

**Determination of the genetic basis for
successful fermentation in high sugar media**

by

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Thesis summary

Yeast (*Saccharomyces cerevisiae*) plays a key role in the completion of several fermentations including those used for beverage and bioethanol production. In the wine industry, slow or incomplete alcoholic fermentation is still a challenging problem and often results in increased costs of production and decreased wine quality. One of the reasons for the persistence of this problem could be the trend towards rising sugar concentrations in grape musts. What is already a high sugar concentration fermentation ($\sim 200 \text{ g L}^{-1}$ or more) has increased by some $20 - 40 \text{ g L}^{-1}$ due to climate warming and winemaker pursuit of ripeness. In this project we aim to gain a better understanding of how wine yeast cope in high sugar fermentations (HSF) to help develop strategies for managing these types of grape musts.

With the availability of collections of laboratory yeast including gene deletion and overexpression libraries and the development of techniques used for whole genome analysis, it is now possible to investigate yeast biology under oenological conditions with a systems biology approach. A number of genome-wide studies of yeast have previously been conducted to identify yeast genes involved in sensitivity to individual stresses present during fermentation. However, in reality many of these stresses are often present at the same time, or sequentially throughout the phases of fermentation. This highlights an important gap in current research, that being identification of those genes important for maintenance of fermentation efficiency throughout a complete cycle of fermentation, and in particular an environment which has high initial sugar content such as that found in grapes used to make quality wines. We expected these genes to be related to wine yeast adaption, survival and maintenance of fermentative metabolism.

In this study 93 genes were identified as important for the successful completion of high sugar fermentation as deletants of these resulted in either protracted or incomplete fermentation. We have named this gene set the Fermentation Essential

Genes (FEGs). A gene ontology (GO) analysis of these revealed that vacuolar acidification (VA) is an important biological process required for efficient completion of a high sugar fermentation: 20 of the 93 FEGs annotate to this GO term (vacuolar acidification). Also, this gene set is highly represented in the FEGs since these 20 FEGs represent 77% of all genes annotated to this same GO term. In this study we also report 18 genes (also all FEGs), not previously associated with VA, of which deletants have VA defects. This was achieved through examination of the VA of 93 FEGs using the vacuolar specific probe 6-carboxyfluorescein diacetate (6-CFDA), microscopic and Fluorescence Activated Cell Sorting (FACS) analysis.

It was shown that, nine FEGs were seen to be particular critically to fermentation progression and completion. Their deletion result in the extreme phenotype of arrested or ‘stuck’ fermentation. Amongst these, featured two genes involved in trehalose biosynthesis. The disaccharide trehalose is an enigmatic compound accumulated in *Saccharomyces* and known to be associated with survival under environmental stress conditions. Deletion of either *TPS1* or *TPS2*, encoding enzymes involved in trehalose biosynthesis, resulted in incomplete fermentation. This phenotype could be reversed by the over-expression of *HXK2* (a paralog of *HXK1* encoding hexokinase isomer 2) in $\Delta tps1$ and introduction of the phosphotrehalase gene (TreA), from *Bacillus subtilis*, in $\Delta tps2$. *HXK2* over-expression increased the fermentation rate of $\Delta tps1$ and the parent BY4743 which actually demonstrated a shorter fermentation duration than the parent having blank plasmid.

To further investigate fermentation of yeast in HSF we sought to examine the fermentation performance of a gene overexpression library, which was constructed in this study by transformation of a Yeast Genomic Tiling Collection into a haploid wine yeast strain; ISOC9d Δ leu2. The construction of this library was performed in collaboration with two other PhD students (Mrs Jade Haggerty and Ms Jin Zhang). The clonal identity, degree of plasmid retention and development of methodologies to allow fermentation in high sugar chemically defined grape juice medium (CDGJM) were achieved. However, due to time constraints further evaluation of this library was not possible within the current project.

The collective findings from this project have provided greater insight into the mechanism by which yeast cope with HSF as well as providing direction if not specific gene targets for exploitation in strain improvement programs.

Declaration of Authorship

This thesis 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, contains no material previously published or written by another person, except where reference has been made in the text.

Essentially all of the work detailed in Chapter 4 has been submitted for publication.

This thesis may be made available for loan or photocopying.

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Abbreviations

AGRF	Australian Genome Research Facility
ATP	adenosine triphosphate
bp	base pairs of DNA
°C	degrees centigrade
CDGJM	chemically defined grape juice medium
CDGJM+PP	CDGJM enriched with polyphenol extract
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
FACS	Fluorescence Activated Cell Sorting
FAN	free amino acid nitrogen
GO	Gene Ontology
HOG	high osmolarity glycerol
HPLC	high performance liquid chromatography
HSE	heat shock elements
HSF	high sugar fermentation
kb	kilo bases of DNA
LB	Luria-Bertani
LiAc	lithium acetate
M	molar
mL	millilitre
mM	millimolar
mol	mole
NAD ⁺	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide reduced form
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	nicotinamide adenine dinucleotide phosphate reduced form
NOPA	o-Pthaldialdehyde/N-acetyl-L-cysteine
OD	optical density
OE	over-expression
ORF	open reading frame
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PEG	polyethylene glycol
RI	refractive index
RNA	ribonucleic acid
rpm	revolutions per minute
SDS	sodium dodecyl sulphate
STRE	stress responsive element
TAE	tris acetate EDTA
TCA	tri-carboxylic acid
TE	tris EDTA
Tre6P	trehalose-6-phosphate
IPTG	isopropyl β -D-1-thiogalactopyranoside
UDP-Glucose	uridine diphosphate glucose
UTR	untranslated region

URS	upstream regulatory sequence
VA	vacuolar acidification
μL	microliter
μM	micromolar
v/v	volume per volume
w/v	weight per volume
YAN	yeast assimilable nitrogen
YEPD	yeast extract peptone dextrose
X-Gal	5-bromo-4-chloro-indolyl-galactopyranoside

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