Science World Journal Vol. 18(No 4) 2023
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

DOI: https://dx.doi.org/10.4314/swj.v18i4.13

Full Length Research Article

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

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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 cellulosic 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.

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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.

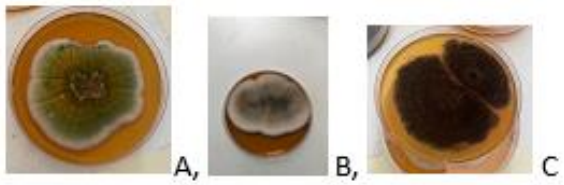
RESULTS

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) respectively.

Table 1: Morphological and cultural characteristics of fungal isolates

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



Plates: A – *Aspergillus flavus*
B – *Aspergillus terreus*
C - *Aspergillus niger*

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.

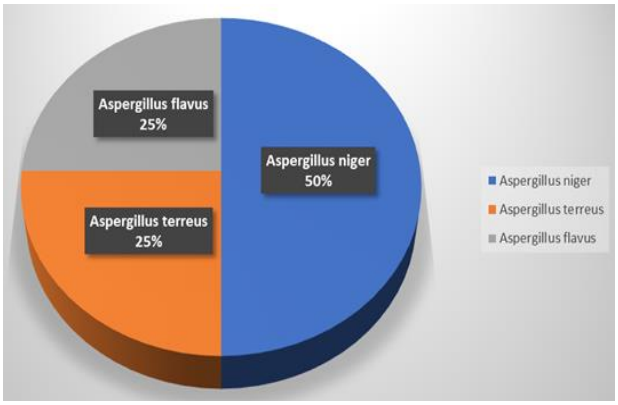


Figure 1: Frequency of Occurrence of Fungal isolates

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)

Fungal isolates	Diameter of zone of clearing (mm)
Aspergillus flavus	18.50±0.50
Aspergillus Niger	25.50±0.50
Aspergillus terreus	0.00

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 Okpara (2022), 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.

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