Isolation and Characterization of Antibiotic Producing Fungi from Soil

ABSTRACT

This study was aimed to isolate and characterize antibiotic producing fungi from the soil environment within Ahmadu Bello University main campus Samaru, Zaria. Soil samples were collected from five different locations within Ahmadu Bello University main campus for the Isolation of fungi. Spread plate method involving serial dilution technique was used for the Isolation using Saboraud Dextrose Agar (SDA). Six species of fungi were isolated from the soil samples and then characterized microscopically and macroscopically. The fungi were *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus* sp, *Penicillium* sp, *Fusarium* sp (P14) and *Fusarium* sp (P15). Sensitivity test using *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* as test pathogens was employed to determine the ability of the fungal isolates to produce antimicrobials. All the fungal isolates were found to inhibit the growth of at least one of the test pathogens except *Fusarium* sp (P14). *Aspergillus niger* produces zones of inhibition of 9mm, 5mm and 6mm against Staphylococcus aureus, *Escherichia coli*, and *Klebsiella pneumoniae*, *Aspergillus fumigatus* produces zones of inhibition of 5mm against Escherichia coli and *Klebsiella pneumoniae*. *Aspergillus* sp produced zones of 6mm against *Staphylococcus aureus* and *Escherichia coli*, *Penicillium* sp which produces a zone of 10mm, 7mm and 6mm against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. The findings of this study show that antibiotic-producing fungus are prevalent in the soil of Ahmadu Bello University's main campus in Samaru, Zaria, and that these strains could be used by pharmaceutical companies to produce antibiotics from local sources.

Keywords: Antibiotic producing; soil fungi; Fusarium sp.

1. INTRODUCTION

1.1 Background of Study

"Soil is a reservoir for a large number of microorganisms such as bacteria, viruses, fungi and protozoa" [1]. "Microbes of soil increase the soil fertility, maintain ecosystem sustainability, antibiotic and enzyme production and biodegradation are the distinct beneficial effects" [2].

"Fungi are geographically widely distributed and have been observed in a broad range of habitats principally in soil" [3].

The term 'antibiotic' literally means 'against life'. According to Talaro and Talaro [4] antibiotics are substances produced by natural metabolic processes of some microorganisms that can inhibit or destroy other microorganism. Duta [5] defined "antibiotics as the miracle drugs of modern times that act as magic bullet shooting down infective organisms that have invaded the human body and caused infections".

"In 1928, the first clinical trial of penicillin was carried out on humans by an English Bacteriologist Late Sir Alexander Fleming. This antibiotic was obtained from a blue green mould of the soil called *Penicillium notatum"* [5].

"Subsequent to the identification of penicillin production by *Penicillium notatum*, screening experiments revealed that *Penicillium chrysogenum* was a superior producer of penicillin. A typical fermentation yields three types of penicillin namely, penicillin F, penicillin G and penicillin V" [6,7,4].

"As more antibiotics were discovered, designed and studied; scientists found that they had different properties. Some of these properties include their source, range of activity and their kinds. These were used to classify them [8]. The ability to produce antibiotics has been found mainly in fungi of the group Aspergillales, and in a few other bacteria" [7].

"Sir Alexander Fleming discovered in 1928 that an isolate of Penicillium notatum produced a chemical capable of destroying gramme positive bacteria, which was maybe one of the few most important discoveries on the beneficial usage of fungus for humans" [5] (Walsh, 2003) [9]. This antibiotic was the first to be found in the E-lactam

class of antibiotics. These substances work by preventing bacteria from making peptidoglycans, and their use has lowered the role of gram-positive bacteria as a cause of disease [10,11].

Fungi (20 percent of isolated antibiotics), Actinomycetes (70 percent), and Eubacteria (10 percent) are among the soil-dwelling microorganisms that have yielded antibiotic medicines [12,13]. A number of Aspergillus-related patents have been issued for medicinal compounds. Lovastatin produced by *Aspergillus terreus* was one of the first commercially cholesterol-lowering drugs lam, 1983. “A number of antibiotics, antitumor and antifungal agents have been derived from Aspergillus metabolites. The experimental anti-Candida drug, Cilofungin, is a semi-synthetic drug, derived from a chemical modification of echinocandin B which is produced by *Emericella nidulans*/ *rogulosa* “ [14].

