not have been possible if it wasn't for the Antarctic CRC team at the University of Tasmania. Thank you to Drs David Nichols and Kevin Sanderson for allowing

---

not only access to the Antarctic samples, but full use of their lab. Also, thanks to Drs Paul Holloway and John Bowman for sequencing of the Antarctic yeasts. Both of my trips to Hobart were wonderful experiences thanks to the generosity of these researchers.

I was fortunate during my candidature to spend two weeks at the Australian Institute of Marine Science in Townsville, Qld. The hospitality of my host, Dr Walter Dunlap was unsurpassed. The opportunity to work in such a place was certainly rewarding.

A special thank you to a special friend – Victoria Dunne. Your enthusiasm and interest [real or otherwise] meant that I always had someone on the outside I could bounce ideas off. You often had more faith in my abilities than I did. For your unconditional support I will be forever grateful.

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 waticus* sp. nov. [type culture = CBS 9496<sup>T</sup>, NRRL Y-27556]. Sequence analyses of the D1/D2 region [26S] ribosomal DNA placed *Cr. waticus* 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 margaritifera*. 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 mucilaginoso*, *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<sub>8</sub> ratio (CoQH<sub>2</sub>/CoQH<sub>2</sub> + 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<sub>2</sub>/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<sub>10</sub> (CoQ<sub>10</sub>) 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<sub>10</sub> (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<sub>10</sub>.

## 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 waticus* 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 *9<sup>th</sup> 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

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

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

# Table of Contents

DECLARATION .....	1
<b>ABSTRACT .....</b>	<b>2</b>
PUBLICATIONS ARISING FROM THIS THESIS .....	4
ASPECTS OF THESIS PRESENTED AT CONFERENCE PROCEEDINGS .....	5
ABBREVIATIONS .....	6
TABLE OF CONTENTS .....	8
LIST OF FIGURES .....	13
LIST OF TABLES .....	15
<b><u>CHAPTER 1: INTRODUCTION</u> .....</b>	<b>16</b>
THE ORIGINS OF TAXONOMY .....	16
<i>Kingdom Fungi</i> .....	18
Definition and classification of Fungi .....	19
Ascomycota .....	20
Basidiomycota .....	21
Yeasts .....	22
<i>Fungal Taxonomy</i> .....	25
Nomenclature .....	26
Identification & classification methods .....	27
Morphology .....	27
Physiological and biochemical techniques .....	28
Fatty acid composition .....	28
Cell wall composition .....	29
Protein composition .....	29
Coenzyme Q systems .....	30
Molecular techniques .....	31
Ribosomal complex .....	32
5S rDNA .....	33
18S rDNA .....	33
26S rDNA .....	33
<i>Phylogenetic analysis</i> .....	34
ANTARCTICA .....	37
<i>The Vestfold Hills</i> .....	40
Ultraviolet light .....	41
Free radical damage .....	41
Antioxidants .....	43

---

<i>Effect of UVR on Antarctic organisms</i> .....	44
Carotenoids and UV .....	45
<i>Heat shock response</i> .....	47
Heat shock response to UV .....	50
THESIS AIMS .....	51

## **CHAPTER 2: MATERIALS AND METHODS.....53**

MATERIALS .....	53
<i>Chemicals</i> .....	53
<i>Media</i> .....	55
General.....	55
Solutions .....	56
METHODS .....	57
<i>Yeast maintenance</i> .....	57
<i>Yeast cultures</i> .....	57
<i>Protein Analysis</i> .....	57
Protein extraction.....	57
Protein assay .....	58
<sup>35</sup> S-methionine labelling of proteins in yeast .....	58
SDS-polyacrylamide gel electrophoresis.....	58
SDS-polyacrylamide gel silver staining .....	59
SDS-polyacrylamide gel Fast Blue staining .....	59
Polyacrylamide gel drying.....	60
Autoradiography.....	60
<i>Fatty acid analysis</i> .....	60
Fatty acid extraction .....	60
Fatty acid Analysis – Gas chromatography.....	61
<i>rDNA Sequencing</i> .....	61
DNA extraction .....	61
Phylogenetic analysis .....	62
<i>Assimilation Tests</i> .....	62
Procedure .....	62
<i>Diazonium Blue B (DBB) test</i> .....	64
<i>Colony photographs and micrographs</i> .....	64
<i>Ubiquinone analysis</i> .....	64
Ubiquinone extraction .....	64
Ubiquinone analysis – High performance liquid chromatography (HPLC).....	65
NAD(P)H: quinone oxidoreductase activity .....	65
<i>Stress response</i> .....	65
Thermotolerance.....	65
UV-irradiation .....	66
<i>Statistical analysis</i> .....	66

