LIPASE: CULTURE OPTIMIZATION OF *ASPERGILLUS* sp. ARC3 AIMING AT BIODIESEL SYNTHESIS

Biatriz V. A. Santos[1], David L. Nelson[1], Vivian M. Benassi[3]*

[1]*Instituto de Ciência e Tecnologia/Universidade Federal dos Vales do Jequitinhonha e Mucuri/Diamantina/Minas Gerais/Brasil.*
** Corresponding author's email address: vivian.benassi@ufvjm.edu.br*

ABSTRACT

The study investigated the production of lipases by the fungus *Aspergillus* sp. ARC3 in submerged culture with a focus on optimizing cultivation conditions. Different culture medium, cultivation time, initial pH of the medium, organism growth temperature, and nitrogen sources were evaluated to determine their impact on lipolytic activity. The results revealed that M5 medium provided the highest enzymatic activity, while the fifth day of cultivation proved to be ideal for maximum lipase production, with an initial pH of 5.5, and the fungus maintained stationary at 30°C, while temperatures above 35°C and alkaline pH reduced lipolytic activity. Additionally, the microorganism demonstrated a preference for mixed nitrogen sources. It is concluded that the lipase produced by *Aspergillus* sp. ARC3 exhibited significant activity and potential applicability in biodiesel synthesis.

Keywords: Biotechnology. Enzymes. Filamentous Fungi. Prospecting.

1 INTRODUCTION

Many technological and industrial processes employ catalysts in chemical conversion, whether synthetic or biological. The global enzyme market has grown significantly, reflecting interest and demand for these biomolecules due to their high specificity, biodegradability, and ability to operate in controllable temperature and pH environments. Among the most utilized enzymes are microbial lipases. Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are a class of enzymes widely used in industry, capable of catalyzing the hydrolysis of triglycerides into various products such as diglycerides, monoglycerides, glycerol, and free fatty acids in organic-aqueous systems. Additionally, these enzymes can catalyze synthesis reactions such as esterification, transesterification (interesterification, alcoholysis, and acidolysis), aminolysis (amide synthesis), and lactonization (intramolecular esterification).

The versatility and importance of lipases highlight the need to explore and better understand their sources, production, and applications in various industrial and biotechnological contexts. These biological organic substances have a variety of applications in industrial processes, including dairy, bakery, pharmaceuticals, cosmetics, biodiesel, and other sectors. In this regard, this study aimed to standardize the cultivation of *Aspergillus* sp. ARC3, isolated from macaúba husks, with the purpose of increasing lipase production in submerged culture for future application in biodiesel synthesis.

MATERIAL & METHODS

This work was carried out at the Laboratory of Mycology, Enzymology and Product Development (LMEDP), at the Federal University of the Jequitinhonha and Mucuri Valleys (UFVJM) JK campus, Diamantina, Minas Gerais, Brasil. The microorganism *Aspergillus* sp. ARC3 was collected from macaúba husks and registered in the National System for Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under number A64AD93.

2.1 Otimização do cultivo do *Aspergillus* sp. ARC3 em meio submerso

The influence of different submerged culture media on lipase production by the filamentous fungi ARC3 was analyzed by inoculating two 1 cm diameter discs of the microorganism into 25 mL of three different culture media: Carvalho-Peixoto (CP) 4, M5 5, and Khanna 6, with an initial pH of 5.0 and soybean oil ABC® as the carbon source, maintained for six days at 30°C, stationary in a bacteriological incubator. Subsequently, the optimal fungal growth time for maximum lipolytic production in the pre-selected submerged medium using soybean oil as the carbon source, initial pH 5.0, was analyzed over seven days at 30°C, stationary, with enzymatic activity assessed every 24 hours.

Next, to verify the influence of initial pH of the culture medium and fungal growth temperature, a total of nine experiments were conducted, varying the initial pH at 5.0, 5.5, and 6.0, while the organism's growth temperature was set at 30°C, 35°C, and 40°C, in medium containing soybean oil as the carbon source, maintained for five days stationary in a bacteriological incubator.

