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Utilization of Agricultural Byproducts for Production of Industrially Important Enzymes by *Aspergillus spp.*

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Abstract: ***The present study was aimed to utilize different agricultural byproducts such as wheat bran, rice bran and chickpea husk for production of industrially important enzymes (amylase, cellulase, xylanase) in submerged fermentation carried out at 30°C, pH 5 and 120 rpm. A. flavus and A. niger showed highest amylase activity 0.5 U/ml and cellulase activity 0.5 U/ml, respectively in the fermentation medium containing wheat bran as substrate. However, the A. niger showed significantly high amount of xylanase enzyme production 200 U/ml using wheat bran as substrate. Further studies in terms of characterization of the enzyme, medium optimization, genetic improvement would lead the xylanase to be used in the industrial level.***

Keywords: A. niger, wheat bran, xylanase, amylase, cellulose

1. Introduction

Microorganisms such as filamentous fungi are important sources of enzymes used in different industrial areas. *Aspergillus*, one of the most important genera of filamentous fungi, is explored in industrial application. *Aspergillus* spp. have different characteristics such as the presence of a secretory pathway, the possibility of genetic manipulation, and high productivity using different fermentative processes which made them advantageous and favorable for different purposes. Production of different industrial enzymes (amylase, cellulase, xylanase) has been reported for different *Aspergillus* species (Guimaraes and Souza, 2017). The use of enzymatic processes have been implanted in broad range of industries in recent decades because they are specific, fast in action and often save raw materials, energy and chemical compared to conventional processes (Kumar et al, 2017). Amylases which hydrolyze the starch molecules have potential applications in a wide number of industrial sectors such as food, textile, paper and detergent. The production of α-amylases has generally been carried out using submerged fermentation using bacterial or fungal strains (de Souza PM, de Oliveira Magalhães P, 2010). Another important enzyme is microbial cellulase which shown its potential application in various industries including pulp and paper, textile, laundry, biofuel production, food and feed industry, brewing, and agriculture. Due to the complexity of enzyme system and immense industrial potential, cellulases have been a potential candidate for research by both the academic and industrial research groups (Kuhad et al., 2011). Xylanases are produced from different microorganisms (fungi and bacteria) and they are widely used in pulp and paper industries. It has also potential application in fruit juice processing and bakery processes. Xylanase enzyme was found to be effective in getting enhanced sugar extraction from fruit juices, clarification of fruit juices, and substantial dough-raising in bakery (Kumar et al, 2017).

Agroindustrial wastes such as rice bran, wheat bran, wheat straw, sugarcane bagasse, and corncob are cheapest and plentifully available natural carbon sources for the production of industrially important enzymes. Utilization of agricultural wastes offers great potential for reducing the production cost and increasing the use of enzymes for industrial purposes (Bharathiraja et al., 2017). In this study, the main objective was to find out the best combination of substrate and *Aspergillus* sp. to carry out the submerged fermentation for production of the industrial enzyme in a significant level.

2. Materials and Methods

Fungi for enzyme production

Three species of *Aspergilllus*, *A. niger*, *A. flavus* and *A. terreus* kept in the laboratory were selected for screening of enzyme production using the agricultural substrates.

Selection of substrate for enzyme production

Different agro-industrial byproducts (Chick pea husk, rice bran, wheat bran) were used to determine a suitable substrate for the highest production of enzymes.

Enzyme production media

Production medium contained (g/L) yeast extract 5.0 gm, $MgSO_4$ 0.5 gm, K_2HPO_4 1.0 gm, NaCl 5.0 gm, (NH4)2SO4 5.0 gm and agro-industrial byproduct 10.0 gm. 50 ml of media were taken in 250 mL conical flasks. The flasks with media were sterilized in an autoclave at 121°C for 15 min.

Inoculum preparation

The fungal strains were grown on Potato Dextrose Agar (PDA) Medium for seven days. Then the whole fungal

mycelia were scrapped off from the medium and added to 30 ml of sterile 1% NaCl to prepare the inoculum.

Enzyme production

The flasks containing the fermentation media were inoculated with 2% fungal inoculum prepared. The inoculated media were incubated at 30°C, pH 5 and 120 rpm in a shaker incubator (Excella E25, NBS, USA) for 72h. After fermentation, the culture media were centrifuged at 6000 rpm for 10 min and the supernatants were used as enzymes.

Enzyme assay

Enzyme activities were measured by determining the reducing sugar content following the method described by Miller (1959). Briefly, a reaction mixture composed of 0.5 ml of crude enzyme solution and 1.0 mL of 1% carboxymethyl cellulose (CMC) solution in Citrate buffer (pH 5.2) for cellulase assay, 1% starch solution in Phosphate buffer (pH 6.8) for amylase assay or 1% xylan solution in Sodium acetate buffer (pH 4.5) for xylanase assay was incubated at 50°C in a shaking water bath for 30 min. The reactions were terminated by adding 3 ml of DNS reagent. The color was then developed by boiling the mixture for 15 min. OD of samples were measured at 540 nm against a control solution containing all the reagents except the crude enzymes. One unit of the activity is the amount of enzyme necessary to produce 1 µmol reducing sugar per min under the standard assay conditions.

