



Research Article

Screening of Some Fungi Associated with Maize Cob Degradation for Cellulase Activity

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ABSTRACT

Fungi involved in the biodegradation of maize- cob were isolated and screened for cellulase activity using glucose and carboxymethyl cellulose as carbon sources. The effect of the carbon sources on cellulase production was determined.

Eleven fungi species were isolated which include *Rhizopus oryzae*, *Aspergillus flavus*, *Mucor racemosus*, *Aspergillus niger*, *Penicillium atrovenetum*, *Penicillium expansum*, *Botryotrichum piluliferum*, *Penicillium chrysogenum* and *Penicillium restrictum*. Effect of carbon source on cellulase production was also reported. The highest reducing sugar production for all fungi isolate was obtained on day 14 when carboxymethyl cellulose (CMC) was used as the carbon source; while the highest reducing sugar production was obtained on day 7 when glucose was used as the carbon source. *Aspergillus niger* had the highest production of reducing sugar (1.68mg/ml and 0.82/m) in CMC and glucose respectively while the lowest production was recorded for *Mucor racemosus* (0.69mg/ml and 0.01mg/ml). All *Aspergillus* species and *Penicillium* species isolated had very high reducing sugar production in CMC: *Aspergillus flavus* 1.24 mg/ml; *A. oryzae* 1.08mg/ml, *P. expansum* 1.60 mg/ml, *P. atrovenetum* 1.43mg/ml except *Penicillium chrysogenum* and *Penicillium restrictum* with 0.78mg/ml and 0.81 mg/ml, respectively.

Aspergillus and *Penicillium* species are good cellulase producers especially those that are associated with degrading maize cob. These organisms are considered suitable to increase the nutrient of maize- cob, an agro industrial waste and consequently make them useful for animal feed production.

INTRODUCTION

Agricultural wastes which include maize- cob are among the causes of environmental pollution. Their conversion to useful products may ameliorate the problems they cause. Proper biotechnological utilization of these wastes in the environment will eliminate pollution and convert them into useful byproducts (Milala, 2005).

Cellulose and Hemicellulose which form about 85 percent of maize cob (Tuah and Oskor, 1990) is commonly degraded by an enzyme called cellulase. This enzyme is produced by several microorganisms commonly bacteria and fungi (Shin *et al.*, 2006; Immanuel *et al.*, 2006).

In this study fungi species were actually isolated from degrading corn cobs and tested for their cellulase activity by measuring the amount of reducing sugar produced by the fungi species.

MATERIALS AND METHODS

Collection of Samples: Corn cobs were collected from refuse dumps in a local market in Ajegunle area of Oyo town in Oyo state, Nigeria. Isolation and Identification of Isolates. Fungi species were isolated using potato dextrose Agar (PDA) and Yeast extract agar (YEA) using pour plate and spread plate techniques. Identification of fungal isolates were done by microscopic observation using methylene blue stain and compared to standard diagram of already identified fungi in fungi compendium.

Growth of fungi and Production of Cellulase

The fungal species were separately grown and tested for production of cellulase in submerged culture in a chemically defined media composed of KH_2PO_4 (1gl^{-1}), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5gl^{-1}), Yeast extract (1gl^{-1}), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.14gl^{-1}), thiamine (0.0025gl^{-1}), glucose (1%) (Vahidi *et al.*, 2004). The cultures were grown at 32°C for 21 days. Culture broths were sampled at different days during growth to determine enzyme- production by Carboxyl methyl cellulose (CMC) hydrolysis.

Effect of Carbon Source on Cellulase Production

One percent (1%) carboxyl methyl cellulose (CMC) was used to replace glucose as the carbon source in the chemically defined medium and the cultures were grown at 32°C for 21 days. Culture broths were sampled for enzyme production.

Enzyme Assay

Cellulase activity was assayed by the determination of reducing sugar released from carboxyl methyl cellulose (CMC) using the method of Reese and Mandels (1963). The assay medium was 0.55% CMC in 0.55M acetate buffer (pH 5.5) and 9ml of this were incubated with 1ml of the fungus filtrate for 1 hour at 32°C . The filtrate of the uninoculated control was also obtained and similarly assayed. The reducing sugar produced was assayed by dinitrosalicylic acid (DNSA) method using glucose as the sugar standard and a standard glucose graph was constructed. The amount of reducing sugar produced by one ml of fungus filtrate was calculated from this graph. Cellulolytic activity of the filtrate was then expressed in terms of the amount of total Reducing Sugar R.S. per ml.

