EFFECT OF CHITIN PRETREATMENT IN THE PRODUCTION OF CHITINOLIGOSACCHARIDES BY CHITINASES OF *Lecanicilium lecanii*

G.VILLA-LERMA[1], M. GIMENO [2], H. GONZALEZ-MARQUEZ [1], A. LOPEZ-LUNA[1] and K. SHIRAI[1*]

[1] 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
[2] UNAM, Facultad de Química, Ciudad Universitaria, C.P. 04510,
Key words: Chitin, Chitinases, Steam explosion, Sonication, Chitinoligosacharides

Introduction. *N*-acetyl-*D*-glucosamine and *D*-glucosamine with β 1-4 bonds [1]. Furthermore chitinases are enzymes that catalyze the hydrolysis of chitin; they have been classified into 2 main categories endochitinases (Endo) and Exochitinases comprising Chitobiosidases and *N*-acetylhexosaminidases (Nhase). Usually chitoligosacharides are obtained by treating chitin with acids and heating. This treatment causes deacetylation and presence of contaminants [2]. It has been reported that chitinoligosaccharides (COS) with high molar concentration of acetylated units (F_A) presented antibacterial and antitumor activities as well as inmunostimulating effects [3]. Therefore, enzymatic hydrolysis using Endo and Nhases represents an interesting alternative in the production of COS with higher F_A than those obtained with chemicals.

The aim of this study was the purification of chitinases and their application in the hydrolysis of pretreated chitin for the production of COS.

Methods. Chitinases were obtained using Czapeck medium and varying pH from 5 to 8. Crude enzyme was purified by salting out and size exclusion chromatography. Purified enzymes were used for the hydrolysis of chitin pretreated by steam explosion (SE), sonication and deacetylation [4][5][6]. The enzymatic hydrolysis was carried out as described in previous work [7]. COS were analyzed by MALDI-TOF [7].

Results. Chitin hydrolysis by chitinases presented the highest yielding when substrate was pretreated by steam explosion reaching 1.7 and 0.81 mmol/L for treatments using 0.1 g/mL 8min and 0.4 g/mL 5min respectively (Figure1), COS with DP of 2,3,4 and 5, these were identified by MALDI-TOF (Figure 2A), commercial chemically produced chitinoligosaccharides were also analyzed by this technique showing the same polymerization degree but neither of the oligosaccharides turned out to be acetylated (Figure 2B).

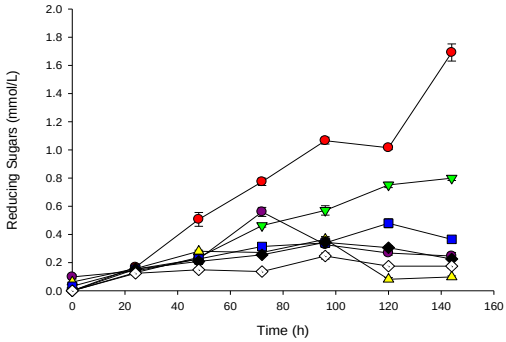

Figure 1. Kinetics of enzymatic hydrolysis of chitins. Sonicated (purple), SE 0.1g/mL 8 min (red), SE 0.4g/mL 5 min (green), DA 23 (blue), DA 0.03 (yellow), DA 52 (black) and native chitin (white)

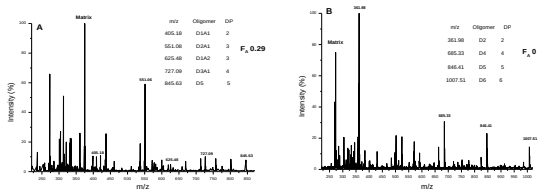

Figure 2. MALDI-TOF spectra of COS: A) SE chitin (0.1 g/mL 8 min) B) Commercial KYTOLIFE

Conclusion. Pretreatment of chitin improves enzymatic hydrolysis. the combination of steam explosion and this enzyme-mediated process does not involve deacetylation allowing the production of *N*-acetylated oligomers.

Acknowledgment: The authors acknowledge the research funding of CONACYT Mexico (Project No. 105628) and scholarship grant (GVL), also the grant for attending to this conference to ICYTDF and UAM.

References [1] Shirai K. (2006). Advances in Agricultural and Food Biotechnology. 289-304. [2]Trombotto, S.; Ladavière, C.; Delolme, F.; Domard, A. Biomacromolecules, 2008, 9,1731–1738. [3] Kuroiwa T. (2009). Process Biochemistry, 44:283-287. [4] Jiebing L.,Gunnar H., Göran G. (2007). *Bioresource Technol*. 98, 3061-3068 [5] Barreto-Cardoso M., Signini R., Campana-Filho P., (2001). *Polym Bull*. 47:183-190. [6] Pacheco N., Garnica-González M., Gimeno M., Bárzana E., Trombotto S., Shirai K., (2011). Biomacromolecules. 12:3285-3290. [7] Ramírez-Coutiño L, Espinosa-Marquez J., Peter M., Shirai K. (2010).Bioresour. Technol. 101: 9236-9240