F.62. DETERMINATION OF PRIMERS EFFICIENCY IN THE DETECTION OF *PEDIOCOCCUS* IN WINES

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Abstract. *Pediococcus spp.* are lactic acid bacteria which are considered spoilage in the wine making processes. They are responsible for fast production of diacetyl and glucan, causing undesirable olfactive changes in wines, instabilities and increasing viscosity levels (wine ropiness). Early detection can minimize the negative effects of *Pediococcus spp.* on wine quality and prevent the development of advanced spoilage stages. The goal of this work was development and testing of a fast and efficient Real Time Polymerase Chain Reaction (RT-PCR)-based method for easy detection of *Pediococcus spp*. strains in wine in the early stages of wine spoilage. To achieve this goal, primer sets for the RT-PCR were designed to allow for correct and efficient detection of *Pediococcus spp.*, and experimentally tested. Total DNA from the red wine produced from endogenous grapevine variety Feteasca neagra infected with *Pediococcus spp.* was isolated. Specific primers were designed based on the DNA sequences available in public databases. The primers were tested in RT-PCR reaction with the isolated DNA as a template. The melt curves for each primer set were built. The melt curves for each primer set showed a single well pronounced peak of the expected melting temperature. The efficiency of the primer sets was determined using the serial dilutions of the template and the appropriate calculations. The best primer pair showed the efficiency of 100.36%. The average Cq/Ct value obtained for the wine sample was equal to 22.51. All tested primer sets can be used for detection of *Pediococcus spp.* in wine in the

early stage of wine spoilage, with one primer set showing the best efficiency and capability of detecting *Pediococcus spp.* in the template with higher dilution factor. Significance and Impact of the Study: The developed primer sets can be successfully used for early detection of *Pediococcus spp*. in wines by RT-PCR, as an alternative to the traditional culture-based methods which are time and labor consuming.

Keywords: Real time PCR, wines spoilage, lactic bacterium, dilutions, microbiological DNA