THE MOLECULAR DETECTION OF BRETTANOMYCES WILD YEAST IN RAW WINES

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Brettanomyces is the yeast commonly associated with red wine spoilage through the production of volatile phenol compounds. If not managed properly, it can cause severe economic loss. Timely detection of *Brettanomyces* in raw wine is essential for ensuring wine quality preservation.

In this work, home-designed primers for detection of *Brettanomyces* in wine by Sybr Green I-based real-time PCR (Polymerase Chain Reaction) were developed. Primers were tested both in silico and in vitro. The primers were designed using the sequence of *Brettanomyces internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence*, available in GeneBank. Primer specificity was checked by aligning the primers against the sequences available in GeneBank using BLAST. Primer performance was tested using positive (wine sample infected with Brettanomyces) and negative controls.

The developed primers were used for analyzing 5 raw wines (Pinot Noir Taraclia, Merlot Taraclia, Merlot Nisporeni, Malbec Romanești, Cabernet-Sauvignon Taraclia), produced in the winery of the Technical University of Moldova, for the presence of *Brettanomyces*. Two methods of DNA extraction from wine were tested and compared, DNAsol-based method and SDS-PVP-based method of DNA extraction.

In the result, four out of five samples (Merlot Taraclia, Merlot Nisporeni, Malbec Romanești, Cabernet-Sauvignon Taraclia) were positive for Brettanomyces, when the DNA was extracted using SDS-PVP-based method, and three out of five (Merlot Taraclia, Malbec Romanești, Cabernet-Sauvignon Taraclia) were positive, when DNA was extracted using DNAsol-based method. Moreover, the Ct values of all positive samples extracted by SDS-PVP method were lower than of those extracted by DNAsol, indicating higher amount of Brettanomyces DNA in those samples. This confirms that SDS-PVP DNA extraction method from wine was more efficient, and allows to detect *Brettanomyces* even in the samples with low abundance.

The developed primers for Sybr Green I-based qPCR detection of *Brettanomyces* allow for a rapid and reliable detection of this wine spoilage yeast in raw wines and prevent the economic loss. Also, our findings highlight the importance of the DNA extraction method of choice for the successful PCR detection of *Brettanomyces* in wine, especially in the wines with relatively low level of Brettanomyces infection.

Keywords: Brettanomyces, DNA extraction, primers, PCR, Sybr Green, wine

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