### **CHAPTER 3: THE BASIDIOMYCETES..... 67**

INTRODUCTION .....	67
<i>Urediniomycetes</i> .....	67
<i>Ustilaginomycetes</i> .....	68
<i>Hymenomycetes</i> .....	68
CRYPTOCOCCUS SPECIES .....	78
<i>Cryptococcus walticus</i> sp. nov.....	78
<i>Cryptococcus nyarrowii</i> .....	86
<i>Cryptococcus gilvescens</i> & <i>Cr. gastricus</i> .....	89
<i>Cryptococcus victoriae</i> .....	94
RHODOTORULA SPECIES .....	98
<i>Rhodotorula mucilaginosa</i> .....	98

### **CHAPTER 4: THE ASCOMYCETES..... 101**

INTRODUCTION .....	101
<i>Archiascomycetes</i> .....	101
<i>Euascomycetes</i> .....	102
<i>Hemiascomycetes</i> .....	102
CANDIDA SPECIES.....	106
<i>Candida parapsilosis</i> .....	106
<i>Candida norvegica</i> .....	110
<i>Debaryomyces hansenii</i> .....	113
UNE116c .....	116

### **CHAPTER 5: THE BIODIVERSITY OF ANTARCTIC YEASTS ..... 119**

INTRODUCTION .....	119
THE ANTARCTIC NICHE.....	122
SIGNIFICANCE OF MICROBIAL DIVERSITY .....	124
<i>Biodiversity as a bioresource</i> .....	127
YEASTS IN THE ANTARCTIC .....	128
<i>Yeast biodiversity in the Vestfold Hills</i> .....	129
<i>Environmental impact on yeast biodiversity</i> .....	137
CONCLUSION .....	139

### **CHAPTER 6: STRESS RESPONSE OF RHODOTORULA MUCILAGINOSA ..... 141**

INTRODUCTION .....	141
LIFE UNDER STRESS .....	142
<i>Heat shock proteins &amp; thermotolerance</i> .....	142
<i>Ubiquinone</i> .....	145

<i>Fatty acid composition</i> .....	146
RESULTS .....	148
<i>Molecular &amp; physiological analyses</i> .....	148
<i>Stress tolerance</i> .....	150
DISCUSSION .....	162
<i>Phylogenetic &amp; physiological analysis</i> .....	162
<i>Lipid analysis</i> .....	162
<i>Expression of hsp's following heat and UVA stress</i> .....	163
<i>UVR response</i> .....	164
Coenzyme Q redox balance following UVA.....	165

