Appendix I

The following is a copy of the original data accompanying the samples

Bar code:	Antarctic CRC code number
Location:	Where the sample was taken from
Date:	Date of collection
Comments:	Brief description

100107397S	Lichen Valley	05-Dec-97	011937	Snow petrel carnage	
100107497S	Lichen Valley	05-Dec-97	011937	Soil	
100107597S	Lichen Lake	05-Dec-97	017939	Soil	
100107697S	Lichen Lake	05-Dec-97	017939	Soil	
100107797S	Moss Cirque	09-Dec-97	027933	Moss, soil, lichen	
100107897S	Marine Plain	01-Dec-97	860826	Soil	
100107997S	Marine PLain	01-Dec-97	861827	Soil underneath pink quartz rock	S A/F
100108097S	Marine Plain	01-Dec-97	862829	Soil	
100108197S	Gardner Island	19-Dec-97	898726		
100108297S	Abraxas Lake	21-Dec-97	998891		
100108397S	Lichen Valley	05-Dec-97	014944	Soil, lichen	
100108497S	Lichen Valley	05-Dec-97	014944	Soil, lichen	
100108597S	Lichen Valley	05-Dec-97	014945	Soil, lichen	
100108697S	Lichen Valley	05-Dec-97	014945	Soil	
100108797S	Lichen Valley	05-Dec-97	014945	Soil	
100108897S	Lichen Valley	05-Dec-97	014945	Soil, lichen	
100108997S	Ekho Lake	22-Dec-97	963893		
100109097S	Moss Cirque	09-Dec-97	026933	soil, guano	
100109397S	Moss Cirque	09-Dec-97	026935	Soil	
100109497S	Moss Cirque	09-Dec-97	025937	Soil	
100109597S	Moss Cirque	09-Dec-97	025937	Lichen growing on snow petrel carcass	
100109697S	Gardner Island	19-Dec-97	898726	Soil	
100109797S	Moss Cirque	09-Dec-97	028933	Soil	
100109897S	Moss Cirque	09-Dec-97	028930	Soil	
100109997S	Moss Cirque	09-Dec-97	025934	Soil	
100110097S	Moss Cirque	09-Dec-97	025937	Soil	
100110197S	Moss Cirque	09-Dec-97	025935	Soil	
100110297S	Moss Cirque	09-Dec-97	026936	Soil	
100110397S	Moss Cirque	09-Dec-97	024936	Soil	
100110497S	Ekho Lake	22-Dec-97	963893		
100110597S	Pointed Lake	22-Dec-97	964897		
100110697S	Abraxas Lake	21-Dec-97	998891		
100110797S	Abraxas Lake	21-Dec-97	998891		
100110897S	Abraxas Lake	21-Dec-97	998891		
100110997S	Moss Cirque	09-Dec-97	027933	Moss, soil, lichen	
100111097S	Marine Plain	01-Dec-97	856819	Soil,lichen	
100111197S	Tryne Island	18-Nov-97	130935	penguin guano	S A/F
100111297S	Lucas Island	18-Nov-97	976755	Soil, penguin guano	S A/F
100111397S	Marine Plain	01-Dec-97	850831	Soil underneath orange quartz	
100111497S	Marine Plain	01-Dec-97	853831	Soil	
100111597S	Tierney Falls	17-Nov-97	833907	Soil	
100111697S	Moss Cirque	09-Dec-97	027932	Lichen, soil	
100111797S	Marine Plain	01-Dec-97	859819	Soil	
100111897S	Abraxas Lake	21-Dec-97	998891		
100111997S	Ekho Lake	22-Dec-97	963893		
100112097S	Ekho Lake	22-Dec-97	963893		
100112197S	Marine Plain	01-Dec-97	848828	Soil	
100112297S	Marine Plain	01-Dec-97	847828	Soil	
100112397S	Marine Plain	01-Dec-97	855821	Soil	
100112497S	Marine Plain	01-Dec-97	855822	Soil	
100112597S	Marine Plain	01-Dec-97	849831	Soil	
100112697S	Marine Plain	01-Dec-97	850830	Soil	
100112797S	Marine Plain	01-Dec-97	855829	Soil	
100112897S	Marine Plain	01-Dec-97	851831	Soil	
100112997S	Grace Lake	18-Nov-97	082954	Benthic algal mat and soil. Obtained from pond near lake	
100113097S	Moss Cirque	09-Dec-97	027933	Soil, benthic, algal mat	
100113197S	Moss Cirque	09-Dec-97	027933	Soil	
100113297S	Moss Cirque	09-Dec-97	027933	soil, lichen	
100113397S	Lichen Valley	05-Dec-97	011937	Soil, lichen	
100113497S	Marine Plain	01-Dec-97	857820	Soil,lichen	
100113597S	Marine Plain	01-Dec-97	849829	Soil	
100113697S	Marine Plain	01-Dec-97	849829	Soil underneath rose quartz	
100113797S	Mossell Lake	17-Nov-97	835902	Soil	
100113897S	Mossell Lake	17-Nov-97	835902	Soil, lichen	
100113997S	Mossell Lake	17-Nov-97	835902	Soil, lichen	
100114097S	Mossell Lake	17-Nov-97	835902	Soil	
100114197S	Mossell Lake	17-Nov-97	835902	Soil, lichen	
100114297S	Mossell Lake	17-Nov-97	835902	Soil	
100114397S	Chelnok Lake	17-Nov-97	828917	Benthic mat stuck on ice top	S A/F

