AQUEOUS MESQUITE EXTRACT AS MEDIUM FOR FUNGAL PECTINASES PRODUCTION

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ABSTRACT

The use of aqueous extract of mesquite (*Prosopis juliflora* (Sw) DC) as a substrate for biotechnological processes becomes an interesting strategy because of its rich composition containing carbohydrates and proteins. The objective of this study was to verify the ability of three fungal isolates (*Aspergillus* sp. NA01, *Paecilomyces* sp. TP08 and *Penicillium* sp.) to synthesize pectinases from the aqueous extract of mesquite. For this purpose, the cultivation medium consisted of 1% (v/v) aqueous extract of mesquite with the pH adjusted to 5.8. For the cultures, a 10% (v/v) aliquot of a spore suspension at $1x10^7$spore/mL was added to each of the cultivation flasks to a final medium volume of 100 mL. Samples were taken every 24 hours to verify the enzymatic activity for each of the fungal isolates. Pectinolytic activity was determined using 0.6% (w/v) citrus pectin in acetate buffer (pH 5.5) as the specific substrate. The results indicated that among the three fungal isolates analyzed, *Aspergillus* sp. NA01 showed the highest pectinase activity (2.460 U/mL) and the total consumption of the substrate at the end of four days of the process. Therefore, this isolate demonstrated greater ability to hydrolyze the pectin present in the mesquite extract, making it a potential producer of pectinases with a non-conventional substrate under the analyzed cultivation conditions.

Keywords: fungal enzymes, enzymatic activity, *Aspergillus* sp., *Penicillium* sp., *Paecilomyces* sp.

1 INTRODUCTION

The mesquite pods, due to their nutrient-rich composition, can be used as a source of substrate for the growth of microorganisms of industrial interest. The aqueous extract of mesquite, as a substrate for biotechnological processes, is an interesting alternative, as there are few reports in the national (MUNIZ, 2009; PEDREIRA et al., 2022) and international (NJOKWENI et al., 2019; SALEH, ABU DIEYEH, 2021; MACEDO et al., 2023) literature of its use to obtain bioproducts. Due to its properties, there is an interest in using its residues as a way of producing enzymes that add value to the industry, such as the production of pectinolytic enzymes.

The pectinases are part of the hydrolase family and are responsible for the degradation of pectic substances, which are polysaccharides found in plant cell walls (ANDRADE et al., 2018). These enzymes are of great importance to the industry, accounting for a total of 25% of the global market share in the food and beverage sector (AMIN; BHATTI; BILAL, 2019). Due to their ability to degrade pectin, they are mainly used in the clarification processes of fruit juices and wines (WAGH et al., 2022). In addition, regarding the production of these enzymes, it can occur through sources such as plants and/or microorganisms, but industrially speaking, production through microorganisms, mainly through filamentous fungi, is more advantageous due to the low production cost and the ease of the process. According to Ahmed et al. (2016), the genera that stand out the most for this production include *Aspergillus*, *Penicillium*, *Rhizopus*, and *Trichoderma*.

Due to all the aforementioned factors, the primary purpose of this study was to develop pectinase enzymes through liquid-state fermentation, using mesquite pod extract as a substrate, with an emphasis on the performance of the filamentous fungi *Penicillium* sp. TP01, *Paecilomyces* sp. and *Aspergillus* sp. NA01.

2 MATERIAL & METHODS

Substrate:

The substrate used for enzymatic production was a 10% (v/v) aqueous extract of mesquite with the pH adjusted to 5.8. The extraction of the aqueous mesquite extract was based on the methodology described by Silva (2009). The cultivation medium

was characterized for total soluble solids content (SANTOS et al., 2021), total reducing sugars (VASCONCELOS, 2013), pH, and pectin content (ANDRADE et al., 2018).

Microorganisms:.

The fungal isolates used were *Paecilomyces* sp. TP08 (isolated from diesel oil/Gas Station/João Pessoa/Paraíba – 2014), *Aspergillus* sp. NA01 (isolated from leaf-cutting ants from Buraquinho Forest/João Pessoa/Paraíba) and *Penicillium* sp. (isolated from anatomical specimens preserved in 10% formalin at the Anatomy Laboratories Complex of the Department of Morphology of the Health Sciences Center (CCS)), all provided by the Molecular Biology and Plant Biotechnology Laboratory of the Biotechnology Center of the Federal University of Paraíba. For maintaining the fungal isolates, the fungi were maintained on solid Sabouraud-Dextrose Agar 2.0% (w/v) and incubated at 30 °C for 10-15 days for mycelial development. After growth, they were stored at 4 °C. For inoculum preparation, 10 mL of sterilized distilled water was added to Petri dishes containing the fungi, and with the aid of a platinum loop, the spores were superficially scraped and suspended. They were then counted using a Neubauer chamber to establish a concentration of $1.0x10^7$ spores/mL.

Enzymatic production:

The cultures were established in Erlenmeyer flasks (250 mL), containing 100 mL of 10% (v/v) aqueous mesquite extract, under the following process conditions: 30°C, 200 rpm, pH 5.8, an aeration rate of 0.4, and a spore concentration of $1.0x10^7$ spores/mL. Samples were taken every 24 hours to determine pectinolytic activity and substrate consumption.

