Production of Cellulase from *Aspergillus niger* using Rice Husk as Substrate by Solid State Fermentation.

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Introduction

Cellulases are enzymes that effectively degrade cellulose into glucose. There are types of cellulase enzymes namely endoglucanases (EC 3.2.1.4, endo-1, 4-β-D-glucanases), cellobiohydrolases (exo- 1, 4-β-D-glucanase EC3.2.1.91) and β-glucosidases or Cellobiases (EC 3.2.1.21; synonyms: cellobiase). One of the most well-known and efficient producers of cellulolytic enzymes is the filamentous fungus, *Aspergillus niger*. This fungus secretes a complex array of degradative enzymes to hydrolyze cellulose efficiently and it is an important commercial source of cellulase, especially in the food, textile and pharmaceutical industries (de Vries and Visser, 2001; Coleman, *et al*., 2007). Fungi carry out extracellular digestion and secrete digestive enzymes into their substrates and absorb only digested food into their hyphae, as such, they produce cell free enzyme. Fungi are the main cellulase-producing microorganism in which *Aspergillus sp*. are known to hydrolyse both soluble and insoluble cellulose (Sridevi *et al.,* 2009). Since the production of cellulase enzyme is a major factor in hydrolysis of cellulosic material, it is important to make the process economically viable. This study therefore investigated the bioconversion of agricultural waste rice husk which could cause pollution to the environment into a more useful product, that is, cellulase using *Aspergillus niger*.

Materials and Methods

Fungi were isolated from plantain garden soil using ten-fold serial dilution and pour plate method on Potato Dextrose Agar. Isolates were screened for the ability to produce cellulase on Carboxymethylcellulose Agar. The cellulase hyper-producers were characterized morphologically (colonial and cellular) and biochemically. Cellulase was produced through solid state fermentation using rice husk as the sole carbon source over a 96-hour incubation period at 25° C. Cellulase was assayed according to the method of Olaniyi, *et a*l. 2014, by adding 0.5mL of 1% Carboxymethylcellulose substrate into 100µL of the enzyme and incubated at 50ºC for 15 mins.100µL DNSA solution was added and mixed well, boiled for 5 mins in a water bath.Thereafter, 1000µL distilled water was added and the optical density (OD) was read at 540 nm against the blank. The amount of glucose released per ml was estimated from a standard curve prepared with known glucose concentration. One unit of cellulase activity was expressed as the amount of protein that liberated reducing sugar equivalent to 1 g of glucose per minute under assay condition, The unit of enzyme activity was presented as µmol/ml/min. The specific enzyme activity was expressed as the unit of enzyme activity per mg of protein. The unit of enzyme specific activity was presented as µmol/min/mg of protein.

Abstract

Background: Cellulases are hydrolytic enzymes that catalyze the breakdown of cellulose into glucose. They can be produced from plants, animals and microorganisms; however, microorganisms have been reported as better producers in terms of yield. The choice of a fungus for this study is based on previous results in literature that they are the most common and highest yield producers of extracellular cellulase compared to bacteria. We report findings from the use of *Aspergillus niger* for the production of cellulases using rice husk as substrate. **Materials and Methods:** Fungi were isolated from plantain garden soil using ten-fold serial dilution and pour plate method on Potato Dextrose Agar. Isolates were screened for the ability to produce cellulase on Carboxymethylcellulose Agar. The cellulase hyper-producer was characterized morphologically (colonial and cellular) and biochemically. Cellulase was produced through solid state fermentation using rice husk as the sole carbon source over a 96-hour incubation period at 25° C. **Results and Conclusion:** Fourteen strains of fungi were isolated, however only one fungus was able to hydrolyze carboxymethylcellulose efficiently and gave the largest zone of clearance. The fungus was identified morphologically and biochemically as *Aspergillus niger.* Our results reveal the maximum enzyme activity of the crude cellulase as 9.15 µmol/min/ml at 72 hours into the autolysis phase of *Aspergillus niger* and the specific activity of 0.76 µmol/min/mg of protein. This result shows that rice husk is a good carbon substrate for *Aspergillus niger* in cellulase production.

Results & Discussion

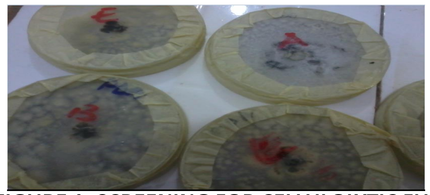

FIGURE 1: SCREENING FOR CELLULOLYTIC FUNGI ON CARBOXYL METHYL CELLULOSE AGAR PLATE.

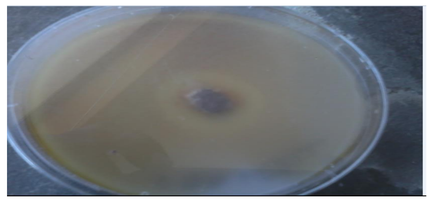

FIGURE 2: QUALITATIVE ASSAY FOR CELLULASE ACTIVITY.

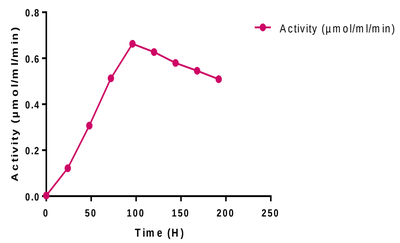

FIGURE 3: GROWTH CURVE OF *ASPERGILLUS NIGER* ON CARBOXYLMETHYLCELLULOSE (CMC).

TABLE 1: IDENTIFICATION OF MOULDS

ISOLATE CODE	COLONIAL MORPHOLOGY	MICROSCOPIC MORPHOLOGY
FR20	Growth colony spreads rapidly well within incubation periods of 3 to 4 days with fluffy and velvety texture with aerial mycelium white at first stage of the growth and frequently developing into dark brown to black conidial heads with no colour at the reverse (i.e. at the back of the petri dish culture plates)	Conidial heads were round or globose large and also radiated as they grow splitting into loose columns of conidia chains with age ,conidiophores arising from the substratum, mostly colourless to brown, smooth splitting when crushed like a pieces of cane-vessicle, metallae and foot cells are usually present

TABLE 2: FUNGAL COUNT (CFU)

SAMPLE CODE	X10² cfu/g	X10³ cfu/g	X10⁴ cfu/g
Soil sample 1	$29x10^2$	$27x10^3$	$24x10^4$
Soil sample 2	$14x10^2$	$18x10^3$	$22x10^4$
Soil sample 3	$20x10^2$	$23x10^3$	$26x10^4$
Soil sample 4	$25x10^2$	$26x10^3$	$28x10^4$

Conclusions

This result shows that rice husk is a good carbon substrate for *Aspergillus niger* in cellulase production.

This study has also shown that *Aspergillus niger* grown on rice husk could serve as a very cheap source of cellulase with desirable physical-chemical properties.

In addition, it will be a sort of an environmental control measure, whereby rice husk which form a nuisance to the community may be converted to a very useful raw material in the industries.

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