100114597S	Lichen Valley	05-Dec-97	014942	Soil	
100114697S	Lichen Valley	05-Dec-97	014940	Dried algal mat	
100114797S	Mossell Lake	17-Nov-97	835902	Lichen/soil	S A/F
100114897S	Mossell Lake	17-Nov-97	835902	Soil, lichen	
100114997S	Mossell Lake	17-Nov-97	835902	Soil, lichen	
100115097S	Mossell Lake	17-Nov-97	835902	Soil, lichen	
100115197S	Mossell Lake	17-Nov-97	835902	Lichen	
100115297S	Mossell Lake	17-Nov-97	835902	Soil	
100115397S	Mossell Lake	17-Nov-97	835902		
100115497S	Mossell Lake	17-Nov-97	835902	Lichen	
100115597S	Mossell Lake	17-Nov-97	835902	Soil, lichen	
100115697S	Marine Plain	01-Dec-97	859824	Soil	
100115797S	Lichen Valley	05-Dec-97	014940	Dried algal mat	S A/F
100115897S	Lichen Valley	05-Dec-97	014942	Soil, lichen	
100115997S	Lichen Valley	05-Dec-97	014942	Soil	
100116097S	Lichen Valley	05-Dec-97	014942	Soil, lichen	
100116197S	Lichen Valley	05-Dec-97	014950	Soil	S A/F
100116297S	Moss Cirque	09-Dec-97	024935	Soil	
100116397S	Moss Cirque	09-Dec-97	026935	Soil, lichen	
100116497S	Moss Cirque	09-Dec-97	024935	Soil	
100116597S	Lichen Valley	05-Dec-97	014943	Stromatilite/soil	S A/F
100116697S	Tryne Island	18-Nov-97	130935	Adelie penguin guano	
100116797S	Mossell Lake	17-Nov-97	835902	Soil	
100116897S	Mossell Lake	17-Nov-97	835902	Lichen/Soil	
100116997S	Moss Cirque	09-Dec-97	026934	Soil, moss, lichen	
100117097S	Moss Cirque	09-Dec-97	026935	Soil, lichen	
100117197S	Moss Cirque	09-Dec-97	026935	Soil	
100117297S	Moss Cirque	09-Dec-97	027932	Lichen, soil	
100117397S	Lichen Valley	05-Dec-97	013947	Soil	
100117497S	Moss Cirque	09-Dec-97	028930	Soil	
100117597S	Lichen Valley	05-Dec-97	013949	Soil	
100117697S	Lichen Valley	05-Dec-97	013948	Soil	
100117797S	Moss Cirque	09-Dec-97	028929	Soil	
100117897S	Moss Cirque	09-Dec-97	028930	Soil	
100117997S	Moss Cirque	09-Dec-97	028931	Soil	
100118097S	Moss Cirque	09-Dec-97	026933	Soil	
100118197S	Lichen Valley	05-Dec-97	014941	Wet soil	
100118297S	Moss Cirque	09-Dec-97	026934	Soil	
100118397S	Lichen Valley	05-Dec-97	011937	Soil	
100118497S	Moss Cirque	09-Dec-97	026933	Soil	
100118697S	Moss Cirque	09-Dec-97	028929	Soil	
100118797S	Lake Fletcher	21-Dec-97	045874	Soil	
100118897S	Lake Fletcher	21-Dec-97	045875	Soil	
100118997S	Lake Fletcher	21-Dec-97	044873	Soil	
100119097S	Lake Fletcher	21-Dec-97	042877	Algal Mat on top of Ice	
100119197S	Lake Fletcher	21-Dec-97	045875	Lichen/Soil	
100119497S	Gardner Island	19-Dec-97	898726		
100119697S	Gardner Island	19-Dec-97	898726		
	Lake Dingle	30-Dec-97	912798	Old Shoreline	
	Lake Stinear	30-Dec-97	921831		
	Lake Dingle	30-Dec-97	913799		
	Lake Stinear	30-Dec-97	921831	20 m above water level	
	Brookes Hut	30-Dec-97	939850		
	Brookes Hut	30-Dec-97	934844		
	Brookes Hut	30-Dec-97	941853		
	Brookes Hut	30-Dec-97	941853	Brackish puddle behind Brookes Hut	
	Davis Station	25-Dec-97	897764	Toilet Block	
	Davis Station	25-Dec-97	897764	Ops Building	
	Davis Station	25-Dec-97	897764	Met Office	
	Davis Station	25-Dec-97	897764	SMQ	
	Davis Station	25-Dec-97	897764	Smokers Hut	
	Davis Station	25-Dec-97	897764	Science Lab	
	Davis Station	25-Dec-97	897764	Dieso Workshop	
	Wilkes Station	07-Jan-98		Refer Casey Map	
	Lake Nella	31-Dec-97		Larsemann Hills 69• 23.4 S 76• 22.4E	

New Soil Samples

25/02/98

Bar Code	Location	Date	Grid Ref	Comments	Examined	
100100197S	Ekho Lake	22-Dec-97	963893		A/F	1
100100297S	Ekho Lake	22-Dec-97	963893		A/F	2
100100397S	Ekho Lake	22-Dec-97	963893		A/F	3
100100497S	Ekho Lake	22-Dec-97	963893		A/F	4
100100597S	Ekho Lake	22-Dec-97	963893		A/F	5
100100697S	Ekho Lake	22-Dec-97	963893		A/F	6
100100797S	Ekho Lake	22-Dec-97	963893		A/F	7
100100897S	Ekho Lake	22-Dec-97	963893		A/F	8
100100997S	Abraxas Lake	21-Dec-97	998891			9
100101097S	Abraxas Lake	21-Dec-97	998891			10
100101197S	Abraxas Lake	21-Dec-97	998891			11
100101297S	Lake Fletcher	21-Dec-97	045874	Soil		12
100101397S	Lake Fletcher	21-Dec-97	045875	Soil		13
100101497S	Lake Fletcher	21-Dec-97	045874	Soil		14
100101597S	Lake Fletcher	21-Dec-97	045875	Soil		15
100101697S	Lake Fletcher	21-Dec-97	044873	Soil/ Bird Guano		16
100101797S	Watts Lake	14-Nov-97	882868	Soil		17
100101897S	Watts Lake	14-Nov-97	882868	Soil		18
100101997S	Watts Lake	14-Nov-97	882868	Soil, shells		19
100102097S	Watts Lake	13-Nov-97	882868	Soil		20
100102197S	Watts Lake	14-Nov-97	882868	Soil		21
100102297S	Watts Lake	14-Nov-97	882868	Stromatilite, shell		22
100102397S	Watts Lake	13-Nov-97	882868	Stromatilite		23
100102497S	Watts Lake	14-Nov-97	882868	Calcareous worm tubes, soil		24
100102597S	Watts Lake	14-Nov-97	882868	Soil/stromatalite	S A/F	25
100102697S	Paltcha Hut	11-Nov-97	981983	Soil (fine, brown)	S A/F	26
100102797S	Platcha Hut	11-Nov-97	981983	Soil with guano covering	S A/F	27
100102897S	Pioneer Crossing	11-Nov-97	005923	Soil	S A/F	28
100103097S	Gardner Island	19-Dec-97	898726			30
100103197S	Magnetic Island	01-Dec-97	933735	Soil, shit, guano, feathers	S A/F	31
100103897S	Teat Lake	13-Nov-97	855912	Lichen near entrance.		38
100103997S	Teat Lake	13-Nov-97	855912	Lichen (Alectoria minusula ?)	S A/F	39
100104097S	Watts Lake	14-Nov-97	882868	Soil		40
100104197S	Watts Lake	14-Nov-97	882868	Soil		41
100104297S	Organic Lake	16-Nov-97	845037			42
100104397S	Lichen Valley	05-Dec-97	013947	Soil, lichen		43
100104497S	Organic Lake	16-Nov-97	036848	Soil	S A/F	44
100104597S	Lichen Valley	05-Dec-97	013947	Soil		45
100104697S	Lichen Valley	05-Dec-97	014946	Soil		46
100104797S	Watts Lake	14-Nov-97	882868	Stromatilite, soil		
100104897S	Watts Lake	13-Nov-97	882868	Soil		
100104997S	Watts Lake	14-Nov-97	882868	calcareous worm tubes	S A/F	
100105197S	Watts Lake	14-Nov-97	882868	Soil		
100105297S	Watts Lake	14-Nov-97	882868	Stromatilite, soil		
100105397S	Watts Lake	14-Nov-97	882868	Stromatilite		
100105497S	Watts Lake	14-Nov-97	882868	Soil		
100105597S	Watts Hut Door	14-Nov-97	882868	Soil. Should be contaminated	S A/F	
100105697S	Teat Lake	13-Nov-97	855912	lichen (Buellia frigida). Obtained from na	S A/F	
100105797S	Marine Plain	01-Dec-97	859825	Soil		
100105897S	Marine Plain	01-Dec-97	860829	Soil		
100105997S	Marine Plain	01-Dec-97	861830	Soil		
100106097S	Marine Plain	01-Dec-97	860830	Soil		
100106197S	Marine Plain	01-Dec-97	861829	Soil		
100106297S	Moss Cirque	09-Dec-97	028929	Soil, lichen		
100106397S	Marine Plain	01-Dec-97	861830	Soil,guano		
100106497S	Lichen Valley	05-Dec-97	011937	Soil, moss		
100106597S	Marine Plain	01-Dec-97	863830	Soil		
100106697S	Marine PLain	01-Dec-97	861828	Soil underneath green quartz rock	S A/F	
100106797S	Marine Plain	01-Dec-97	859826	Soil		
100106897S	Marine Plain	01-Dec-97	859824	Soil		
100106997S	Marine Plain	01-Dec-97	856829	soil/lichen		
100107097S	Marine Plain	01-Dec-97	849831	Soil		
100107197S	Marine Plain	01-Dec-97	857829	Soil		
100107297S	Marine Plain	01-Dec-97	858830	Soil		

Appendix II

Copy of abstracts and posters arising from this thesis presented at conferences.

