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Full Length Research Article

CELLULOLYTIC POTENTIALS OF ASPERGILLUS ORYZAE AND STREPTOMYCES GRISEUS ISOLATED FROM WASTE DUMP SOIL IN NILE UNIVERSITY OF NIGERIA, ABUJA

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ABSTRACT
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. The aim of this research is to assess the cellulose degrading potentials of two microorganisms, *Aspergillus oryzae* and *Streptomyces griseus* using cellulose Congo red agar media. Soil sample collected from waste dump was serially diluted and inoculated in starch casein agar and SDA to isolate *S. griseus* and *A. oryzae* respectively. To assess their potentials to utilize cellulose, each of the two microorganisms was inoculated on cellulose Congo-red media and incubated at 30 ºC for 7days. A zone of clearing around the colonies after incubation confirms the secretion of extracellular cellulase, and was used as an indication for cellulose utilization. The zone of clearing was measured with a meter rule. In the results obtained, both microorganisms demonstrated cellulose utilization ability with *Aspergillus oryzae* showing a zone of clearing of 30.50 ± 0.50 mm while *Streptomyces griseus* showed a wider zone of clearing of 60.00 ± 1.00 mm. The results indicate that both microorganisms can be potent producers of the enzyme cellulase, with *Streptomyces griseus* having a higher cellulase-producing ability.

Keywords: Cellulose, Congo-red, waste-dump, *Streptomyces griseus*

INTRODUCTION

Background of study

Cellulose is the principal component of plant cell walls and the most abundant organic compound in terrestrial ecosystems (Book *et al*., 2016). It is insoluble in water and exists as crystals. Its degradation is a critical process especially in soil ecosystems, playing a vital role in nutrient cycling and organic matter decomposition (Datta, 2024). A combination of a chemical (or thermochemical) and biochemical processes is used to degrade such polysaccharide biomass on an industrial scale, but the processes have many problems as special equipment are needed because of issues arising from acid or base corrosion, high temperature, desalting from neutralized solution, and the difficulty in the controlling of the reaction. The biochemical aspect of the process is a more environmental-friendly and mild method compared to the chemical or thermochemical process, but does not produce enough yield (Sato *et al*., 2020), hence, the need for microbial activities. Also, regarding the production of various fuels and chemicals from biomass, especially cellulosic materials, rather than fossil fuels, cellulose is considered the most suitable feedstock for the production of biofuels and renewable feedstock chemicals, because 10^{11} - 10^{12} tons of cellulose are produced each year (Wakai *et al*., 2019).

The complete solubilization of cellulose and hemicellulose into simple sugar monomers are facilitated by some complex enzymes known as cellulases (Legodi *et al*., 2023). Some microorganisms can produce these cellulolytic enzymes, which play important roles in natural biodegradation processes in which plant lignocellulosic materials are effectively degraded (Bakare *et al*., 2019). Begum & Alimon, (2020) reported that the enzymes are widely used for the extraction of valuable compounds from plant cells, improving nutritional values of animal feed and in preparing plant protoplasts for genetic research. The production of cellulase has been reported from a wide variety of bacteria and fungi. However, filamentous fungi are preferred for commercial enzyme production, because the level of the enzymes produced by these cultures is higher than those obtained from yeast and bacteria (Mrudula & Murugammal, 2011).

Almost all fungi of the genus *Aspergillus* are capable of producing extracellular enzymes, cellulase. Ezeagu *et al.*, (2023), reported that *Aspergillus* species are capable of degrading cellulose and synthesizing large quantities of extracellular cellulases that are more efficient in depolymerizing the cellulose substrate.
Aspergillus oryzae, a member of the *Aspergillus* family and considered as GRAS (generally recognized as safe), has been applied for years in the making of “tuong ban” (or Vietnamese fermented soybean paste) in Vietnam or miso in Japan. Their ability to produce cellulase enzyme at appropriate conditions and substrates was reported by Nguyen *et al*., (2022). However, there are limited studies focused on their ability to produce cellulase using different cultured media as compared to many other species of *Aspergillus.*

Streptomyces on the other hand, are ubiquitous in a variety of ecological niches but are commonly found in soil and are ecologically significant due to their role in decomposing cellulose (Cuebas-Irizarry & Grunden, 2023). The genus is arguably most known for secondary metabolite production (Alam *et al*., 2022) and are well studied as sources for antibiotic production in the pharmaceutical industry. However, studies of complete *Streptomyces* genome sequences have revealed a huge number of genes encoding enzymes for the utilization of divergent nutrient sources, such as cellulose, chitin, xylan, and their hydrolysis products (Marushima *et al*., 2009). Although a large number of *Streptomyces* species can grow on plant biomass, only a small percentage (14%) have been shown to efficiently degrade crystalline cellulose (Takasuka *et al.,* 2013). Datta, 2024, reported

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eight species of *Streptomyces* which have the ability to secret cellulase enzyme but *S. griseus* was not included in the number. This can be taken as an indication that *S. griseus* has not received a very wide report as a cellulose degrader when compared to other *Streptomyces* species. This study is therefore aimed at assessing the cellulose degrading potentials of two microorganisms, *Aspergillus oryzae* and *Streptomyces griseus* using cellulose Congo-red agar media.

