EXTRACELLULAR LIPASE PRODUCTION BY A NOVEL *Geotrichum candidum* STRAIN IN CORNCOB CELLULOSIC HYDROLYSATE

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ABSTRACT

An agricultural by-product, corncob is now considered a raw material capable of generating value-added products. Subjected to different pretreatment techniques, this material releases fractions rich in cellulose and hemicellulose that can be converted into fermentable sugars, which in turn, can be used in fermentative processes for the production of biotechnological products. Employing a new strain of *Geotrichum candidum* MG-Y7142, this study aimed to evaluate the productive potential of lipases (intra and extracellular) using cellulosic corncob hydrolysate medium (CCH). The maximum hydrolytic activity observed (26 U/mL) was detected at 24 hours in the cellulosic corncob hydrolysate medium, with optimal conditions being 30 ºC and pH 5.0.

Keywords: *Geotrichum candidum*. Cellulosic hydrolysate. Lipase. Biochemical characterization.

1 INTRODUCTION

Brazil is currently the second-largest producer of corn in the world, with a projected production of 119.1 million tons in 2024. For each ton produced, an estimated 2.3 tons of by-products are generated. Globally, this value can reach 822 million tons, of which 13% consist of corncob ([1],[2]). The typical composition of corncob includes approximately 33-43% cellulose, 35-36% hemicellulose, and 15-21% lignin. Cellulose and hemicellulose are the main fractions from which fermentable sugars are obtained, which can be converted into value-added products, such as biofuels and enzymes. However, the conversion of these fractions is hindered by the complex and highly recalcitrant chemical structure of the biomass ([3]).

To overcome these challenges, various pretreatment methods are available. Physical methods, such as extrusion, grinding, and irradiation, help reduce particle size and increase material porosity. Chemical treatments, such as the use of acids, alkalis, or organic solvents, promote the breakdown of chemical bonds in the biomass structure, facilitating enzymatic action. Biological methods employ specific microorganisms or enzymes to degrade lignocellulose. The combination of different pretreatments, known as a hybrid approach, can enhance the effects of each method, providing greater efficiency in converting biomass into fermentable sugars ([4]).

Among value-added biotechnological products, lipase stands out with a potential market value of USD 428.6 million by 2025 ([5]). A microorganism notable for its high enzymatic potential is *Geotrichum candidum* sp. Considered an excellent enzyme producer, this fungus can produce at least four types of lipases with different substrate specificities, both intra and extracellularly, in the presence of an inducer. Inducers, such as vegetable oils and fatty acids, are essential for maximizing lipase production by stimulating the gene expression of the enzymes and improving their yield ([6]).

Intracellular enzymes contribute to achieving better enzyme yields. However, during the recovery phase, the cell disruption process leads to an increase in impurities in the medium and, consequently, more steps in the downstream process. Considering this, selecting strains and optimizing extracellular lipase production processes can be a current challenge ([7]). The present study aims to determine the potential for producing intra and extracellular lipases using cellulosic hydrolysate medium from corncob obtained through a hybrid pretreatment, which involves extrusion followed by enzymatic hydrolysis, as an alternative carbon source. A newly isolated strain of *Geotrichum candidum* MG-Y7142 was employed, highlighting its role in efficient enzyme production. This study emphasizes the importance of value-added products in biotechnology, contributing to the valorization of agricultural by-products and the sustainable development of the sector.

2 MATERIAL & METHODS

Microorganism: The new strain of *Geotrichum candidum* MG-Y7142 used in this study was provided by the Collection of Microorganisms, DNA, and Cells of the Department of Microbiology at the Federal University of Minas Gerais (UFMG).

Obtaining the cellulosic hydrolysate from corncob: The enzymatic hydrolysis of corncob pretreated by extrusion was performed in 125 mL Erlenmeyer flasks using sodium citrate buffer (50 mmol.L^{-1}, pH 4.8) at 200 rpm and 50 °C, with a solid-to-liquid ratio of 1:10. Tween 80 (10% w/w) and the enzyme complex Cellic® CTec2 (25.25 FPU/g) were added. With 96 hours, the hydrolysate was heated at 85 °C for 15 minutes to inactivate the enzymes, centrifuged, filtered, and the supernatant stored at -20

°C for subsequent cultivation of *G. candidum* MG-Y7142 as a carbon source. The sugar concentration in the hydrolysate was determined by High-Performance Liquid Chromatography (HPLC) ([8]).

