



## Rhizopus oligosporus AND Lactobacillus spp FERMENTATIONS FOR CHITIN EXTRACTION FROM CRUSTACEAN WASTES AND AGRICULTURAL BY PRODUCTS.

 <u>R. ARANDAY</u><sup>1</sup>, A. LOPEZ<sup>1</sup>, EB. GUTIERREZ- CIRLOS<sup>2</sup>, <u>K. SHIRAI</u><sup>1\*</sup>
 <sup>1</sup> Universidad Autónoma Metropolitana, Dpt. Biotecnología Lab. Biopolímeros. Av. San Rafael Atlixco 186. Col. Vicentina, México City. C.P.09340. \*E-mail: smk@xanum.uam.mx
 <sup>2</sup>Facultad de Estudios Superiores Iztacala, UNAM, Unidad de Biomedicina, Lab. 2. Av. de los Barrios 1, Col. Los Reyes Iztacala, Tlalnepantla, Estado de México, C.P. 54090 *Key words: chitin, agricultural by products, chitosan*

Introduction. The biological extraction of chitin from fisheries by-products, such as shrimp, is performed by bacterial cultures, which purifies the biopolymer by production of organic acids with a very low consumption of water and energy. This process is claimed as an alternative to chemical extraction.<sup>1</sup> Highly produced agricultural by product, corn cobs are rich in sugars that are used as carbon fermentation.<sup>2</sup> source for Herein the combination of lactic acid bacteria as acidifying microorganisms; as well as proteolytic fungi Rhizopus oligosporus (R) have been studied for the biological extraction of chitin using corn steep liquor (CSL).

Methods. Fermentations were carried out at 30 ℃ during 192 h with shrimp wastes and varying the carbon source for levels of CSL and glucose (10%,20% and 30% wt/wt). The type of starter was evaluated using one stage of Rhizopus oligosporus (10<sup>5</sup> spores/ml) (72 h) or in two stages fungi during the first 72 h and second stage with Lactobacillus spp (L) (5% v/wt) for other 120 h. The sugars in CSL were determined by HPLC.<sup>3</sup> Demineralization and deproteinization (DP) (DM) were determined by measuring ash and protein contents, respectively. pH and total titratable acidity (TTA), sugars and organic acids (HPLC) were determined. As well, chitin was characterized by ATR-FTIR spectroscopy.

**Results.** The sugar composition in the CSL was glucose (24%), arabinose (6%) and galactose (70%). On the contrary of previous studies, galactose was found as the main sugar contained in CSL instead of xylose.<sup>2, 3, 4</sup>



Fig. 1. DM percentages determined in fermentations with R, L starters in one or two stages at various levels of sugar (10%, 20% and 30%). Fermentations with added 20% of sugar (glucose or its equivalent in CSL) and R *in* one stage of 72 h and R followed by L (120 h) produced the lowest pH and the highest DM, DP, lactic acid and acetic acid concentrations (Fig. 1, Table 1).

 Table 1. Chemical composition of shrimp fermentations

 with added 20% of glucose employing one stage of R or

 in two stages with L.

Treatme	ent Fermentation time	DP(%)	DM(%)	Lactic acid (mmol/g)	Acetic acid (mmol/g)
R/L	72 h/ 120 h	79.6 <u>+</u> 9.6	64.11 <u>+</u> 0.72	0.487 <u>+</u> 0.01	0.002 <u>+</u> 0.0005
R	72 h	78 <u>+</u> 6.1	66.27 <u>+</u> 0.16	0.306 + 0.04	0.0018 <u>+</u> 0.005

The sugar consumption was higher than 98% for all the conditions tested in the fermentations (Fig 2).



Fig. 2. Sugar consumption within fermentation at various levels of sugar

**Conclusions.** The use of a highly proteolytic microorganism such as *Rhizopus oligosporus*, alone or in combination with lactic acid bacteria achieved DP and DM.

Acknowledgements. The authors thank Institute of Science and Technology of Mexico City (ICyTDF) (Project No. PICSA 11-69 and PICSO12-152) for funding and to CONACYT for scholarship grant (RA).

## References

1. Cira, L.A., Huerta, S., Hall, G.M., Shirai, K. (2002) Pilot scale lactic acid fermentation of shrimp wastes for chitin recovery. *Process Biochemistry*. 37:1359-1366.

2. Wang, L., Yang, M., Fan, X., Zhu, X., Xu, T., Yuan, Q. (2011). *Process Biochemistry*. 46:1619-1626.

3. Wang, G. S., Lee, J., Zhu, J.Y. (2011). *Appl. Biochem. Biotechnol.* 163: 658-668.

4. Bai-Yan, C., Jing-Ping, G., Hong-Zhi, L., Ke-Ke, C. Wen-Xiang, P. (2010). *Biomass and bioenergy.* 36:250-257.