SELECTION OF BASIDIOMYCETOUS FUNGI WITH ENDOGLUCANASE ACTIVITY AND EVALUATION OF ITS ENZYMATIC PROFILE

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Introduction. Lignocellulosic materials have an enormous potential for being used as raw material in the generation of a vast quantity of products with industrial applications, such is the case of second-generation biofuels. Is in this point where cellulose degradation and enzymes involved in the process play a key role.
Because of this, in this work we analyzed endoglucanase activity of 7 strains of basidiomycetous fungi grown in lignocellulosic material and we detect the presence of isoenzymes by zymography analysis.

Methods. Fermentation was carried out in 250 ml Erlenmeyer flasks, with wheat straw and mineral solution as culture medium. Flasks were inoculated with 5 mycelia fragments (5mm) and incubated by 15 days. Endoglucanase activity was determined at days 5, 10 and 15, and it was evaluated by measuring the quantity of reducing sugars released during 45 min (1). Isoenzymes detection was assayed through zymography analysis (2). Fungal strains were identified by amplification and sequencing of ITS regions from rDNA.

Results. The highest endoglucanase activity detected was 0.88 U/ml at day 10, and it was produced by a strain identified as *Phanerochaete chrysosporium*, and the lowest activity was 0.4 U/ml, detected at day 5 in a strain identified as *Pycnoporus sanguineus*. Endoglucanase zymogram, demonstrated the presence of 10 isoenzymes with high activity (Mrf from 13.2 kDa to <100 kDa) in the enzyme crude extract (ECE) from *P. chrysosporium* and 7 isoenzymes in the ECE from a *P. sanguineus* (Mrf from 21.65 kDa to 40.52 kDa) (Fig **1**). The expression profile of enzymes varied in relation to the fermentation day, and it is interesting to highlight that molecular mass ranges reported in other studies for endoglucanases from *P. chrysosporium* is between 22-45 kDa (3), while our results showed a variety of isoenzymes with lower and higher molecular masses.
Moreover, genomic analysis of *P. chrysosporium* reveals that exist around 40 genes that could encode for endoglucanases (4). In addition of this results, we found that number of isoenzymes detected in supernatants from both strains is bigger than the number detected in previous studies.

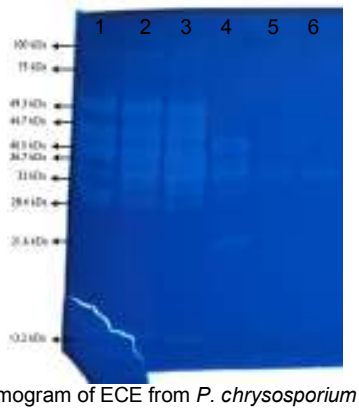

Fig.1 Zymogram of ECE from *P. chrysosporium* and *P. sanguineus* with endoglucanase activity. Lanes 1-3, ECE from *P. chrysosporium* at day 5,10 and 15 respectively; lanes 4-6 ECE from *P. sanguineus* at day 5, 10, 15 respectively.

Conclusions. From the fungal strains evaluated, *P. chrysosporium* showed the highest activity at day 10 of fermentation.
In both of the strains analyzed by zymography is noticeable a different expression pattern, and number and intensity of bands in the zymogram coincide with days in which maximum and minimum activity levels were detected. Number and size of some of the isoenzymes detected differs with currents reports.

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