RESULT

Eleven fungi species designated as FA, FB, FC, FD, FE, FF, FG, FH, FJ, and FK were isolated from the degrading corn cobs. Table 1 shows the morphological, growth characteristics and identification of the fungal isolates. The cellulase activity of the fungi when glucose was used as the carbon source is seen in figure 1. *Aspergillus niger* has the highest reducing sugar production of 0.82mg/ml followed by *Aspergillus oryzae* (0.75mg/ml). The lowest value of 0.01mg/ml was recorded for *Rhizopus oryzae*, *Mucor racemosus* and *Geotrichum candidum*. Figure 2 shows the cellulase activity of fungi when carboxyl methyl cellulose (CMC) was used as the carbon source. The highest reducing sugar value of 1.68mg/ml was produced by *Aspergillus niger* while the lowest value of 0.69mg/ml was produced by

Mucor racemosus.

Table 1: Morphological, growth characteristic and identification of fungal isolates

Isolate	Surface growth on laboratory media	Description of the characteristic feature of fungi	Growth rate	Reverse coloured or uncoloured	Identification
FA	White to grayish brown colonies about 1cm with tendency to collapse. Black	Colonies are raised, about 1-2cm high. Stolon hyaline sporangiophore arising directly from stolon or aerial lyphae. Sporangia black	Fast	Cream	Rhizopus oryzae
FB	Dirty green colonies	Colonies are raised conidiophore with large globose vesicle, metulae are inflated and club shaped and are borne on the	Fast	Cream	Aspergillus flavus
FC	White fluffy Cottonwool - like	Colonies are raised. Intact conidia heads, non septate hyphae	Fast	Cream	Mucor racemosus
FD	Chocolate brown colonies	Intact conidia heads conidiophore arising from thick walled conidia conidiophore hyaline to brown, mostly smooth walled conidia at maturity globose irregularly roughened with conspicuous ridges.	Fast	Brown	Aspergillus niger
FE	Grey colonies with white surrounding raised colonies	Densely branched conidiophore straight elevate to ellipsoids. Branches arising fairly low in the conidiophore. Conidia globose, conspicuously roughened	Slow	Cream	Penicillium atrovirens

Table 1 Continues

FF	Greyish green mycelium	Colonies fast growing, reaching 5-5cm dia after 10 days conidia sub globose or ellipsoidal penicilli two-three stage branched with numerous usually somewhat appressed metulae	Fast	Cream	Penicillium expansum
FO	Light brown powdery mycelium white hyphae	Colonies spreading broadly with abundant aerial mycelium late became bluff from production of rough walled sterile sepha.	Fast	Light brown	Botryotrichum piluliferum
FH	Bluish green mycelium	Large globose conidiophore are arising from long broad thickened foot cell	Fast		Aspergillus oryzae
FI	White fluffy Mycelium	Fast growing white colonies white butyrous and membranous advancing hyphae dichotomously branched conidia chain mostly aerial and erect.	Fast	^ - "	Geotrichum candidum
FJ	Bluish green mycelium	Conidia globose to ellipsoidal smooth walled colonies blue, grey or grey green, velvety reverse, intensely yellow	Slow	Yellow	Penicillium chrysogenum
FK	Grey colonies	Brownish grey with much aerial mycelium and rather few conidiophore, reverse yellow conidiophore arising from creeping aerial hyphae short, mostly monoverticillate, few small phialides with thin conidia bearing necks, conidia in short chains globose and conspicuously roughened.	Slow	Yellow	Penicillium restrictum

DISCUSSION

Eleven fungal species were isolated from the degrading maize cobs which include *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus oryzae*, *Rhizopus oryzae*, *Mucor racemosus*, *Botryotrichum piluliferum* and *Geotichum candidum*; others are *Penicillium expansum*, *Penicillium atrovenetum*, *Penicillium chrysogenum* and *Penicillium restrictum* (Table 1). All the fungal isolates have their highest production of reducing sugar on day 7 when glucose was used as the carbon source (figure 1). While the highest production of reducing sugar when carboxyl methyl cellulose was the carbon source for growth was day 14. This shows that carbon source affect enzyme production. This was also observed by Milala *et al.* (2005) where the time course for enzyme production by *Aspergillus niger* differ depending on the substrate differs; guinea corn day 5, maize day 3, millet day 4, and rice day 3.