Determination of Pectinase Activity:

The analysis of the produced pectinases was conducted based on adaptations of the methodology by Pinheiro (2007). This method involves preparing a solution of citrus pectin (P.A.) at 0.6% in acetate buffer (w/v) with a pH of 5.5 as the specific substrate. In test tubes, 2 mL of the pectin solution and 0.25 mL of the produced enzyme broth were added, then incubated at 35°C for 30 minutes to catalyze the enzymatic reaction. Next, 0.5 mL of the reaction mixture was removed and mixed with 0.5 mL of DNS reagent, heated in a thermostatic bath at 100°C for 5 minutes and then analyzed using a spectrophotometer (EVEN) at 540 nm. The pectinolytic activity of the enzyme broth was determined based on the increase in reducing sugar/monomer generated by the action of the pectinases on the citrus pectin. One unit of enzymatic activity corresponds to the amount of enzyme that releases one µmol of galacturonic acid per minute, expressed in U/mL.

3 RESULTS & DISCUSSION

The aqueous extract of mesquite showed a total soluble solids (TSS) content of 28 °Brix, indicating that the solution contains sufficient fermentable carbohydrates to be used as a substrate in microbial transformation processes. The determination of total reducing sugar (TRS) at 210 g/L of complex sugars confirms the amount of sugars present in the sample in terms of glucose. The amount of pectin in the form of calcium pectate found in this study was 0.262%, as shown in Table 1.

Table 1. Characterization of Aqueous Mesquite Extract

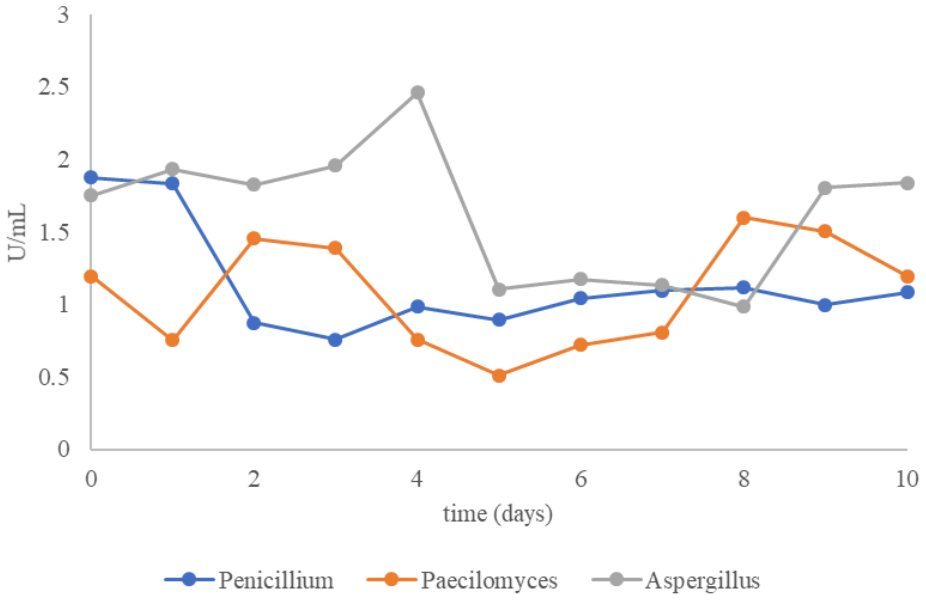
Cultivation medium	pH	SST (ºBrix)	TRS (g/L)	[Pectin] (%)
Mesquite extract 10%(v/v)	3,0	3,2	8,5	0,262

The results of the cultivation with the *Penicillium* sp. isolate in a 10% (v/v) aqueous mesquite extract medium over 240 hours showed an initial substrate concentration, determined by total reducing sugars (TRS), of 8.04 g/L, with maximum consumption observed between 48 and 120 hours of cultivation. For the cultivation with the *Paecilomyces* TP08 sp., the initial substrate concentration was 8.10 g/L, and maximum consumption was identified in just 72 hours of cultivation, resulting in a biomass growth of 2.39 g/L and a maximum enzymatic production of 1.60 U/mL of pectinolytic activity. Finally, the cultivation with *Aspergillus* NA01 sp. had an initial substrate concentration of 9.55 g/L, with maximum substrate consumption also occurring at 72 hours of cultivation, fungal biomass growth at the end of cultivation reaching 2.43 g/L, and a maximum pectinase production of 2.46 U/mL.

Regarding pectinase production, the results indicate that *Aspergillus* sp. NA01 showed a production of 2.460 U/mL on the fourth day. Along with the rapid consumption of sugars, it demonstrated the best results in pectinase production, followed by *Penicillium* sp. and *Paecilomyces* sp. TP08, respectively, as shown in Figure 1. Therefore, the results indicated that *Aspergillus* sp. NA01 was the isolate that achieved the best results in terms of pectinase production.

In Figure 1, it is possible to better observe the pectinase production for each of the fungal species and which one showed the best pectinolytic activity.

Figure 1. Pectinase production comparison in assays with *Penicillium* sp., *Paecilomyces* sp. TP08 and *Aspergillus* sp. NA01



The use of mesquite by-products as a nutrient source for enzyme production can be even more advantageous when combined with solid-state fermentation techniques. This approach allows for the efficient use of agro-industrial residues and offers advantages such as lower water consumption and reduced effluent generation (SILVA et al., 2019).

4 CONCLUSION

The mesquite extract can be used as a substrate for the production of pectinolytic enzymes with the fungal isolates used, *Penicillium* sp., *Paecilomyces* TP08 sp. and *Aspergillus* NA01 sp., which demonstrated the ability to produce pectinolytic enzymes induced by the substrate under the analyzed process conditions.

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