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**CELLULOLYTIC *ASPERGILLUS FLAVUS* ISOLATED FROM ENVIRONMENTAL
SAMPLE IN GHANA**

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ABSTRACT

Cellulases are enzymes of great industrial value especially in the hydrolysis of the abundant lignocellulosic materials in Ghana into cheap bioethanol. Forty fungal species isolated from soil, corn dough and cassava flour were screened for cellulase activity. The screening was carried out on modified Mandel's agar media and their radial clearing zone diameters (RCZD) were recorded as indices of their cellulose activities. All the isolates screened exhibited cellulolytic activities and there was significant difference ($p < 0.05$) in their activities. Isolates GN020, YW027, WE023, GN034, BN037, BL038, BL029, BN025, WE024, YW030, GN039, BL040, WE026 and BL022 recorded RCZD of 81.5 ± 4.24 mm, 76 ± 1.41 mm, 74.75 ± 1.06 mm, 74.75 ± 9.55 mm, 74.75 ± 2.48 mm, 74.75 ± 1.06 mm, 74 ± 1.41 mm, 73.5 ± 1.41 mm, 73 ± 3.56 mm, 73 ± 2.83 mm, 70 ± 1.41 mm, 69.75 ± 4.60 mm, 69.75 ± 4.24 mm and 68.75 ± 3.89 mm respectively after 144 hours of incubation on Mandel's agar medium, and they were significantly different from the rest. Total cellulase activities of the GN020, YW027 and WE023 which showed highest activity upon screening on modified Mandel's agar plates were determined by Filter paper assay. GN020, YW027, WE023 recorded 0.495 ± 0.06 , 0.397 ± 0.07 and 0.384 ± 0.09 FPU/ml. FPA of GN020 was significantly higher ($p < 0.05$) whilst no significant difference was recorded between YW027 and WE023 (lsd = 0.0702). Morphological identification of macroscopic and

microscopic features of these isolates with significantly high cellulose activities revealed belong to genera *Aspergillus*(93.3%) and *Trichoderma* (6.7%). These isolates have the potential of producing low-cost cellulase for bioethanol production.

Keywords: Cellulase, Filter Paper Activity, Bioethanol, Fungi

INTRODUCTION

Cellulases are enzymes which hydrolyze the β -1, 4- glycosidic linkage of cellulose [1]. Complete enzymatic hydrolysis of cellulosic materials require the three major types of cellulase namely endoglucanase, $1,4\text{-}\beta$ -D-glucan-4-glucanohydrolase (EC 3.2.1.4), exocellobiohydrolase 1, $4\text{-}\beta$ -D-glucanglucohydrolase (EC 3.2.1.74) and β -glucosidase (D-glucosideglucohydrolase; (EC 3.2.1.21). Cellulases are employed in animal feed, food, textiles, detergents paper and bioethanol industries [2, 3, 4, 5, 6, 7]. With the dwindling fossil fuel reserves, there is a rising need to find alternative sources of energy. This has led to a renewed interest in the bioconversion of lignocellulosic biomass using cellulases and other enzymes to bioethanol [2].

In Ghana, lignocellulosic materials such as wood, grass, agricultural residues, forestry wastes, and solid municipal wastes are abundant and are promising feedstock for the production of bioethanol. However, technical challenges still hamper the economic feasibility of lignocellulosic bioethanol production. The cost of cellulases represents a significant part in the overall production costs [8].

A number of fungi and bacteria capable of utilizing cellulose as a carbon source have been identified [5]. Screening for efficient cellulase-producing microorganisms is important in reducing cost of cellulases. Forty fungal isolates were previously isolated from environmental samples: soil, corn dough and cassava flour. The objectives of this work were to screen these isolates for cellulolytic activities and identify the potential cellulolytic isolates.

MATERIALS AND METHODS

Screening for Cellulase Activity

Potato Dextrose Agar (PDA) and modified Mandel's media were used in the work. The following chemicals in g/L (urea, 0.3; $(\text{NH}_4)_2\text{SO}_4$ 1.4; KH_2PO_4 , 2; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.4; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3; peptone 1.0; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.005; $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0016; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0014, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.002, Tween 80, 2 ml and Avicel cellulose, 10) were dissolved in 1 L distilled water to constitute the modified Mandel's broth media. Mandel's agar was prepared by adding 20 g/l of agar to the broth.

The stock cultures of the isolates were plated on modified PDA plates and incubated at 25 °C. A cork borer with diameter of 4 mm was used to transfer a 7 day old pure culture of each of the 40 isolates and placed at the middle of replicate modified Mandel's agar plate and incubated at 25°C. After 144 hours of incubation, the radial clearing zone diameters (RCZD) formed around colonies were measured and taken to represent the cellulolytic activities of the fungal isolates.

Identification of the Isolates

A cork borer with diameter of 4 mm was used to transfer a 7 day old pure culture of each isolates and placed at the middle of PDA plate and incubated at 25°C. Colony morphological features such as texture, pigmentation, elevation, margin and form as well as microscopic features such as hyphae, conidiophores, conidial heads, conidia or spores and other fruiting bodies were observed for the isolates identification.

