THE DETECTION OF MYCOTOXIGENIC MICROORGANISMS IN GRAPE MARC USING REAL-TIME PCR

Rodica STURZA[1]
Valentin MITIN[2]
Irina MITINA[2]
Dan ZGARDAN[1]*
Emilia BEHTA[1]

[1]*Technical University of Moldova, 168, Stefan cel Mare Bd., Chisinau, RM*
[2] *Institute of Genetics, Physiology and Plant Protection, 20, Padurilor st., Chisinau, RM*

**Corresponding author:* Dan Zgardan, dan.zgardan@enl.utm.md

Wine production generates a considerable amount of winemaking waste (grape marc). The rich content of bioactive substances in the composition of grape marc, derived from grape skin, remaining pulp, seeds and stems is makes it a good choice for use in the pharmaceutical industry, cosmetics and, especially, in the food industry. Thus, grape marc could have various applications, if it is confirmed to be free of unwanted microorganisms, for example, producers of mycotoxins. In this work, we developed a Real-Time Polymerase Chain Reaction (Real-Time PCR) methodology for testing the presence of potentially mycotoxogenic fungal species capable of producing *ochratoxin A* (OTA), which could be applied before grape marc processing.

The eight grape marc samples of Feteasca Neagra, Cabernet-Sauvignon, Merlot, Pinot Noir varieties were collected from different geographical zones. We also used as a positive control DNA extracted from wheat grains with visual signs of fungal infection.

Total deoxyribonucleic acid (DNA) was extracted from wine using SDS-based DNA extraction methods with some modifications. In a Real Time PCR we used SYBR Green I nonspecific dye as the fluorescent agent and 40 cycles of DNA amplification.

During this work, two pairs of primers (P183-184; P185-186) based on *Vitis vinifera* 26S ribosomal RNA gene sequence were developed. Using these primers in PCR reaction can confirm that the extracted DNA is of PCR quality and is free of PCR inhibitors. Besides, these primers allow normalizing the amount of the input DNA in PCR reaction for estimation of the amount of pathogen DNA relative to plant DNA in different samples.

Primers for detection of the potential producers of OTA were designed based on the sequence of OTA non-ribosomal *peptide synthetase* gene. Four pairs of primers (P71-72; P73-74; P75-76; P77-78) can recognize the following pathogens containing OTA non-ribosomal peptide synthetase gene in their genome, and thus potentially capable of producing OTA: *Aspergillus nidulans*, *Aspergillus tubingensis*, *Aspergillus tamarii*, *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus carbonarius*, *Penicillium viridicatum*, *Penicillium carneum*, *Penicillium aurantiogriseum*, *Penicillium melanoconidium*, *Penicillium nordicum*, *Penicillium verrucosum.*

SDS-based method of DNA extraction was adapted for purifying the DNA from grape marc.

Though the grape marc DNA was of a sufficient quality for PCR reaction, no DNA belonging to potential OTA producers from genera *Aspergillus* and *Penicillium* was detected. This can speak in favor of grape marc biological safety for further use.

The proposed Real-Time PCR method for verifying the presence of mycotoxigenic microorganisms in grape marc would avoid further contamination of grape marc, derived products and grape marc extracts, obtained from this by-product of vinification.

Keywords: *Mycotoxin, OTA, Real-Time PCR, grape marc, Aspergillus, Penicillium*

Acknowledgments: This work was supported by Moldova State project 20.80009.5107.09 ”Improvement of food quality and safety by biotechnology and food engineering”, running at Technical University of Moldova.