Aspergilli are known for their ability to secrete a variety of biologically chemical compounds including antibiotics, mycotoxins, immune-suppresants, and cholesterol- lowering agents [15].

1.2 Statement of Research Problem

Treatment of infectious diseases caused by pathogenic bacteria was one of the most traditional problems in the clinical field (Mehrgan et al., 2008). In addition, antimicrobial resistance presents as major challenge in the effective treatment of infectious disease, resulting in increased patient morbidity and mortality [16].

1.3 Justification of the Study

As more and more bacteria continue to develop resistance to currently produced antibiotics, research and development of new antibiotics continue to be important. The discovery of penicillin in 1928 by Alexander Fleming, showed its efficacy in laboratory cultures against many diseases producing bacteria. This discovery marked the beginning of the development of antibacterial compounds produced by living organism [7,8].

This research will help to reveal if strains of antibiotic-producing fungi is present in the soil that could be harnessed by pharmaceutical industry for modification to produce new high-yielding strains for the production of new antibiotics.

1.4 Aim of Study

The aim was to isolate and characterize antibiotic-producing fungi from the soil within Ahmadu Bello University main campus Samaru, Zaria.

1.5 Objectives

1. To determine the physico-chemical properties of the soil samples.
2. To isolate and Identification of fungi from soil samples.
3. To assess the antibacterial activity of the fungal isolate metabolites against *Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus.*

2. LITERATURE REVIEW

2.1 General Characteristics of Fungi

Fungi are eukaryote with a nuclear membrane and cell walls. As heterotrophs, they depend on living or dead organic materials for both their carbon and their energy. Fungi are aerobic organisms, although, some can tolerate the rather low oxygen concentrations and high levels of carbon dioxide found in wet or compacted soils. Strictly speaking, fungi are not entirely microscopic, since some of these organisms such as mushrooms, form macroscopic structures that can easily be seen without magnification [17].

The fungi constitute a group of living organisms devoid of chlorophyll. They resemble green plants in that, with few exceptions, they have definite cell walls, they are usually non-motile, although they may have motile reproductive cells, and they reproduce by means of spores. They do not possess stems, roots, or leaves, nor have they developed a vascular system, as have the more advanced types of plants. Fungi are usually filamentous and multi-cellular; their nuclei can be demonstrated with relative ease [18].

The kingdom Fungi is enormous, the identified species and those not yet classified are up to over 300,000 species. The majority of these species are Microscopic fungi (yeast and molds) often used for the production of antibiotics. A relatively small number of species have reproductive systems known as "mush rooms" that can be easily observed in the field, fungi of various shapes and colours can be observed. Examples of common shapes and appearances are the saddle or honey comb shapes, corals shape appearance, umbrella shaped (with a cap and a stipe). Similar variation exists in the colour,

taste and smell of fungi. However, such macroscopic observations are not sufficient to achieve a proper scientific identification and classification of fungi and consequently, microscopic studies are also necessary (Sarad et al., 2016).

Fungi belong to their own kingdom (Kingdom fungi). They obtain their nutrition through a range of ways including degradation of organic material and symbiosis (as lichen) among others [19]. As such, they are categorized as heterotrophic because they are unable to synthesize their own food (they lack chlorophyll). They can reproduce sexually or asexually with a majority of fungi being spore producers. Examples include Moulds, yeast, mushrooms to larger multicellular capable of forming hyphae threads of false roots. For this reason, fungi are classified according to their mophorlogies. Fungi are very important microorganisms because of their variety of uses which include mycorrhizal relationship between some of the fungi and plants that benefits various plants in many ways. For instance, whereas the fungi obtain nutrients from the plants, the fungi may make various materials such as phosphates readily available for the plant. Some others help in degradation of organic materials [20].

