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

Lorena Farías-Rosales; Alma Laura Díaz-Pérez y Jesús Campos-García. Universidad Michoacana de San Nicolás de Hidalgo, Laboratorio de Biotecnología Microbiana, Instituto de Investigaciones Químico-Biológicas, Edif. B-3, C.U., C.P. 58030, Morelia, Mich., México.
lorena.farias.ibq@hotmail.com

Saccharomyces cerevisiae, acetate esters, volatiles in beverages.

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 AATTCG) -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 analyzes 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).

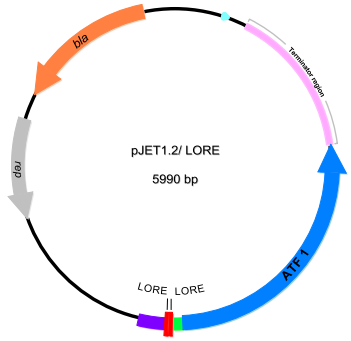

Fig. 1. Construction of plasmid pJET1.2/LORE. The vector contains the LORE element tandem repeat twice fused to *ATF1* gene promoter.

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.

Acknowledgements. To CONACyT by scholarship 258944. CONACyT-106567, FOMIX-C01-117130, and C.I.C. 2.14/UMSNH grants.

References.

1. Saerens S, Delvaux F, Verstrepen K, Thevelein J. (2012). *Microl Biotechnol*. 3(2):65-177.
2. Cordente A, Curtin C, Varela C, Pretorius I. (2012). *Appl Microbiol Biotechnol*. 96:601-618.
3. Vasconcelles M, Jiang Y, McDaid K, Gilooly L, Wretzel S, *et al. (*2001). *J. Biol. Chem.* 276:14374–14384.
4. López-Alvarez A, Díaz-Pérez A, Sosa-Aguirre C, Macías-Rodríguez L, Campos-García J. (2012). *J. Biosci. Bioeng.* 113:614-618.