A comprehensive molecular study of the Basidiomycetes incorporating new Antarctic yeasts

Skye Thomas-Hall[1], **Sharon Guffogg[1]**, Ken Watson[1] and Jack Fell[2]
[1]School of Biological Sciences, Human Biology, University of New England, Armidale, Australia 2351;
2Rosensteil School of Marine and Atmospheric Science, Key Biscayne, Florida, USA 33149.

Sequence analysis of the D1/D2 region of the large ribosomal DNA (26S) has been used to construct comprehensive phylogenetic trees for all known Ascomycetes and Basidiomycetes (Kurtzman & Robnett, 1998; Fell *et al.*, 2000). The adjacent internal transcribed spacer DNA (ITS1-5S-ITS2) region has also been used to identify strains within a species as has, to a lesser extent, the highly variable intragentic spacer (IGS) region. For this project, we illustrate the 26S ribosomal DNA region of the phylogenic tree for all known Basidiomycetes with enlargement of the clades to include the newly isolated Antarctic yeasts. In the case of the latter isolates, we also include molecular sequencing data for the ITS1-5S-ITS2 region. Carbon assimilation tests, 1D-proteome analyses and morphological characteristics also supplemented the molecular analyses. The vast biodiversity of yeasts isolated from Antarctica is illustrated by the identification of 11 new Basidiomycetes and 18 genetic variant strains, from just 36 isolates. The latter had been previously screened by 1D-proteome analyses and temperature growth profiles as psychrophilic yeasts, with a maximum growth temperature of <25°C. With further taxonomic studies in progress, this figure could be just the tip of the iceberg.

Kurtzman C.P. & Robnett C.J. 1998. Identification and phylogeny of ascomycetes yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie van Leeuwenhoek 73: 331-371.

Fell, J.W, Boekhout, T., Fonseca, A., Scorzetti, G. & Statzell-Tallman, A. 2000. Biodiversity and systematics of basidiomycetous yeasts as determined by large subunit rD1/D2 domain sequence analysis. Int J Syst Evol Microbiol. (in press).

Resistance to UV-B radiation in Antarctic yeasts

Masego Tsimako, **Sharon Guffogg**, Skye Thomas-Hall and Ken Watson
School of Biological Sciences, Human Biology, University of New England, Armidale, Australia 2351

Many of the most harmful, acute and cumulative effects of solar UV have been attributed to damaging free radicals, generated by a number of physiological and biochemical processes. Free radicals are believed to be important in skin ageing and in the development of skin cancer. Results from a number of animal studies, and a limited number of human studies, have indicated that antioxidants may help protect cells from free radical-related damage. In the present studies, we have used yeast cells as a model system to study the interrelationship among free radicals, antioxidants and UV-B and UV-A induced cell damage. A Vilber-Lourmat Bio-Sun apparatus was used to generate known amounts of radiation at calibrated wavelengths for UV-A (355-375 nm, calibrated at 365 nm) and UV-B (280-320 nm, calibrated at 312 nm). Yeast cells (*Saccharomyces cerevisiae*) were exposed to UV-A or UV-B radiation (50-500 mJ/cm^2) at 22^oC and cell viability measured by dilution plate count. Cells were relatively resistant to UV-A but sensitive to UV-B. In marked contrast, Antarctic yeasts (*Cryptococcus* sp.) were remarkably resistant to UV-A and UV-B. It was noteworthy that many of the Antarctic yeasts were pigmented, ranging from pale yellow to orange-red in colour and we speculate that protection against UV-radiation in these yeasts may be related to pigmentation. In this respect, recent reports have suggested that Antarctic plants and mosses have the ability to block out UV possibly by increased production of UV-absorbing pigments. Work is currently in progress on the effects of growth temperature and manipulation of membrane lipid composition with respect to sensitivity to UV-B radiation.

Resistance to UV-B radiation in Antarctic yeasts

Masego Tsimako, Sharon Guffogg, Skye Thomas-Hall and Ken Watson

School of Biological Sciences, Human Biology, University of New England, Armidale, NSW

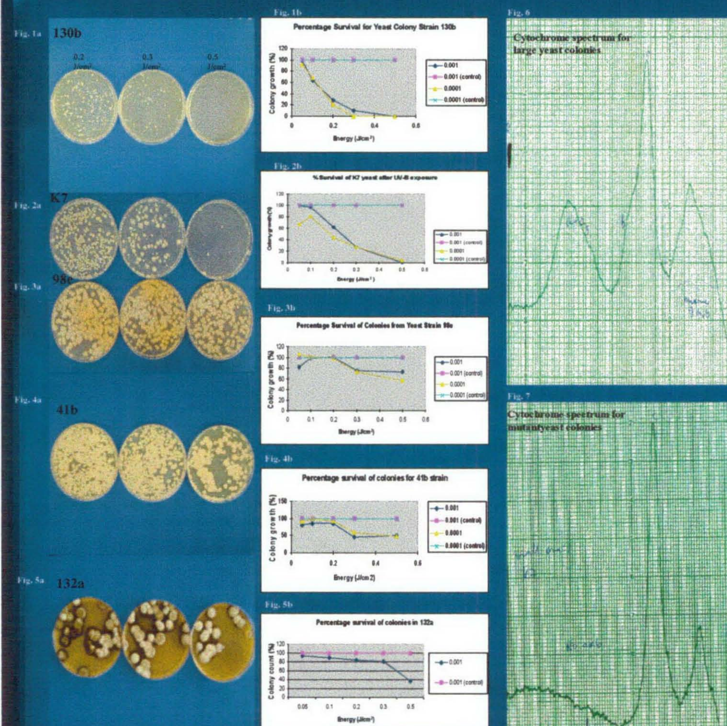
ntroduction

olar radiation especially in the ultraviolet range of 280-380 nm is one of the most important stress agents affecting human skin, causing sunburn, premature skin aging and cancer. Free radicals are most likely to play a role in biological effects elicited by ultraviolet radiation on the skin. Several studies have shown that skin exposure to UV-radiation increases levels of reactive oxygen species and reactive nitrogen species resulting in oxidative damage to lipids, proteins and DNA. A number of studies on animal model systems, and limited number of human studies, have indicated that antioxidants may help protect cells from free radical-related damage. In the present studies, we have used yeast cells as a model system to study the interrelationship among free radicals, antioxidants and UV-A and UV-B induced cell damage.

Aims

Compare the effects of UV-radiation on cell survival in Antarctic (psychrophilic) and normal (mesophilic, *Saccharomyces cerevisiae*) yeasts.

