

**Investigation into the mechanism of action and biological role of
Saccharomyces cerevisiae mannoproteins which reduce visible
haziness in white wine**

by

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TABLE OF CONTENTS

Declaration	i
Thesis summary.....	ii
Acknowledgements	iii
Abbreviations	iv

CHAPTER ONE

INTRODUCTION	1
1.1 INTRODUCTION.....	1
1.2 HAZE.....	1
1.2.1 Types of Haze	1
1.2.2 Heat Unstable Proteins	2
1.3 REMOVAL OF HEAT UNSTABLE PROTEINS	3
1.3.1 The use of bentonite as the protein fining agent.....	3
1.3.2 Alternatives to the use of bentonite for protein fining	4
1.4 HAZE PROTECTIVE FACTOR.....	4
1.5 THE SECRETORY PATHWAY IN YEAST.....	6
1.5.1 The Yeast Cell Wall	7
1.5.2 Sorting of proteins through the secretory pathway.....	7
1.5.3 Glycosylation in the endoplasmic reticulum	8
1.5.4 Glycosylphosphatidylinositol (GPI) anchors	9
1.5.5 Further glycosylation in the Golgi apparatus	10
1.6 GPI ANCHORED CELL WALL/PLASMA MEMBRANE PROTEINS IN <i>SACCHAROMYCES CEREVISIAE</i>	11
1.7 CONCLUSIONS AND AIMS OF THE PROJECT	13

CHAPTER TWO

MATERIALS AND METHODS	14
2.1 MOLECULAR BIOLOGY TECHNIQUES	14
2.1.1 Enzyme treatment of DNA.....	14
2.1.2 Competent <i>E. coli</i>	14
2.1.3 Isolation of plasmid DNA from <i>E. coli</i>	15
2.1.4 Preparation of chromosomal DNA from yeast.....	15
2.1.5 Preparation of <i>S. cerevisiae</i> for transformation and introduction of DNA into <i>S. cerevisiae</i>	15

2.2	YEAST MATING, SPORULATION AND MICRODISSECTION.....	15
2.3	GENERATION OF DELETION STRAINS USING SHORT FLANKING HOMOLOGY POLYMERASE CHAIN REACTION (SFH-PCR).....	15
2.4	AMPLIFICATION OF HPF GENES AND CLONING INTO p415GAL1	17
2.5	AMPLIFICATION OF HPF GENES FOR 6XHis TAGGING AND CLONING INTO PYES2/GS	18
2.6	FERMENTATION IN CHEMICALLY DEFINED GRAPE JUICE MEDIUM	19
2.7	ETHANOL PRECIPITATION FROM FERMENTATION SUPERNATANTS	20
2.8	MANNOSE ASSAY	20
2.9	HEAT TEST FOR PROTEIN HAZE POTENTIAL	21
	2.9.1 Preparation of wine for the assay	21
	2.9.2 Preparation of samples for the ‘heat test’ assay	21
2.10	STATISTICAL ANALYSIS	21
2.11	OVEREXPRESSION OF 6XHis-HPF IN <i>S. CEREVISIAE</i>	22
2.12	PURIFICATION OF 6XHis-HPF USING IMMOBILISED METAL AFFINITY CHROMATOGRAPHY	22
2.13	SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE).....	23
2.14	PERIODATE-SCHIFF’S STAIN FOR DETECTION OF CARBOHYDRATE ON SDS-PAGE GELS	23
2.15	COOMASSIE BLUE STAINING FOR DETECTION OF PROTEINS ON SDS- PAGE GELS.....	23
2.16	TRANSFER OF PROTEINS TO NITROCELLULOSE.....	24
2.17	WESTERN BLOT ANALYSIS.....	24
2.18	PHENOTYPIC ANALYSES OF <i>HPF</i> OVEREXPRESSION AND DELETION MUTANTS.....	25
	2.18.1 Growth at various temperatures.....	25
	2.18.1.1 Plate assay	25
	2.18.1.2 Liquid assay.....	25
	2.18.2 Growth whilst under an osmotic stress.....	25
	2.18.3 Ethanol tolerance	25
	2.18.4 Oxidative stress	26
	2.18.5 Sensitivity towards Calcofluor white and Congo red.....	26

2.18.6 Zymolyase sensitivity.....	26
2.18.7 Killer phenotype	26
2.18.8 Electron microscopy.....	27
2.18.9 Cell integrity-MAP kinase mediated signal transduction pathway involvement	28
2.18.9.1 Detection of alkaline phosphatase.....	28
2.18.9.2 Caffeine sensitivity.....	28
2.18.10 Oleic acid utilisation	28
2.18.11 Competitive growth assay	28
2.18.12 α -factor growth arrest	28
2.18.13 Shmoo formation.....	29
2.18.14 Agglutination assay.....	29
2.18.15 Mating efficiency	29
2.18.15.1 Liquid assay	29
2.18.15.2 Limited filter assay	30
2.18.16 Frequency of zygote formation using a cytoplasmic mixing assay	30
2.18.17 Direct Interference Contrast Microscopy of mating cells.....	31
2.18.18 Transmission Electron Microscopy of mating cells.....	31
2.18.19 Indirect immunofluorescent localisation of Hpf1p and Hpf1'p.....	31

