Characterisation of glycosidase enzymes of wine Lactic Acid Bacteria

By

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To my family,
who supported and encouraged me
in this adventure.

Abstract

Many compounds important for wine quality are in the glycosidic form, that is a sugar moiety is attached, through a glycosidic linkage, to the alcoholic group of the compound. Such glycosidic compounds comprise several groups, all having great relevance to wine quality and can be broadly grouped as the aromatic compounds and compounds that contribute to colour. The former group, when in the glycosidic form are, unfortunately, odourless and do not contribute to the aroma of wine. However, once the sugar moiety is cleaved, they regain their aromatic characteristics. This is the reason why glycosyl-terpenols, for example, are considered a potential source of aroma in wine. On the other hand, anthocyanins need to be in the glycosidic form to contribute to colour in wine, especially red wines. Once de-glycosylated, these compounds tend to lose a substantial colour capacity (at least in wine conditions, particularly at low pH) and become more chemically reactive. In red wines this outcome may be undesirable but, conversely, for rose or 'blanc de noir' wines, decolourisation might be beneficial.

Of the many methods to increase the amount of aroma or manipulate colour, enzymatic hydrolysis seems to be the most appropriate for wine since it has lesser drawbacks compared to methods such as acidic hydrolysis or heating. Given the particular nature of glycosides in wine, attention has to focus on five glycosidase enzymes: β-D-glucopyranosidase, α-D-glucopyranosidase, α-L-rhamnopyranosidase, α-L-arabinofuranosidase and β-D-xylopyranosidase. This project has investigated the presence and distribution of glycosidases amongst 40 isolates of Lactic Acid Bacteria (LAB) (22 of which being *Oenococcus oeni* and the remaining 18 equally represented by *Lactobacillus* and *Pediococcus*), as a start to investigating their potential application in winemaking. Three lines of research were followed in this study: a) a biochemical investigation of glycosidase activities using artificial (*p*-nitrophenol-linked) substrates, b) an investigation of the enzymatic effect of LAB on wine anthocyanins, and c) identification and cloning of a putative β-glucosidase gene from *Oenococcus oeni*.

From this work it was clear that all *O. oeni* strains studied showed activity against most of the glycosides tested, both under optimized and wine-like conditions. Most importantly, some strains showed an increase or no effect by the presence of glucose and a few cases glycosidases, such as α-arabinosidase and α-rhamnosidase,

were in fact highly stimulated when fructose was present in the assay medium. By comparison, *Lactobacillus* and *Pediococcus* isolates showed activity only against selected glycosidase substrates. Highest enzymatic activities were observed for all tested strains at pHs nearer neutral with the nature and magnitude of such activities being highly strain-dependent.

In order to increase understanding of the interaction of LAB glycosidases with natural substrates, the ability of arbutin, salicin and the anthocyanin, malvidin-3-glucose, to induce these activities were studied. In subsequent experiments, the ability for anthocyanins to be decolourised when LAB isolates were present in the incubation media was examined, with the resulting reduction in colour intensity be obvious to the naked eye. While loss of malvidin-3-glucoside was monitored by HPLC analysis, the appearance of the expected breakdown product(s) could not be confirmed.

β-D-glucopyranosidase is a well characterised enzyme in many organisms, including several LAB. With this information it was possible to locate three β-D-glucopyranosidases in the *Oenococcus oeni* genome, which has recently been sequenced and published on GenBank. These enzymes were inserted in what, most likely, seemed operons of the phosphotransferase system (PTS) of the carbohydrate catabolism. Two of these enzymes were in the same operon, which showed a higher PTS structure than the other. In fact there was a gene codifying for putative a transcriptional regulator, most probably of the PTS domain EII, representing a cellobiose permease. Genomic DNA was extracted from strain Oen2, being the bacterium that showed the highest glycosidase activity. One of the b-D-glucopyranosidase enzyme was PCR amplified and sequenced, showing 25 nucleotide modifications that produced 6 amino acid substitutions.

Wine LAB therefore may represent a valid alternative as a source of enzymes for use in winemaking and other food industry processes. They clearly possess and range of glycosidase activities, which in some cases appear to overcome many of the drawbacks (e.g. inhibition by pH, ethanol and/or sugar) found with enzymes derived from other wine organisms or fungi commonly exploited in food technology. Wine LAB also influence the persistence of malvidin-3-glucoside in incubation assays, which may have important repercussions for wine quality.

Declaration 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 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.

Components of Chapter 1 and essentially all of the work detailed in Chapters 3 and 4 has been published in peer-reviewed scientific journals:

Matthews, A., Grimaldi, A., Walker, M., Bartowsky, E., Grbin, P.R. and Jiranek, V. (2004) Lactic acid bacteria as a source of enzymes for use in vinification. *Applied and Environmental Microbiology*. **70**, 5715-5731.

Grimaldi A., Bartowsky, E. and Jiranek, V. (2005) A survey of glycosidase activities of commercial strains of *Oenococcus oeni*. *International Journal of Food Microbiology*. **105**, 233-244.

Grimaldi, A., Bartowsky, E. and Jiranek, V. (2005) Screening of *Lactobacillus* spp. and *Pediococcus* spp. for glycosidase activities that are important in oenology. *Journal of Applied Microbiology*. **99**, 1061-1069.

Copies of these papers are included in Appendix 11.

I give consent to this copy of my thesis being made available in the University Library. The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

Antonio Grimaldi

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