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Research Article

Screening for Antibiotic-Producing Fungi from Soil in Caleb University, Imota, Lagos, Nigeria

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ABSTRACT

Antibiotics are chemical substances of biological, synthetic, or semi-synthetic origin, playing important roles in culture techniques, molecular biology, biochemistry, microbiology, genetics and pharmacology. Natural antibiotics are produced by certain microorganisms that can inhibit or suppress the growth of other microorganisms. Antibiotics are the most important microbial secondary metabolites and as a result, antibiotics are the most commercially exploited microbial secondary metabolites with obvious relevance to modern medicine and biotechnology. Since the discovery of antibiotics, over-dependence on antibiotics has led to the emergence of antibiotic-resistant microorganisms. Hence, there is an increasing demand for antibiotic-producing fungi from soil. Soil samples were sourced from seven (7) different sites and the collected soil samples were labeled J, C, DS, L, AB, G, F, LG and SIG. Isolation and screening of isolate were performed using standard procedures. This study reveals that Samples from the Laboratory garden and the school's inner garden had colonies that were too numerous to count while samples from the laboratory garden had the lowest number of colonies. Results from growth on plates show that fungi were abundantly distributed in both clay and loam soils. A total of 7 fungal isolates were selected. Antimicrobial activities against clinical isolates of *Staphylococcus aureus* and *Escherichia coli* were confirmed. In conclusion, it was deduced from this study, that *Penicillium* and *Aspergillus* species were isolated from soils sourced from Caleb University However, penicillium had no inhibitory activity against *Staphylococcus aureus* and *Escherichia coli*.

Keywords: Antibiotics; Fungi; Soil; P*enicillium*; *Staphylococcus aureus*

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INTRODUCTION

Since the discovery of secondary metabolite penicillin, the global use of antibiotics has rapidly increased and has led to the discovery of thousands of secondary metabolites of microbial origin used in modern medicine (Nordenfjäll, 2014). Although thousands of antibiotics have been discovered over the past ten decades, none has produced a definitive solution to the apparent challenge of microbial resistance (Okudoh, 2010). The success of antibiotics has led to their massive

consumption and distribution in healthcare (Nordenfjäll, 2014). However, indiscriminate use and overconsumption of the products as well as their frequent release into the environment during ecological interactions, has led enormous selective pressure on microorganisms which have been forced to adapt to the antibiotics, causing them to either gain resistance (Nordenfjäll, 2014). This resulted in the emergence of antibiotic-resistant microbial strains as an adaptive strategy and thus, has birthed a critical problem in human medicine (Rafiq *et al.,* 2018). The World Health Organization in 2021 stated that antibiotic resistance is one of the greatest challenges not only to public health, but to the environment at large due to the ubiquitous nature of microorganisms as many antibiotics today are becoming increasingly ineffective in treating microbial infections. Hence, there is an increasing demand for new antibiotics to combat such resistant strains (Rafiq *et al.,* 2018).

Although there are so many potential sources where antibiotics can be discovered, soil remains the most important target for researchers in an effort to discover novel antibiotics with pharmaceutical value (Anokhee *et al.,* 2017). This is because soil is a loose naturally-occurring mixture of mineral and organic particles in which many microorganisms, especially bacteria, reside with the ability to produce biologically active secondary metabolites such as useful antibiotics (Anokhee *et al.*, 2017). In their natural habitats, such microbes are able to utilize the antibiotics they produce to inhibit the growth or invasion of other bacterial species as a protective adaptive strategy during competition Ismail and Ahmed (2021). Due to the diversity of soil microbial communities, not all taxa in soil have been well-identified and described, hence offering good potential for the discovery of new and useful microbial products.

MATERIALS AND METHODS

Sample Area

This research was conducted in Caleb University, located in the Imota district of Ikorodu Local Government Area of Lagos State located in the North-Eastern region of Lagos State, with latitude and longitude coordinates; 6.729°N and 3.6737°E respectively. It covers a total area of 0.828km^2, with an annual temperature between 26-31°C.

Sample Collection

Using the method employed by Anokhee *et al* (2017), the debris from the surface of the soil was first removed before sample collection commenced. Then following the method of Rafiq *et al* (2018), soil samples were collected from seven (7) different spots within the school by random sampling. Samples were collected from a depth of 1-5 cm Sura *et al.* (2017) using sterile spatulas and a calibrated ruler. The samples were then placed in labeled sterile sample collection containers, labeled as J, C, DS, LAB G, F, LG and SIG which were sealed and then immediately transferred to the laboratory at the Department of Biotechnology and Biological Sciences at Caleb University, for analyses.

