GENE EXPRESSION OF ATF1 IN Saccharomyces cerevisiae UMARN3 AND ITS EFFECT ON THE CONTENT OF VOLATILE ESTERS PRODUCED BY FERMENTATION

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Introduction. During fermentation process, the yeast Saccharomyces cerevisiae produces a wide range of volatile compounds, being organic esters the most abundant. Such substances are responsible of the highly desired fruity and candy aromas in the fermented beverages (1). The alcohol acetyltransferases I and II encoded by the ATF1 and ATF2 genes, respectively, catalyzes the synthesis of acetate-fatty acids esters (2). Overexpression of ATF1 gene during fermentation produces a significant increase in acetate esters (3). ATF1 transcription is regulated by the low oxygen response element (LORE), which functions as a cis element for transcriptional gene activation in hypoxic conditions (4). The aim boarded in this work focus in to increase the acetate esters production by increment of the ATF1 gene expression in the industrial-used S. cerevisiae UMARN3 yeast. Parameters as growth rate, yield and content of volatile compounds including acetate esters will be evaluated.

Methods. Oligonucleotides were designed for amplify the ATF1 gene by PCR, including its regulatory, promoter and terminator sequences. Yeast genomic DNA was used as template. DNA fragments were cloned into the pJET1.2/blunt vector and transferred into the Escherichia coli JM101 strain. Plasmid pJET1.2/LORE contains a tandem repeat of LORE sequences (GCCAACCCAACAAA AATTTCG) -83 nt upstream to ATG star codon in the ATF1 promoter. S. cerevisiae UMARN3 competent cells were transformed with linearized recombinant plasmid for homologous integration into the ATF1 gene. For screening transformed cells, the yeast clones were grown on YPD medium under fermentative conditions and the acetate esters were determined in the cells-free medium by the ferric hydroxamate test. Selected recombinant clones were submitted to further analyses such as determination of the acetyltransferases activity, fermentation parameters and volatile compounds content by GC-MS analyses.

Results. To induce ATF1 gene expression and obtains increased levels of acetyltransferase activity in the yeast used for fermentation, the LORE cis-element was duplicated into the promoter region of the ATF1 gene (Figure 1).

The results obtained at the date correspond to DNA plasmids constructions with the cis transactivation LORE element duplication incorporated into the promoter region of the ATF1 gene. S. cerevisiae UMARN3 was transformed with the plasmid obtained and five putative clones have been selected for further analyses. The results of acetyltransferases activity, fermentation parameters and determination of volatile compounds by GC-MS will be showed. Results obtained will contribute to improving the organoleptic quality of fermented beverages, favored by increasing of the volatile compounds content such as acetate esters.

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References.