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Wine yeasts of New Zealand

An investigation into their distribution,
contribution to Sauvignon Blanc aroma
and interaction in co-ferments

Nicole Anfang

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degree of Doctor of Philosophy in Biological Sciences

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Abstract

Sauvignon Blanc is the most important grape variety for New Zealand's economy with exports of ~\$740M in 2008. The two varietal thiols 3-mercaptohexanol (3MH) and 3-mercaptohexyl acetate (3MHA), reminiscent of tropical aromas like passion fruit and grapefruit, have been identified as key aroma compounds in Marlborough Sauvignon Blanc. These compounds are released during fermentation due to the metabolic action of yeast. This thesis investigates the contribution of natural New Zealand yeast isolates to the characteristic aroma of Sauvignon Blanc. Natural *Saccharomyces* and non-*Saccharomyces* isolates were screened for their 3MH and 3MHA production. Four non-*Saccharomyces* yeasts from two species, *Candida zemplinina* and *Pichia kluyveri*, were identified as predominantly producing either 3MH or 3MHA. In subsequent co-fermentation experiments with commercial *S. cerevisiae* strains, the combination of VL3 and *P. kluyveri* I at a ratio of 1:9 showed remarkable increase in 3MHA production. Analysis of nitrogen usage in this co-ferment revealed that *P. kluyveri* I was able to grow on proline as sole nitrogen source in a synthetic medium. Proline is one of the most abundant amino acids in grape juice but cannot be utilized by *S. cerevisiae* as its degradation pathway requires oxygen. Nothing is known about proline utilization in *P. kluyveri* but the data presented here suggests a different utilization pathway than that found in *S. cerevisiae*.

In addition, a *S. cerevisiae* population was isolated for the first time from a New Zealand vineyard, mainly from soil samples. Furthermore, *S. cerevisiae* strains found in a nearby apiary matched genotypes found in the vineyard, providing evidence that bees are transport vectors for *S. cerevisiae*.

*Around here, however, we don't look backwards for very long.
We keep moving forward, opening new doors, and doing new things,
because we're curious and curiosity keeps leading us down new paths.*

Walt Disney

Für Opi

(19.03.1916 – 1996)

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Abbreviations

#	number
(NH ₄) ₂ SO ₂	ammonium sulfate
°C	degrees Celsius
3MH	3-mercaptohexan-1-ol
3MHA	3-mercaptohexyl acetate
4M2M2MB	4-methoxy-2-methyl-2-mercaptobutane
4MMP	4-mercapto-4-methylpentan-2-one
AA	amino acid(s)
AWRI	Australian Wine Research Institute
BC	before christ
BHA	butylated hydroxyanisole
bp	base pair
CBS	Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands)
cfu(s)	colony forming unit(s)
CO ₂	carbon dioxide
DMDC	dimethyl dicarbonate
DNA	deoxyribonucleic acid
dNTP(s)	deoxynucleotide triphosphate(s)
ESR	Environmental Science and Research Institute
FRST	Foundation for Research, Science and Technology

gt	genotype
HCl	hydrochloric acid
ITS	internal transcribed spacer
Kg, g, µg, ng	kilogram, gram, microgram, nanogram
L, ml, µl	litre, millilitre, microlitre
MgCl ₂	magnesium chloride
min	minutes
n	number
Na-acetate	sodium acetate
OD	optical density
p	probability value
PCR	polymerase chain reaction
pHMB	sodium-4-(hydroxymercuri)benzoate
rDNA	ribosomal DNA
rpm	revolutions per minute
s.e.m.	standard error of the mean
SD medium	synthetic defined medium
SO ₄	sulfate
TA	titratable acidity
YAN	yeast available nitrogen
YNB –aa-c-n	yeast nitrogen base, without amino acids, carbon and nitrogen source
YPD	yeast extract, peptone, dextrose rich medium