Sample Preparation

Seven (7) sets of 10 test tubes were filled with 9mL each of distilled water and sterilized at 121ºC for 15 minutes. Following the modified method of Rafiq *et al.* (2018), the first soil suspension was made by adding 1g of the first soil sample (J) in 9 mL of the first test tube of the series, labeled "J stock", and mixed vigorously using a vortex mixer to give a uniform suspension. This gave the stock solution. Then 1 mL of the stock was transferred into the second test tube of the set, labeled as J10^{-1}giving the first dilution. This was carried out using the sterile syringe. Then 1 mL from the second test tube was transferred into the third test tube labeled "J10^{-2}", to give the second dilution. This procedure was continued for the rest of the test tubes in the set to give the 10-fold dilution, and this was repeated for all other sets till all the samples had been serially diluted.

Media Preparation

Potato Dextrose Agar (PDA) was used for the isolation of fungi in this study. 19.50 g of PDA was weighed and dissolved in 500 mL of distilled water, in strict compliance with the manufacturer's instructions, sealed using a stopper. The prepared medium was then sterilized in an autoclave at 121ºC for 15 minutes.

Isolation of Antibiotics Producing Fungi from Sample

The methodology for the isolation of soil antibiotic-producers followed a modified method of isolating antibiotic-producing fungi, employed by Rafiq *et al*., (2018) and Njenga *et al.,* (2018). 0.5 mL each of the dilutions 10^{-2} and 10^{-5} were transferred into separately labeled sterile plates using sterile syringes and then molten Potato Dextrose Agar (PDA) which was incorporated with 4 mL of chloramphenicol (250 mg/mL) was poured into the Petri dishes containing the samples. The inoculated plates were allowed to solidify. The plates were then inverted and incubated at 28°C for 5 days.

Sub-culturing

Discrete fungal colonies were sub-cultured on freshly prepared sterile PDA plates and incubated at 28ºC for 3 days. The pure fungal colonies were then stored at 4°C as stock culture in PDA slants for subsequent studies, in accordance with the method conducted by Ismail and Ahmed (2021).

Microscopic and Macroscopic Examination of Fungal Isolates

This was carried out following the method of Rafiq *et al.* (2018). Microscopic examination was carried out by

picking fungal mycelia with the help of a sterilized needle and placing it on a slide containing a drop of lactophenol cotton blue stain. The slide was then covered with a cover slip and viewed using the x40 objective lens of the microscope.

The macroscopic examination of fungi was conducted by comparing the fungal isolates with the second edition of Tsuneo Wantanbe's pictorial atlas of seed and soil fungi.

Screening for Antimicrobial Activity

Test organisms *E. coli* and *S. aureus* isolates, obtained from a private clinical laboratory were first confirmed using Gram staining and biochemical tests. For antibiotic production, the method conducted by Ismail and Ahmed (2021) was relied upon. Isolated and sub-cultured fungal colonies were inoculated in test tubes containing 5 ml of nutrient broth and incubated at 37 °C for 24 hrs. 38 g of Mueller-Hinton agar was then dissolved in 1000 mL of distilled water and autoclaved at 121°C for 15 minutes. Afterward, the media was cooled, poured in Petri dishes and allowed to solidify. The test organisms (*E. coli and S. aureus*) were inoculated in test tubes containing 2 mL of sterilized physiological saline. Cell concentrations equivalent to 0.5 McFarland standard of all the test bacteria were inoculated onto the Muller-Hinton agar plates using sterilized cotton swabs. A sterile cork borer was used to bore the wells on the inoculated Mueller-Hinton agar. About 0.1 mL of the fungal isolates were introduced into the wells and ketoconazole (antifungal disc) as control was also placed on the agar. The plates were then incubated at 37ºC for 24 hrs and inhibition zones were observed and measured.

RESULTS

Table 1 shows the physical description of soil samples collected from Caleb University, including the texture and color of the soil samples, ranging from clay to loam soil and from light brown to dark brown respectively as well as the mean viable fungal plate count (CFU/g). Samples from Laboratory garden behind school building and school inner garden had colonies that were too numerous to count while sample from the laboratory garden had the lowest number of colonies. Results from growth on plates show that fungi were abundantly distributed in both clay and loam soils. After appropriate incubation of the fungal isolates on appropriate media, colonial morphology was observed (Table 2). Each distinct colony present on the primary culture was sub-cultured on fresh plates and the colonies were observed and described in Table 2. The sub-culture was carried out to obtain pure colonies of each fungal colony from the primary culture. The fungal isolates were stained with lactophenol blue cotton stain for identification. Microscopic characteristics of fungal isolates were compared with the pictorial atlas of seed and soil fungi. A total of seven (7) fungal isolates were selected, isolated, and identified to test for inhibitory properties. Table 3 presents results for screening of fungal isolates for antimicrobial activities against confirmed clinical isolates of *Staphylococcus aureus* and *Escherichia coli.* Results showed no antimicrobial activity by our isolates against *S. aureus* and *E. coli*.