Media and cultivation conditions and analysis of enzymatic activity: The assays were conducted in duplicates at 30 ºC and 150 rpm in 125 mL Erlenmeyer flasks containing 50 mL of different cultivation media: GYMP (Malt Extract 1%, Yeast Extract 0.5%, Monobasic Sodium Phosphate 0.2%, Glucose 1%) and GYMP with glucose replaced by Cellulosic Hydrolysate of CornCob (CCH) rich in glucose as an alternative carbon source. In both cases, olive oil was used as an inducer. The cultivation media were inoculated using the agar-inoculum cut technique according to the methodology optimized by Maldonato et al. (2014). Samples were taken at 24, 48, and 72 hours. For the analysis of intracellular enzymes, the cells were washed with distilled water and subjected to six cycles of 5 minutes of cell disruption in a vortex with intervals in an ice bath, using beads and sodium phosphate buffer. Hydrolytic activities were determined according to the methodology proposed by Soares et al. (1999), expressed in lipase units (U/mL), corresponding to 1 mol of fatty acid released per minute (Equation 1).

$$A(\frac{U}{mL}) = \frac{(va - vb).N.10^3}{t.vc} \quad (1)$$

Where: va is the volume of the titrated sample (mL); vb is the volume of the titrated blank (mL); vc is the volume of the sample used in the reaction (mL); t is the time; and N is the normality of the NaOH solution.

Biochemical characterization of the lipase: The intra- and extracellular lipases of *G. candidum* MG-Y7142 were characterized using an olive oil emulsion. The effect of temperature was evaluated in the range of 25 to 60 ºC in 100 mM sodium phosphate buffer at pH 7.0. The effect of pH was evaluated in the ranges of 4.0 to 5.5 using 100 mM sodium citrate buffer, and 6.0 to 8.0 using 100 mM sodium phosphate buffer ([11]).

3 RESULTS & DISCUSSION

In the analysis of lipase production using olive oil as an inducer, it was observed that *G. candidum* MG-Y7142 strain is capable of producing the enzyme with both tested carbon sources: semi-defined and CCH media (Figure 1).

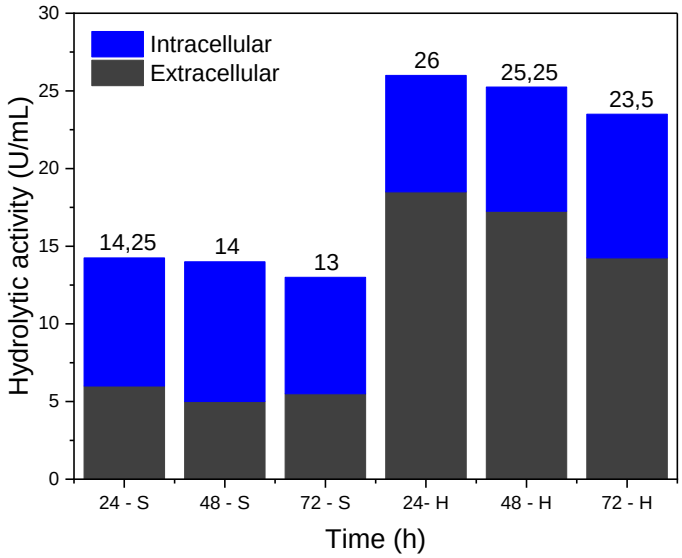

Figure 1: Hydrolytic activity of lipase (Intra and Extracellular) in different fermentation media (S: semi-defined medium, H: CCH medium) at 24, 48, and 72 hours.

Figure 1 shows that the highest hydrolytic activity in both tested media was recorded in the first 24 hours, with only small variations thereafter. However, there is a distinct activity profile: while the highest activity in the semi-defined medium is intracellular (8.24 U/mL), in the CCH medium with the alternative carbon source, the highest activity is extracellular (18.5 U/mL).

Comparing the enzyme activity produced in CCH medium with literature data for the same fungal species reveals a strong potential for its use. Ramos et al. (2015) reported an extracellular activity of 22.91 U/mL in 48 hours at 30 ºC using a semi-defined medium. Similarly, Maldonato et al. (2014) achieved a maximum activity of 15.8 U/mL in 48 hours using the same medium.

Figure 2 presents the biochemical characterization of the enzymes. The intra- and extracellular lipase enzymes have identical optimal conditions for pH and temperature when produced in the same medium. However, when comparing lipases produced in different media, those in the CCH medium have lower optimal temperature and pH (30 ºC and 5.0) compared to those in the semi-defined medium (35 ºC and 5.5).

