DETERMINATION OF CELLULOLYTIC POTENTIALS OF ASPERGILLUS SPECIES ISOLATED FROM CENTRAL WASTE DUMP SITE OF NILE UNIVERSITY OF NIGERIA

Ezeagu G.G., Sanusi U.R., Wali U.M. & Mohammed S.S.D.

Department of Biology, Microbiology and Biotechnology, Nile University of Nigeria, Abuja, Nigeria

*Corresponding Author Email Address: gloria.ezeagu@nileuniversity.edu.ng

ABSTRACT
A large number of microorganisms are capable of degrading cellulose but only a few of these microorganisms produce significant quantities of enzymes capable of completely hydrolyzing cellulose. Fungi are the main cellulase-producing microorganisms. This study was aimed to determine the cellulolytic potentials of Aspergillus species isolated from the central waste dump site of Nile University of Nigeria. In this study, fungal species were isolated from soil samples obtained from waste dump site using pour plate technique. The isolates were characterized using cultural and morphological features as well as microscopic examination. Aspergillus flavus, Aspergillus niger, and Aspergillus terreus, which were isolated were further screened on carboxymethylcellulose agar for their ability to degrade cellulose. Screening of fungal isolates was performed by plate method. Cellulolytic fungi were evaluated after 5 days for the production of cellulolytic enzymes by staining with 1% Congo red. The diameter of clear zone on fungal plates, gave an approximate indication of cellulase activities. Aspergillus niger had a zone of clearing of 25.50 mm while Aspergillus flavus had 18.50 mm. Aspergillus terreus did not show any cellulolytic activity. Aspergillus niger had the highest occurrence rate of 50%. Aspergillus flavus and Aspergillus terreus both had 25% occurrence rate.

Keywords: Cellulose, Aspergillus terreus, Congo red, enzymes, hydrolysis

INTRODUCTION
Background of the Study
Fungi are one of the dominant groups of microorganisms present in soil, which strongly influence ecosystem structure and functioning and thus play a key role in many ecological services (Frac et al., 2018). At the ecosystem scale, extracellular enzyme activity is influenced by organic matter abundance and composition (Sinsabaugh et al., 2008).

Recently, increased attention has been paid towards the use of agricultural wastes for the large-scale production of various industrial enzymes using microorganisms. The potential of using microorganisms as biological sources of industrially economic enzymes has stimulated interest in the exploitation of extracellular enzymatic activity in several microorganisms (Pandey et al., 2000). Also, the introduction of microbial enzymes as an alternative to harsh chemical technologies has led to intensive exploration of natural microbial biodiversity to discover microbial enzymes with possible application in waste recycling under appropriate conditions (Lynd et al., 2002, Okpara, 2022).

Almost all fungi of the genus Aspergillus are capable of producing extracellular enzymes (cellulase) responsible for degradation of cellulose present in refuse dumps. Aspergillus species are capable of degrading cellulose and synthesize large quantities of extracellular cellulases that are more efficient in depolymerizing the cellulose substrate. Cellulolytic enzymes play an important role in natural biodegradation processes in which plant ligno-cellulosic materials are effectively degraded by cellulolytic fungi (Bakare et al., 2019). This enzyme (cellulase) is known as the key enzyme for the conversion of cellulotic materials into simple sugars which can serve as feed-stock for the production of different chemicals and fuels via anaerobic fermentation. A large volume of cellulase enzyme is required for the industrial processes to break down cellulose (Siva et al., 2022). Cellulases have wide range of applications including production of chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, and pulp and paper. However, from a commercial point of view, the cost of cellulose-degrading enzymes is a major barrier to the economical production of biochemicals and second generation biofuels (Patyshakuliyeva et al., 2016). The availability of huge amounts of cellulosic materials in Nigeria underlines the need to exploit the potentials of organisms which utilize lignocellulosic materials for their carbon and energy sources or the conversion of these wastes into products that are beneficial to mankind (Sinsabaugh et al., 2008).

This research project focused on the determination of cellulolytic potentials of Aspergillus species isolated from central waste dump site of Nile university of Nigeria.

MATERIALS AND METHODS
Collection of Samples
Soil samples were taken with sterilized spatula from topsoil at different areas of the central waste dump site of Nile University of Nigeria into sterile polythene bag. They were transported to Microbiology laboratory of the University for further study.

Isolation of Fungi
One gram of the soil sample was transferred to 9mL sterile distilled water in test tube. It was shaken vigorously at constant speed for 15min. This was the stock culture used for the investigations. The soil suspension was then subjected to serial dilutions up to sixfold dilution. PDA (potato dextrose agar) media was prepared with the addition of 50µg/L of erythromycin and 1mL of the suspension was poured into sterile petri plates in duplicates, using pour plate technique and an uninoculated plate served as the control. The plates were incubated for 5 days at 30°C. After the incubation, the well-grown colonies of fungi were picked up and subcultured on sterile potato dextrose agar (PDA) plates and pure cultures were obtained.
Identification of Fungi
The fungal isolates were identified using cultural and morphological features such as the growth pattern, conidial morphology, colony colour/pigmentation in accordance with Samson and Varga, (2007). The microscopic identification was carried out following standard laboratory techniques using lactophenol cotton blue as described by Montanari et al. (2012). A small portion of the aerial mycelia from the fungi cultures was removed with the aid of a mounting needle and placed in a drop of lactophenol cotton blue stain on a clean slide. The mycelium was well spread on the slide with the needle. Carefully, the stain was covered with a clean sterile coverslip and was viewed under the light microscope with x10 and x40 objective lenses. The morphological characteristics and appearance of the fungal organisms seen were identified in accordance with (Chinedu et al., 2010).

