## ISOLATION OF YEASTS WITH INCREASED CAPACITY OF FERMENTATION FROM TEQUILA INDUSTRY IN MEXICO

<u>José Humberto Valenzuela-Soto</u>, Susana De La Torre-Zavala, Montserrat Orencio-Trejo, José Luis Hernández-Flores, Osmar Martínez-Castro, Esmeralda Beltrán-Guerrero, Santos García-Aguilar, Jessica Torres-Granados, Arelis Rangel-Lara, Edmundo Lozoya-Gloria. Laboratorio de Vinculación, Departamento de Ingeniería Genética, CINVESTAV-IPN Unidad Irapuato. Km 9.6 Libramiento Norte Carretera Irapuato-León, C.P. 36821, Irapuato Gto. México. johuvaso@gmail.com

Keywords: yeasts, ethanol, yields, pentoses.

Introduction. Among the major challenges to increase ethanol yields is to obtain the most adequate yeast strains. With this goal, we isolated yeasts from samples taken from the Magellan Handicraft Cooperative Society in the mountain chain of Pénjamo, a tequila factory since about 200 years ago. Samples were from places to store agave residues and fluids from different stages of tequila production. The purpose of the present work was to isolate and select yeast that had high yields of ethanol from tequila industry.

**Methods.** Initial screening was done in YPS medium (20% sucrose, 8% ethanol) to isolate carbenicillin resistant yeasts (1). The isolated colonies were grown in YPS medium supplemented with 2, 4, 6 and 8% ethanol at 28°C. Then they were grown in 10 ml YPS just with 2% sucrose to evaluate ethanol production. The initial inoculum was set at DO<sub>600</sub>= 0.4 and incubated at 28°C for 48 hours with gently agitation. The supernatants were obtained by centrifugation at 3000 rpm for 30 minutes at 4°C. Selected and isolated yeasts were identified by PCR utilizing ITS primers (Internal Transcribed Spacer) (2) and sequenced later on.

Results. Ethanol production from isolated strains after 24 hours was compared to that obtained from a commercial strain of Saccharomyces cerevisiae. We selected 7 isolated yeasts and all have been identified as typical fermenting yeast such as Saccharomyces cerevisiae, Lachancea thermotolerans and Torulaspora delbrueckii.

**Conclusions.** Yeasts with increased capacity of fermentation were isolated from tequila industry in Guanajuato, México. Our goal is to increase more the ethanol yield in some selected yeasts by genetic manipulation, in order to utilize xylose efficiently in pentose pathway.

## References.

- 1. Aparecida Oliveira, V., Araújo Vicente, M., Gomes Fietto, L., de Miranda Castro, I., Xavier Coutrim, M., Schüller, D., Alves, H., Casal, M., de Oliveira Santos, J., Dias Araujo, L., Alves da Silva, P.H. and Lopes Brandão, R. 2008. Appl. Environ. Microbiol. 74: 693-701.
- 2. Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W. and Fungal Barcoding Consortium. 2012. PNAS. 109: 6241-6246.