## **CHAPTER 7: EFFECTS OF DIETARY INTAKE OF COENZYME Q<sub>10</sub> ON HEALTHY INDIVIDUALS.....167**

INTRODUCTION .....	167
<i>The role of Coenzyme Q</i> .....	167
METHODS .....	171
<i>Study population</i> .....	171
<i>Lymphocyte extraction</i> .....	171
<sup>35</sup> S-methionine labelling of proteins.....	172
Stress response.....	172
Protein extraction.....	172
Protein assay .....	172
SDS-polyacrylamide gel electrophoresis.....	172
Autoradiography .....	173
<i>Western immunoblot</i> .....	173
Detection of antibody .....	173
<i>Enzyme-linked immunosorbent assay [ELISA]</i> .....	174
<i>Erythrocyte haemolysis</i> .....	174
<i>Protein carbonyl assay</i> .....	175
<i>Lipid peroxidation</i> .....	175
<i>Total antioxidant status</i> .....	176
RESULTS .....	177
<i>Erythrocyte haemolysis and CoQ<sub>10</sub> supplementation</i> .....	177
<i>Heat shock protein synthesis in lymphocytes</i> .....	177
<i>Western immunoblot</i> .....	185
<i>The effect of CoQ<sub>10</sub> on oxidative stress parameters in plasma</i> .....	187
DISCUSSION .....	189
<i>The effect of CoQ<sub>10</sub> supplementation on erythrocyte haemolysis</i> .....	191
<i>Expression of heat shock protein synthesis in lymphocytes</i> .....	193

## **CHAPTER 8: SUMMARY & FUTURE DIRECTIONS .....196**

YEAST TAXONOMY.....	196
<i>Potential commercial applications of Antarctic yeasts</i> .....	197
<i>Yeast biodiversity</i> .....	198

<i>RHODOTORULA MUCILAGINOSA</i> .....	199
COENZYME Q .....	200
REFERENCES.....	202
APPENDIX I.....	236
APPENDIX II .....	237
APPENDIX III .....	243



# List of Figures

<b>FIGURE 1.1</b>	Phylogenetic tree showing evolutionary distances among the three domains, <i>Archaea</i> , <i>Bacteria</i> and <i>Eucarya</i> .....	16
<b>FIGURE 1.2</b>	Unrooted phylogenetic tree showing the relative positions of fungal clades.....	17
<b>FIGURE 1.3</b>	Diagrammatic representation of coenzyme Q (ubiquinone) where <i>n</i> = number of side chain isoprenoid.....	29
<b>FIGURE 1.4</b>	Arrangement of ribosomal genes in yeast.....	31
<b>FIGURE 1.5</b>	Map of Antarctica.....	39
<b>FIGURE 1.6</b>	Scheme showing penetration of the ozone layer by UVR.....	40
<b>FIGURE 1.7</b>	Schematic overview of cellular oxidative stress.....	42
<b>FIGURE 1.8</b>	Chemical structures of astaxanthin, lycopene, zeaxanthin and $\beta$ -carotene.....	45
<b>FIGURE 3.1</b>	Classification of the Basidiomycetes.....	69
<b>FIGURE 3.2</b>	<i>Cryptococcus watticus</i> consensus tree of the D1/D2 region.....	80
<b>FIGURE 3.3</b>	<i>Cryptococcus watticus</i> consensus tree of the ITS region.....	81
<b>FIGURE 3.4</b>	Fatty acid profiles for <i>Cr. watticus</i> strains.....	82
<b>FIGURE 3.5</b>	Colony and cell formation of <i>Cr. watticus</i> .....	82
<b>FIGURE 3.6</b>	1D-SDS-PAGE of Basidiomycetous yeast isolates.....	83
<b>FIGURE 3.7</b>	1D-SDS-PAGE of Basidiomycetous yeast isolates.....	84
<b>FIGURE 3.8</b>	Colony formation of <i>Cr. nyarrowii</i> .....	86
<b>FIGURE 3.9</b>	Fatty acid profiles for <i>Cr. nyarrowii</i> strains.....	87
<b>FIGURE 3.10</b>	<i>Cr. gilvescens</i> and <i>Cr. gastricus</i> consensus tree of the D1/D2 region.....	90
<b>FIGURE 3.11</b>	<i>Cr. gilvescens</i> and <i>Cr. gastricus</i> consensus tree of the ITS region.....	91
<b>FIGURE 3.12</b>	Fatty acid profiles for <i>Cr. gilvescens</i> and <i>Cr. gastricus</i> .....	92
<b>FIGURE 3.13</b>	Colony formation of <i>Cr. gilvescens</i> .....	92
<b>FIGURE 3.14</b>	Fatty acid profiles for <i>Cr. victoriae</i> .....	94
<b>FIGURE 3.15</b>	Colony formation of <i>Cr. victoriae</i> .....	94
<b>FIGURE 3.16</b>	<i>Cryptococcus victoriae</i> consensus tree of the D1/D2 region.....	95
<b>FIGURE 3.17</b>	<i>Cryptococcus victoriae</i> consensus tree of the ITS region.....	96
<b>FIGURE 3.18</b>	<i>Rhodotorula mucilaginosa</i> consensus tree of the D1/D2 region.....	98
<b>FIGURE 3.19</b>	<i>Rhodotorula mucilaginosa</i> consensus tree of the ITS region.....	99
<b>FIGURE 4.1</b>	Classification of the Ascomycetes.....	102
<b>FIGURE 4.2</b>	Colony and cell formation of <i>Candida parapsilosis</i> .....	107
<b>FIGURE 4.3</b>	Fatty acid profiles for <i>C. parapsilosis</i> .....	107
<b>FIGURE 4.4</b>	Fatty acid profile for <i>C. norvegica</i> .....	107
<b>FIGURE 4.5</b>	<i>Candida parapsilosis</i> consensus tree of the D1/D2 region.....	108
<b>FIGURE 4.6</b>	<i>Candida norvegica</i> consensus tree of the D1/D2 region.....	110
<b>FIGURE 4.7</b>	1D-SDS-PAGE of Ascomycetous yeast isolates.....	111
<b>FIGURE 4.8</b>	Fatty acid profile for <i>Debaryomyces hansenii</i> .....	113
<b>FIGURE 4.9</b>	Colony formation of <i>D. hansenii</i> .....	113
<b>FIGURE 4.10</b>	<i>D. hansenii</i> consensus tree of the D1/D2 region.....	114
<b>FIGURE 4.11</b>	Fatty acid profile for UNE116c.....	116
<b>FIGURE 4.12</b>	Colony and cell formation of UNE116c.....	116
<b>FIGURE 4.13</b>	UNE116c consensus tree of the D1/D2 region.....	117
<b>FIGURE 5.1</b>	Species diversity.....	119
<b>FIGURE 5.2</b>	Colony formation of various species on agar.....	120
<b>FIGURE 5.3</b>	Map of territorial claims of Antarctica.....	124
<b>FIGURE 5.4</b>	Map of the Vestfold Hills, Davis Base, Antarctica.....	131
<b>FIGURE 6.1</b>	Physiological factors that activate hsp70 within a cell.....	141

