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Bioprospecction of fungi from Cerrado soil aiming lignocellulosic biomass degradation.

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ABSTRACT

Due to the climate change and the environmental pollution caused by fossil fuels combustion, the use of renewable energy sources and fuels, such as the second generation bioethanol, has been considered as an important alternative to avoid global warming and increased levels of pollution. Therefore, the present study main goal is evaluate filamentous fungi from Cerrado biome soil as producers of hydrolytic enzymes capable of degrading lignocellulosic biomass during growth in the presence of sugar cane bagasse as carbon source. At first a total of 20 isolates were screened for xylanolytic and cellulolytic activity in solid media containing xylan and carboxymethyl cellulose, as carbon source. Seven isolates exhibiting higher growth rate were chosen to carry growth in liquid media and to evaluate production of hollocelulotyic enzymes. Further experiments are in development to quantify production of pectinases; xylanases; avicelases; mananases; and endoglucanases by the isolated fungi, as well as, aiming their identification using molecular and morphological parameters.

Keywords: Sugarcane bagasse, xylanase, Cerrado, lignocellulosic biomass

INTRODUCTION

Cerrado's soil has a variety of microorganisms which hasn't been well characterized in respect of their ability to produce biomolecules applied to biotechnological processes. For example in lignocellulosic biomass deconstruction to obtain fermentable sugars or other molecules presenting industrial interest (Dias *et al*., 2012). A different set of enzymes are required to reach a complete hydrolysis of lignocellulosic wastes such as cellulases, pectinases and xylanases (De Siqueira *et al*., 2010). In this context, the present study was carried out to evaluate the use of fungi from Cerrado soil as a source of plant cell wall deconstructing enzymes during growth on sugar cane bagasse as carbon source.

MATERIAL AND METHODS

A total of 20 fungal isolates previous obtained from Cerrado soil were re-isolated using cultivation in MYG agar media. Isolated fungi were screened for their ability of growing in minimal media containing carboxymethylcellulose and xylan, as carbon source. Seven isolates named as: 7, 8, 9, 10, 15 and 16 fungi were chosen based on their growth under the previous described condition.

Production of plant cell wall degrading enzymes (xylanases, endoglucanases, pectinases, cellobiohydrolases, and mannanases) was carried out in liquid minimal media at 28^{o}C and under rotation of 121 rpm, spore suspensions (1x10^{6}) obtained from a pure MYG plate culture was used as inoculums. Enzymes production was monitored during the time of growth for five days, fungal cultures were filtered using filter paper N^{o} 5, centrifuged at 10000*g* for 20 minutes, and the supernatant storaged at 4^{o}C until be used as source of proteins. Xylanase, endoglucanase, pectinase, manannase, and cellobiohydralases activities were quantified as described in Hamann et al (2015).

RESULTS AND DISCUSSION

A total of 20 isolates were able to grow in solid media containing CMC and xylan as carbon source, however 7 isolated named: ISO3,ISO7,ISO8,ISO9,ISO10,ISO15,ISO16 presented higher growth rates. Then, they were chosen for further experiments of production of plant cell wall degrading enzymes on liquid media supplemented with sugarcane bagasse 0.5% (w/v).

Highest xylanase activity (3.2 IU ml^{-1}) was found for ISO16 after two days of growth. Regarding pectinases, ISO9 presented the highest activity (0.5 IU ml^{-1}). Highest CMCase titers were obtained for ISO3 and ISO7. Avicelase and mannanase activities were detected for all isolates with respective highest activities 0.13 IU ml^{-1} for ISO3 and 0.11 IU ml^{-1} for ISO10.

In general, all isolates were capable of secreting plant cell wall degrading enzymes after 1 day of growth in the presence of sugar cane bagasse as carbon source, which is in agreement to previously showed for *Emericella nidulans* grown in the presence of sugar cane bagasse (SILVA, C.O.G., 2014).

Currently,ISO3,ISO7,ISO8,ISO9,ISO10,ISO15 are in process of identification, the genomic DNA was already extracted and PCR products of ITS1 and ITS2 regions will be sequenced in order to molecularly identify these new fungi isolates obtained from Cerrado's soil.

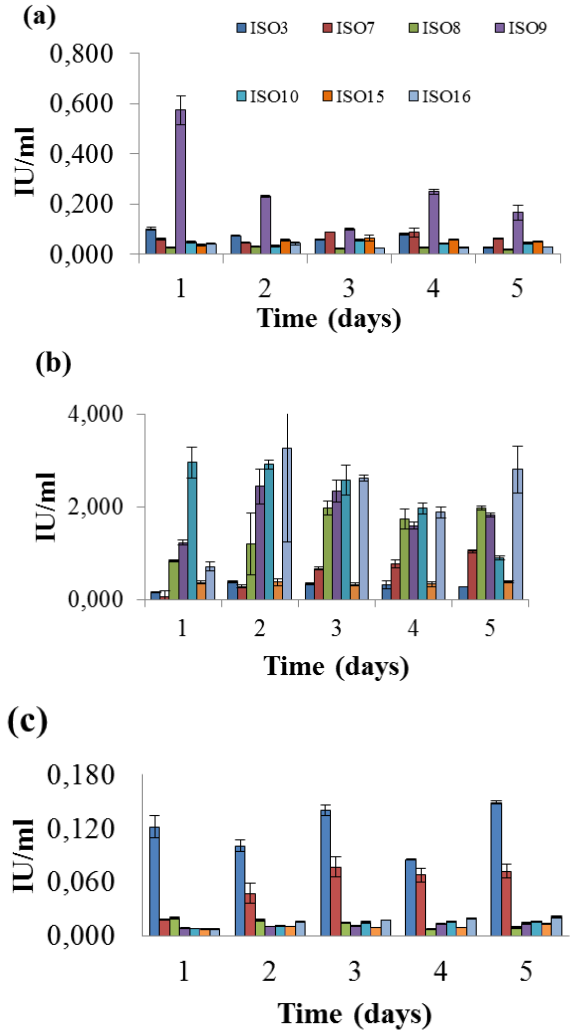

Figure 1: Time course production of plant cell wall degrading enzymes by the fungal isolates ISO3, ISO7, ISO8, ISO9, ISO10, ISO15 and ISO16 (a) Pectinase activity (b) Xylanase activity (c) CMCase activity.

CONCLUSION

Highest activity values were obtained for xylanase, CMCase and pectinase activities produced by the isolates ISO16, ISO9, ISO3 and ISO7. These isolates will be further studied in order to develop enzymatic blends aiming hydrolysis of sugarcane bagasse and production of molecules which could be further applied in biotechnological processes.

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