Determine the effects of cell membrane lipid composition on sensitivity to UV-radiation.



Methods

Cells were grown at 15ºC (Antarctic yeast, psychrophilic) or at 25ºC (mesophilic) to late exponential phase. Suitable serial dilutions were plated onto yeast extract-peptone agar plates (YEP) and plates exposed to UV-radiation. A Vilber-Lourmat Bio-Sun apparatus was used to generate known amounts of radiation at calibrated wavelengths for UV-A (355-375 nm, calibrated at 365 nm) and UV-B (280-320 nm, calibrated at 312 nm). Following UV-exposure, plates were incubated at appropriate temperatures and cell viability determined. Cytochrome spectra were analyzed in intact cells by difference spectra and membrane lipid (fatty acids) composition by gas chromatography using a Hewlett Packard 5890 GC flame ionization detector.

Membrane lipid composition was manipulated by incorporation of fatty acids (oleic $C_{18:1}$, linoleic $C_{18:2}$ and linolenic $C_{18:3}$ acids) in the growth media.

Results & Discussion

Under the present experimental conditions, the psychrophilic (Antarctic) and mesophilic (*Saccharomyces cerevisiae*) yeasts were essentially resistant to UV-A radiation (500 mJ/cm²). In the case of UV-B radiation, the percentage survival for mesophilic yeasts were 20-30% at 300 mJ/cm² and < 5% at 500 mJ/cm² (Fig. 2b). Interestingly, exposure to UV-B radiation resulted in three phenotypes on YEP plates, namely, large wild type colonies (as in control, non-irradiated cells), intermediate sized colonies and small colonies.

Cytochrome difference spectra showed that the latter colonies had normal levels of cytochrome c but were deficient in cytochrome $a + a_3$ and b (Fig. 7).

Alterations in membrane lipid composition in mesophilic yeast did not change sensitivities to UV-B radiation. In the case of highly pigmented Antarctic yeasts, survival was approximately 70% and 60% respectively at 300 mJ/cm² (Fig. 3). In a number of non-pigmented Antarctic yeasts, survival was significantly less with 25% and 7% survival respectively at 300 and 500 mJ/cm² (Fig. 1b). Although small colonies were observed in cells exposed to UV-radiation, cytochrome difference spectra revealed the presence of cytochromes $a + a_3$, b and c (Fig. 6). Previous analyses of membrane fatty acid composition of Antarctic yeasts showed high concentrations of C 18 unsaturated fatty acids ($C_{18:1}$, $C_{18:2}$ and $C_{18:3}$) with very small amounts of $C_{16:1}$.

Conclusion

Antarctic yeasts were remarkably resistant to UV-B radiation as compared with mesophilic (*S. cerevisiae*) yeasts.The membrane fatty acid composition was not a determining factor in UV-B resistance. In general, the highly pigmented Antarctic yeasts were particularly resistant to UV-B radiation (Fig. 3a, 4a and 5a) as compared with non-pigmented species (Fig.1a) . However, a number of the non-pigmented Antarctic yeasts were also relatively resistant. Nevertheless, we speculate that the presence of pigments in Antarctic yeasts may offer a degree of protection against UV-B radiation.

Acknowledgements

Antarctic CRC, Hobart Tasmania, Jonathan, Visala and Graham

Antarctic yeasts: Phenotypic and phylogenetic analyses

S.Guffogg[1], S.Thomas-Hall[1], M.Tsimago[1], P. Holloway[2] and K.Watson[1]
[1]School of Biological, Biomedical & Molecular Sciences, University of New England, Armidale, NSW 2351, Australia; [2]School of Agriculture, University of Tasmania, Hobart, TAS 7000, Australia

The Antarctic environment is severe with temperatures ranging from -85°C to -10°C. Nevertheless, microorganisms, including yeasts, have adapted to these extremes. In the present studies, soil and snow samples from the Vestfold Hills, Davis Base, Antarctica, were screened for yeasts. A select number (~200) of isolated yeasts were subject to detailed phenotypic and phylogenetic analyses. The yeasts were initially classified into two main groups, the psychrophiles or psychrotrophs. The former were defined as having a maximum growth temperature of ≤25°C and the latter ≥25°C. Protein fingerprinting by 1D-SDS-PAGE was used to rapidly identify identical or closely related strains.
Phenotypic analyses included classical carbon assimilation tests, morphology by light and scanning electron microscopy and membrane fatty acid composition. Fatty acid analyses indicated that the psychrophilic Antarctic yeasts were enriched in linoleic acid ($C_{18:2}$) and to a lesser extent linolenic acid ($C_{18:3}$) while the psychrotrophs were enriched in oleic acid ($C_{18:1}$). Interestingly, many of the Antarctic yeasts were relatively resistant, as compared with non-Antarctic yeasts, to UVB irradiation. In particular, the highly pigmented yeasts were resistant to high doses of UVB. Preliminary analysis indicated that the pigmented yeasts were enriched in carotenoids. Sequence analyses of the D1/D2 region of the 26S ribosomal DNA and the ITS regions were used to place the isolates into phylogenetic trees of the known Basidiomycetous and Ascomycetous yeasts and to identify a number of new species.

ANTARCTIC YEASTS: A PHENOTYPIC AND PHYLOGENETIC ANALYSES

Sharon P Guffogg[1], Skye Thomas-Hall[1], Masego Tsimako[1], Paul Holloway[2] & Kenneth Watson[1]

[1]School of Biological, Biomedical & Molecular Sciences, University of New England, Armidale, NSW 2351, Australia;

[2]School of Agricultural Science, University of Tasmania, Hobart, Tas 7004, Australia.

Introduction

Numerous taxonomic techniques have been utilised for yeast identification and classification including classical techniques such as assimilation and fermentation tests. Cellular morphology and molecular studies have exposed the limitations of classical taxonomy with misidentification of some strains. Sequencing of the D1/D2 domain of the large ribosomal subunit (rDNA) [Fig 1], which is sufficiently variable to allow for species separation, has been accomplished for all known ascomycete and basidiomycete species, thus allowing newly isolated yeasts to be placed within the phylogenetic tree of known species [Kurtzman & Robnett 1998; Fell et al. 2000].

Figure 1: Arrangement of Ribosomal Genes in Yeast

In the present study, ~200 yeast strains were isolated from soil and snow samples taken from Davis Base, Antarctica [Thomas-Hall & Watson 2002]. Isolates were identified using a combination of classical and molecular methods. The yeast cells were used as a model system to study the interrelationship among free radicals, antioxidants and UV-A and UV-B induced cell damage. Cells were also screened for polyunsaturated fatty acids and extracellular enzyme activities.

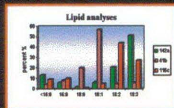


Fig 2. Fatty acids of selected Antarctic yeast

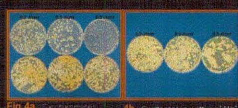


Fig 4a. Saccharomyces cerevisiae [A7] and Cryptococcus ... [26b] **4b.** Cryptococcus watticus [41b]

Methods

Isolation and characterisation

Soil samples from the Vestvold Hills, Davis Base, Antarctica were collected and stored at -10°C. Yeasts were isolated and maintained on yeast-extract-peptone [YEP] plates [2% w/v glucose, 0.5% bacteriological peptone, 0.5% yeast extract, 0.3% KH_2PO_4, 0.3% $(NH_4)_2SO_4$, 1.5% agar]. Isolates were characterised by standard methods described by Yarrow [1998].

