

## TRANSCRIPTOME ANALYSIS OF *ASPERGILLUS FUMIGATUS* GROWN ON SUGARCANE BAGASSE

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FAPESP Process 2014/10466-0 | Term: Nov 2014 to Oct 2016

The biomass of sugarcane is made of lignocellulosic material, primarily hemicellulose and cellulose, polysaccharides which are sugars and high energy that can be converted into ethanol. However, the association between cellulose, hemicellulose and pectin imposes great difficulties in recovering the constituent sugars in the form of monomers with high purity. Due to this recalcitrant characteristic of bagasse, a major challenge in the production of second generation biofuels is the conversion of lignocellulosic substrates in fermentable sugars. In nature, bacteria and fungi play an important role in the degradation of plant biomass, since secrete specific enzymes for the constituent polysaccharides. However, little is known about the response for fungi to different lignocellulosic materials and the production of enzymes and accessory proteins required for the breakdown of plant biomass. The most studied enzymes are from fungi such as *Aspergillus niger* and *Trichoderma reesei*. Other members of the genus *Aspergillus* are able to secrete hydrolytic enzymes of greatest importance, among them the *A. fumigatus*. Although *A. fumigatus* is a pathogenic fungus, is considered an important enzymes producer such as cellulases, xylanases and lipases, whose synergistic effect increases the efficiency of hydrolytic enzymes secreted, but little is known about these enzymes, facts that emphasize the importance of better understanding of this mechanism in *A. fumigatus*. Accordingly, the analysis of the transcriptional profile of *A. fumigatus* when grown in the presence of sugarcane bagasse and the identification of secreted enzymes in the medium (secretome) may bring new knowledge of hydrolytic enzymes that support their industrial applicability, with emphasis on second generation ethanol.

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The main objective of this project is the identification and characterization of hydrolytic enzymes from *A. fumigatus* capable of degrading sugarcane bagasse. The *A. fumigatus* conidia were incubated in medium containing fructose (control) and exploded sugarcane bagasse (SEB) and the activity of main hydrolytic enzymes such as xylanase and cellulase were determined by different culture times. We observed an increase of 40X for xylanase (24 h) and 5X cellulase (72h) activities when the fungi were incubated in the presence of SEB. Besides, we observed a gradual increase over the time in reducing sugars as determined by the DNS method, suggesting that hydrolysis in sugarcane bagasse is occurring and monosaccharides are been released in the culture medium. Based on this data, we characterized the secretome profile under the same conditions. The proteins were separated by SDS-PAGE electrophoresis, the bands were digested and the peptides sequenced using a nanoAcquity UPLC system (Waters Corp) coupled to a Synapt G2 HDMS high resolution accurate mass tandem mass spectrometer (Waters Corp.) via a nanoelectrospray ionization source. We identified diverse group of hemicellulases and cellulases including, endo-1,4-beta-xylanase, beta-xylosidase, alpha-1,2-Mannosidase, endo-arabinase, 1,4-beta-D-glucan cellobiohydrolase, alpha-galactosidase and many other ones. Enzymes involved in lignin degradation like laccase, isoamyl alcohol oxidase and etc., were also identified. In addition this study identified several peptidases and proteases, which can be directly or indirectly associated with sugarcane bagasse hydrolysis.

The transcriptional profiles have been characterized through RNAseq techniques. RNAseq libraries were generated from RNA extracted from both cultures and a reference transcriptome was generated by assembly of all Ion Torrent PGM sequencing data. Raw reads (3,000,430 in total) were initially filtered for host nucleotide contamination and ribosomal RNAs. The sequences reads were analyzed using TopHat2 program. The calculation of the difference of expression, normalization and data analysis were performed through the Edger program. From these data, we identified a few genes expressed only in SEB, such as those coding 3 endo-1,4- $\beta$ -xylanase (AFUA\_3G15210, AFUA\_3G00470, AFUA\_6G13610), endoglucanase (AFUA\_7G06150, AFUA\_7G06740), cellobiohydrolase (AFUA\_3G01910) and carrier hexose (AFUA\_6G14560). To get more information about the transcriptome, a new sequencing is being conducted using Illumina platform. For better characterization of the function and activity of the genes and proteins identified, it will be expressed in a heterologous strain, such as of *S. cerevisiae*. Based on these results, it is suggested that *A. fumigatus* has great potential as a hydrolytic enzymes producer that may contribute to the development of more efficient enzyme cocktails and with low cost.

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