FUNCTIONAL SOIL METAGENOMICS OF SUGARCANE FIELDS FROM THE PAPALOAPAN BASIN: ENZYMES WITH CELLULASE AND ESTERASE ACTIVITY

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Introduction. Enzymes have been used for decades to perform chemical and biochemical reactions useful in food, chemical and pharmaceutical industries [1, 2]. Currently there is considerable interest in the production and marketing of cellulases and esterases, since they are used as additives in food and cleaning products, as well as in paper, textile, agricultural, pharmaceutical and biofuel industry [1, 3]. The hydrolysis of cellulose is carried out by a group of cellulases. Endo-β-1,4-glucanase (1,4- β-D-glucanohydrolase E.C.3.2.1.4) acts on glycosidic internal bonds β-1,4 present between glucose units forming the cellulose molecule. Exo-β-1,4-glucanase (1,4- β-D-glucan cellobiohydrolase E.C.3.2.1.91) breaks 1,4 β-D-glucan bonds at the non-reducing end of cellulose and cellodextrins. Finally, β-1,4-glucosidase (β-D-glucoside glucohydrolase E.C.3.2.1.21) hydrolyses cellobiose to glucose [4]. Esterases catalyze break down and synthesis of ester bonds. Feruloyl esterases degrade plant cell walls hydrolyzing ester groups involved in binding ferulates inside hemicelluloses and between hemicellulose and lignin [3]. Metagenomics is a new tool for studying genomes of a particular microorganism community in a habitat. The use of metagenomics allows the isolation of new enzymes. This is done by extraction, cloning and analysis of genetic material from nature. Enzymatic activities can be found by screening metagenomic libraries or by high throughput sequencing [1, 5].
Given the demand for new and better enzymes, the objective of this work is to search and functionally characterize cellulases and esterases in agricultural soil metagenomic libraries from the Papaloapan region of Oaxaca.

Methods. DNA was isolated from soil with MOBIO Ultra *Clean Mega Soil DNA isolation kit*. Isolated DNA was digested with *Sau 3AI*, and cloned in to ZAP Express vector. Cloned fragments were packaged into phage λ. Cellulase activity screening was performed in 0.5% CMC-Na and 0.1% Congo red. Esterase activity was screening on naphthyl acetate (20 mg / mL), fast blue RR (80 mg / mL) and 0.4% agarose.

Results. A 1.89 Gbp soil metagenomic library was isolated with 210.000 pfu and a mean fragment size of 9 kbp. The screening techniques used to identify cellulase and esterease activity were performed on two phases. For cellulase activity 2.156 clones were analyzed. First, 11 positive clones were obtained, and 7 proved positive on confirmation screen. Five clones that showed higher cellulase activity were selected. A screening of 1.494 clones to identify esterase activity was performed, first screening detected 5 positive clones however, on confirmation screening no clones were obtained, possibly because they were false positives. Currently, clones that showed cellulase activity are being cloned and sequenced.

Conclusions. Cellulase activity could be detected on 0.33% of the clones isolated from soils cultivated with sugarcane at the Papaloapan region of Oaxaca. On the other hand, no esterase activity could be detected.

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References.

1. Ferrer, M., Beloqui, A., Timmis, K. y Golyshin, P. (2008). *J. Mol. Microbiol. Biotechnol*. 16:109-123.
2. Lorenz, P. y Eck, J. (2005). *Nat. Rev. Microbiol*. 3(6):510-516.
3. Bornscheuer, U. (2002). *FEMS Microbiol. Rev*. 26(2002):73-81.
4. Avitia, C., Castellanos-Juárez, F., Sánchez, E., Téllez-Valencia, A., Fajardo-Cavazos, P., Nicholson, W. y Pedraza-Reyes, M. (2000). *Eur. J. Biochem*. 267:7058-7064.
5. Øvreås, L. y Torsvik, V. (2002). *Curr. Opin. Microbiol*. 5(3):240-245.