

Characterisation of glycosidase enzymes of wine Lactic Acid Bacteria

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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 β -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 a 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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TABLE OF CONTENTS

ABSTRACT	iii
DECLARATION OF AUTHORSHIP	v
ACKNOWLEDGEMENTS	vi
1 LITERATURE REVIEW	1
1.1 Introduction	1
1.2 Glycosides as precursors of aroma	2
1.2.1 Types of aglycones and sugars	5
1.3 Presence of glycosides and their aglycones in plants	5
1.3.1 The plant kingdom	5
1.3.2 Grapevine and cultivated grape varieties	6
<i>1.3.2.1 Sugar moieties of grape glycosides</i>	6
<i>1.3.2.2 Aromatic aglycones of grape varieties</i>	9
<i>1.3.2.3 Grape glycosides important for wine colour</i>	13
1.4 Methods used to liberate aromatic compounds from glycosides in wine	18
1.4.1 Acidic hydrolysis and heating	18
1.4.2 Enzymatic hydrolysis	19
1.5 Studies of glycosidases in wine and grape juice	21
1.5.1 Grapevine glycosidases	21
1.5.2 Fungal glycosidases	22
<i>1.5.2.1 Immobilised enzymes extracted from fungi</i>	24
<i>1.5.2.2 Commercial enzymatic preparations having glycosidase activity</i>	24
1.5.3 Enzymes from yeasts	27
1.5.4 Glycosidases of wine bacteria	29
<i>1.5.4.1 Lactic Acid Bacteria in wine</i>	29
<i>1.5.4.2 Glycosidases of wine Lactic Acid Bacteria</i>	30
1.6 Conclusions	31
2 RESEARCH PLAN	32
2.1 Significance of the project	32
2.2 Objectives of the project	33

3	BIOCHEMICAL CHARACTERISATION OF <i>OENOCOCCUS OENI</i> GLYCOSIDASE ENZYMES	34
3.1	Introduction	34
3.2	Materials and methods	34
3.2.1	Bacterial strains and cultivation	34
3.2.2	Determination of glycosidase activity of <i>O. oeni</i>	35
3.2.3	Temperature dependence of glycosidase activity	37
3.3	Results	37
3.3.1	pH and substrate interactions	38
	3.3.1.1 <i>β</i> -D-glucopyranosidase	38
	3.3.1.2 <i>α</i> -D-glucopyranosidase	39
	3.3.1.3 <i>β</i> -D-xylopyranosidase	39
	3.3.1.4 <i>α</i> -L-rhamnopyranosidase and <i>α</i> -L-arabinofuranosidase	39
3.3.2	Temperature optima of glycosidase activities	44
3.3.3	Influence of ethanol, glucose or fructose on glycosidase activity	47
3.3.4	Influence of multiple parameters on glycosidase activity	52
3.4	Discussion	54
4	BIOCHEMICAL CHARACTERISATION OF <i>LACTOBACILLUS</i> AND <i>PEDIOCOCCUS</i> GLYCOSIDASES	58
4.1	Introduction	58
4.2	Materials and methods	59
4.2.1	Bacterial strains and cultivation	59
4.2.2	Determination of glycosidase activity	59
4.3	Results	61
4.3.1	Effect of pH	61
4.3.2	Effect of ethanol	64
4.3.3	Effect of glucose and fructose	66
4.3.4	Effect of temperature	69
4.3.5	Effect of combined parameters	69
4.4	Discussion	72

5	INFLUENCE OF LACTIC ACID BACTERIA ON WINE PIGMENTS	75
5.1	Introduction	75
5.2	Materials and methods	80
5.2.1	Anthocyanin extraction	80
5.2.2	Induction of β -glucopyranosidase activity by natural glycosides	80
	5.2.2.1 <i>LAB growth</i>	80
	5.2.2.2 <i>Induction assay conditions</i>	80
5.2.3	Action of LAB β -D-glucopyranosidase on wine anthocyanins	83
	5.2.3.1 <i>Anthocyanin medium and incubation conditions</i>	83
	5.2.3.2 <i>HPLC analysis of hydrolysed anthocyanins</i>	83
	5.2.3.3 <i>Colour measurements at 520 nm</i>	84
5.3	Results	84
5.3.1	Induction of LAB β -D-glucopyranosidase activity	84
5.3.2	Action of LAB β -glucopyranosidase on wine anthocyanins	87
5.4	Conclusions	96
6	GENETIC CHARACTERISATION OF <i>OENOCOCCUS OENI</i> GLUCOSIDASES	98
6.1	Introduction	98
6.2	Materials and methods	102
6.2.1	DNA extraction and purification from <i>Oenococcus oeni</i>	102
6.2.2	PCR conditions	102
6.2.3	Design of primers	102
6.2.4	Sequencing of <i>O. oeni</i> β -D-glucopyranosidase genes	103
	6.2.4.1 <i>Nucleotide analysis</i>	103
6.3	Results	106
6.3.1	Localisation of the putative genes for β -D-glucopyranosidase in <i>Oenococcus</i> and <i>Pediococcus</i>	106
	6.3.1.1 <i>Operon 1</i>	110
	6.3.1.2 <i>Operon 2</i>	110
6.3.2	Sequencing of putative <i>O. oeni</i> β -D-glucopyranosidase genes	116
6.4	Conclusions	121

7	GENERAL CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS	122
APPENDIX 1	Influence of pH on the α -L-rhamnopyranosidase activity of <i>O. oeni</i> .	131
APPENDIX 2	Influence of pH on the α -L-arabinofuranosidase activity of <i>O. oeni</i> .	132
APPENDIX 3	Influence of pH on the β -D-xylopyranosidase activity of <i>Pediococcus spp.</i>	134
APPENDIX 4	Influence of pH on the β -D-xylopyranosidase activity of <i>Lactobacillus spp.</i>	135
APPENDIX 5	Influence of pH on the α -L-rhamnopyranosidase activity of <i>Pediococcus spp.</i>	136
APPENDIX 6	Influence of pH on the α -L-rhamopyranosidase activity of <i>Lactobacillus spp.</i>	137
APPENDIX 7	Influence of pH on the α -L-arabinofuranosidase activity of <i>Pediococcus spp.</i>	138
APPENDIX 8	Influence of pH on the α -L-arabinofuranosidase activity of <i>Lactobacillus spp.</i>	139
APPENDIX 9	Homology between <i>Oenococcus oeni</i> transcriptional regulator Oen610 with similar bacterial genes.	140
APPENDIX 10	Classes of terpenols found in wine.	141
APPENDIX 11	Publications arising from this study.	142
9	BIBLIOGRAPHY	181