CHAPTER THREE

PHENOTYPIC ANALYSES OF *HPF* DELETION AND OVEREXPRESSION MUTANTS..... 32

3.1 INTRODUCTION.....	32
3.2 RESULTS.....	34
3.2.1 Construction of <i>HPF</i> overexpression strains and <i>hpf</i> Δ deletion strains.....	34
3.2.2 <i>HPF</i> deletion strains show no phenotypes linked to cell wall signalling responses.....	35
3.2.3 <i>hpf1</i> Δ , <i>hpf1'</i> Δ and <i>hpf2</i> Δ deletion mutants do not have a visible structural cell wall defect.....	36
3.2.4 <i>hpf1</i> Δ <i>hpf1'</i> Δ deletion mutants are not affected by the use of oleic acid as their sole carbon source	37
3.2.5 <i>hpf1</i> Δ <i>hpf1'</i> Δ deletion strains have improved growth at low temperature	38
3.2.6 <i>hpf1</i> Δ <i>hpf1'</i> Δ deletion strains have improved growth in the presence of ethanol	39
3.2.7 The <i>hpf1</i> Δ <i>hpf1'</i> Δ mutant out-competes the wild type under laboratory growth conditions.....	39
3.3 DISCUSSION.....	40
3.4 CONCLUSION	45

CHAPTER FOUR

BIOLOGICAL FUNCTION OF THE *HPF* GENE PRODUCTS IN YEAST 46

4.1	INTRODUCTION.....	46
4.2	RESULTS.....	49
4.2.1	Pheromone response elements upstream of <i>HPF1</i> and <i>HPF1'</i>	49
4.2.2	Mating	50
4.2.2.1	Mating efficiency	50
4.2.2.2	The mating defect of <i>hpf1</i> Δ mutants is mating type specific	51
4.2.3	α-factor growth arrest.....	51
4.2.4	Agglutination assays.....	52
4.2.5	Mating projection formation	52
4.2.6	Persistence of a septum between <i>hpf1</i> Δ <i>hpf1'</i> Δ mating partners.....	53
4.2.6.1	Detection of cell fusion defects by a cytoplasmic mixing assay.....	53
4.2.7	Immunofluorescent localisation of Hpf in <i>S. cerevisiae</i>	54
4.3	DISCUSSION.....	54
4.4	CONCLUSION	60

CHAPTER FIVE

THE EFFECT OF DELETING AND OVEREXPRESSING *HPF* GENES ON HAZE

PROTECTIVE ACTIVITY 61

5.1	INTRODUCTION.....	61
5.2	RESULTS.....	62
5.2.1	The laboratory yeast, S288c, expresses haze protective factor	62
5.2.1.1	Comparative growth and glucose metabolism of the laboratory strain, S288c, and the wine strain, AWRI838, in chemically defined grape juice medium.....	62
5.2.1.2	Haze protective activity of material isolated from the laboratory yeast strain, S288c, ferments	64
5.2.2	Deletion and overexpression of <i>HPF</i> genes from yeast provides evidence that these genes code for Hpfs.....	64
5.2.2.1	Deletion of yeast <i>HPF</i> genes reduced the haze protective activity of the supernatant material	64
5.2.2.2	Overexpression of <i>HPF</i> genes in yeast results in increased haze protective activity of supernatant material.....	65
5.2.3	Expression of 6xHis- <i>HPF</i> in yeast confirms that <i>HPF2</i> encodes a HPF	66

5.2.3.1	Expression levels of 6xHis-Hpf1p, 6xHis-Hpf1'p and 6xHis-Hpf2p.....	66
5.2.3.2	Purification of 6xHis-Hpf2p.....	67
5.2.3.3	Addition of purified 6xHis-Hpf2p to wine in a heat test affords significant haze protective activity.....	67
5.2.4	Purification of 6xHis-Hpf1p and 6xHis-Hpf1'p	68
5.3	DISCUSSION.....	68
5.4	CONCLUSION	72
CHAPTER SIX		
IDENTIFICATION OF THE ACTIVE COMPONENT OF HPF		73
6.1	INTRODUCTION.....	73
6.2	RESULTS.....	75
6.2.1	Removal of <i>N</i> -linked oligosaccharides from 6xHis-Hpf2p affects its haze protective activity	75
6.2.1.1	QIAGEN Ni-NTA is the optimal IMAC resin for purifying native and partially deglycosylated 6xHis-Hpf2p.....	75
6.2.1.2	6xHis-Hpf2p is de- <i>N</i> -glycosylated equally under native and denaturing conditions.....	75
6.2.1.3	The haze protective activity of Endo H treated 6xHis-Hpf2p is less than that of native 6xHis-Hpf2p.....	77
6.2.2	6xHis-Hpf2p expressed in glycosylation mutants has altered haze protective activity compared to the wild type	77
6.2.3	Glycosylation mutants and their effect of haze protective activity of HPF	78
6.3	DISCUSSION.....	78
6.4	CONCLUSION	83
CHAPTER SEVEN		
SUMMARY AND PERSPECTIVE FOR FUTURE WORK.....		86
APPENDIX ONE		
DNA SEQUENCES		89
APPENDIX TWO		
SOLUTIONS		105
APPENDIX THREE		
OLIGONUCLEOTIDE PRIMERS		113