The longer day required by CMC based media may be due to the lower rate of growth by the organism in the media compared to that of glucose. Industrially useful enzymes either associated with the microbial cells or exoenzymes are often synthesized by microorganism during balanced growth (Prescott *et al.*, 2008).

A high reducing sugar production of 1.68 mg/ml was obtained for *Aspergillus niger* when CMC was the carbon source compared with the value 0.82 mg/ml when glucose was the carbon source. Immanuel *et al.* (2007) also obtained a higher level of cellulase enzyme production from sawdust than coir ret. The amount of

cellulase produced varied because of the influence of carbon source and growth of cellulolytic organism (Mandels and Reese, 1985). All species of *Aspergillus* and most of the *Penicillium* species has very high reducing sugar production; A niger (1.68 sugar); A flavus (1.24mg/ml); A oryzae (1.08mg/ml); P expansion (1.60mg/ml); P atrovenetum (1.43mg/ml). Fungal of the genera *Aspergillus* and *Penicillium* are good cellulase producer (Immanuel, 2005; Milala *et al.*, 2005; Hoffman and Wood, 1985).

Mucor racemosus had the lowest reducing sugar production, both CMC and glucose as carbon source (0.6mg/ml & 0.01mg/ml respectively). Several species of *Mucor* which include *Mucor pusillus* and *Mucor michei* are not cellulolytic (Tausey, 1971).

Screening fungi associated with corn- cob degradation has shown that not all the fungi species are cellulolytic; however various species of *Aspergillus* and *Penicillium* will be useful in further degradation of corn-cob into more nutritive product for animal feed.

CONCLUSION

The cellulase activity of eleven fungal isolate has been studied. The genera *Aspergillus* and *Penicillium* are good cellulase producers but the level of production is based on the carbon source used in the medium. Fungi are good cellulase producers and will be useful in the conversion of cellulose in agricultural waste into simple sugar, which will make it easily digestible in animal feed.

