Identification of genomic differences between laboratory and commercial strains of *Saccharomyces cerevisiae*

by

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Declaration

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Thesis summary

The yeast *Saccharomyces cerevisiae* is used in many industrial applications including beer brewing, bread making, and winemaking. Winemaking yeast strains have the ability to convert grape sugars into alcohol and other metabolites consistent with good wine. An exploratory comparative approach was undertaken to identify the genes and corresponding proteins that give wine yeast strains of *S. cerevisiae* their distinctive phenotype, with a focus on studying genes that provide tolerance to ethanol.

A genomic and proteomic approach has been used to identify potential 'wine specific' genes. By using amplified fragment length polymorphism (AFLP) techniques, it has been demonstrated that commercial winemaking strains have genetic sequences within their genome that may have arisen from other *Saccharomyces* sensu stricto yeasts. This is the first known report of a wine strain having *Saccharomyces kudriavzevii* genetic sequences encoded within its genome.

To further explore the phenotypic characters distinguishing wine yeast strains from other *S. cerevisiae* strains, a comparative proteomics approach was taken. A proteomics platform using two-dimensional gel electrophoresis (2D gels) has elucidated target proteins for future research, including a glycolytic protein, Tdh3p (glyceraldehyde 3-phosphate dehydrogenase), as well as a one-carbon pool protein, Shm2p (serine hydroxymethyltransferase). The latter protein was characterised

further to determine its possible role and function in wine strains, with results indicating a potential role in wine flavour. It has also been shown that certain wine strains may have different mechanisms for transcription/translation control of *SHM2*.

Using the comparative proteomic approach above, no differences were seen between laboratory strains and wine strains after exposure to an ethanol stress. To ascertain the genes that enable *S. cerevisiae* strains to counteract the high ethanol concentrations encountered during grape juice fermentation, a continuous culture approach was utilised. Ultimately, this will reveal genes that are important to *S. cerevisiae* strains to acclimatise to a high ethanol environment, as opposed to a short-term ethanol stress. The continuous culture approach identified 34 genes that significantly changed expression in the ethanol-containing cultures, suggesting their involvement in ethanol tolerance of *S. cerevisiae*.

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Abbreviations

2D gel	Two-dimensional polyacrylamide gel electrophoresis
AFLP	amplified fragment length polymorphism
APAF	Australian Proteome Analysis Facility
AWRI	Australian Wine Research Institute
cDNA	complementary deoxyribonucleic acid
DIG	digoxigenin
DNA	deoxyribonucleic acid
FBA1	fructose bis-phosphate aldolase
GC-MS	gas chromatography - mass spectrometry
HPLC	high performance liquid chromatography
HSP	heat shock protein
HSTF	heat shock transcription factor
IEF	isoelectric focusing
ITS RFLP	internal transcribed spacer restriction fragment length polymorphism
MALDI-TOF	matrix-assisted laser desorption ionisation time-of-flight spectrometry
MAP	mitogen activated protein
mRNA	messenger ribonucleic acid
MS/MS	tandem mass spectrometry
NIRS	near infra-red spectroscopy
ORF	open reading frame
PCA	principle component analysis

PCR	polymerase chain reaction
PI	isoelectric point
rDNA	ribosomal deoxyribonucleic acid
RNA	ribonucleic acid
RP	ribosomal protein
RPM	revolutions per minute
SAGE	serial analysis of gene expression
SC	synthetic complete
SDS	sodium dodecyl sulphate
SGD	*Saccharomyces* Genome Database
SHM2	serine hydroxymethyltransferase
SPI1	stationary phase induced
STRE	stress response element
TBS-T	tris buffered saline – Tween 20
TDH3	glyceraldehyde 3-phosphate dehydrogenase
TRIS	tris(hydroxymethyl)aminoethane
WUGSC	Washington University Genome Sequencing Centre
YPD	yeast extract/peptone/dextrose medium