Purification and characterization of a *p*-coumarate decarboxylase and a vinylphenol reductase from *Brettanomyces bruxellensis*

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Abstract

The presence of *Brettanomyces* bruxellensis has been correlated with an increase of phenolic aromas in wine. The production of these aromas results from the metabolization of cinnamic acids, present in the wine, to their ethyl derivatives. Hence, the participation of two enzymes has been proposed: a *p*-coumarate decarboxylase (CD) and a vinylphenol reductase (VR). Both enzymes were purified and characterized from *B. bruxellensis*. In denaturing conditions, the CD enzyme had a molecular mass of 21 kDa, while in native conditions its mass was 41 kDa. The optimal activity was obtained at a temperature of 40 °C and a pH of 6.0. For *p*-coumaric acid, the K_m value and V_{max} were 1.22 ± 0.08 mM and 98 ± 0.15 μmol/min mg, respectively. The VR enzyme had a molecular mass of 37 kDa in SDS-PAGE, while in natural conditions its mass was 118 kDa. The K_m value was > 3.37 ± 2.05 mM and its V_{max} was 107.62 ± 50.38 μmol/min mg for NADPH used as a cofactor. Both enzymatic activities were stable at pH 3.4, but in the presence of ethanol the CD activity decreased drastically while the VR activity was more stable. This is the first report that shows the presence of a CD and a VR enzyme in *B. bruxellensis*.