Thesis submitted for the award Doctor of Philosophy

Submitted for examination November 2011

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Thesis title:

Genes and mechanisms responsible for β-glucoside metabolism in the oenologically important lactic acid bacterium *Oenococcus oeni*

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Abstract

The lactic acid bacterium *Oenococcus oeni* plays a pivotal role in winemaking by carrying out malolactic fermentation (MLF), which results in the decarboxylation of L-malic acid to L-lactic acid. It is the species commonly inoculated for MLF but also it will often spontaneously develop after alcoholic fermentation because of its superior tolerance to wine conditions such as high alcohol (up to 16% v/v), low pH (from 3.0 to 4.0) and little or no residual sugar. A marked increase in aroma has been reported after the completion of MLF. This increase has been principally attributed to enzymatic modifications by lactic acid bacteria. In accordance with this *O. oeni* has been reported to possess β-glucosidase activity. The hydrolysis of β-glucosides in wine can have a significant impact on the sensory profile of a wine by conferring an increase in aroma. Many aroma compounds in wine and must are found in the glycosidic form (i.e. linked to a sugar) and are only perceivable in their non-glycosidic form. For this reason it is of interest to characterise such activities, particularly in *O. oeni*.

Comparative sequence analyses of lactic acid bacteria suggest that six open reading frames (AG1 and ORFs 1 to 5) from the sequenced *O. oeni* PSU-1 are involved in the hydrolysis of β-glucosides. The ORFs 1 to 3 demonstrated homology to glycosyl hydrolase family (GHF) 1 β-glucosidase/β-glucanase/phospho-β-glucosidase N-terminal and active site signature sequences, whilst AG1 and ORF 4 were lacking the N-terminal signature sequence. Glycosyl hydrolase family 3 β-glucosidase signature sequences

were identified in ORF 5. ORF 1 (subsequently designated *bglD*) was characterised as a GHF 1 phospho-β-glucosidase and found to be part of a phosphoenolpyruvate phosphotransferase system (PEP-PTS) β-glucoside metabolising operon, *bgl*. Site directed mutagenesis identified a single amino acid responsible for the affinity of BglD towards phosphorylated substrates, providing insight to the catalytic mechanism for all GHF 1 enzymes. ORF 2 and 3 (designated *celD* and *celC*) were also characterised as GHF 1 phospho-β-glucosidases and are components of a second PEP-PTS β-glucoside metabolising operon, *cel*. Neither AG1 nor ORF 4 could be expressed as soluble proteins and it is speculated that the lack of the GHF 1 N-terminal signature sequence is responsible for this. ORF 5 was found to be a GHF 3 β-glucosidase. Transcriptional analysis indicates that these β-glucosidase metabolising operons may be regulated by carbon catabolite repression and transcriptional anti-termination.

Given the potential impact of β-glycosidases on the sensory profile of wine, it is hoped that the characterization of β-glycosidase systems from *O. oeni* will provide information to aid winemakers in tailoring wine aroma, colour and overall complexity where grape quality may otherwise be compromised due to adverse weather conditions or poor viticultural practices.

Acknowledgments

To my supervisors, I could not have made a better choice. I would make the same choice again. My three supervisors all went above and beyond to provide guidance, direction, support, each with his/her own unique complementing skill set.

To my principal supervisor Vladimir Jiranek, your vision, vast knowledge and expectation of excellence has enabled me to get to where I am.

To my co-supervisor and very good friend Michelle, I already miss your guidance, advice, calming presence and many cups of tea.

To my co-supervisor Chris Ford, having the ability to access your specialised knowledge and refer to you for matters where I needed a third opinion was priceless.

A special mention to my colleague and good friend Krista, who started her own PhD at the same time with much more experience than myself, took me under her wing, displayed an unmeasurable amount of patience and availability to help me. I cannot thank-you enough.

To Tommaso, Frank and Vix. Tommaso kept me laughing and Frank fixed everything. Vix helped me on many aspects of my PhD but is also a very special friend. I thank you all.

To an academic in our laboratory, Paul, who has been a constant point of reference for all wine and research related queries. I will also miss his dedication to Friday afternoon beers.

To the entire wine microbiology and biotechnology lab, I very much enjoyed my time which can be attributed the people in it.

To my parents for their love, absolute support and encouragement for as long as I can remember.

To my Dad for being my mentor.

To my mum for teaching me the skills which have enabled me to manage all aspects of life.

Lastly,

To my rockstar future husband.

His support, love and encouragement have made this journey an awesome life experience.

Statement of Authorship

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to **Alana Capaldo** and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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