EFFECT OF THE SALT ADDITION AND WASHING OF BARLEY STRAW IN THE PRODUCCTION OF *Trichoderma harzianum* ENZYMES AND SPORES

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Key words: Trichoderma harzianum, barley straw, enzymes

Introduction. The straw of herbal crops is a carbon source that can be used in many industrial processes and as growth medium for microorganisms in biotechnological processes, because it has a great amount of biomass and can be renewed every year (Dinis et al, 2009). The use of *Trichoderma harzianum* (T.h.) as biological control agent has been studied in many types of crops and as antagonist in a wide variety of phytopathogen agents (Ezziyyani et al, 2004). Hence, the culture of this microorganism is important in the agricultural industry. T.h. reproduction is easy because it can grow in different agricultural residues. These agricultural residues are used by T.h. as support and carbon source because they have a great hydrolytic enzyme complex as cellulase and xylanase, among others. Furthermore, these hydrolytic enzymes help to T.h. to degrade complex polysaccharides in solid state fermentation (SSF). (Agamez-Ramos et al., 2008).
Hence, the aim of this study is to evaluate the T.h. growth in barley straw in SSF with different conditions: washed and unwashed barley straw; also with and without salt.

Methods. The T.h. culture was done during 10 days using 5 g of straw contained in flasks of 100 mL in culture conditions of 75% humidity and 28°C, and tripled measurements were done every 24 h. In order to obtain the extract, 50 mL of sterilize distilled water were added to each flask, and they were shaken to 150 rpm during 120 minutes. Then, a direct counting of spores in a Neubauer camera was done, and the xylanase and cellulase activity was measured by quantification of released reductive sugar using the DNS method and the protein quantification was done by the Bradford method (Membrillo et al., 2008)

Results. In the culture with added salt and washed straw was observed the biggest xylanase and cellulase enzymatic activity (Table 1). However, in the unwashed Straw this activity was in less time. These activities were significantly different ($p<0.05$) only with cultures without added salt. The difference in the production of total protein is significant between the washed straw and the unwashed straw. In the last case, the production of total protein was bigger and in less time with the salt addition. On the other hand, the results of spores production do not present a significant difference ($p<0.05$) among the different treatments; however, without salt addition the maximum production of spores was in less time.

Table 1. Maximum values gotten in each treatment.

Time/ Máximum activity	Water / unwashed straw	Water / washed Straw	Addition of salt / washed straw	Salt addition / unwashed straw
Xylanase AU/dmg*	168 h 29.1 ± 0.4	96 h 28.7±1.3	192 h 33.5±0.2	72 h 32.7±0.7
Celulase AU/dmg	120 h 4.5 ± 0.5	96 h 5.6 ± 0.2	144 h 10.9±0.3	72 h 10.2± 0.3
Total Protein AU/dmg	168 h 1129.2±2.7	192 h 875± 25.5	168h 977.6±48.1	72 h 1010.2±46.5
Spores /mL	192 h $8.33X10^8$	168 h $5.95X10^8$	216 h $1.59X10^9$	216 h $8.8X10^8$

*dmg: Dry matter gram.

Conclusions. Based on these results, the effect of salt addition is the increasing of enzymatic activity and the use of unwashed straw decrease the time of spore production.

Acknowledgements. To CONACYT for the financial support to the project FINNOVA-2011-03-173702

References.

1. Agamez-Ramos, E. Y., Zapata-Navarro, R. I., Oviedo-Zumaqué, L. E. & Barrera-Violeth, J. L., (2008). Evaluación de sustratos y procesos de fermentación sólida para la producción de esporas de *Trichoderma sp*. *Revista Colombiana de Biotecnología*. (10) 2, 23-34
2. Dinis, M., Bezerra, R., Nunes, F., Diaz A., Guedes, C., Ferreira, L. M., Cone, J., Marques, G., Barros, A. & Rodrigues, M. (2009). Modification of wheat straw lignin by solid state fermentation with white-rot fungi. *Bioresource Technology*. 100, 4829–4835.
3. Ezziyyani, M., Pérez-sánchez, C., Sid, A., Requena, M. E. & Candela, M. E. (2004). *Trichoderma harzianum* como biofungicida para el biocontrol de *Phytophthora capsici* en plantas de pimiento (*Capsicum annuum* L.). *Anales de Biología*. 26, 35-45.
4. Membrillo, I. Sánchez, C., Meneses, M., Favela, E. & Loera, O. (2008). Effect of substrate particle size and additional nitrogen source on production of lignocellulolytic enzymes by *Pleurotus ostreatus* strains. *Bioresourse Technology*. 99, 7842-7847.