

Captan and Folpan inhibition of PCR amplification of a 375 bp DNA fragment specific for the 18S region of *Saccharomyces*

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Abstract:

The off-flavor metabolites produced by nutritionally fastidious yeasts can be economically disastrous to the wine producer since they render the wine unpalatable, decreasing the quality of the wine, and resulting in a lower sale price. These microorganisms are difficult to be detected by traditional culture media since these techniques involve complex enrichment. The polymerase chain reaction (PCR) is a well established molecular technique that uses tiny amounts of genetic material to be amplified, it does not depend on culture media for cell growth and so it can overcome several difficulties. This essentially enzymatic reaction can be employed to detect microorganisms in foods and beverages but it can also be susceptible to inhibitors. Molecular systematic investigation of members of the ascomycetous yeast genera or monitoring spoilage microorganisms in wine may not be an easy task due the presence of inhibitor compounds. Besides the presence of wine naturally occurring inhibitors, cultural practices, like fungicide treatments can also contribute to add external inhibiting factors if residues of these compounds are present in wine. The inhibition is likely to be dependent upon the residue levels. Captan and Folpan are widely employed in Brazilian vineyards to control fungal threats. The purpose of this work was to detect the action of these compounds upon Taq polymerase using a forward primer Fungf (5'-CTTAGTTGGTGGAGTGATTTG-3') and a reverse primer Fungr (5'-GGTACTAGCGACGGGCG-3') that amplify a 375 bp DNA fragment specific for the 18S region of fungi. The efficiency of PCR and yeast metabolism were evaluated with the strain *Saccharomyces cerevisiae* Embrapa 1vvt/97 (BRM-4687) in the presence of different concentrations of Captan (1200-recommended dose, 600, 300, 150, 75, and 37.5 µg/mL) and Folpan (1350-recommended dose, 675, 337.5, 168.8, 84.4 and 42.2 µg/mL). The amplification was observed only at 75, and 37.5 µg/mL Captan. Negligible amplification was obtained at 84.4 µg/mL Folpan and visible amplification was possible only at 42.2 µg/mL Folpan. Captan and Folpan are, depending on the concentration, strong Taq polymerase inhibitors. The presence of both fungicides can make the hard work of monitoring process of spoilage microorganisms in wine even harder. These fungicides also showed to be strong inhibitors of the cell yeast metabolism.

Key words: Fermentation, fungicides, PCR, Taq polymerase, yeast