Screening of filamentous fungi from the Atlantic Forest biome producing enzymes of the pectinolytic complex

Triagem de fungos filamentosos do bioma Mata Atlântica produtores de enzimas do complexo pectinolítico

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ABSTRACT
Fungi are the major producers of enzymes such as cellulases, pectinases and xylanases, so these microorganisms were selected in the present work to analyze the ideal conditions to improve the production of the activity of the pectinase enzyme. A total of 36 fungi previously collected from the Atlantic Forest of Paraná were cultivated in a test tube containing 10 to 15 mL of BDA medium for 7 days at 40 °C. Among all analyzed, the filamentous fungus Aspergillus terreus strain PA3A5T was the best producer of pectinases, whose greatest enzymatic activity occurred at a temperature of 60 °C and at pH 5. The ideal culture medium for inducing production Enzyme was the defined medium Vogel supplemented with passion fruit peel at 1% (w/v) as a carbon source and incubated for 96 hours. To our knowledge, this is the first time that these data are reported in the literature.

Keywords: enzymes, pectinase, enzymatic activity.

INTRODUCTION
Fungi are major producers of several enzymes. The important characteristics are fast cycle and large-scale production. Among numerous enzymes produced by fungi, the one that gained great prominence within this research was pectinase, an enzyme that degrades pectin, a substance present in plant cells. For pectinase production to occur with great efficiency, it is necessary to find the ideal conditions for cultivation and consequently its production (Maia et al., 2005). This research focused on the enzymatic characterization of temperature, pH, enzyme production medium, growth curve and carbon source.
2 MATERIAL AND METHODS

In the present work 36 filamentous mesophilic and thermophilic fungi isolated from forest fragments of the Atlantic Forest of Paraná were used and cultivated in sterile test tubes containing 10 to 15 mL of BDA medium (potato dextrose agar) in an oven at 40 ºC for 7 days. The liquid cultures were optimized by inoculating 1.0 mL of a spore suspension in 25 mL in different culture media (Vogel, Czapeck, SR, Khanna, Klausen and Adams), supplemented with 1% (w/v) of different carbon sources, incubated at varying temperatures, under stationary regime, for different periods.

The carbon sources tested were: citrus pectin, glucose, sugarcane bagasse, corn cob, lemon peel, orange peel, wheat straw, corn straw, passion fruit husk, rice straw, apple husk and soy straw. The cultures obtained from the liquid media were vacuum filtered in Büchner funnel and Whatman filter paper nº1, obtaining a cell-free filtrate and a mycelium. The filtrate (crude enzymatic extract) was used to determine the enzymatic activity through the reaction with DNS (Miller, 1959), being used as substrate 1% citric pectin, carboxymethylcellulose and xylan, to measure pectinase, cellulase and xylanase. Protein quantification was performed according to the methodology described by Bradford (1976), using bovine serum albumin (BSA) as a standard. The absorbances of samples were determined in microplate by spectrophotometry at 595 nm.

The molecular identification of the fungal strain PA3A5T was performed by the company Helixxa® (Paulinia, SP) according to the sequencing methodology of the ITS (Internal Transcribed Spacer) region of the fungal ribosomal RNA and comparison of the consensus sequence obtained against the bank gene database using the BLAST-N algorithm (http://www.ncbi.nlm.nih.gov/).

The effect of temperature on pectinolytic activity was verified by measuring pectinase activity at 30 ºC to 80 ºC. The pH effect was verified by quantifying the pH enzyme from 3 to 8 in McIlvine 0.1 M buffer.

3 RESULTS AND DISCUSSION

During the selection of the best enzyme-producing fungus, different strains were inoculated in Czapek liquid medium and the enzymatic extract obtained from these cultures was used to screen for the best producer of pectinase, cellulase and xylanase. Among the mesophilic strains, the fungus PA3S16MC was the best producer of cellulase, the best producer of pectinase was the strain PA3S18MV and finally it can be observed that the strain PA3SMM was the largest producer of xylanase. Oumer and Abate (2018) screened pectinase-producing microorganisms from coffee pulp and observed that 13.68% of the isolates are fungi, indicating that these microorganisms are major...
enzyme producers. Furthermore, Corrêa et al. (2019) isolated and evaluated a total of 181 fungal species for cellulases and xylanases production and described 74% were classified as good enzymatic producers. According to Sarsaiya et al. (2018), the fungi Trichoderma viride, A. niger, A. fumigatus and Fusarium oxysporum have a high production capacity for the cellulase enzyme, but the fungi T. viride and A. niger are the ones that have the greatest potential for enzymatic activity of cellulose. From the studies of Uday and his collaborators (2017) it was discovered that the fungus Aspergillus niger has a high production of the enzyme xylanase.

The results obtained to the thermophilic fungi showed that the fungus PA3A5T was the best producer of pectinase, PA4S4T was the best producer of cellulase and the best producer of xylanase was PA4S3T. Abdullah and colleagues conducted studies on cellulase and found that one of the largest producers of this enzyme is the thermophilic fungus Aspergillus fumigatus BBT2. The researchers Brito Cunha and his collaborators (2013) used the fungus Streptomyces sp to carry out research, due to its great capacity to produce xylanase. On the other hand, Cavello and his collaborators (2017) carried out research with fungi, Cystofilobasidium infirmominiatum, Cryptococcus adeliensis and G. pullulans and found that they are excellent producers of pectinase. According to all results, the fungus selected to continue the experiments with pectinases was the PA3A5T strain.

The molecular identification results obtained showed that the fungus of the PA3A5T strain showed 99% identity with the species Aspergillus terreus. To optimize the production of pectinases, the best inducing medium was Vogel (Vogel, 1964) (Figure 1A), with 96 hours of incubation (Figure 1B). In fact, according to Santi (2005), the ideal time for incubation and production of the enzyme pectin lyase is 96 hours.

The carbon source that induced the highest enzyme production was the passion fruit peel at 1% (w/v) (Figure 2). According to Maller (2008), the passion fruit peel stood out for the production
of pectin lyase and pectinolytic activity. The experiment to check the best temperature for the enzymatic reaction showed that the best condition for pectinase is at 60 °C. According to Trindade et al. (2016), the fungus *Rhizomucor pusillus* A13.36 obtained better pectinase production at a temperature of 61 °C. Then the experiment was carried out to find the ideal pH for the enzymatic activity. It was concluded that the best pH is 5.0. According to Rezende et al. (2009), he found that the ideal pH for degrading pectic substances from the enzyme polygalacturonases is 5.0.

Figure 2 – The best carbon source for pectinase production by the fungus *A. terreus* (PA3A5T).

4 CONCLUSION

The molecular identification of the PA3A5T strain showed that it corresponds to a representative of the *Aspergillus terreus* species with high pectinase activity. The activity for pectinase was better at pH 5 and at 60 °C. The ideal medium for incubating this fungus was Vogel with a carbon source of passion fruit peel for 96 hours.

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