<b>FIGURE 6.2</b>	Ubiquinone to ubiquinol chemical reaction.....	145
<b>FIGURE 6.3</b>	Structure of saturated, monosaturated and polyunsaturated fatty acids.....	146
<b>FIGURE 6.4</b>	<i>Rhodotorula mucilaginosa</i> consensus tree of the D1/D2 region.....	148
<b>FIGURE 6.5</b>	Fatty acid profile of <i>R. mucilaginosa</i> .....	149
<b>FIGURE 6.6</b>	Induced thermotolerance of <i>R. mucilaginosa</i> .....	149
<b>FIGURE 6.7</b>	Colonies of <i>R. mucilaginosa</i> following UVC radiation.....	151
<b>FIGURE 6.8a</b>	Colonies of <i>R. mucilaginosa</i> following UVA radiation.....	151
<b>FIGURE 6.8b</b>	Colonies of <i>S. cerevisiae</i> following UVA radiation.....	151
<b>FIGURE 6.9</b>	Cytochrome difference spectra of <i>R. mucilaginosa</i> following UVR.....	152
<b>FIGURE 6.10</b>	Cytochrome difference spectra of <i>S. cerevisiae</i> following UVR.....	152
<b>FIGURE 6.11</b>	Autoradiograph of labelled proteins following UVA.....	153
<b>FIGURE 6.12</b>	Autoradiograph of labelled proteins following heat shock.....	154
<b>FIGURE 6.13</b>	CoQ redox balance in <i>R. mucilaginosa</i> during UVA.....	157
<b>FIGURE 6.14</b>	CoQ <sub>8</sub> analysis of <i>Mrakia gelida</i> .....	158
<b>FIGURE 6.15</b>	CoQ <sub>9</sub> analysis of <i>Cr. nyarrowii</i> (UNE180e).....	158
<b>FIGURE 6.16</b>	CoQ <sub>10</sub> analysis of <i>Cr. nyarrowii</i> (UNE64a).....	159
<b>FIGURE 6.17</b>	CoQ analysis of UNE116c.....	159
<b>FIGURE 6.18</b>	Reductive response in cellular CoQ balance in <i>R. mucilaginosa</i> during UVA.....	160
<b>FIGURE 6.19</b>	NAD(P)H: quinone oxidoreductase reaction in <i>R. mucilaginosa</i> during UVA.....	160
<b>FIGURE 7.1</b>	Chemical structure of coenzyme Q.....	167
<b>FIGURE 7.2</b>	Location of coenzyme Q in the inner mitochondrial membrane.....	168
<b>FIGURE 7.3</b>	Principle of the Calbiochem Total Antioxidant Status Assay.....	175
<b>FIGURE 7.4</b>	Mean erythrocyte haemolysis in males following CoQ <sub>10</sub> .....	177
<b>FIGURE 7.5</b>	Mean erythrocyte haemolysis in females following CoQ <sub>10</sub> .....	177
<b>FIGURE 7.6</b>	Effect of heat shock on induction of hsps in lymphocytes - females, baseline.....	178
<b>FIGURE 7.7</b>	Effect of heat shock on induction of hsps in lymphocytes - females, supplemented .....	179
<b>FIGURE 7.8</b>	Effect of heat shock on induction of hsps in lymphocytes - females, wash-out.....	180
<b>FIGURE 7.9</b>	Effect of heat shock on induction of hsps in lymphocytes - males, baseline.....	181
<b>FIGURE 7.10</b>	Effect of heat shock on induction of hsps in lymphocytes - males, supplemented.....	182
<b>FIGURE 7.11</b>	Effect of heat shock on induction of hsps in lymphocytes - males, wash-out.....	183
<b>FIGURE 7.12</b>	Western immunoblot for hsp70 – male and females, baseline and supplemented..	184
<b>FIGURE 7.13</b>	Malondialdehyde concentration in males – baseline, supplemented and wash-out.....	187
<b>FIGURE 7.14</b>	Malondialdehyde concentration in females – baseline, supplemented and wash-out .....	187
<b>FIGURE 7.17</b>	Reduction of ubiquinone to ubiquinol via a free radical intermediate.....	191