2.1.1 Occurrence of soil fungi

Soil fungi comprise of an extremely diverse group of micro-organisms. Tens of thousands of species have been identified in soils, representing some 170 genera. Although their numbers are usually somewhat smaller than bacterial, their relatively large sizes result in their dominating the biomass and metabolic activities in many soils. Their biomass is commonly ranges from 1,000 to 15,000kg/ha in the upper 15cm [17].

Surprisingly, fungi may be the most abundant eukaryotic members of some deep-sea sediment [21]. A key question is the extent to which these taxa are active to these hostile environments, as opposed to surviving in highly resistant, dominant spore stages following introduction by wind or by other vectors [22].

The most commonly measured characteristics of soil fungi are their biomass, elemental stoichiometry, growth rates and efficiencies and activities of their extracellular enzymes [23-25]. These parameters define the size of fungal C and nutrient pools and rates of fungal substrates by enzymatically degrading complex organic matter outside the cell. However, control of the of the organisms. Hence, a substantial fraction of the enzymatic potential of soils is a legacy of enzymes that are spatially and temporally displaced from their origins [26]. In some cases, particularly for oxidative enzymes, these enzymes catalyze degradation and condensation reactions secondary to their original functions [27].

Soil-inhabiting fungi are an important and wide spread component of most terrestrial ecosystems and play a key role in micro-ecosystem (Jackson et al., 2000). Fungi have profound influences on biogeochemical cycles through their growth habits, which include external digestion of food resources using a powerful arsenal of degredative enzymes and secondary metabolism [28]. Fungi are prominent and present in all soils. At broad phylogenetic scale, the aphorism “everything is everywhere” does seem to apply to fungi; beyond soils, they are also found in nearly every other habitat on earth, including deep sea hydrothermal vents and sediments, sub glacial sediments, ancient permafrost, sea ice, hot and cold deserts, and soils of dry valleys of antarctica [29].

Fungal biomass varies widely within and across biomes in relation to litter composition, root density, and nutrient availability. Fungi may comprise up to 20% of the mass of decomposing plant litter. Although fungal abundance and ratios of fungal to bacterial biomass tends to increase as soil pH decreases [30,31]. Other studies suggest that fungal distributions are more influenced by nitrogen and phosphorous than pH [32,33].

2.1.2 Structure of fungi

The body or vegetative structure of a fungus is called a thallus (pl., thalli). It varies in complexity and size, ranging from the single-cell microscopic yeasts to multi-cellular molds, macroscopic puff balls, and mushrooms. The fungal cell usually is encased in a cell wall of chitin. Chitin is a strong but nitrogen-containing polysaccharide consisting of N-acetyl glucosamine residues [4].

2.1.3 Division of soil fungi

Fungi may be divided into three groups which are yeast, mushrooms and moulds. Yeasts which are single-celled organisms live principally in water logged, anaerobic soils. Molds and mushroom are both considered to be filamentous fungi, because they are characterized by long, thread-like, branching chains of cells. Individual group of filaments called hypha are often twisted together to form mycelia that appear somewhat like woven ropes. Fungal mycelia are often visible as

thin, white or colored strands running through decaying plant litter. The filamentous fungi reproduce by means of spores, often formed on fruiting bodies which may be microscopic (i.e. molds) or macroscopic (i.e. mushrooms) Brady and Weil [17].

The molds are distinctively filamentous, microscopic or semi-macroscopic fungi that play a much more important role in soil organic matter breakdown than the mushroom fungi. Molds develop vigorously in acid, neutral, or alkaline soils. Some are favored rather than harmed, by lower pH. Consequently, they may dominate the micro-flora in acid surface soils, where bacteria and actinomycetes offer only mild competition. The ability of molds to tolerate low pH is especially important in decomposing organic residues in acid forest. Many genera of molds are found in soils. Four of the most common are *Penicillium*, *Mucor*, *Fusarium*, and *Aspergillus*. Species from these genera occur in most soils. Soil conditions determine which species are dominant [17].

