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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DECLARATION

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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 *MAT*α 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	*N*-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-*N*-(acetyl-β-glucosaminyl) 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