## List of Tables

<b>TABLE 1.1</b>	Comparison of classification schemes.....	17
<b>TABLE 1.2</b>	Classification of Fungi.....	24
<b>TABLE 1.3</b>	Conditions that induce heat shock proteins.....	48
<b>TABLE 2.1</b>	Bio-Rad molecular weight protein standards.....	58
<b>TABLE 2.2</b>	Biochemical tests used in yeast classification.....	62
<b>TABLE 3.1</b>	Basidiomycetous yeasts phylogenetically analysed.....	75
<b>TABLE 3.2</b>	UNE Basidiomycetous yeast isolates phylogenetically analysed.....	76
<b>TABLE 4.1</b>	Ascomycetous yeasts phylogenetically analysed.....	104
<b>TABLE 5.1</b>	Indigenous Antarctic yeast species.....	134
<b>TABLE 5.2</b>	Antarctic yeast species found in other locations.....	135
<b>TABLE 6.1</b>	Location and function of representative yeast hsps.....	142
<b>TABLE 6.2</b>	Ubiquinol/ubiquinone redox status in Antarctic yeast.....	156
<b>TABLE 7.1</b>	Fold increase of hsp70 Western blot following CoQ <sub>10</sub> supplementation....	185
<b>TABLE 7.2</b>	Oxidative stress parameters following CoQ <sub>10</sub> supplementation.....	185