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ANAIS DO 10º SIMPÓSIO DE RECURSOS GENÉTICOS PARA A AMÉRICA LATINA E O CARIBE

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COMPARISON OF MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY (MALDI-TOF MS) AND PCR-RAPD AS POTENTIAL TOOLS TO DIFFERENTIATE SACCHAROMYCES CEREVISIAE STRAINS

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Saccharomyces cerevisiae plays an important role in wine industry as the main yeast to conduct the vinification. This species besides being responsible for converting sugars into alcohol has also an essential function on the release of byproducts that enhance wine aroma and complexity. The presence and the levels of these byproducts in wine are specific of each strain, which impact directly in the product quality. The use of autochthonous yeast can increase wine typicity and this regional character can contribute with the achievement of Geographical Indications for wines. In this sense, it is important to discriminate the genetic variability of the strains used on winemaking in order to authenticate them and, consequently, ensure product quality. The objective of this work is to evaluate the use of MALDI-TOF MS and PCR-RAPD fingerprinting for the distinction of Saccharomyces cerevisiae strains. Five strains were used in this study. Four of them (1B84, 26B84, 91B84 and 1VVT97) belong to vineyards from Bento Gonçalves (RS, Brazil) and the last one is a commercial strain (K1, Lallemand). For PCR-RAPD analysis, primers (GTG)₅, (GAC)₅, (GACA)₄ and M13 were used to discriminate the strains. Gel electrophoresis were conducted on 1,5% agarose and the band pattern were used to cluster analysis. For MALDI-TOF MS, a protein extraction was performed for each strain and were deposited in triplicate to minimize random effects. A consensus spectrum was produced and a main spectra projection (MSP) dendrogram was constructed using the specific function present on Biotyper software (v. 3.0.1, Bruker Daltonics, Germany). Concerning the potential for discriminating between the five strains, the primer GACA was not able to distinguish between K1, 26B84 and 1B84. The remaining primers discriminated 91B84 from the other strains. The primer M13 produced three different profiles, differentiating K1 and 26B84 from 1VVT97 and 1B84; and 91B84 presented a pattern completely different from the others. No correlation between PCR-RAPD and MALDI-TOF MS clustering was found. MALDI-TOF MS spectra are mainly composed of ribosomal proteins and may allow a strain-specific typing based on phenotypes. The dendrogram constructed from the average spectra of the strains has discriminated the commercial strain, K1, from the other four strains isolated from South Brazil. PCR-RAPD is a widespread technique for investigating genetic variability and its potential were confirmed for the strains analyzed. In the other hand, MALDI-TOF MS appears to be a powerful tool for determining the distributions of the strains such as its geographic locations. The combining techniques might bring a higher discriminatory ability for strain authentication purposes. This study is being expanded to more strains from different geographical regions.

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