Slide cultures were prepared and incubated at 25 °C for 3 days for microscopic identification after which they stained with lactophenol cotton blue and observed under light microscope (Leica ZOOM 2000). [10, 11] and other aids such as [12] and [13] were used as guide in the identification.

Production of Crude Cellulase Extract

Ten milliliters of sterile distilled water was poured onto a 7 days old sporulating slants cultures of GN020, YW027 and WE023 respectively. The spores were obtained by scraping the surface with an inoculating loop. It was then shaken to obtain homogenous spores suspension under aseptic conditions. Haemocytometer (Improved Nubauer Haemocytometer) was used to determine the spore concentration of the suspension. Modified Mandel's broth media containing 10 g/L of Avicel cellulose was used as fermentation media. Fifty milliliters of fermentation medium was dispensed into 250 ml conical flask and sterilized at 121°C for 15 minutes in an autoclave and allowed to cool to room temperature. Inoculum of 1.2×10^7 spores were used to inoculate each flask and plugged with cotton. The flasks were incubated at 30 °C and agitated for 5 minutes at 24 hours interval. Each treatment was replicated.

The broths were centrifuged at 10 000 rpm for 10 minutes at -4 °C in centrifuge (P. Selecta Medifriger) after 168 hours of incubation. The supernatant were kept in sterile centrifuge tubes and stored at -20°C for filter paper assay.

Determination of Total Cellulase Activities by Filter Paper Assay

Filter paper assay as described by [14] and [15] were used to determine the total cellulase activities of the isolates. The absorbances of the analytes were determined at 540 nm using spectrophotometer (Jenway 6405 UV/Vis). One filter paper unit (FPU) is defined as mg of reducing sugar liberated in 60 minutes at assay conditions of pH 4.8 and 50 °C.

RESULTS AND DISCUSSION

Cellulases have numerous economic values especially in the hydrolysis of the abundant lignocellulosic materials in Ghana into cheap bioethanol. This study was undertaken to screen and identify cellulase-producing fungi isolated from environmental samples namely soil, corn dough and cassava flour. Forty isolates were screened to determine their cellulolytic ability by plating them on the modified Mandel's agar plates which contained Avicel cellulose as the only source of carbon and the radial clearing zone diameters (RCZD) were used as measure of cellulase activities. **Figure 1** shows a graph of RCZD of the isolates after 144 hours of incubation. All the isolates exhibited some cellulolytic activity but fourteen isolates recorded significantly ($p < 0.05$) high activities. These isolates were GN020, YW027, WE023, GN034, BN037, BL038, BL029, BN025, WE024, YW030, GN039,

BL040, WE026 and BL022 with RCZD of 81.5 ± 4.24 mm, 76 ± 1.41 mm, 74.75 ± 1.06 mm, 74.75 ± 9.55 mm, 74.75 ± 2.48 mm, 74.75 ± 1.06 mm, 74 ± 1.41 mm, 73.5 ± 1.41 mm, 73 ± 3.56 mm, 73 ± 2.83 mm, 70 ± 1.41 mm, 69.75 ± 4.60 mm, 69.75 ± 4.24 mm and 68.75 ± 3.89 mm respectively. The intrinsic ability of these isolates to exude extracellular cellulase with all the three components to hydrolyze the cellulose might account for their significantly high RCZD.

The 14 isolates were cultured on PDA pH 5.6 at 25 °C and identified by their colony morphologies as well as their microscopic characteristics with the aid of manual by [10, 11] and literature at [12] and [13]. Colony morphological features such as texture, pigmentation, elevation, margin and form were observed. Slide cultures stained with lactophenol cotton blue of the isolates were observed under microscope to characterize microscopic structures such as conidia, hyphae and fruiting bodies. **Table 1** shows the observed characteristics. These were identified as *Aspergillus flavus* (GN020), *A. terreus* (YW027), *A. niger* (WE023), *A. flavus* (GN034), *A. terreus* (BN037), *A. niger* (BL038), *Trichoderma sp.* (BL029), *A. niger* (BN025), *A. terreus* (WE024), *A. terreus* (YW030), *A. flavus* (GN039), *A. terreus* (BL040), *A. terreus* (WE026), *A. flavus*

(BL022). *Aspergillus* and *Trichoderma* species constituted 93.3 % and 6.7 % respectively of the isolates with significantly high cellulase activity. *Aspergillus* and *Trichoderma* have been reported to exhibit significant cellulase activity [16]. [17] reported that *Aspergillus spp.* were the abundant species isolated from environmental samples such as soil, air and infected wheat plants. Cellulases produced by *Aspergillus* species have received extensive studies by several researchers [18]. *Trichoderma spp.* have also been reported to possess strong cellulose-degrading activity [19, 20].

The total cellulase activities of the GN020, YW027 and WE023 which showed highest

activity upon screening on modified Mandel's agar plates were determined by Filter paper assay. Supernatants of the fermentation broth after 168 hours of incubation were used as crude enzyme extract. The mean filter paper activity, FPA of the isolates *Aspergillus flavus* (GN020), *A. terreus* (YW027), *A. niger* (WE023) recorded 0.495 ± 0.06 , 0.397 ± 0.07 and 0.384 ± 0.09 FPU/ml. FPA of GN020 was significantly higher ($p < 0.05$) whilst no significant difference was recorded between YW027 and WE023. This is comparable to similar work. [12] reported the highest cellulase activities of 0.163, 0.3 and 0.33 for *A. flavus*, *A. terreus* and *A. niger* respectively.