2.1.4 Activities of fungi

As decomposers of organic materials in soil, fungi are the most versatile and persistent of any group. They usually dominate in the upper horizons of forested soils, as well as in very acid or sandy soils. The carryout the largest share of the decomposition in many cultivated soils as well. Fungi become more efficient than bacteria that they assimilate into their tissues a larger proportion of the organic materials they metabolize. Soil fungi can synthesize a wide range of complex organic compounds in addition to those associated with soil humus. It was from a soil fungus, *Penicillium* species, that the first modern antibiotic drug, penicillin, was obtained. By killing bacteria, such compounds probably help fungi to out compete rival microorganisms in the soil [17].

2.1.5 Importance of fungi to man

The human use of fungi for food preparation or preservation and other purposes is extensive and has a long history. The study of the historical uses and sociological impact of fungi is known as ethno mycology. Because of the capacity of this group to produce enormous range of natural products with antimicrobial or other biological activities, many species have long been used or are being developed for industrial production of antibiotics, vitamins, anti-cancer and cholesterol-lowering drugs. More recently, methods have been developed for the genetic engineering of fungi, enabling metabolic engineering of fungal species. For example, genetic modification of yeast species [34] which are easy to grow at fast rates in large fermentation vessels has opened up ways of pharmaceutical production that are potentially more efficient than production by the original source organisms [14].

2.2 The Genus *Penicillium*

2.2.1 Occurrence

The *Penicillium* species are as common and cosmopolitan as the *Aspergilli*. They are the so-called green molds and blue molds which are so frequently found on citrus and other fruits, and on other food stuffs that have become contaminated with their spores. The conidia of *Penicillium*, like those of *Aspergillus*, are everywhere in the air and in the soil [18].

2.2.2 Economic importance

Many species of *Penicillium* are capable of producing organic acids, such as citric, fumaric, oxalic, gluconic, and gallic. Industrially, the penicillia are important in making cheese and antibiotics. *Penicillium notatum* and *Penicillium chrysogenum* have come to prominence as sources of the now famous antibiotic, Penicillin. Although other species of *Penicillium* also produce this antibiotic, certain strains of the latter species have proved to be most efficient in its manufacture and are being used exclusively in the commercial preparation of this drug. Of great interest in this connection is the ability of mycologists to produce new high-yielding strains of *Penicillium chrysogenum* by subjecting conidia to ultra-violet irradiation and testing the colonies originating from the conidia for Penicillin production. Yields of penicillin have been increased enormously in this way, and this has been the primary factor in the price reduction which has made this first “wonder drug” available to all [18].

2.2.3 Morphology

The life history of a typical *Penicillium* is very much like that of *Aspergillus*, but the morphology of the structures differs considerably. The mycelium produces simple, long erect conidiophores which branch about two thirds of the way to the tip, in symmetrical and asymmetrical, broom-like fashion. The conidiophores commonly refered to as the brush, is technically known as the penicillus (pl. *Penicilli*; l; *Penicillium* = small brush). The conidia are globule to ovoid, and under the microscope resemble glass beads. They are formed in the same manner as in *Aspergillus*. The enormous

quantities of greenish, bluish, or yellow conidia which are produced are chiefly responsible for the characteristic colony colour of various species of *Penicillium*. The conidia germinate easily into germ tubes from which mycelia develop [18].

2.3 The Genus Aspergillus

2.3.1 Occurrence

Aspergillus is a genus of anamorphic fungi reproducing by production of phialospores (conidia borne on phialides). It is a large genus with over 180 recognized species (Pitt et al., 2000).

Thom and Raper (1945) recognized 78 species of Aspergillus. The fungus *Aspergillus niger*, also known as black mould, belongs to the black aspergilli group. The genus is found all across the world, from the arctic to the tropics. The conidia of these organisms appear to be present in the air everywhere. Aspergilli spores can also be found in the soil. Because of the high number of enzymes produced by aspergilli, they are capable of utilising a wide range of substances for nourishment. Indeed, finding a substance with some organic matter and a little moisture that the aspergilli cannot thrive on is challenging [18].

