Investigations into L-Methionine Degradation During Wine Fermentation and the Role of Yeast *ARO* Genes in the Formation of 3-(Methylthio)-1-Propanol

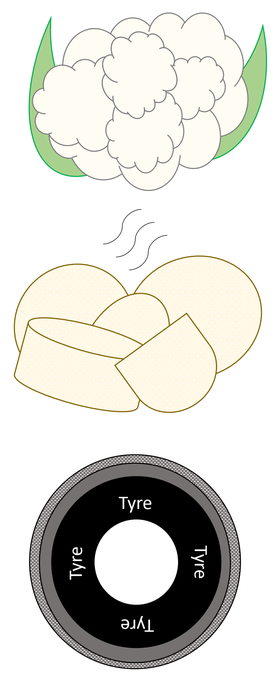
Rebecca C. Deed[*1,2], Ruoyu Hou[2], Matias I. Kinzurik[2], Richard C. Gardner[1] and Bruno Fedrizzi[2]

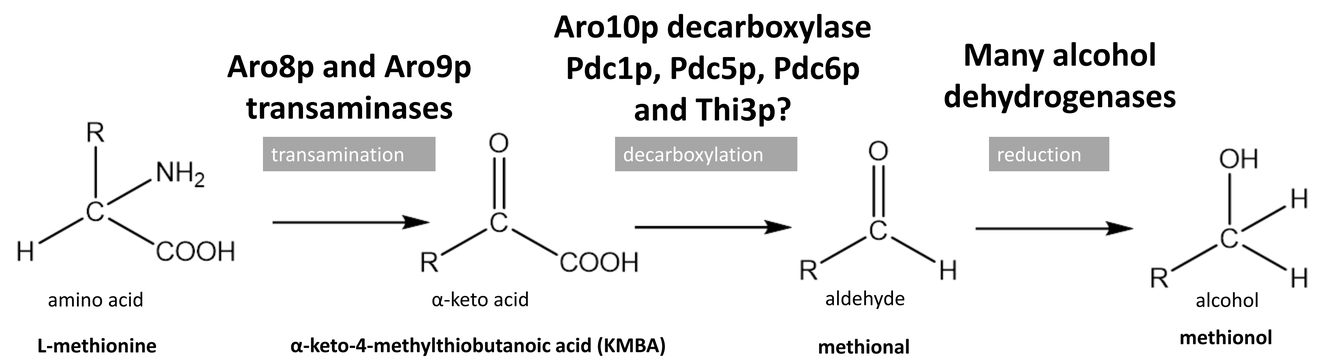
School of Biological Sciences[1] and School of Chemical Sciences[2], University of Auckland, Private Bag 92019, Auckland, New Zealand