APPENDIX FOUR
STRAIN LIST..... 117

APPENDIX FIVE
PLASMIDS 121

BIBLIOGRAPHY 128

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Shauna L Brown

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THESIS SUMMARY

Heat induced protein haze is a common problem in white wine. Grape derived pathogenesis related proteins slowly denature and aggregate during wine storage and this gives rise to light dispersing haze. Protein haze formation is currently prevented by removing proteins using bentonite, an aluminium silicate clay, but this method has drawbacks. A potential alternative or complementary method is the use of haze protective factors (HPF), specific mannoproteins from *Saccharomyces cerevisiae* that visually reduce protein haze.

Hpf1p was originally isolated from Muscat Gordo Blanco wine and Hpf2p from a synthetic grape juice ferment. Based on partial amino acid sequences, putative structural genes, *HPF1* and *HPF2*, for these proteins were identified. *HPF1* has a homologue, *HPF1'*, (71% similarity) in *S. cerevisiae*. Sequence analysis suggests that Hpf1p, Hpf1'p and Hpf2p are localised to the cell wall or plasma membrane.

This study aimed to determine the biological function of the *HPF* genes in *S. cerevisiae*. *HPF* overexpression and deletion strains were constructed and analysed for cell wall related phenotypes. Under a number of conditions, including cold temperature and ethanol stress, the *hpf1Δ hpf1'Δ* strain was more tolerant than the wild type strain. However, mating efficiency of the *hpf1Δ hpf1'Δ* strain was significantly less than the wild type strain and this was found to be correlated with the persistence of a septum between the mating partners. The decreased mating efficiency was also mating type specific, only occurring in *MATα* cells.

This study also aimed to establish conclusively that the *HPF* genes do indeed encode proteins with haze protective properties. Haze protective activity of the material from ferment supernatants was assessed. Material from the *HPF* deletion strains exhibited significantly less haze protective activity than the wild type. Moreover, material derived from *HPF1* and *HPF1'* overexpressors was more active than material from the wild type. A 6xHis-tagged Hpf2p was expressed and purified using immobilised metal affinity chromatography. This Hpf2p had significant haze protective activity. Modification of *N*-glycans of 6xHis-Hpf2p by Endoglycosidase H decreased its haze protective activity.

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ABBREVIATIONS

6xHis	six consecutive histidine amino acids
AWRI	Australian Wine Research Institute
BCIP	5-bromo-4-chloro-3-indolyl-phosphate
cDNA	complementary deoxyribonucleic acid
CDGJM	chemically defined grape juice medium
Da	Dalton
DIC	direct interference contrast
DNA	deoxyribonucleic acid
Endo H	Endoglycosidase H
ER	endoplasmic reticulum
FARA	flexible approach to random analysis
FITC	fluorescein isothiocyanate
GFP	green fluorescent protein
GluNAc	<i>N</i> -acetylglucosamine
GPI	glycosylphosphatidylinositol
HPF	haze protective factor
IMAC	immobilised metal affinity chromatography
kb	kilobase
kDa	kilo Dalton
mA	milli-Amps
Man	mannose
MAP	mitogen activated protein
MEN	mitotic exit network
M-Pol	mannan polymerase
M _r	relative molecular weight
mRNA	messenger ribonucleic acid
NBT	nitro blue tetrazolium
Ni-NTA	nickel-nitrilotriacetic acid

ORF	open reading frame
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pI	isoelectric point
PI(4,5)P ₂	phosphatidylinositol-4,5-bisphosphate
PNGase F	peptide- <i>N</i> -(acetyl- β -glucosaminy) asparagine amidase
PR	pathogenesis related
PRE	pheromone response element
rpm	revolutions per minute
rRNA	ribosomal ribonucleic acid
SC	synthetic complete
SDS	sodium dodecyl sulphate
SDS PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
TBS-T	tris buffered saline-Tween 20
TEM	transmission electron microscopy
TFA	trifluoroacetic acid
Tris	tris(hydroxymethyl)aminoethane
V	Volts
YPD	yeast extract/peptone/dextrose medium or Yeast Protein Database