2.3.2 Economic importance

Because of their great enzymatic activities, aspergilli are employed in several industrial processes. Citric and gluconic acid are manufactured commercially by the use of *Aspergillus niger*. Many other acids and various other chemicals are produced in large or small quantities by members of this genus. Enzyme preparations are made commercially through the use of these fungi, and a number of antibiotics have been isolated from aspergillus cultures, though none has proved equal to Penicillin or to the actinomycete product in therapeutic properties. In Japan, *Aspergillus oryzae* is used to make sake, an alcoholic beverage concocted from rice, and to manufacture various fermented foods. In Java, *aspergillus wentii* is employed in processing soybeans because of its ability to loosen the hard tissue of the bean [18].

The greatest positive economic impact of the *Aspergilli* has been in the exploitation of the enzymes and acids produced a number of species. Two of the most important industrial products produced by *Aspergilli* are amylase and citric acid.' The Koji molds' (*Aspergillus oryzae, Aspergillus sojae and Aspergillus awamoni*) have been used for more than a thousand years to produce a number of a mold strain grown on steamed grain. The mold produce amylases which break down the starches and contribute to the flavour and colour of the product (Hara et al., 1992). Citric acid production by *Aspergillus niger* was discovered in 1916. By the mid-1920's over three quarters of the citric acid, used worldwide was produced by fungal fermentation. It is still used today to produce more than 500,000 tons per year (Roehr et al., 1992).

Unfortunately, the economic benefits of Aspergilli and their metabolites are outweighed by the disadvantages. Aspergilli is a key cause of agricultural product degradation both before and after harvest. Some species are diseases for humans and animals, and others are allergens. They also create a wide range of mycotoxins, which are secondary metabolites that are toxic to both people and animals [35]. Mycotoxins are products of secondary metabolism indicating that their production is not necessary for the survival of the fungus. Unlike antibiotics which are toxic only to microorganisms, mycotoxins are harmful to humans and or animals. Aflatoxin is the most economically important mycotoxin in the world. The most toxic form of aflatoxin is aflatoxin B. Aflatoxin is formed in oil seed crops (cotton seed, corn and peanuts) and tree nuts under drought conditions in the field. It can form in virtually any stored grain that can support fungal growth (Diener et al., 1987).

2.3.3 Somatic structures of *Aspergilli*

The mycellium of *Aspergilli* resembles that of other fungi. The hyphae are well developed, profusely branched, septate, and hyaline; their cells are, as a rule, multinucleate [18].

Aspergillus is characterized by its distinctive conidiosphore. The base of the conidiosphore usually forms 'T' or 'L' shape where it connects with the vegetative hyphae. This is commonly called the 'foot cell' even though it is not a separate cell. The stipe extends from the foot cell and may be quite short (50 micro meter or less) to several millimeters in length. The Apex of the stipe expands into a vesicle. The vesicles may have various characteristic shapes. In some species, the conidia-bearing phialides arise directly from the vesicle. This form is called uniseriate. In other species, there is a second layer of cells between the vesicle and the phialides. These cells are called 'metulae' and *Aspergilli* with metulae are referred to as biseriate species. An important character that distinguishes *Aspergillus* from several closely

related genera is that the phialides/metulae arise simultaneously on the vesicle [35].

2.4. Test Organisms

2.4.1 *Escherichia coli*

Escherichia coli is a gram negative, rod shaped bacteria and a typical coliform of the intestinal tract. It is important:

(a) As a natural commensal (inhabit the intestine of humans and animals, thus belonging to the group of microorganisms known as *Enterobacteria*.
(b) As a pathogen, particularly of the urinary tract, and also in diarrhea, appendicitis, peritonitis, neonatal meningitis and pyelitis.
(c) In proving evidence of faecal contamination of water supplies.

It grows well in most media and it is typically indole-positive, produce gas from glucose and haemolysis is seen on blood agar. It has a complex antigenic structure [36, 37].

2.4.2 *Klebsiella pneumoniae*

Klebsiella pneumonia is a gram negative, non-motile, encapsulated rod. it is lactose fermenting, producing mucoid pink colonies on macConkey agar, yellow mucoid colonies on CLED medium and grey white, usually mucoid colonies on blood agar. It is also urease positive, citrate positive and utilizes malonate.