DNA sequence analysis

PCR products were obtained using forward primer ITS-1 [5'-TCCGTGTTTCATGAACCTGCGG-3'] and reverse primer ITS-4 [5'-TCCTCCGCTTATTGATATGC-3'].The sequence of the D1/D2 domain was determined using forward primer MLF [5'-GCATATCAAGCGGAGGAAAAG-3'] and reverse primer MLR [5'-GGTCCGTGTTTCAAGACGG-3']. Sequences were obtained with the Beckman CEQ2000 sequencer and visually aligned with BioEdit. Phylogenetic relationships were aligned with MegAlign [DNAStar]. Phylogenetic analyses were calculated using the maximum parsimony program of PAUP 4.0 [Sinauer Associates] with heuristic searches and random stepwise additions.

Fatty acid analysis

Cell extracts were analysed with a Hewlett Packard 5890 series II gas chromatograph equipped with a flame ionisation detector. Retention times were used to identify fatty acids relative to appropriate standards and expressed as percentage fatty acid composition.

UV-radiation & cytochrome analyses

A Vilber-Lourmat Bio-Sun apparatus was used to generate known amounts of radiation at calibrated wavelengths for UV-A (355-375 nm, calibrated at 365 nm) and UV-B (280-320 nm, calibrated at 312 nm). Following UV-exposure, plates were incubated at appropriate temperatures and cell viability determined. Cytochrome spectra were analyzed in intact cells by difference spectra.

Results & Discussion

The yeast isolates were grouped according to morphological similarities and growth profiles [psychrophilic with a max growth temperature <20°C or psychrotrophic with a max growth temperature >20°C]. Sequence analyses of the isolates identified new as well as established yeast strains. Previous studies from our laboratory identified 12 new species and 18 sub-species from a selection of 120 isolates. The present study examined 85 isolates from which 47 distinctly different strains were more closely examined.

Fatty acid analysis. Previous studies on psychrophilic yeasts have shown a predominance for C_{18} unsaturated fatty acids. This study has confirmed these results with some isolates having significant quantities of the omega fatty acids [omega-3 $C_{18:3}$ and omega-6 $C_{18:2}$][fig 2].

UV-radiation & cytochrome analyses. All cells were essentially resistant to UV-A [up to 0.5 J/cm^2]. In the case of UV-B radiation, pigmented Antarctic yeast were essentially resistant compared with their mesophilic counterparts [fig 4a & 4b]. Interestingly, exposure of *Saccharomyces cerevisiae* to UV-B radiation resulted in three phenotypes on YEP plates, namely, large wild type colonies, intermediate sized colonies and small colonies. Cytochrome difference spectra showed that the latter colonies had normal levels of cytochrome c but were deficient in cytochrome a + a_3 and b.

DNA sequence analysis. Two new species of Antarctic yeasts were identified based on sequencing analysis of the D1/D2 domain of the large ribosomal subunit (rDNA). Although both strains, designated *Cryptococcus watticus* and *Candida davisiana* were described from a single isolate thus impeding any knowledge of natural distribution or strain variability, their description is nonetheless beneficial in the overall knowledge of yeast biodiversity, in particular that of Antarctica.

The ability of these organisms to survive at such extreme temperatures has attracted special attention and thus their potential exploitation as unique enzyme producers warrants further investigation. Preliminary results suggest that a number of the Antarctic yeast isolates produce extracellular protease, an enzyme with potential industrial application in e.g. detergent additives. Screening for other enzymes such as, cellulase, lipase, pectinase, lichenase and amylase is currently taking place.

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Acknowledgements

Antarctic CRC, Hobart Tasmania

University Research Grant, UNE

David Nichols, Kevin Sanderson, Tom McMeekin; University of Tasmania

Graham Jones, Linda Agnew, Visala Rao, Shanchita Khan and Srdjan Mijajlovic; UNE

Stress Response in Novel Antarctic Yeast

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[2] Marine Biotechnology, Australian Institute of Marine Science, Townsville QLD 4810, Australia

Yeasts isolated from extreme environments such as Antarctica, offer a unique opportunity to exploit characteristics not found in other microorganisms. The focus of this study was 60 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. To date, 3 novel species have been identified as well a number of established species, not previously isolated from Antarctica.

Rhodotorula mucilaginosa is a highly pigmented red yeast collected in 1994. Novel growth characteristics of this Antarctic strain enabled further studies to investigate heat shock protein expression. Cell viability, measured after a 3 hr heat shock at 52 degrees C, declined sharply after 30 min of exposure. However, a mild thermal pre-treatment (37 degrees C for 1 hr) protected the cells against subsequent lethal temperatures. SDS-PAGE utilising ^{35}S-methionine labeling was used to identify heat shock induced proteins. Cells were subjected to UVA-radiation (355-375 nm, calibrated at 365 nm, measured output 4.0 – 4.5 x 10^{-4}J cm^{-2} s^{-1}) for 2, 3 and 4 hr and cell viability measured by serial dilution plate count. Cell viability of *R. mucilaginosa* measured as percent survivors, remained at ~100% after 4 hr exposure as compared with *Saccharomyces cerevisiae* with no surviving colonies after 2 hr. A number of other recently isolated Antarctic yeast produced similar results.

The coenzyme Q_8 ratio (ubiquinol: ubiquinone) of *R. mucilaginosa*, a marker of oxidative stress, was measured in cells by HPLC over a 4 hr period of exposure to UVA followed by a further 2 hr in the absence of UVA. The ubiquinol/ubiquinone ($CoQH_2$: CoQ) ratio varied from 54:45 at time 0 to 90:10 after 4 hours of UVA exposure and remained steady for a further 2 hours following the removal of the stimulus. The regulation of these processes to maintain sufficient levels of the reduced form of coenzyme Q appears to be a novel cellular response in *R. mucilaginosa* to UVA photooxidative stress not observed in other Antarctic or mesophilic yeast.

The University of New England AUSTRALIA

Stress Response in Novel Antarctic Yeasts

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INTRODUCTION

Taxonomic techniques for yeast identification and classification include classical methods such as assimilation reactions, fermentation tests and cellular morphology. However, molecular studies have shown the limitations of these methods which have resulted in the misidentification of some strains. Sequencing of the D1/D2 region of the large ribosomal subunit (rDNA), which is sufficiently variable to allow for species separation, has been accomplished for all known ascomycete and basidiomycete species, thus allowing newly isolated yeasts to be placed within the phylogenetic tree of known species (Kurtzman & Robnett, 1998; Fell *et al.*, 2000). Analysis of sequence data from newly isolated Antarctic yeasts indicated a number of unidentified yeast strains. Physiological tests, morphological descriptions as well as fatty acid and coenzyme Q analyses supported the sequence data. The yeast cells were used as a model system to study the interrelationship among free radicals, antioxidants and UVA induced photo-oxidative damage.

METHODS

Isolation and characterisation

Soil samples from the Vestfold Hills, Davis Base, Antarctica were collected and stored at -10°C. Yeasts were isolated and maintained on yeast-extract-peptone [YEP] plates [2% w/v glucose, 0.5% bacteriological peptone, 0.5% yeast extract, 0.3% KH_2PO_4, 0.3% $(NH_4)_2SO_4$, 1.5% agar]. Isolates were characterised by standard methods described by Yarrow [1998].

