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The effect of polysaccharide-degrading wine yeast transformants on the efficiency of wine processing and wine flavour

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Abstract

Commercial polysaccharase preparations are applied to winemaking to improve wine processing and quality. Expression of polysaccharase-encoding genes in *Saccharomyces cerevisiae* allows for the recombinant strains to degrade polysaccharides that traditional commercial yeast strains cannot. In this study, we constructed recombinant wine yeast strains that were able to degrade the problem-causing grape polysaccharides, glucan and xylan, by separately integrating the *Trichoderma reesei XYN2* xylanase gene construct and the *Butyrivibrio fibrisolvens END1* glucanase gene cassette into the genome of the commercial wine yeast strain *S. cerevisiae* VIN13. These genes were also combined in *S. cerevisiae* VIN13 under the control of different promoters. The strains that were constructed were compared under winemaking conditions with each other and with a recombinant wine yeast strain expressing the endo-β-1,4-glucanase gene cassette (*END1*) from *B. fibrisolvens* and the endo-β-1,4-xylanase gene cassette (*XYN4*) from *Aspergillus niger*, a recombinant strain expressing the pectate lyase gene cassette (*PEL5*) from *Erwinia chrysanthemi* and the polygalacturonase-encoding gene cassette (*PEH1*) from *Erwinia carotovora*. Wine was made with the recombinant strains using different grape cultivars. Fermentations with the recombinant VIN13 strains resulted in significant increases in free-flow wine when Ruby Cabernet must was fermented. After 6 months of bottle ageing significant differences in colour intensity and colour stability could be detected in Pinot Noir and Ruby Cabernet wines fermented with different recombinant strains. After this period the volatile composition of Muscat d'Alexandria, Ruby Cabernet and Pinot Noir wines fermented with different recombinant strains also showed significant differences. The Pinot Noir wines were also sensorial evaluated and the tasting panel preferred the wines fermented with the recombinant strains.

Keywords: Recombinant wine yeast; Glucanase; Xylanase; Pectinase; Wine processing; Wine colour; Aroma

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1. Introduction

Throughout the winemaking process, various biochemical reactions are catalysed by enzymes originating from the grapes, yeasts and other microbes associated with the vineyard and winery (for a review see Van Rensburg and Pretorius, 2000). The addition of desired enzymes to the fermentation can help to improve wine processing and quality. In wine production, maceration refers to the breakdown of grape solids following grape crushing. The addition of macerating enzymes during this period is common practice (Gil and Vallés, 2001). The cell wall of a grape berry cell has a common set of structural polysaccharides, with the main constituents being pectin, cellulose and hemicellulose (Barnavon et al., 2001). The commercial enzyme preparations used during maceration are typically blends of pectinases, cellulases, hemicellulases and other enzymes with carbohydrase activities (Gump and Haight, 1995).

Degradation of the structural polysaccharides by carbohydrases can result in an improvement in juice yield, clarification and filterability during winemaking (Colagrande et al., 1994; Haight and Gump, 1994; Van Rensburg and Pretorius, 2000). The action of these enzymes also facilitates the liberation and solubilisation of phenolic compounds and glycosydic precursors from the cells of the skins, seeds and flesh of the grape berry (Ganga et al., 2001; Günata et al., 1985; Gump and Haight, 1995; Watson et al., 1999). Increased extraction of phenolic compounds, such as tannins, leads to more polymeric pigments being formed in aged red wine, resulting in increased colour intensity and stability (Haight and Gump, 1994; Watson et al., 1999). Terpenols are important constituents of the fruity aroma of Muscat grapes (Günata et al., 1985). The terpenols are present as non-volatile, glycosydically bound precursors and as free volatile forms (Mateo and Jiménez, 2000). The glycosydically bound terpenols are located mainly in the grape skins (Günata et al., 1985). Enzymatic degradation of the cell wall contributes to the release of bound precursors from the berries. The hydrolysis of these precursors during fermentation can result in an improvement in wine aroma (Zoecklein et al., 1997).

Most commercial pectinase and glucanase preparations are derived from *Aspergillus* and *Trichoderma*, respectively (Canal-Llauberes, 1993). Commercial enzymes are typically crude fungal preparations and, besides containing the desired enzymes, impurities such as mucilage, proteins and enzymes with undesired side-activities might be present (Wightman et al., 1997). Since the endogenous polysaccharase activity of *S. cerevisiae* is very limited, the heterologous expression of specific polysaccharase genes in wine yeast can improve the winemaking process. Therefore, it might be possible to produce higher quality wines without the addition of expensive commercial enzyme preparations. Since only the desired enzymes are secreted by the recombinant strain, there will be no undesired side activities detrimental to wine quality.

In this paper, we describe the construction of four polysaccharide-degrading wine yeast strains, expressing the *Trichoderma reesei* endo-β-1,4-xylanase gene, *XYN2* (La Grange et al., 1996) and the *Butyrivibrio fibrisolvens* endo-β-1,4-glucanase gene, *END1* (Van Rensburg et al., 1994). Expression of these genes was regulated by combining the genes with different promoter regions. For constitutive expression the $ADH1_P$ and $TEF1_P$ were used. For regulated expression the $ADH2_P$ was used for expression. The $YG100_P$ ($SSA1_P$) used has a low basal level of expression and expression can be induced by shock conditions. The four recombinant wine yeast strains were constructed by integrating the cassettes into the *ILV2* locus of the commercial wine yeast strain, VIN13 (Anchor Yeast SA). The cassette $TEF1_P$-*XYN2*-$ADH2_T$ was integrated into VIN13, now designated strain VIN13-DLG29; $ADH1_P$-$MF\alpha1_S$-*END1*-$TRP5_T$ was integrated into VIN13, now designated VIN13-DLG30; $ADH1_P$-$MF\alpha1_S$-*END1*-$TRP5_T$ and $ADH2_P$-*XYN2*-$ADH2_T$ were integrated into VIN13, now designated VIN13-DLG33; and $ADH1_p$-$MF\alpha1_S$-*END1*-$TRP5_T$$YG100_P$-*XYN2*-$ADH2_T$ was integrated into VIN13, now designated VIN13-DLG39. Wine was made with the four recombinant strains of VIN13 (VIN13-DLG29, VIN13-DLG30, VIN13-DLG33, VIN13-DLG39), as well as with the following strains: a pectolytic strain, designated VIN13-PPK expressing the *Erwinia chrysanthemi* pectate lyase gene *pelE* [the *PEL5* gene cassette described by Laing and Pretorius (1992) and the *Erwinia carotovora* polygalactoronase gene *peh1* [the *PEH1* gene cassette described by Laing and Pretorius (1993)]. A glucanase- and xylanase-secreting strain, VIN13-PEX expressing the endo-β-1,4-glucanase gene *end1* gene from *B. fibrisolvens* together with the endo-β-1,4-xylanase gene *xyn4* from *Aspergillus*