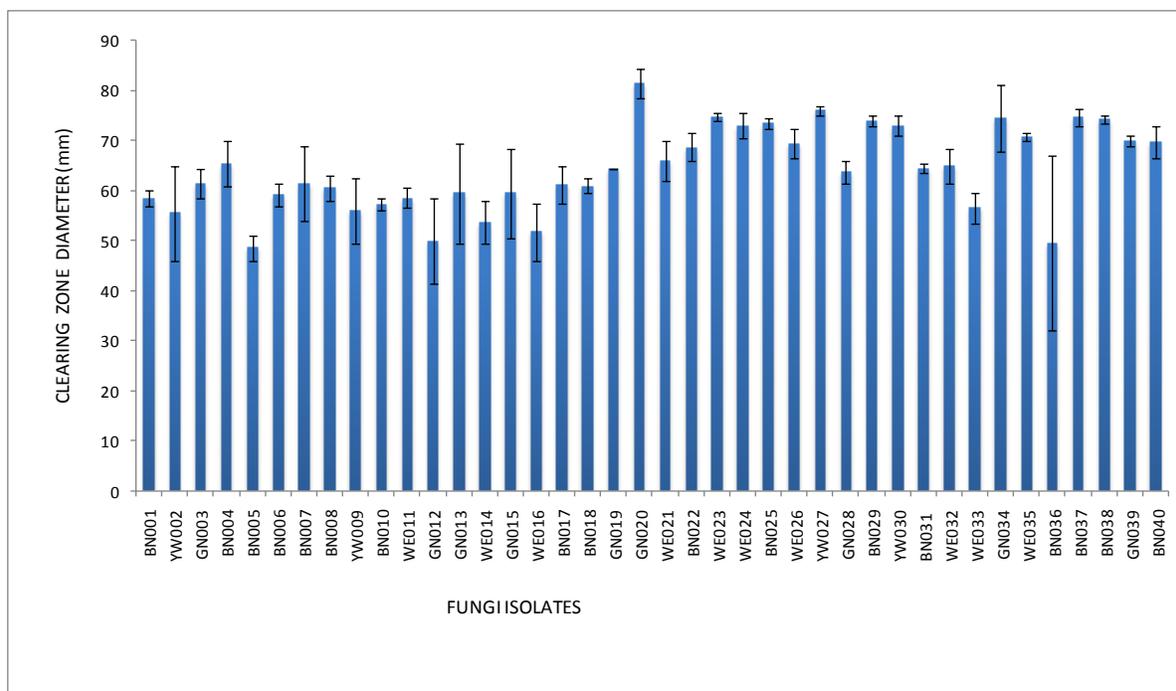


Figure 1: Graph of Radial Clearing Zone Diameter (RCZD) of the Isolates After 144 Hours of Incubation

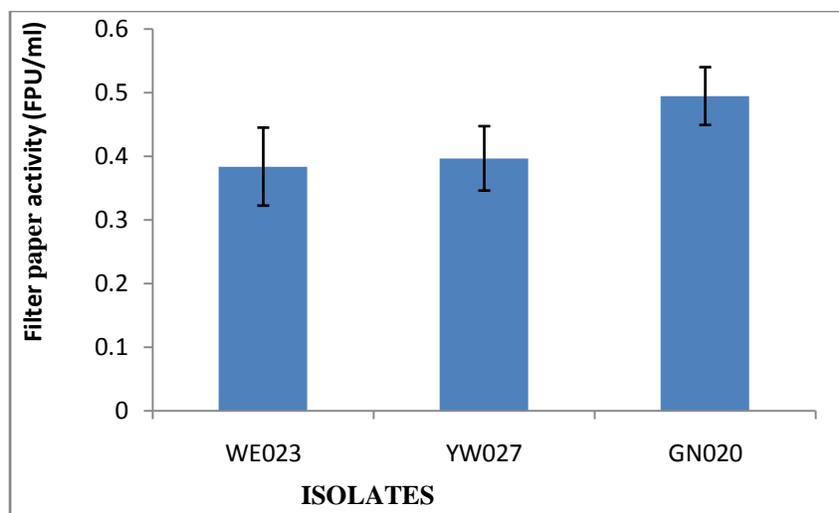


Figure 2: A Graph of Mean FPA of Crude Enzyme Extract From the Isolates

CONCLUSION

It could therefore be concluded that 14 of the isolates screened possessed significant cellulase activities. *Aspergillus* and *Trichoderma* species constituted 93.3 % and 6.7 % respectively of the 14 isolates with significantly high cellulase activity. *Aspergillus flavus* (GN020) with significantly high total cellulase activity of 0.495 ± 0.06 FPU/ml is a potential candidate for improvement in cellulosic bioethanol production in Ghana.

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