PECTINASE PRODUCTION BY ASPERGILLUS NIGER IOC 4003 BY SOLID-STATE FERMENTATION USING CAJA DEPULPING RESIDUE

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ABSTRACT – The purpose of this study was to assess pectinase production through solid-state fermentation by Aspergillus niger IOC 4003 using caja depulping residue. Enzyme production was carried out in 250 mL Erlenmeyer flasks containing 5 g of washed caja residue or unwashed caja residue supplemented with KH₂PO₄ (1.5 g/L) and (NH₄)₂SO₄ (3.3 g/L). Initial pH and moisture were adjusted for 4.5 and 40%, respectively. SSF was started after inoculation of 1 x 10⁷ spores/g residue of A. niger and incubated at 30°C. The microorganism was assessed during 240 hours of cultivation where at every 24 hours the complex enzymatic were extracted with acetate buffer (pH = 4.5, 200 mM) at ratio 1:10 (g residue:mL buffer). There were measured polygalacturonase, pectin lyase and total cellulase activity as well as quantification of total protein and total reducing sugars. The best residue was WCR where it was obtained the maximum enzymatic production of PFase, PGase and PNLase activity of 3.49 ± 0.08 IU/g (48 h), 38.2 ± 0.63 IU/g (72 h), 8.58 ± 0.78 IU/g (48 h), respectively. Initial encountered TRS was 66.0 ± 0.2 mg/g for WCR. Therefore, washed caja residue demonstrated a great potential for the production of pectinolytic enzymes and using these enzymes in industrial applications.

1. INTRODUCTION

Pectinases are a heterogeneous group of enzymes capable of hydrolyzing pectic substances. Therefore, given this capacity, pectinases are valuable and required in several areas of industry (Suhaimi et al, 2016). These enzymes are used in several industrial processes including fruit juice clarification, coffee and tea fermentation, vegetable oil extraction, degumming and retting of plant fibers, paper and pulp industry.

Many studies have already demonstrated the potential and ability of filamentous fungi to be added into processes, especially in secretion and production of pectinolytic enzymes. The A. niger species stands out for the versatility of its metabolism allowing the production of organic acids and different types of industrial enzymes according to available substrate under various environmental circumstances. In view of this wide range of substrates, it is easy to observe the strong dependence that production of pectinase through A. niger has on the composition of the culture medium (Suhaimi et al, 2016).
The main advantage of using solid-state fermentation to produce pectinases when compared to submerged fermentation is the high availability of agro-industrial by-products as a substrate where high volumetric yields may be achieved and to avoid catabolic repression even in more concentrated medium. From these conveniences, it is reasonable to foresee reductions of cost for industrial operations (Pitol et al, 2016). In this context, this study aimed to analysis pectinase production through solid-state fermentation (SSF) by Aspergillus niger IOC 4003 using caja depulping residue.

2. MATERIAL AND METHODS

2.1. Microorganism

The fungus Aspergillus niger IOC 4003 was donated by Coleção de Culturas de Fungos Filamentosos (CCFF) from Oswaldo Cruz/Fiocruz Institute to Biochemical Engineering Laboratory (LEB) – Federal University of Rio Grande do Norte (UFRN). The growth of microorganism occurred in potato-dextrose agar (PDA) medium at 37°C on incubator during 7 days and propagation of spores was performed in Erlenmeyer (125 mL) containing 4.6 g of ground corn cob (Peptone – 56 g/L, KH2PO4 - 0.76 g/L, ZnSO4 - 0.02 g/L, FeSO4 - 0.02 g/L) as a way of diminishing adaptation phase. The concentration of spores was determined by Neubauer chamber.

2.2. Enzymatic Production by Solid State Fermentation

Fermentation in the solid state was carried out using caja depulping residue as a carbon source. Unwashed caja residue (UCR) means that the residue was dried and ground as received from industry and washed caja residue (WCR) before drying and grinding was rinsed with distilled water 5 times (1 kg residue/2 L water). Then, residue (5 g) was submitted to autoclave (121°C for 20 min) in Erlenmeyers (250 mL) (Ahmed et al, 2016). Afterwards, A. niger was inoculated into culture medium at concentration of 10⁷ spores/g residue and incubated for 240 hours (ten days). Also, at temperature of 30 °C, initial pH of 4.5 and moisture of 40% (Santos et al., 2008).

2.3. Enzymatic Extract

The enzymatic extracts were obtained with acetate buffer (pH = 4.5, 200 mM) at ratio 1:10 (g residue:mL buffer) into fermented cultivation at every 24 hours. After, the mixture passed through filtration utilizing filter paper. The filtrate was centrifuged at 3,500 rpm and temperature of 4 °C for 15 min. Samples were stored at -20 °C for further enzymatic quantification, total protein (TP) and total reducing sugars (TRS). All experiments were performed in triplicate and data are presented as the mean ± standard deviation.

2.4. Enzymatic Analysis

The activities of polygalacturonase (PGase) and total cellulase (FPase) were determined by standard methodology provided by Couri (1995) and Ghose (1987), respectively. Activity of pectin lyase (PNLase) was based by Couri (1995) with modification [acetate buffer pH 4.5, 0.8 mL of pectin citric (1%, w/v) and 0.5 mL of extract, 30 min at 35 °C, 0.5 mL of reacting
mixture is determined by DNS-protocol, reading absorbance at 575 nm. One (IU) unit of polygalacturonase activity, pectin lyase and FPase was defined as the amount of enzyme released per 1 μmol of reducing sugar per min under assay conditions.

2.5. Analysis of total protein and total reducing sugars

Protein concentration were determined by Bradford protocol (Bradford, 1976) where standard curve have followed 0.2 mL bovine serum albumin as protein at several concentration (0.02 – 0.14 g/L) mixed with 2 mL of Bradford reagent; readings performed at 595 nm. Total reducing sugars were based by Ghose’s protocol (1987).

3. RESULTS

3.1. Assessment of Enzymatic Production

Unwashed caja residue: As shown in Figure 1, it was not possible to verify any tendency of growing microorganism through consuming sugar or any increasing of the desired enzymatic complex due to enzymatic quantification. Two main reasons are proposed to justify such behavior: the caramelization attributed to autoclave process and sugar inhibition caused by high initial total reducing sugar of about 140 mg/g.

Washed caja residue: In Figure 2, it is possible to observe the maximum activity for PGase occurred at the third day of cultivation in the value of 38.2 ± 0.63 IU/g confirming that A. niger is great at the production of polygalacturonase (Suhaimit et al, 2016). Also, the production of PNase and FPase reached a maximum activity at 48 hours of fermentation with 8.58 ± 0.78 IU/g and 3.49 ± 0.08 IU/g, respectively. The results revealed that WCR supported a pectinase production within 72 h and the reason might be the presence of pectin, vitamins, different proteins and other sugars contents, indicating that this waste may be utilized to induce the pectinases production after wash.
4. CONCLUSION

The process of solid-state fermentation for production of pectinase by *Aspergillus niger* IOC 4003, using caja residue as an alternative carbon source, has been shown to be a promising path for the production of pectinolytic enzymes.

5. REFERENCES

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