MATERIALS AND METHODS

Collection of Samples

Soil samples were collected at 10 cm depth from different points at the central waste dump site in Nile University of Nigeria, Abuja, into clean polythene bags and transported to Microbiology laboratory for the isolation of microorganisms.

Isolation of Microorganisms

The soil sample was serially diluted and inoculated on Starch casein agar (SCA), containing (Starch 10.0 g, Casein 0.3 g, KNO3 2.0 g, NaCl 2.0 g, K2HPO4 2.0 g, MgSO4 7H2O 0.05 g, CaSO4 0.02 g, FeSO4 0.01 g, Agar 20.0 g, distilled water 1L) and was incubated at 30 ºC for 5 days to isolate *Streptomyces*. The same inoculation was made on Sabouraud dextrose agar, (SDA) and was incubated at 30ºC for 5days to isolate *Aspergillus* species according to Gautam & Bhadauria, (2012) and Singh *et al*. (2014).

Characterization and Identification of Microbial Isolates

The bacterial isolate was characterized based on cultural and morphological characteristics as well as biochemical tests according to methods outlined by Baker & Breach, (1980). *Aspergillus* species was characterized based on the colour of aerial and substrate mycelium, nature of hyphae, shape and kind of asexual spore, appearance and characteristics of spore head. A wet mount was prepared using Lactophenol cotton blue and observed under the microscope. The identification was carried out using the scheme of Samson & Varga, (2007).

Screening of Microbial Isolates for Cellulose Utilization

Microbial isolates were inoculated in duplicates on cellulose Congo-red agar media with the following composition: KH_2PO_4 0.5g, $MgSO_4$ 0.25g, cellulose powder 2g, agar 15g, Congo-red 0.2g, Gelatin 2g, distilled water 1L and at a pH 7.0. The plates were incubated at 30 ºC for 7 days. The colonies showing discolouration of Congo-red after incubation were regarded as positive cellulose-degradation (Gupta *et al.,* 2012). The areas with decolouration were measured for each duplicate plates, using transparent ruler. The mean values were taken and the deviation from the mean was also established.

RESULTS

Streptomyces griseus isolated in this study showed gray white coloured colonies on SCA (Plate I). It appeared Gram positive, and was catalase and sucrose positive (Table 1). *Aspergillus oryzae* showed yellowish colours of aerial and substrate hyphae on SDA (Plate II). The macroscopic and microscopic characteristics of the isolate (Table 2), when compared with a reference *A. oryzae* used by Elbashiti *et al.,* (2010) was identical.

Plate I: *Streptomyces griseus* colonies on SCA

Plate II: Growths of *Aspergillus oryzae* on SDA

Table 1: Morphological and biochemical characteristics of bacteria isolated from waste dump

Isolate	SS-1
Colony characteristics	Chalky white
Cell shape	Rod
Gram reaction	+
Mobility	-
Lactose	-
Fructose	+
Sucrose	+
Glucose	+
Maltose	+
Arabinose	+
Galactose	+
Mannitol	-
MR	+
VP	-
Citrate	-
Catalase	+
Indole	-
H_2S	+
Organism	*Streptomyces griseus*

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Table 2: Cultural and morphological characteristics of fungal isolates from waste dump

Isolate	FS- 1
Colour of aerial hyphae/Colony	Greenish yellow
Colour of substrate hyphae	Brownish yellow
Structure of hyphae	Septate
Shape and kind of asexual spore	Oval shaped conidia
Presence of special structure	Foot cell present
Appearance of sporangiophore or conidiophore	Long, erect, non-septate conidiophore
Characteristics of spore head	Swollen vesicle with chains of conidia
Organism	*Aspergillus oryzae*

Cellulose Utilization by Microbial Isolates

The isolated microorganisms that were able to utilize cellulose included *Aspergillus oryzae* and *Streptomyces griseus* (Table 3). A zone of clearing around the colonies was used as an indication for cellulose utilization. *Aspergillus oryzae* showed a zone of clearing of 30.50 mm while *Streptomyces griseus* showed a wider zone of clearing of 60.00 mm on cellulose Congo red agar plate (Plates III& IV).

