MOLECULAR IDENTIFICATION OF SACCHAROMYCES CEREVISIAE STRAIN ISOLATED FROM SPONTANEOUS FERMENTATION OF MANGO (Mangifera indica L.), PULP.

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ABSTRACT

Traditionally, the identification of yeasts have been made through the physiological, biochemical and morphological characteristics. However, the number of phenotypic tests carried out to identify a yeast is very large, leading to a lengthy and costly identification, and not always manages to get the level of species. In the identifying using phenotypic testing, reproducibility can be questionable, since it often depends on the physiological state of the cells and often has no parallel with those determined from the analysis of gene sequences. In this work the aim was, through molecular biology, identify yeasts isolated in the process of spontaneous fermentation of mango, aiming the production of brandy. The spontaneous fermentation of mango occurred for 7 days and every 24 hours aliquots were taken and diluted in saline solution 0.9% performing successive dilutions until 10⁻⁷ and for each dilution was made in Middle GPYágar cultivation and incubated at 30° C for 3 days. The colonies were counted and differentiated according to your morphology and representative types. Were purified by three times stretch marks in plates containing Middle GPY agar and incubated under the same conditions. The extraction of DNA from purified colonies 20 was performed with phenolchloroform and the fragments purified DNA with HIMEDIA kit. The amplification by PCR primers used ITS-1 (5-TCC ´GTA GGT GAA CCT GCG G-3 ´) and ITS-4 (5 '-TCC TCC GCT TAT TGA TAT GC-3 ') and were viewed in 1.5% agarose gel. Sequencing reactions of only 2 samples were carried out using the DYEnamic ET dye Terminator kit cycle sequencing (Amersaham). The sequences were compared to the genbank database using the Blast. The sequences with high rate of identity (> 98%) were considered for phylogenetic reconstruction, using 7 MEGA and the evolutionary history is implied by using the method of maximum likelihood. The analysis of the sequences showed high identity with sequences corresponding to Saccharomyces cerevisae species. The S. cerevisiae species is the main yeast responsible for alcohol production, through alcoholic fermentation and when the yeast is selected from a region, it is already adapted to that environment, and may facilitate the introduction of these strains in the fermentation process.

Key Words: Yeast. Molecular Biology. Fermentation.