Subsequently, nitrogen sources were varied in the cultivation of Aspergillus sp. ARC3 for increased lipase production. The following were analyzed: 1.3% (w/v) ISOFAR® peptone, 1.3% (w/v) VETEC QUÍMICA® yeast extract, 1.3% (w/v) PERFYL TECH® urea, and 1.3% (w/v) CIAVICCO® ammonium acetate, with the control being the original composition of the M5 medium and M5 medium without nitrogen sources, containing soybean oil as the carbon source, at an initial pH of 5.5, at 30°C, for five days of stationary cultivation in a bacteriological incubator.

After fungal growth, the separation of the crude extract containing the lipases from the fungal mycelium was performed by vacuum filtration. Enzymatic activity was determined using 0.03 g of p-nitrophenyl palmitate in 10 mL of isopropyl alcohol as the substrate 7. The reaction was initiated by adding 50 µL of the enzyme extract to 450 µL of the reaction mixture (225 µL of substrate with 225 µL of the solution mixture I, containing 0.01 g of gum arabic added to 50 µL of Triton X-100 and 18 mL of 100 mM sodium acetate buffer pH 6.0), and the reaction was incubated in a water bath at 50°C for 10 minutes. The reaction was stopped by adding 500 µL of a saturated solution of sodium tetraborate. The p-nitrophenol released by hydrolysis in alkaline pH forms p-nitrophenolate, a yellow-colored compound, which was quantified using a colorimeter at 405 nm against a blank with boiled enzyme, determining the spontaneous hydrolysis of the substrates under the assay conditions. Enzymatic activity is determined in U/mL, with one unit (U) defined as the amount of enzyme required to hydrolyze one micromole of substrate per minute (µmols/min) under the assay conditions.

3. RESULTS & DISCUSSION

The lipolytic activity in different submerged media was evaluated to investigate their performance in different medium compositions. The analysis of the results revealed significant variations in enzymatic activity due to the culture medium used. It was possible to identify that the highest lipolytic activity was obtained by cultivating the microorganism in M5 medium, with 5.157 U/mL, followed by CP medium with 4.815 U/mL and Khanna medium with 3.748 U/mL. The results were further analyzed using statistical analyses, specifically the Tukey method, which demonstrated no significant difference in the means between the two media with the highest enzymatic activity.

After standardizing the culture medium to M5, the analysis of the cultivation time aiming for higher lipolytic activity revealed a complex dynamic of lipase production over time. The fifth day showed an activity of 5.481 U/mL, making it evident that the fifth day was the best cultivation time for *Aspergillus* sp. ARC3 for lipase production.

With the culture medium and cultivation time defined for maximum lipase production, tests were conducted to analyze the influence of fungal growth temperature and initial pH of the submerged M5 culture medium. Through the Tukey method, the samples showed means with significant differences, where pH 5.5 at 30°C presented the best cultivation conditions for ARC3 fungus for higher lipase production with 5.531 U/mL, while the second observed lipolytic activity was at pH 5.0 at 30°C. These results indicated that temperatures above 35°C and basic pHs inhibited the production of the enzyme of interest significantly due to adverse effects on growth.

The results obtained in our studies disagree with those obtained for lipase production by *Penicillium* sp. F0, whose optimal enzymatic production time was in submerged cultivation for seven days with agitation [8]. Likewise, Mendes et al. (2019) [9] also obtained the sixth day as the best result for lipolytic activity by the filamentous fungus *C. cylindracea* in submerged Vogel medium at 28°C.

With parameters such as temperature, pH, cultivation time, and submerged medium defined, the final test was to investigate the influence of nitrogen sources on lipase production to determine the impact of these nutrients on enzyme induction performance. Therefore, the results obtained demonstrated that the microorganism showed a greater preference for mixed nitrogen sources (yeast extract, ammonium acetate, and peptone), that is, the control medium, which resulted in 5.539 U/mL of lipolytic activity.

From the reported results, it can be observed that the optimization of enzymatic production by the selected filamentous fungi is of great value, as up to now there has been a 7.4% increase in lipolytic activity by *Aspergillus* sp. ARC3.

2 CONCLUSION

The lipase produced by the fungus *Aspergillus sp.* ARC3 in submerged M5 medium with five days of stationary cultivation, in a bacteriological incubator, at 30ºC, and an initial culture medium pH of 5.5, revealed maximum lipase production. Regarding nitrogen sources, the microorganism showed a preference for mixed sources, due to the presence of a mixture of salts and nitrogen. Therefore, these results demonstrated that the produced lipase exhibited significant activity and potential applicability of this enzyme in biotechnological processes.

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