Primary Screening for Cellulose utilization
The isolates were grown on basal salt media supplemented with 5% carboxymethylcellulose (CMC) according to (Guatam et al., 2010). The pure cultures were inoculated in the centre and incubated at 30 °C until substantial growths were observed. The Petri plates were then flooded with Congo red solution (0.1%), and after 5min, the Congo red solution was discarded, and the plates were washed with 1M NaCl solution, and were allowed to stand for 20 minutes. The clear zone around the colony indicates cellulose utilization. Cellulolytic fungi were screened based on their ability to hydrolyze cellulose by forming diameter zone of clearance around the fungal colony.

RESULTS
IDENTIFICATION OF FUNGAL SPECIES
The macroscopic and microscopic characteristics (Table 1) of Aspergillus species isolated from the waste dump soil are indicative of the identities of Aspergillus flavus, A. terreus and A. niger (Plates A, B and C).

<table>
<thead>
<tr>
<th>Macroscopic Appearance</th>
<th>Microscopic Appearance</th>
<th>Fungi identified</th>
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<tbody>
<tr>
<td>Powdery masses of yellowish-green fluffy mycelium/ spores on the upper surface and reddish-gold on the lower surface</td>
<td>Septate hyphae with filamentous structure. The conidiophores appeared as rough.</td>
<td>Aspergillus flavus</td>
</tr>
<tr>
<td>Aspergillus terreus is a rapid grower producing a colony with a cinnamon brown surface.</td>
<td>Septate hyphae often bear solitary conidia, or aleuroconidia. Short conidiophores are smooth. Biseriate phialides form on the upper half of vesicles and bear chains of round conidia.</td>
<td>Aspergillus terreus</td>
</tr>
<tr>
<td>Colonies with loose white mycelium rapidly becoming dark brown to black on the development of conidia</td>
<td>The conidiospores are large with Aspergillus niger septate hyphae.</td>
<td>Aspergillus niger</td>
</tr>
</tbody>
</table>

Table 1: Morphological and cultural characteristics of fungal isolates

Determination of the Frequency of Occurrence
The frequency of occurrence of the fungal isolates was shown in Figure 1, where A. niger had 50% occurrence, while A. flavus and A. terreus had 25% occurrence each.

Cellulolytic Activity of fungal isolates
Among the three fungal isolates, two isolates were identified as cellulase producers. A zone of clearing around the colonies was used as an indication for cellulose utilization. Aspergillus niger had a zone of clearing of 25 mm, while Aspergillus flavus had 18 mm. Aspergillus terreus however, did not show any cellulolytic activity (Table 3).

Table 3: The Diameter of zone of clearing on cellulose Congo-red agar for cellulose-utilizing fungi (Mean ± SD)

<table>
<thead>
<tr>
<th>Fungal isolates</th>
<th>Diameter of zone of clearing (mm)</th>
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<tbody>
<tr>
<td>Aspergillus flavus</td>
<td>18.50±0.50</td>
</tr>
<tr>
<td>Aspergillus Niger</td>
<td>25.50±0.50</td>
</tr>
<tr>
<td>Aspergillus terreus</td>
<td>0.00</td>
</tr>
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DISCUSSION
Fungi are known agents of decomposition of organic matter in general and of cellulosic substrates in particular (Lynd et al., 2012). The isolation of Aspergillus species from waste dump site has been
reported by many researchers and their abundance could be because the dumpsite accommodates numerous and diverse microorganisms (Sangale et al., 2019; Ogbuji et al., 2021). Reanprayoon and Pathomsiriwong (2012) established that members of the genera Aspergillus are dominant fungi in tropical soils and are ubiquitous in nature. Obire et al. (2012) also reported to have isolated A. niger and A. flavus from waste dump while isolation of Aspergillus terreus was reported by Gautam et al. (2012).

Aspergillus niger had the highest frequency of occurrence than others in the present study as also reported by Oshoma et al. (2017) and Bala et al. (2020), and this might be due to its unique characteristics. Apart from being ubiquitous in nature, A. niger also has extensive metabolic diversity, including its non- fastidious nutritional requirements (Bakare et al., 2019; Zhao et al., 2020). The cellulose utilization ability of A. niger was reported by Opara (2012), who stated that cellulases for food industry application can be produced from fungi such as Aspergillus niger. This result agrees with the work of Panda et al. (2012), who reported Aspergillus niger with the highest cellulolytic activity followed by Aspergillus flavus. The inability of Aspergillus terreus to show any cellulolytic activity in the present study contrasts with that of Ogbonna et al. (2015), who reported that Aspergillus terreus was found to possess cellulolytic activity. The result of this study could be that although some strains of A. terreus possess genome known to contain genes with conserved domains that encode several putative cellulose-degrading enzymes, the expression of endoglucanases and cellobiohydrolases is still very poor from some Aspergillus species (Kumar & Parikh, 2015). The present study indicates that the isolates, Aspergillus niger and Aspergillus flavus are capable of inhabiting various cellulolytic wastes, producing extracellular cellulases.

**Conclusion**

The results and discussions of this study affirmed that the prevalence of Aspergillus species in decaying waste dump site of Nile University of Nigeria may be attributed to their cellulolytic ability and Aspergillus niger has the highest cellulolytic activity. This could mean that it plays greater roles in decomposing the wastes in the dump site than the other Aspergillus species.

**REFERENCES**


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