Email: rebecca.deed@auckland.ac.nz

Introduction

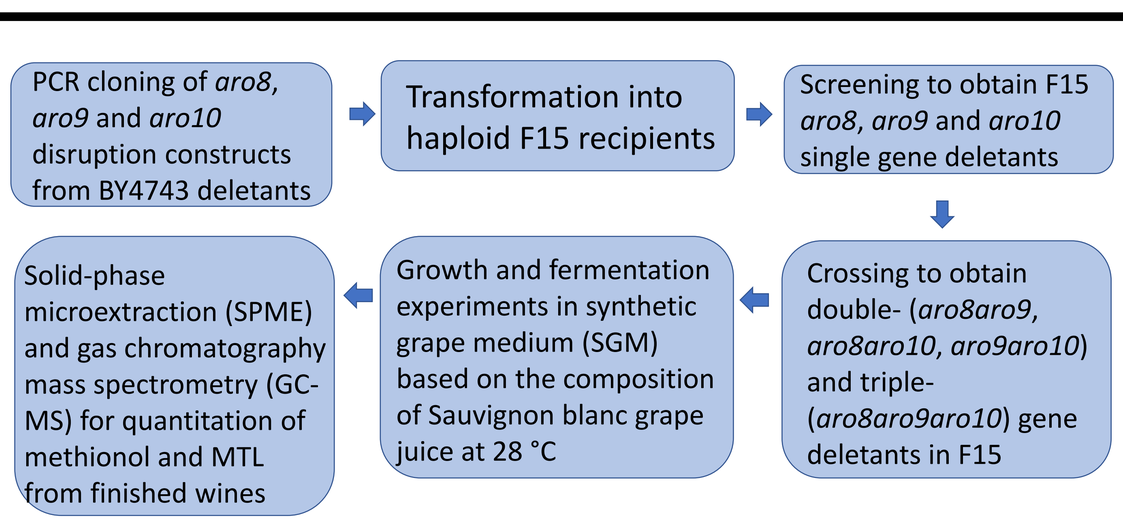
3-(Methylthio)-1-propanol (methionol) is a volatile sulfur compound (VSC) produced by *Saccharomyces cerevisiae* yeast during fermentation, imparting off-odours of rubber, cauliflower and boiled potato to wines [1]. Methionol is produced via the step-wise degradation of L-methionine (L-Met) via the Ehrlich pathway for yeast to scavenge nitrogen. In *S. cerevisiae* lab yeast strains, the *ARO8* and *ARO9* genes have been shown to encode enzymes which can perform the transamination step from L-Met to α-keto-4-methylthiobutanoic acid (KMBA) [2]. *ARO10* encodes a decarboxylase with broad specificity which can convert KMBA into the aldehyde, methional. Additional decarboxylases such as Pdc1p, Pdc5p, Pdc6p, and Thi3p, could play complementary or competing roles with Aro10p. The last step of methionol formation from methional is likely catalysed by numerous alcohol dehydrogenase enzymes.



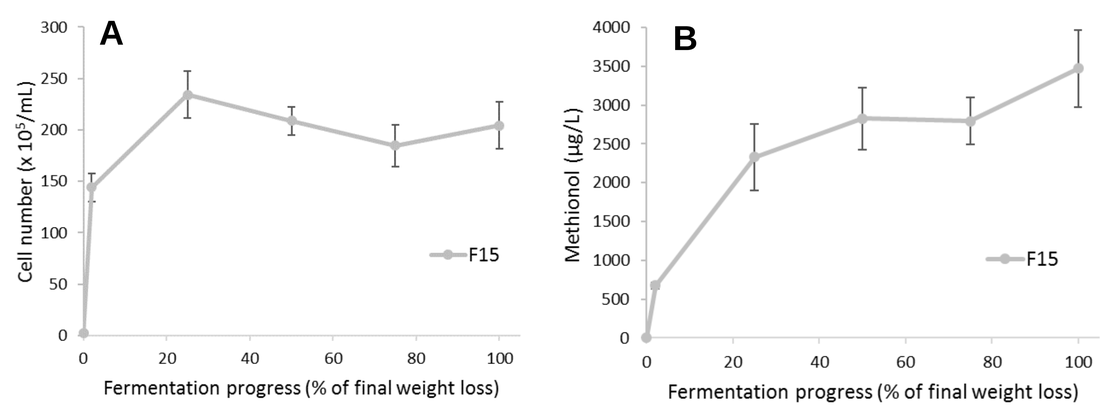


This research looked at the role of *S. cerevisiae* *ARO* genes, using multiple deletions, in the production of methionol from L-Met in the wine yeast strain, F15. We also investigated the possibility of an alternative pathway for L-Met degradation, via demethiolation of L-Met or KMBA, by quantitating the accumulation of a VSC, methanethiol (MTL), which could arise from direct demethiolation, and by measuring methionol concentrations in an F15 *str3* mutant.