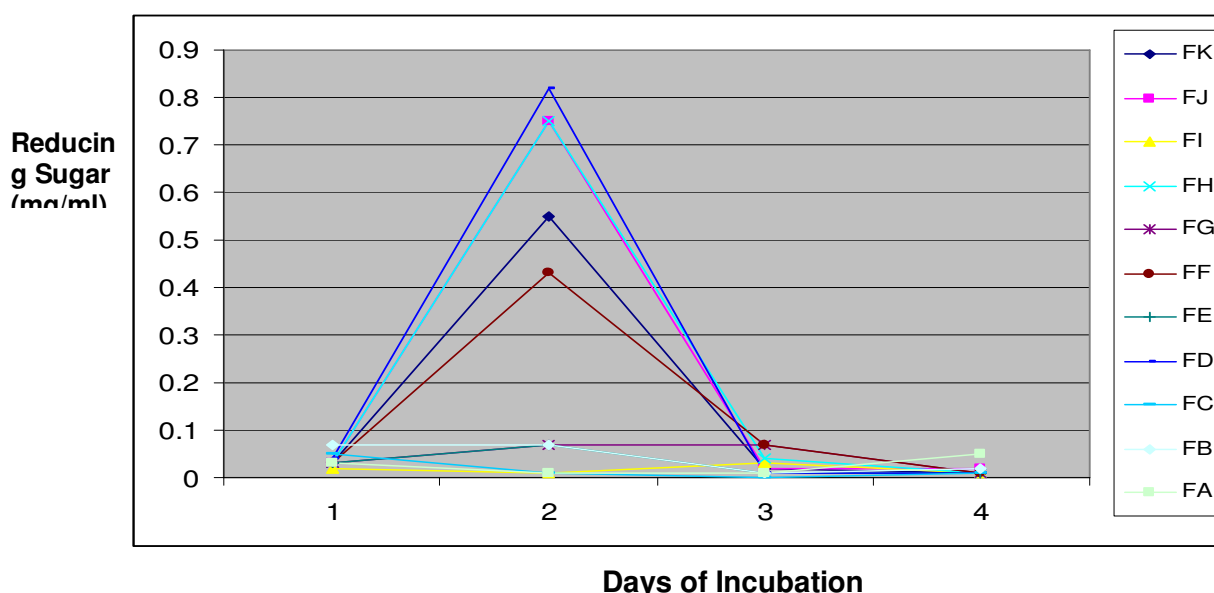


Figure 1: Cellulase activity of fungi at different days of incubation using glucose as carbon source

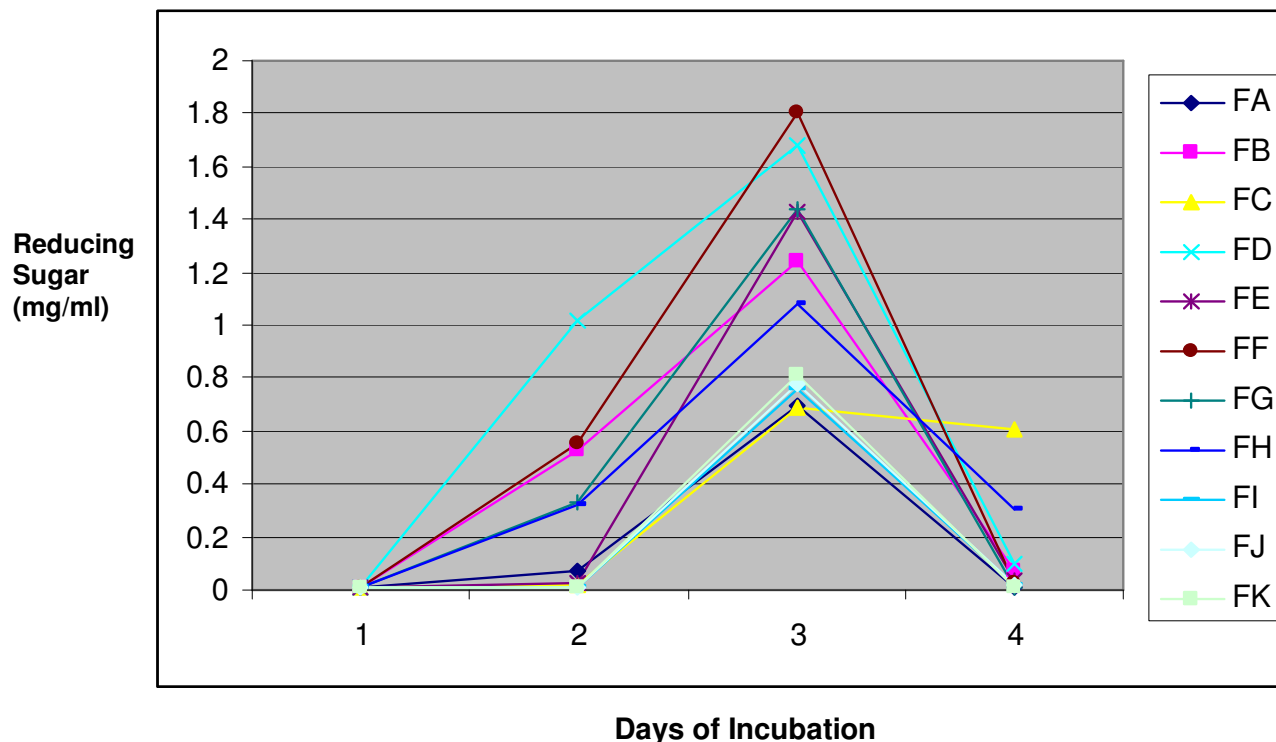


Figure 2: Cellulase activity of fungi at different days of incubation with CMC as carbon source

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- NOTE: The following authors were cited in the content but not listed as reference:
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