DNA sequence analysis

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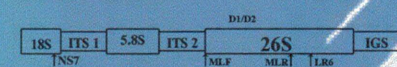

Fatty acid analysis

Cell extracts were analysed with a Hewlett Packard 5890 series II gas chromatograph equipped with a flame ionisation detector. Retention times were used to identify fatty acids relative to appropriate standards and expressed as percentage fatty acid composition.

UVA-irradiation/NAD(P)H: quinone oxidoreductase [NQR] assay

Cells were subjected to UVA-radiation with twin UVA lamps (Phillips TL20 measured output 4.0 – 4.5 x 10^{-4}J cm^{-2} s^{-1}) for 2, 3 and 4 hours. Following UV-exposure, plates were incubated at appropriate temperatures and cell viability determined. NQR enzyme activity was determined spectrophotometrically at 600 nm.

Whole cell protein analysis

1D-PAGE was performed on protein samples (10 mg) run on 10% resolving acrylamide gels, followed by either Fast-Blu staining or autoradiography.

Coenzyme Q analysis

Methanol/isopropanol cell extractions were injected onto a phenomenex ODS (2) column followed in-line by a platinum reduction column (Type RC – 10, Irica), with amperometric electrochemical detection operating at an oxidation potential of +600mV (vs $Ag/AgCl_2$) on a glassy carbon electrode. The mobile phase consisted 50 mM-sodium perchlorate in methanol/isopropanol (60/40 v/v) delivered at a flow rate of 1.0 ml/min.

Heat Shock/Thermotolerance

Intrinsic thermotolerance was measured by rapidly increasing the temperature of cells grown at 25 °C to a heat stress temperature of 52 °C. Induced thermotolerance was measured by exposing cells to a mild heat shock of 37 °C for 1 hr prior to a heat stress of 52 °C for 3 hr. Cell viability was measured over time by serial dilution plate assay at 15 °C. Similar conditions were used to measure hsp synthesis. Proteins were labeled with 35S-methionine prior to heat treatment and analysed by SDS-PAGE and autoradiography.

RESULTS

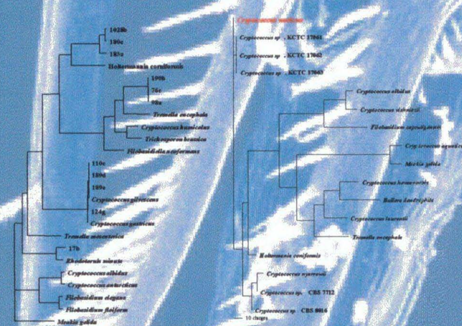

Fig 1. ITS tree showing placement of newly isolated strains among related basidiomycetous yeast. *Cryptococcus aquaticus* and *Mrakia gelida*, which represent the Cystofilobasidiales, are the designated out group species in this analysis. Labelled clades have representative species only.

Fig 2. Phylogenetic tree showing placement of *C. watticus* among related basidiomycetous yeasts, derived analysis of the 26S rDNA D1/D2 domain. *Cryptococcus aquaticus* and *Mrakia gelida*, which represent the Cystofilobasidiales, are the designated out group species in this analysis. Labelled clades have representative species only.

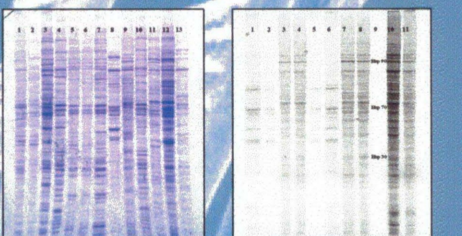

Fig 3. 1-D SDS-PAGE of whole cell protein extracts showing differences in protein banding patterns between isolates. Lane 1 *C. watticus*; lane 2 *C. narrowii*; lane 3 174b; lane 4 *M. frigida*; lane 5 151b; lane 6 98a; lane 7 *C. victoriae*; lane 8 98e; lane 9 180e; lane 10 *R. zellii*; lane 11 1028e; lane 12 1266b; lane 13 *R. mucilaginosa*

Fig 4. Autoradiograph of ^{35}S-methionine labeled proteins isolated from *Saccharomyces cerevisiae* [K7] and *R. mucilaginosa*. Lanes 1 & 6 K7 heat shock, lanes 2 & 5 K7 control, lanes 3, 7 & 10 *R. mucilaginosa* control, lanes 4 & 8 *R. mucilaginosa* heat shock, lane 11 *R. mucilaginosa* UVA, lane 9 molecular markers. Lanes 4 to 4 ^{35}S-methionine added prior heat shock, lanes 5 to 8 ^{35}S-methionine added post heat shock.

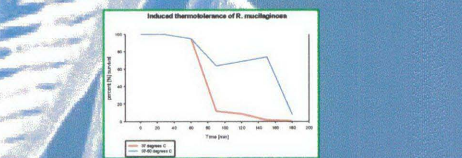

Fig 5. Induced thermotolerance of *R. mucilaginosa* grown at 25°C.

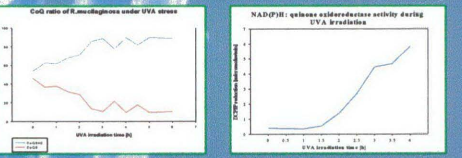

Fig 6. Changes in coenzyme Q_8 ratio (ubiquinol: ubiquinone) of *R. mucilaginosa* during UVA exposure.

Fig 7. Changes in NAD(P)H: quinone oxidoreductase activity of *R. mucilaginosa* during UVA exposure.

DISCUSSION

One-dimensional SDS-PAGE of whole-cell proteins was initially employed to characterize relatedness among unknown isolates by comparing protein banding patterns [fig 3]. Isolates with visually identical or similar protein banding patterns were grouped together.

Sequence analyses of the 26S D1/D2 region of the large ribosomal subunit identified three unique yeast strains. Figure 2 shows the placement of *Cryptococcus watticus*, a basidiomycetous yeast belonging to the hymenomycetous yeasts in a cluster with *Holtermannia corniformis* and *Cryptococcus nyarrowii*. Classic taxonomic methods including biochemical tests, Diazonium blue B test and morphological studies supported the data. Two ascomycetous yeasts were identified and are currently being assessed as to their phylogenetic placement.

Rhodotorula mucilaginosa is a highly pigmented red yeast isolated from Antarctic soil. Cell viability, measured after a 3 hr heat shock at 52°C, declined sharply after 30 min of exposure. However, a mild heat shock (37°C for 1 hr) protected the cells against subsequent lethal temperatures [fig 5]. SDS-PAGE utilising ^{35}S-methionine labeling was used to identify heat shock induced proteins [fig 4]. Cells were subjected to UVA-radiation (355-375 nm, calibrated at 365 nm, measured output 4.0 – 4.5 x 10^{-4}J cm^{-2} s^{-1}) for 2, 3 and 4 hr and cell viability measured by serial dilution plate count. Cell viability of *R. mucilaginosa* measured as percent survivors, remained at ~100% after 4 hr exposure as compared with *Saccharomyces cerevisiae* with few surviving colonies [fig 8]. A number of other recently isolated Antarctic yeasts produced similar results.

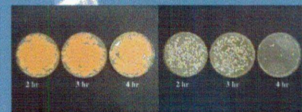

Fig 8. Following UVA-irradiation cell viability of *R. mucilaginosa* measured as percent survivors, remained at ~100% after 4 hr exposure as compared with *Saccharomyces cerevisiae* with few surviving colonies after 4 hr.