Methods

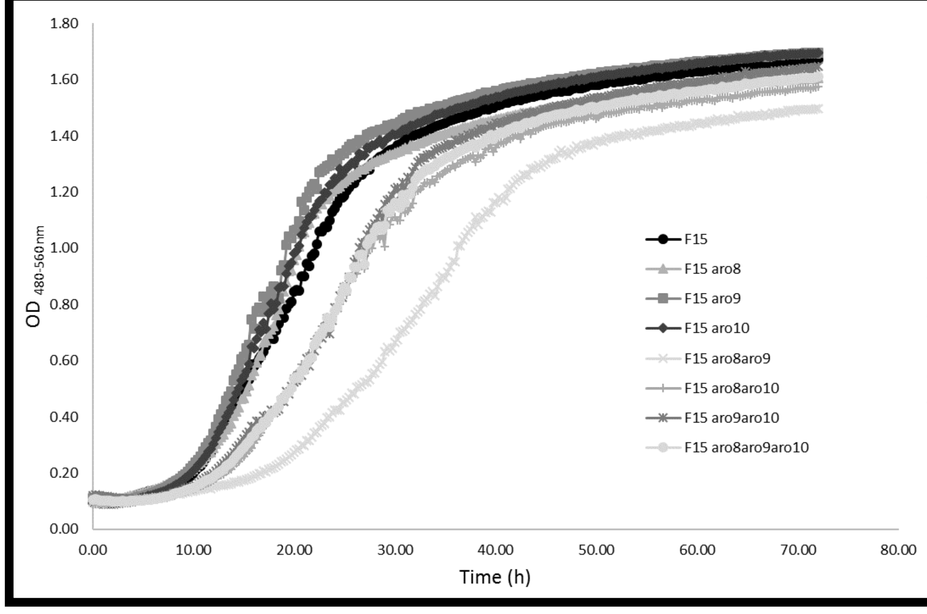


Kinetics of methionol formation during wine fermentation



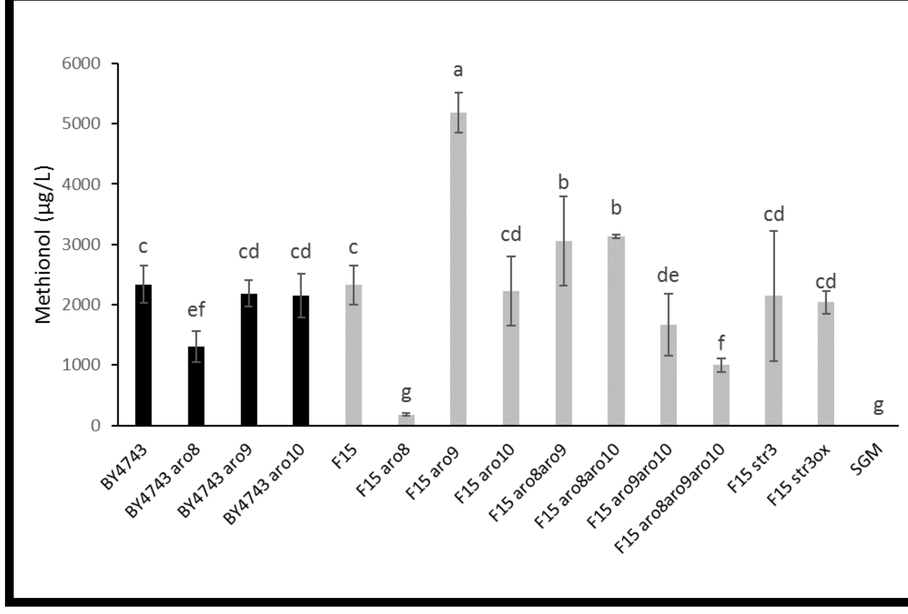
Fermentation progress (% of final weight loss) of F15 in SGM at 28 °C plotted against cell number (× 10^5/mL) (A) and methionol production (µg/L) at five stages of fermentation (B). Error bars represent 95 % confidence intervals, n = 3.

F15 double- and triple-*aro* gene deletants have a reduced growth rate (*aro8aro9* in particular) compared to F15 and F15 single *aro* deletants



