Investigation and Characterisation of Highly Nitrogen Efficient Wine Yeast

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Declaration

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Abstract

Sufficient yeast assimilable nitrogen (YAN) is essential for wine yeast (*Saccharomyces cerevisiae*) to complete alcoholic fermentation. YAN largely consists of alpha-amino acids and ammonium ions. With adequate YAN (as well as other nutrients) in grape juice, yeast cellular processes such as protein synthesis, growth and proliferation occur, allowing alcoholic fermentation to proceed efficiently. By contrast, insufficient YAN may result in sluggish or stuck fermentation and is often coupled with the formation of undesirable aromas, such as hydrogen sulfide, which impact on wine quality. Employment of highly nitrogen efficient (HNE) wine yeast provides an alternative strategy to facilitate the completion of alcoholic fermentation under limited nitrogen conditions. In this study, a group of HNE candidate strains were investigated in both synthetic media and grape juice under different YAN conditions. A mutant with disruption of *ECM33* showed superior fermentation performance under various YAN conditions compared with the wild type. Accordingly, the Δ*ecm33* strain is defined as a HNE strain. The role of *ECM33* was further investigated using the loss-of-function Δ*ecm33* mutant. Growth on agar plates containing Calcofluor White (CFW) or Congo Red (CR) was limited, suggesting that Δ*ecm33* possesses a cell wall defect resulting in increased chitin (target of the antifungals CFW and CR). QRT-PCR results showed that the transcriptional abundance of a group of key genes involved in the cell wall integrity (CWI), high osmolarity glycerol (HOG) and central nitrogen metabolism (CNM) pathways were altered in Δ*ecm33* mutant. In order to understand the HNE mechanism in the Δ*ecm33* strain, two genes *PTP2* and *SLT2* were investigated by overexpression, and also evaluated for their fermentation performance in synthetic media. Results showed that the overexpression of *PTP2* improved yeast fermentation performance in the late stages of fermentation. *SLT2* overexpression was

not found to be helpful for fermentation. Metabolites examined in fermentation samples showed that a higher concentration of citric acid and ethanol were produced in *PTP2* overexpression (OEX) strains. Increased ethanol yield was also observed in the *SLT2* OEX strain. Less acetaldehyde was produced in the Δ*ecm33* background strains during alcoholic fermentation. Based on the observations in this study, it is suggested that the HNE phenotype of Δ*ecm33* might be triggered by activation of a metabolic network(s), including: one or more of the CWI, HOG or CNM pathways, resulting in a more robust yeast cell with good fermentative capability and adaption to the dynamic environment of alcoholic fermentation.

List of abbreviations

Abbreviation	Full term
Δ	Gene deletion
3’	Three prime, of nucleic acid sequence
5’	Five prime, of nucleic acid sequence
A	Adenine
AGRF	Australian Genome Research Facility
Ap^r	Ampicillin resistance
AWRI	Australian Wine Research Institute
bp	Base pairs, of nucleic acid
C	Cytosine
$CaCl_2$	Calcium chloride
Cat #	Catalogue number
CDGJM	Chemically defined grape juice medium
CDGJSM	Chemical defined grape juice starter medium
cDNA	Complementary deoxyribonucleic acid
CDS	Coding sequence
cfu	Colony forming units
CFW	Calcofluor white
Conc.	Concentration
CR	Congo red
CRS	Congo red sensitive
CWI	Cell wall integrity
D	Aspartic acid
DAP	Diammonium phosphate
DNA	Deoxyribonucleic acid
DO	Drop-out
E	Glutamic acid
EDTA	Ethylenediaminetetraacetic acid
EMS	Ethyl methanesulfonate
F/Fwd	Forward
G	Glycine
gDNA	Genomic deoxyribonucleic acid
GPI	Glycosyl-phosphatidylinositol
h	Hour
HNE	Highly nitrogen efficient
HOG	High osmolarity glycerol
I	Isoleucine
KO	Knock out
LWR	Length to width ratio
Lys	Lysine
MAPK	Mitogen-activated protein kinase
Mpa	Megapascal
N	Nitrogen group
N	Asparagine

NCR	Nitrogen catabolite repression
NF	Normalization factor
NREL	Normalized relative expression level
OD	Optical density
OEX	Overexpression
ORF	Open reading frame
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PTP	Tyrosine phosphatase
Q	Glutamine
QRT-PCR	Real-time PCR/quantitative PCR (qPCR)
QTLs	Quantitative trait locus
R	Arginine
R/Rvs	Revers
RNA	Ribonucleic acid
S	Serine
SNPs	Single nucleotide polymorphisms
T	Thymine/threonine
TAE	Tris Acetate EDTA
TOR	Target of rapamycin
Tris	Tris(hydroxymethyl)aminomethane
UV/Vis	Ultraviolet visible
v/v	Volume per volume
WT	Wild type
YAN	Yeast assimilable nitrogen
YPD/YEPD	Yeast extract peptone dextrose