The coenzyme Q_8 ratio (ubiquinol: ubiquinone), a marker of oxidative stress, of *R. mucilaginosa*, was measured in cells by HPLC over a 4 hr period of exposure to UVA followed by a further 2 hr in the absence of UVA. The ubiquinol/ubiquinone ($CoQH_2$: CoQ) ratio varied from 54:45 at time 0 to 90:10 after 4 hr of UVA exposure and remained steady for a further 2 hr following the removal of the stimulus. NAD(P)H: quinone oxidoreductase [NQR] functions to maintain coenzyme Q in its reduced antioxidant state [fig. 9], thereby providing protection from free radical damage. NQR was assayed at 30 min intervals during UVA exposure. NQR activity dramatically increased after exposure for 2 hr [fig. 7].

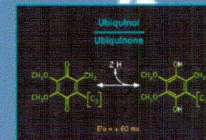

Fig 9. NAD(P)H: quinone oxidoreductase catalyses the conversion of ubiquinone to ubiquinol.

The regulation of these processes to maintain sufficient levels of the reduced form of coenzyme Q appears to be a novel cellular response in *R. mucilaginosa* to UVA photo-oxidative stress not observed previously in yeast.

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Acknowledgements

- Antarctic CRC: Hobart Tasmania
- University Research Grant, Keith & Dorothy Mackay Travel Scholarship: UNE
- John Bowman, David Nichols, Kevin Sanderson: University of Tasmania
- Linda Agnew, Skye Thomas-Hall, Graham Jones: UNE

Antarctic yeast: How to stay cool when the heat is on

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[2] Marine Biotechnology, Australian Institute of Marine Science, Townsville QLD Australia

Antarctica is an extreme environment, with low temperatures, high UV exposure and oxidative stress all potential sources of stress. In the current study, yeasts isolated from Antarctic soil and snow, have been exposed to various stress conditions in order to ascertain their response. One of these isolates, a highly pigmented yeast, was identified as *Rhodotorula mucilaginosa* and this yeast, together with a number of newly identified strains, were examined in relation to heat and UVA-radiation stress.

Cells grown at 25 degrees C and subjected to a mild heat shock (37 degrees C/1 hr) exhibited heat shock-induced thermotolerance to a normally lethal heat stress (52 degrees C, time course). Heat shock induced proteins were identified by ^{35}S-methionine labeling on SDS-PAGE. Response to UVA radiation (355-375 nm, calibrated at 365 nm, measured output 4.0 – 4.5 x 10^{-4}J cm^{-2} s^{-1}) was measured over a time course (2, 3 and 4 hr) and cell viability estimated by serial dilution plate count. Essentially 100% cell survival was observed in the case of the Antarctic yeast *R. mucilaginosa*, as compared with 0% survival for the typical mesophilic yeast *Saccharomyces cerevisiae*. High percentage survivals were also observed for a number of other recently isolated Antarctic yeast.

The coenzyme Q_8 ratio of *R. mucilaginosa* (ubiquinol: ubiquinone), a sensitive measure of cellular redox potential and oxidative stress, was measured in cells by HPLC over the 4 hr period of exposure to UVA followed by a further 2 hr period in the absence of UVA. The ubiquinol:ubiquinone ($CoQH_2$:CoQ) ratio increased from 54:45 at time zero, to 90:10 after 4 hr exposure to UVA. This latter ratio remained relatively constant following the 2 hr recovery period. Elevated ubiquinol to ubiquinone content is indicative of a more efficient antioxidant capability. Importantly, this profile was not seen in any other Antarctic or mesophilic yeast analysed, suggesting a novel stress response. We conclude that Antarctic yeasts in general are relatively resistant to UVA-radiation and that *R. mucilaginosa* in particular has a highly efficient antioxidant capacity which may be associated with an unusual $CoQH_2$:CoQ ratio.

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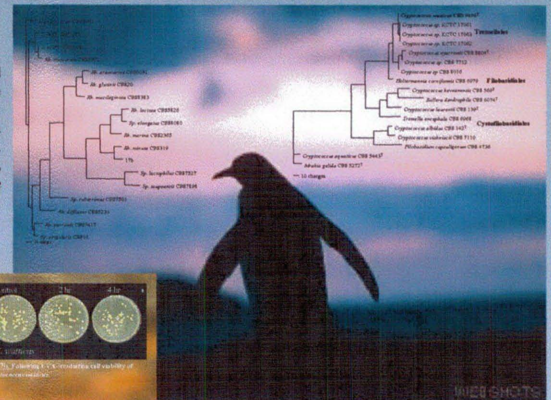
INTRODUCTION

Yeasts were isolated from soil and snow samples taken from the Australian Davis Base, Antarctica in the summer of 1994/1995 and 1997/1998. Sequencing of the D1/D2 region of the large ribosomal subunit (rDNA) has been accomplished for all known ascomycete and basidiomycete species, thus allowing newly isolated yeasts to be placed within the phylogenetic tree of known species (Kurtzman & Robnett, 1998; Fell *et al.*, 2000). Analysis of sequence data indicated a number of new yeast strains including *Cryptococcus watticus*, a basidiomycetous yeast belonging to the hymenomycetous yeasts in a cluster with *Holtermannia corniformis* and *Cryptococcus nyarrowii*. Sequence data was supported by physiological tests, morphological descriptions and fatty acid and coenzyme Q analyses(Guffogg *et al.*, 2004). The newly isolated yeasts, both novel and established, were used as model systems to study the interrelationship among free radicals, antioxidants and UVA induced cell damage.



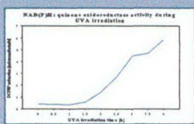


Fig 1. Changes in NAD(P)H: quinone oxidoreductase activity of *R. mucilaginosa* during UVA exposure.

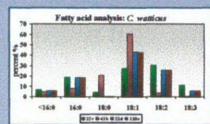
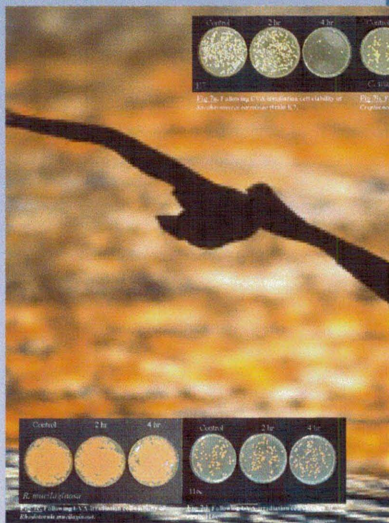
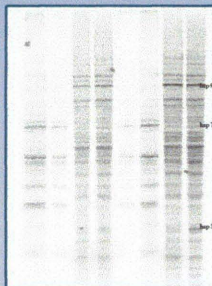


Fig 2. Fatty acid profile of *Cryptococcus watticus* strains 22c, 22d, 41b & 110c.