It is a major pathogen of humans, present in the respiratory tract and faeces of about 5% of normal individuals. It causes a small proportion of (about 1%) of bacterial pneumonia. *Klebsiella pneumonia* causes chest infections and occasionally severe bronchopneumonia with lung abscesses. It occasionally produces urinary tract infections and bacteremia. Infections of *Klebsiella pneumoniae* are often opportunistic occurring in patients with existing diseases or in debilitated patients [36,37].

2.4.3 *Staphylococcus aureus*

Staphylococcus aureus are gram positive, non-sporing, non-motile cocci which forms a regular grape-like clusters. They grow rapidly and abundantly under aerobic condition. They can't operate high concentration of salt and are resistant to heat. They are catalase positive, oxidase negative and have the ability to produce coagulase (Harish et al., 2002).

They cause different diseases, such as septicaemia, boils, styes, toxic food-poisoning, Staphylococcus aureus can survive on dry surfaces for months and infect its host from the skin, mouth and nose [38].

3. METHODOLOGY

3.1 Materials and Methods

3.1.1 Study area

This research was carried out within Ahmadu Bello University main campus Samaru Zaria. The soil samples were gotten from the following Area/sites: Dangote hostel, Sassakawa hostel, Akenzua hostel, Danfodio hostel and Division of Agricultural College (DAC).

3.2 Collection of Samples

Soil samples was collected aseptically from five different locations within Ahmadu Bello University. Spatula cleansed with 70% alcohol was used to collect the soil samples into a transparent sterile polythene bag. The samples were then immediately taken to the Microbiology Laboratory, where they were analyzed. The five locations where the samples were collected includes: Dangote hostel, Sassakawa hostel, Akenzua hostel, Division of Agricultural College (DAC) and Danfodio hostel.

3.3 Determination of Soil Temperature

A thermometer was used to determine the temperature of the soil at the five separate sites. The thermometer was placed into the soil up to a depth of 5cm and left in place for 10 minutes before taking temperature measurements. For each site, the average of three consecutive readings was taken [39].

3.4 Determination of Soil ph

The pH values of the soil were obtained using Watson and Brown's standard procedures in the soil analysis laboratory of the Institute of Agricultural Research (IAR) (1998). 3g of soil sample was weighed into a beaker containing 3ml of distilled water, which was then swirled for five seconds and let to stand for ten minutes using this procedure. The pH metre electrode was then carefully put into the slurry and stirred. For each site, a reading was taken and the mean of the consecutive readings was documented.

3.5 Soil Types

The soil types were determined in Institute of Agricultural Research (IAR), soil analysis

laboratory (Ahmadu Bello University) according to the method of [40](Pettijohn, 2000).

3.6 Isolation and Identification of Fungal Isolates

The soil fungi were isolated by Soil Dilution Techniques using the spread plate method. The Media used for the isolation was Sabouraud Dextrose Agar (SDA) which was prepared according to the manufacturer's instruction. 1g of soil sample was measured using a weighing balance and was then immersed into 9ml of distilled water in a bijou bottle. Serial dilution was then carried out in ten (10) folds in bijou bottles each containing 9 ml of distilled water to obtain 1/10 dilution (stock solution). 1ml of the stock solution was transfered into the first bijou bottle which was shaken vigorously to have a uniform distribution. Another 1ml was taken from the first diluents to the second bottle and 1ml was also taken from the second diluents to the third bottle respectively [41]. 0.1 Aliquot each of 10^{-3} dillution was then taken by the use of a syringe which was then dispensed into a Petri dish and the same was done for all the remaining samples. The Plates were incubated for three (3) to seven (7) days at room temperature. Pure cultures of the fungal isolates were identified using both macroscopic (cultural) and microscopic (morphological) features with reference to Atlas of Mycology.

3.7 Determination of Percentage Occurrence Frequency of Fungal Isolates

The percentage frequency of occurrence for each species of fungus isolated was determined by the methods of Sampo et al.[42]. This was computed by the formula: A/B x 100; where A = Number of plates in which the species appeared, and B = Total number of plates incubated for each sites.

