Cellulolytic and accessory enzymes from brazilian microbiota for biomass deconstruction

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Background

With the increasing global demand for fuels and materials from petroleum, sustainable alternatives are essential to minimize the environmental impact. To complement the growing worldwide demand and reduce the environmental impact of oil exploration, research is being developed. Agro-industrial residues such as sugarcane bagasse can be converted to fermentable sugars. However, the cellulose-hemicellulose-lignin matrix present in biomass is highly recalcitrant and thus the cellulose and hemicellulose present in it are not efficiently converted to sugars by enzymes only. Because of this, a pre-treatment of the lignocellulosic material is commonly used to disrupt the crystal structure, facilitating the access of enzymes that will act on the biomass. The use of enzymes in the lignocellulosic biomass saccharification process is considered more sustainable technology for second-generation ethanol (HORN et al., 2012). The bioconversion of these materials into sugars require not only cellulases but also auxiliary enzymes, for example, lytic polysaccharide monooxygenases (LPMO). These enzymes carry out oxidative cleavage of cellulose and hemicellulose (AGGER et al., 2014.), and now the degradation of these polysaccharides must be considered a hydrolytic and oxidative process (AGGER et al., 2014). In this context, the aim of this study was to characterize enzymatically and molecularly cellulolytic fungi, and evaluate a metagenomic library aiming to discover auxiliary and cellulolytic enzymes for lignocellulosic biomass degradation.

Methods

Enzymatic assays (adapted from GHOSE, 1987) targeting activities FPase, β-glucosidase, endoglucanase and exoglycanase were performed for both fungi isolates, the CBF13 come from Cerrado soil and the 147, endophytic of sugarcane. To accomplish the production of enzymes, it was used Mandel's culture medium (MANDELS; WEBER 1969), with modifications, because the carbon source has been or Avicel®, or bagasse sugarcane *in natura* and pre-treated, or elephant grass *in natura* and pre-treated, or lactose; all at a

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concentration of 1%. The genomic DNA of the fungi was extracted according to the protocol suggested by Cenis (1992). The coding regions ITS1-5.8S-ITS2 and β-tubulin for filamentous fungi were amplified and sequenced, thereby the microorganisms were properly identified by comparing the sequences deposited in GenBank. The isolate CBF13 were also identified by the BIOLOG® system. In order to identify a filamentous fungus monooxygenase a PCR was performed on isolated CBF13. Degenerated primers focused on phyla Firmicutes and Bacterioidetes were constructed with the intention of amplifying bacterial LPMO enzymes in metagenomic library goat rumen. Furthermore, a bacterial LPMO from *Thermobifida fusca* YX already described in literature and named E7 (MOSER et al., 2008) was synthesized (Life-Technologies) in order to be used as a model test of the activity of this accessory enzyme (LPMO). This enzyme was expressed in the pET21α + vector using *E. coli* BL21 DE3, Tuner DE3, Rosetta blue DE3, Pelac I and BL21 DE3 pLIS S strains as a host cell. Then three clones out of each host cell were selected and expressed in a small scale. An electrophoresis SDS PAGE gel was performed and the best clones selected for expression on a large scale to purify the enzyme and then perform enzymatic assays.

Results and Conclusions

FPase and endoglucanase activities were the highest ones for both fungi. Therefore, if it were to be done an enzymatic cocktail from these isolates based on these assays, it is possible to determine that the best substrate to obtain the highest endoglucanase activity is lactose. Although to obtain FPases and β-glicosidases for both isolates, the best substrate is elephant grass and to obtain exoglucanses the best substrate is Avicel® to isolate CBF13. From the analysis of the ITS region and the gene encoding the β-tubulin was possible to determine that these isolates belong to the phylum Ascomycota, eurotiomycetes class, Eurotiales order and Aspergillaceae family. Despite all belong to the same family, they don't belong to the same genera, isolte 147 is an *Aspergillus fumigatus*, a human pathogen; and isolate CBF13 is a *Penicillium citrinum*, a ubiquitous fungi. The biochemical identification method Biolog is a good tool for identification of fungi, ranking isolated CBF13 correctly as belonging to the genus *Penicillium*. Apart from cellulases, another important class of enzymes to the biomass deconstruction are the copper dependent accessory enzymes that include monooxygenases. Isolated CBF13 has a copy of a fungal monooxygenase similar to other *Penicillia* LPMO, it is therefore possible to invest in studies of this accessory enzyme. From three different methods was reached the same identification of the isolated CBF13. To identify bacterial LPMO a screening in a small inserts metagenomics library from rumen goat was performed, these primers were tested in individuals of the Bacillales order and metagenomics goat rumen library, obtaining no success amplifying any bacterial LPMO. An SDS PAGE gel was performed showing the clones that better expressed the synthetic LPMO E7.

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