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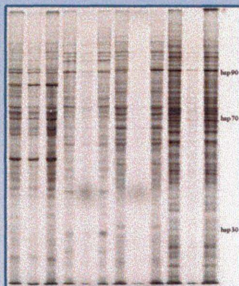
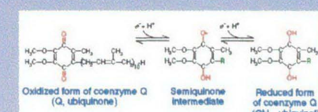


Fig 4. Autoradiograph of ^{35}S-methionine labeled proteins. Lanes 1, 2 & 3 K7 control, K7 UVA, K7 12 hr post UVA; lanes 4, 5 & 6 *R. mucilaginosa* control, *R. mucilaginosa* UVA, *R. mucilaginosa* 12 hr post UVA; lanes 7, 8 & 9 *C. watticus* control, *C. watticus* UVA, *C. watticus* 12 hr post UVA; lanes 10, 11 & 12 116c control, 116c UVA, 116c 12 hr post UVA; lane 12 molecular markers. Lanes 2, 5, 8 & 11 ^{35}S-methionine added prior to UVA exposure, lanes 3, 6, 9 & 12 ^{35}S-methionine added post UVA exposure.



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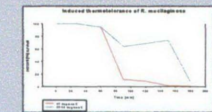

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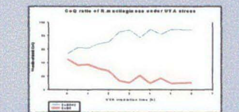

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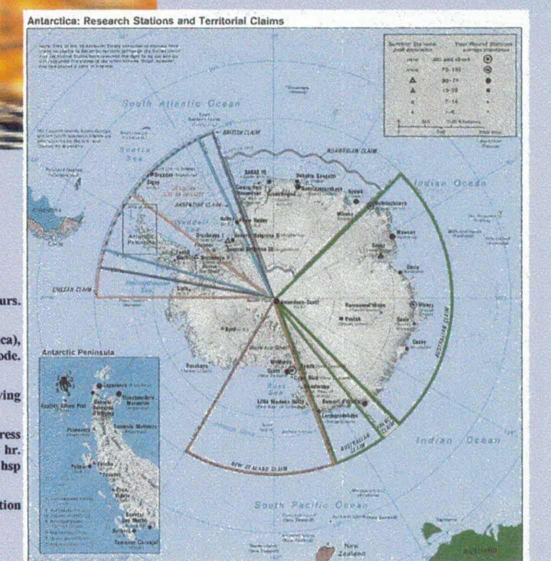

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UVA-radiation: Cells were subjected to UVA-radiation with twin UVA lamps (Phillips TL20 measured output 4.0 – 4.5 x 10^{-4}J cm^{-2} s^{-1}) for 2, 3 and 4 hours. Following UV-exposure, plates were incubated at appropriate temperatures and cell viability determined.

Coenzyme Q analysis: Extractions were injected onto a phenomenex ODS (2) column followed in-line by a platinum reduction column (Type RC – 10, Irica), with amperometric electrochemical detection (Model Σ 985, Irica) operating at an oxidation potential of +600mV (vs Ag/AgCl$_2$) on a glassy carbon electrode. The mobile phase consisted 50 mM sodium perchlorate in methanol/isopropanol (60/40 v/v) delivered at a flow rate of 1.0 ml/min.

Whole cell protein analysis: 1D-PAGE was performed using the standard procedures of Laemmli (1970). Protein samples (10 mg) were run on 10% resolving acrylamide gels, followed by either *fast-blu* staining or autoradiography .

Heat Shock/Thermotolerance: Instrinsic thermotolerance was measured by rapidly increasing the temperature of cells grown at 25°C to a heat stress temperature of 52°C. Induced thermotolerance was measured by exposing the cells to a mild heat shock of 37°C for 1 hr prior to a heat stress of 52°C for 3 hr. 0.5 ml samples were taken at time intervals and spread plated onto YEP plates in duplicate and incubated at 15°C. Similar conditions were used to measure hsp synthesis. Cells were labelled with ^{35}S-methionine prior to heat treatment followed by protein extraction [see above] and autoradiography.

Fatty acid analysis: Cell extracts were analysed with a Hewlett Packard 5890 series II gas chromatograph equipped with a flame ionisation detector. Retention times were used to identify fatty acids relative to appropriate standards and expressed as percentage fatty acid composition.

RESULTS & DISCUSSION

Yeasts were subjected to heat and UVA irradiation stress, typical results for *Rhodotorula mucilaginosa*, a highly pigmented red yeast isolated from Antarctic soil, is shown in Fig. 5. Cell viability, measured after a 3 hr heat stress at 52°C, declined sharply after 30 min of exposure. However, a mild heat shock (37°C for 1 hr) protected the cells against subsequent lethal temperatures [Fig. 5]. SDS-PAGE utilising ^{35}S-methionine labeling was used to identify heat shock and UVA induced proteins. Figure 3 indicated upregulation of small heat shock proteins in the 30 kDa range in *R. mucilaginosa* but not in *Saccharomyces cerevisiae* K7, whereas hsp 70 was only upregulated in K7 suggesting that hsp 70 does not induce thermotolerance in *R. mucilaginosa*. Cells were subjected to UVA-radiation for 2, 3 and 4 hr and cell viability measured by serial dilution plate count. Cell viability of *R. mucilaginosa* measured as percent survivors, remained at ~100% after 4 hr exposure as compared with *S. cerevisiae* with few surviving colonies after 4 hr. A number of other recently isolated Antarctic yeasts produced similar results [Figs. 7a, b. c & d], indicating that Antarctic yeast may be instrinsically resistant to UVA. Figure 4 identified proteins synthesised following 4 hr UVA exposure in *S. cerevisiae, R. mucilaginosa, C. watticus* and strain 116c. UVA exposure was carried out at 6°C to eliminate any heat shock reaction. Both up and down regulation can be seen at a number of protein bands for *R. mucilaginosa, C. watticus* and *S. cerevisiae.* Western blot analysis is currently under way to identify these proteins. Fatty acid analysis of *C. watticus* revealed oleic acid [$C_{18:1}$] to be the predominant fatty acid present together with the polyunsaturated fatty acids $C_{18:2}$ and $C_{18:3}$ [Fig. 2] The fatty acid compositions confirmed previous studies that have shown that psychrophilic Antarctic yeasts, such as species of *Candida, Leucosporidium* and *Mrakia* have a high unsaturated fatty acid content (Watson, 1987). The coenzyme Q_8 ratio (ubiquinol: ubiquinone, a marker of oxidative stress and a measure of cellular redox potential) of *R. mucilaginosa* was measured in cells by HPLC over a 4 hr period of exposure to UVA followed by a further 2 hr recovery in the absence of UVA. The ubiquinol/ubiquinone ($CoQH_2$: CoQ) ratio varied from 54:45 at time 0 to 90:10 after 4 hr of UVA exposure and remained steady for a further 2 hr following the removal of the stimulus [Fig. 6]. The enzyme NAD(P)H: quinone oxidoreductase [NQR] functions to maintain coenzyme Q in its reduced antioxidant state, thereby providing protection from free radical damage [Fig. 8]. NQR was assayed at 30 min intervals during UVA exposure. NQR activity dramatically increased in *R. mucilaginosa* after exposure for 2 hr [Fig. 1].

The regulation of these processes to maintain sufficient levels of the reduced form of coenzyme Q appears to be a novel cellular response to UVA photooxidative stress in *R. mucilaginosa* not observed in other Antarctic or mesophilic yeast.

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Acknowledgements

- Antarctic CRC: Hobart Tasmania
- University Research Grant, Keith & Dorothy Mackay Travel Scholarship: UNE
- John Bowman, David Nichols, Kevin Sanderson: University of Tasmania
- Linda Agnew, Skye Thomas-Hall: UNE