Table 1: Mean Viable Fungal Count

S/N	Sample code	Colour	Texture	CFU/g
1.	J	Light brown	Clay soil	1.5×10^5
2.	CAF	Dark brown	Loam soil	3.3×10^5
3.	DS	Dark brown	Loam soil	3.3×10^5
4.	LAB G	Dark brown	Loam soil	1.2×10^5
5.	F	Light brown	Clay soil	1.3×10^5
6.	LG	Dark brown	Loam soil	TNTC
7.	SIG	Dark brown	Loam soil	TNTC

Key: J=Joshua Hall, C=Cafeteria, DS=Dump site, LAB G=Lab garden, F=Field, LG= Garden behind school building and SIG =School Inner Garden, TNTC = too numerous to count

Table 2: Morphological and Microscopic Characteristics of Fungi Isolated From Soil in Caleb University

ISOLATE CODE	Colony Morphology on PDA	Lactophenol blue cotton staining morphology	Identification
LABG102gP	Large dark green mycelial growth with background yellowish coloration on agar plate	Conidia and phialides observed with conidiophore and septate hyphae (figure 4.3.5)	*Penicillium* spp.
CAF102AP	Large Greenish-blue mycelial growth with white surrounding and yellow coloration on the back of the agar plate.	Conidia seen, displayed a typical *Penicillium*-like structure, as conidia were observed with septate hyphae and conidiophores	*Penicillium* spp.
F10C2P	Greenish round mycelial growth with a white surrounding background	Conidia observed with conidiophores	*Penicillium* spp.
F102GP	Black mycelial growth with a characteristic white background	septate hyphae observed with swollen vesicle and uniseriate phiallide arrangement	*Aspergillus niger.*
SIG105AP	Grayish-green mycelial growth with white margins with yellowish coloration on an agar plate	Septate hyphae seen with conidia distributed in the field.	*Penicillium* spp.
LG102AP	Dark green mold-like mycelial growth with white margins and yellowish coloration on an agar plate	Conidia observed with phialides in biserrate arrangement and septate hyphae	*Penicillium* spp.
LABG105P	Large round black with white mycelial growth	Unbranched conidiophore seen with swollen vesicle and uniserate phialides arrangement	*Aspergillus niger*

Table 3: Screening of Isolates for Antibacterial Activities

ISOLATE CODE	*E. coli*	*S. aureus*
LABG102gP	R	R
CAF10AP	R	R
F102CP	R	R
F102GP	R	R
SIG105AP	R	R
LG102AP	R	R
LABG105P	R	R

S=Sensitive; R= Resistant (No activity)

DISCUSSION

In this study, Fungal species were isolated from soil samples obtained from the study area, Caleb University, Imota, Lagos, sub-cultured to obtain pure colonies and then identified using macroscopic and microscopic characteristics and compared with the second edition of Tseunoe Wantanbe's Pictorial Atlas of Seed and Soil Fungi. The isolates were then tested to check for their ability to produce antibiotics using test organisms *E.coli* and *S.aureus.* The presence of *Penicillium* spp. in soil in this study is in agreement with the findings of Njenga *et al.* (2018) and Rafeeq *et al.* (2018), respectively. However, the negative results from the screening suggest that none of the isolates exhibited any inhibitory activity against either of the test bacteria. This may be due to the fact that the strains isolated might not have the genetic potential to produce antimicrobial metabolites that would inhibit the growth of neighboring susceptible bacteria. This may also be due to the fact that the fungal isolates were not in their

stationary phase of growth. The stationary phase is a phase that most secondary metabolites including compounds capable of inhibiting microbial growth are produced. Sanchez and Demain (2011) reported that microbial secondary metabolites are products of secondary metabolism and are usually produced during the late stationary phase. The results obtained therefore suggest that the fungal isolates from the study area did not produce any metabolite capable of being screened for the production of novel antibiotics.

CONCLUSION

Penicillium and *Aspergillus* species were isolated from soils sourced from Caleb University, however, p*enicillium* showed no antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

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