3. Results and Discussions

Production of amylase enzyme by *Aspergillus* spp. using different substrates

The fungal species *A. terreus, A. flavus, A. niger* produced diminutive amount of amylase enzyme using chickpea husk as a substrate. On the other hand using rice bran as substrate the amylase yields were found 0.13 U/ml, 0.023 U/ml and 0.23 U/ml by *A. terreus, A. flavus and A. niger*, respectively. The fungi *A. terreus, A. flavus and A. niger* produced 0.13 U/ml, 0.5 U/ml and 0.181 U/ml, respectively using wheat bran as substrate. From this experiment it was found that highest amylase activity was achieved by *A. flavus* using wheat bran (Figure 1).

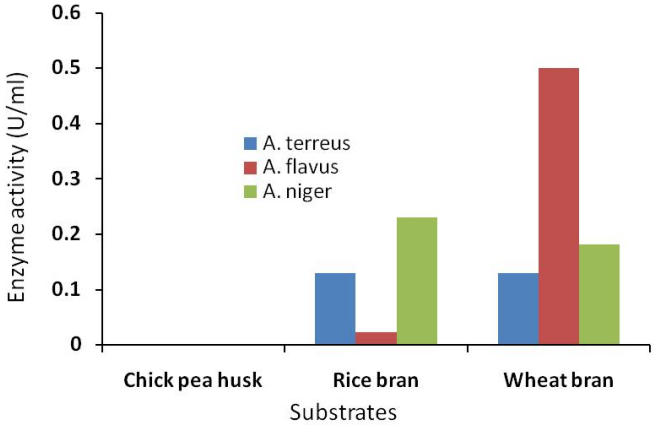

Figure 1: Production of amylase by *Aspergillus* spp. using different substrates

Production of cellulase enzyme by *Aspergillus* spp. using different substrates

Using chick pea husk as a substrate there was found no activity by the fungal stains. In case of rice bran a as substrate only *A. niger* produced very low (0.19 U/ml) amount of cellulase. But wheat bran as a substrate supported higher yield of cellulase enzyme 0.05 U/ml, 0.25 U/ml and 0.5 U/ml by *A. terreus, A. flavus and A. niger*, respectively. It is clear that highest titer of cellulase was produced by *A. niger* using wheat bran (Figure 2).

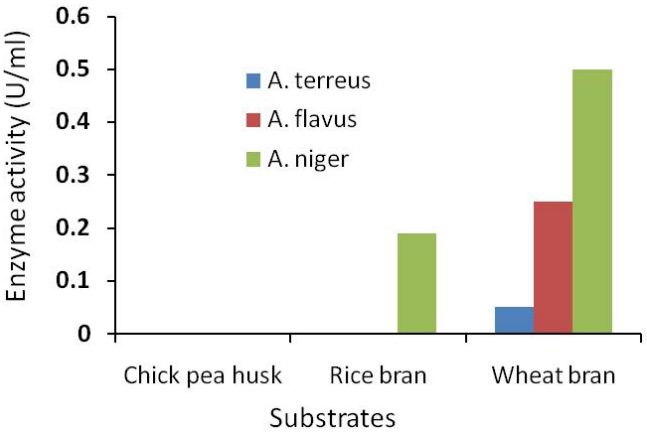

Figure 2: Production of cellulase enzyme by *Aspergillus* spp. using different substrates

Production of xylanase enzyme by *Aspergillus* spp. using different substrates

Xylanase was not produced by the fungal stains using Chick pea husk and rice bran. But *A. niger* produced significantly high amount of xylanase enzyme 200 U/ml using wheat bran as a substrate (Figure 3). Suleman et al., 2016 also found higher amount of xylanase from *A. niger* using wheat bran as a substrate.

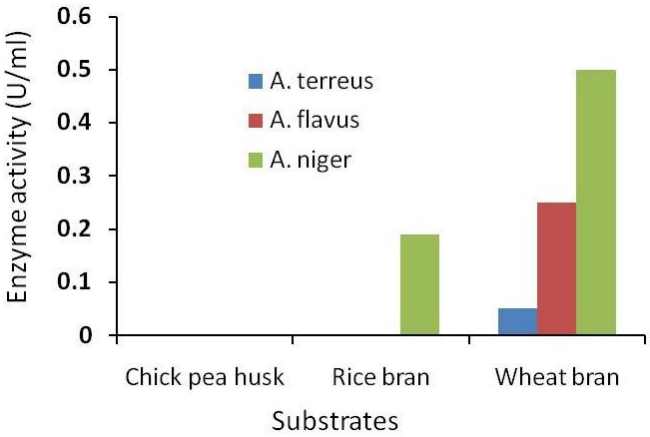

Figure 3: Production of xylanase enzyme by *Aspergillus* spp. using different substrates

From this study, wheat bran was found as the best among the substrates used to support production of the enzymes by *Aspergillus* spp. Many researchers used wheat bran for production of amylase by *A. flavus* (Manivannan et al., 2015), *A. niger* (Kaur et al., 2003), for production of cellulase by *A. niger* (Kumar et al., 2018) and for

production xylanse by *A. niger* (Khonzue et al, 2011, Kumar et al., 2018).

4. Conclusion

In this study, different agro-industrial residues were used for production of industrial important enzymes by *Aspergillus* spp. Among the fungal enzymes (amylase, cellulase and xylanase) xylanase was produced in significantly high level by *A. niger* using wheat bran as substrate. Further investigation in terms of characterization of the enzyme, medium optimization, genetic improvement would lead to produce higher amount of the xylanase which can be used industrially. Thus an agricultural byproduct (wheat bran) might be utilized for production of industrial important enzyme.

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