Table 3: Diameter of zone of clearing on cellulose Congo-red agar for cellulose-utilising microorganisms (Mean ± SD)

Isolate	Diameter of zone of clearing (mm)
Aspergillus oryzae	30.50±0.50
Streptomyces griseus	60.00±1.00

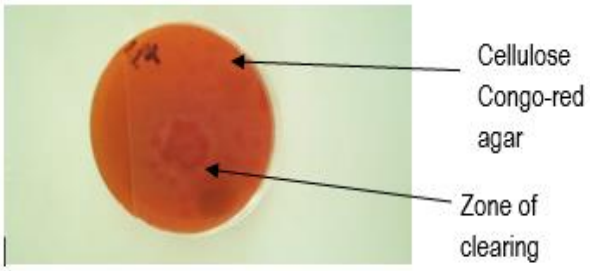

Plate III: Zone of clearing shown by *Aspergillus oryzae* on cellulose Congo red agar medium

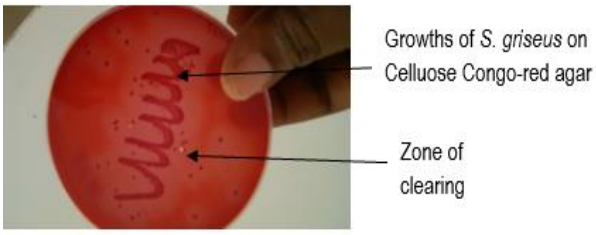

Plate IV: Zone of clearing shown by *Streptomyces griseus* on Cellulose Congo red agar medium

DISCUSSION

Streptomyces griseus, which was isolated from waste dump soil in this study is known to be a soil microorganism. Ram (2014), used starch casein agar for isolation of *S. griseus* from soil sample collected from a park.

Elbashiti *et al.* (2010) isolated *Aspergillus oryzae* from contaminated rice, soybean and wheat, while Lynn *et al.* (2013) also isolated *A.oryzae* from food materials. This could be the reason the microorganism, *A. oryzae,* was isolated from waste dump soil in this study, since contaminated foods are eventually disposed of at the waste dump.

The use of Congo red as an indicator for cellulose degradation in an agar medium provides the basis for a rapid and sensitive screening test for cellulolytic bacteria (Gupta *et al.,* 2012). The formation of clearing zone around the colonies confirms the secretion of extracellular cellulase (Ghimire *et al.,* 2016).

Utilization of cellulose by microorganisms in the present study is in agreement with the reports of some previous researchers. Cellulose utilization by *A. oryzae* has been reported by Youssef, (2011), where variations in the growth conditions were used to determine cellulase production. Yamada *et al.* (2014), also used cellulose as substrate for production of kojic acid by *A. oryzae*. Imran *et al*. (216) also reported cellulose utilization by *A. oryzae* using untreated and pre-treated soybean hulls. In the report of Begum & Alimon, (2020), *A. oryzae* exhibited a maximum clear zone of 40 mm on carboxymethyl cellulose (CMC). ManR, a transcriptional regulator that controls the cellulose utilization system of *A. oryzae* was reported by Ogawa *et al.* (2014).

In the report of Wakai *et al*. 2019, genetically engineered *A. oryzae* strains were used for the production of cellulases, kojic acid, lactic acid, and antibody. The researchers were able to prepare a cellulase cocktail which effectively degraded kraft pulp into glucose. *Aspergillus oryzae* was among the species of *Aspergillus* reported by Datta, (2024) as cellulose utilizers.
Sato *et al*. (2020), reported that insoluble polysaccharide biomass such as cellulose can be catabolized by *Streptomyces* species, which are the most abundant soil bacteria on earth and play an important role in carbon cycle. The report of Djuric *et al*. (2021) also stated that *Streptomyces* species degraded raw cellulose in variable substrates. The degradation ability is because they possess several cellulase-encoding genes. de Melo *et al.* (2018), also reported that *Streptomyces* species genomes have revealed several carbohydrate-active enzymes (CAZymes), including cellulases, hemicellulases, and lytic polysaccharide monooxygenases. However, Ram (2014) reported that *Streptomyces griseus* did not produce the enzyme cellulase in a research test, his report is opposed to the report of the present study, where *S. griseus* demonstrated a high ability to degrade cellulose.

Arora *et al.* (2005), had reported that *Streptomyces griseus* B_1 isolated from soil, when grown on cellulose powder as submerged culture produced high levels of all the three components of the cellulolytic enzyme systems. Also, in the research by Al-Rubaye *et al.* (2023), different strains of *S. griseus* were able to utilize cellulose in variable degrees. The ability of *S. griseus* to utilize cellulose even much more than *Aspergillus*

oryzae, which belongs to a family of well-established cellulose utilizers as reported in the present study, could be lying in the report of Sato *et al.* (2020) which asserts that the genome contains six lytic polysaccharide monooxygenases (LPMO)10-encoding genes that phylogenetically cluster with cellulose or chitin targeting LPMO10s. The *Sc*LPMO10C, which is composed of the same domains as *Sg*LPMO10A, cleaves the cellulose chain and accelerates cellulose degradation by glucoside hydrolases (GHs).

Conclusion

The two microbial species assessed in this study demonstrated appreciable abilities to degrade cellulose in cellulose Congo-ed agar media. Furthermore, *S. griseus* needs to receive more attention with respect to researches in cellulose utilization.

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