Optimal pH and temperature conditions for enzyme activity are typically close to the physiological growth conditions of the microorganism, resulting in a unique profile for each lipase, even among strains of the same species. In a review study, Zavarise and Pinotti (2020) observed that approximately 41% of 31 microorganisms from different genera had an optimal pH range of 6.5 to 7.5, while about 51% had an optimal temperature around 40 ºC. Comparing their findings to this study, it is noted that the enzymes produced by *G. candidum* MG-Y7142 can operate at lower pH levels (Figure 2 – B), D)), than most filamentous fungi analyzed. Additionally, the temperature range for *G. candidum MG-Y7142* was within the expected range, showing greater thermoresistance, with relative activity above 70% in the range of 35 to 55 ºC (Figure 2- A)).

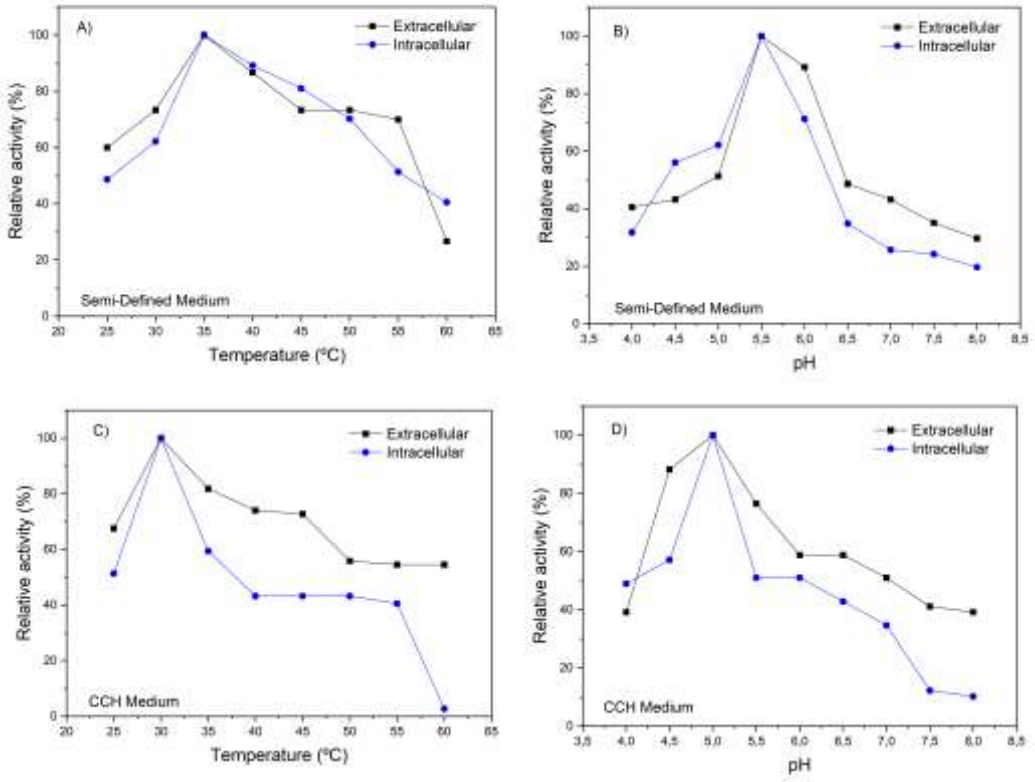

Figure 2 Biochemical characterization of intra- and extracellular lipases from *Geotrichum candidum* MG-Y7142 in the hydrolysis of olive oil emulsion: effect of temperature and pH. Semi-defined medium: a) temperature, b) pH. CCH medium: c) temperature, d) pH.

There are no specific data in the literature for lipases produced in CCH medium. However, it is noted that when compared to those produced in semi-defined medium, these have lower pH and temperature values. This characteristic may represent an advantage for the enzyme's performance in more acidic processes or those occurring at milder temperatures, such as in the detergent industry or wastewater treatment ([14]).

4 CONCLUSION

The use of a medium based on corncob hemicellulosic hydrolysate (CCH) as an alternative carbon source in the production of microbial lipases by the novel strain *G. candidum* MG-Y7142 proved to be efficient, yielding higher activity values compared to those produced in semi-defined medium. Additionally, its use promoted an increase in the secretion of extracellular enzymes. Lipases produced in semi-defined and CCH media exhibit distinct biochemical characterizations.

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