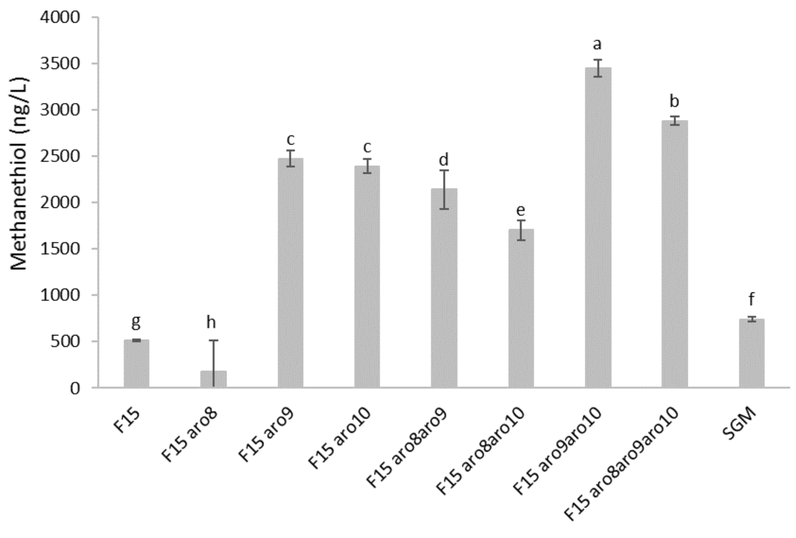
Semi-anaerobic growth (OD 480-560 nm) of F15, and F15 single- double- and triple deletants in *aro8*, *aro9* and *aro10* in at 28 °C in SGM, each line represents the mean of n = 5.

Methionol production in BY4743 and F15 *aro* gene deletants



Methionol production (µg/L) in BY4743, BY4743 *aro* deletants, F15, F15 *aro* and *str3* deletants and F15 *STR3* overexpressing strain (grey bars) fermented at 28 °C in SGM. Error bars represent 95 % confidence intervals, n = 3. Samples not connected by the same letters are significantly different (single factor ANOVA with Fisher's LSD at $p < 0.05$).

Methanethiol production in F15 *aro* gene deletants



MTL production (ng/L) in SGM by F15 single- double- and triple deletants in *aro8*, *aro9* and *aro10* fermented at 28 °C. Error bars represent 95 % confidence intervals, n = 3. Samples not connected by the same letters are significantly different (single factor ANOVA with Fisher's LSD at $p < 0.05$).

Discussion and conclusions

- Double and triple *aro* gene deletions had a negative impact on the growth rate of F15. The double transaminase deletant (F15 *aro8aro9*) had the slowest growth rate, suggesting an impaired ability to utlilise amino nitrogen.
- The single *aro8* deletion reduced methionol formation by 50 % in BY4743 and by 90 % in F15. Therefore, *ARO8* contributes greatly to L-Met transamination and plays a key role in methionol accumulation, particularly in wine yeast.
- The single deletion of *aro9* in BY4743 had no effect on methionol production compared to BY4743, suggesting that *ARO8* is sufficient for transamination. Unexpectedly, deletion of *aro9* in F15 resulted in a 2-fold increase in methionol production, suggesting that the absence of *ARO9* results in an upregulation of *ARO8* and the Ehrlich pathway.
- The *aro10* deletion by itself had no effect on methionol accumulation, suggesting that other yeast decarboxylase enzymes may convert KMBA to methional during fermentation. This result is contrary to findings in the literature [3].
- Patterns of methionol production in double- and triple-*aro* mutants are highly complex and could involve significant feedback regulation and activity of other decarboxylases. Surprisingly, deletion of both transaminases in F15 *aro8aro9* and F15 *aro8aro9aro10* still yielded methionol, suggesting the possibility of another pathway to methionol.
- An appreciable quantity of MTL (739 ng/L) was quantitated in SGM without yeast, indicating that this VSC can be produced chemically and may result from Strecker degradation of L-Met, as demonstrated for Japanese sake [4].
- The modulation of MTL concentrations by yeast indicates a yeast-mediated pathway and the potential demethiolation of KMBA or even methional to MTL.

References

[1] Selli, S. *et al*. (2008). *J. Agric. Food Chem*. **56**, 227-234.
[2] Iraqui *et al*. (1998). *MGG.* **257**, 238-248.
[3] Perpète, P. *et al*. (2006). *FEMS Yeast Res.* **6**, 48-56.
[4] Isogai *et al*. (2009). *J. Agric. Food Chem*. **57**, 189-195.

Acknowledgments

Thanks to New Zealand Winegrowers and the Romeo Bragato Trust for funding this project.