Biochemical and Molecular Characterisation of Oenologically Important Enzymes Identified in Lactic Acid Bacteria

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ABSTRACT

Enzyme concentrates are available for use in commercial wineries to aid in wine processing, or to enhance wine quality. However, pectolytic enzyme remains the sole product routinely used in most wineries. One disadvantage of some of the products currently available commercially is they contain enzymes sourced from microorganisms not usually associated with grape juice or wine, typically fungi such as *Aspergillus* species. As a result, enzymes are inefficient catalysts under the harsh oenological conditions. In addition, some products contain secondary, and potentially undesirable, contaminant enzyme activities. Clearly there is the potential to develop enzyme preparations specifically for use in grape juice and wine. A potential source of such enzymes are the lactic acid bacteria (LAB), the organisms more commonly associated with the conduct of the malolactic fermentation (MLF) during vinification. In this study, the production of cell-associated enzymes with potential oenological applications by LAB was investigated.

A screening of 50 LAB isolates for the production of lipases, esterases, tannases, and polysaccharide-degrading enzymes revealed wine LAB can produce enzymes of oenological importance. In general, activity towards polysaccharide substrates was more frequent among the lactobacilli and pediococci strains. Lipase activity was observed in three lactobacilli, and all strains were found to have tannase activity. Similarly, all strains displayed some esterase activity, although the activity was markedly stronger among the *Oenococcus oeni*.

On the basis of the initial screen, a more detailed characterisation of the esterase activity of selected LAB isolates was conducted. Esterase activity was examined across a range of pH, temperature, and ethanol concentrations - all important oenological parameters. In addition, substrate specificity was determined using six ester substrates. In general, activity was maximal at pH values close to 6.0, and temperatures close to 40°C, although exceptions were observed with some strains. Increases in ethanol concentration resulted in lower activity for most lactobacilli and pediococci, but stimulated the esterase activity of all *O. oeni*.

Work conducted with dairy LAB isolates has suggested esterases may be capable of both hydrolysing and synthesising esters. In the wine industry, the results of some volatile-profiling studies tend to support this theory, with concentrations of esters being reported to both increase and decrease during MLF. Malolactic fermentation trials were conducted in

wine with six strains of *O. oeni* and GCMS was used to quantify particular esters before and after MLF. Some esters were found to increase in concentration during MLF, while others were found to decrease. These findings suggest LAB esterases are in fact capable of both synthesising and hydrolysing ester substrates in wine.

To further dissect the esterase make-up of selected LAB strains, attempts to clone and heterologously express three structural genes for these enzymes were made. Three putative esterase genes were identified in *O. oeni* and cloned. Sequencing was completed and alignment with published esterase sequences used to reveal theoretical proteins of the *O. oeni* genes with high homology with those from other organisms. Of note, key features, such as active site motifs, were conserved in each *O. oeni* sequence. Expression of the recombinant proteins in *E. coli* resulted in higher esterase activity in one of the clones compared to the host. These results indicate that the open reading frame of one esterase gene in *O. oeni* has been identified.