3.8 Test Organisms

The test bacteria that were used for this study were *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus.* The bacterial isolates were obtained from Ahmadu Bello University medical center, Samaru, Zaria, where it was isolated from patients. The Isolates of these organisms were taken to the laboratory of the Microbiology Department Faculty of Life Sciences, Ahmadu Bello University Zaria. The organisms were sub-cultured on Nutrient Agar for reconfirmation.

3.8.1 Reconfirmation of *Staphylococcus aureus* Isolates

3.8.1.1 Catalase test

A drop of hydrogen perioxide was added to the surface of a clean, grease-free glass slide and colonies of the suspected *Staphylococcus aureus* was added to it with a sterile straight wire loop and emulsified. Bubbles formation indicates positive test while absence of bubbles indicates negative result. *Staphylococcus aureus* is catalase positive [37].

3.8.1.2 Coagulase test

A drop of plasma was added to already emulsify distilled water with colonies of the suspected *Staphylococcus aureus* on clean grease-free slide, and then the slide was rocked for 10 seconds. Positive test was indicated by clumping, while negative test shows no clumping. *Staphilococcus aureus* was coagulase positive [37].

3.8.2 Reconfirmation of *Escherichia coli* and *Klebsiella pneumoniae* Isolates

3.8.2.1 Indole test

The indole test was carried out by adding three drops of kovac's indole reagent to 5ml of young broth culture of the isolated bacteria in the test tube and shaken gently for a minute and positive result was indicated by a red layer at the top of the broth culture while a negative result was indicated by lack of colour change at the top of the broth culture. *Escherichia coli* and *Klebsiella pnuemoniae* are indole positive [37].

3.8.2.2 Methyl-red test

Tubes containing Methyl Red-Voges proskauer (MR-VP) broth was inoculated with the suspected isolates and incubated for 24 hours at 37C. Three drops of Methyl-Red solution was added to each tube. After incubation, a distinct red colony indicated a positive test for *Escherichia coli* while absence of colour change indicated negative test for *Klebsiella pneumoniae* [37].

3.8.2.3 Voges-proskauer test

This test uses alpha naphtol and potassium hydroxide to test for the presence of acetyl methyl carbinol (acetoin). Tubes of MR-VP broth

was inoculated with the suspected isolates and incubated for 24 hours at 37C. 1ml of the broth was transferred into another tube, 0.6ml a-naphtol solution and 0.2ml 40% KOH was added and shaked, it was then allowed to stand for 30-60 minutes. A yellowish color is an indication of a negative result while absence of color change indicated a positive result. *Escherichia coli* is VP negative while *Klebsiella pneumonia* is VP positive [37].

***Table* 1. Physio-chemical properties of the soil samples from the different locations within Ahmadu Bello University Campus Samaru, Zaria**

Locations	Soil Types	Soil pH	Temperature (□C)
DAC	Loamy	7.33	30
Dangote	Clay	7.44	27
Danfodio	Loamy	5.92	26
Akenzua	Sandy	6.35	32
Sassakawa	Clay	8.03	28

Table 2. Macroscopic and microscopic characteristics of the fungal Isolates

Sample code	Macroscopic Characteristics	Microscopic Characteristics	*Inference*
P10	Colour: White at first then turns black to dark brown Texture: powdery Elevation: Flat Reverse: White to Yellow	Conidia: Globose and irregularly rough. Stipes: Thick, smooth, slightly brown especially near the apices Vesicles: Nearly spherical.	*Aspergillus niger*
P11	Colour: Dull green colour Texture: Floccose Elevation: plane Reverse: Dull Yellow	Conidia: Globose to broadly ellipsoidal, smooth Stipes: Uncoloured or grayish near the apices, smooth-walled Vesicles: Pyriform.	*Aspergillus fumigatus*
P12	Colour: Green Yellow Texture: slightly granular, wrinkled Elevation: Plane Reverse: Yellow	Conidia: Globose, smooth walled Stipes: Un coloured to pale brown, walls smooth to slightly roughed Vesicles: Subglobose to pyriform.	*Aspergillus sp*
P13	Colour: Blue green Texture: powdery Elevation: plane Reverse: White to Yellowish	Mycelium produces simple long erect conidiophores which branch about two-third of the way with phialides in brush-like clusters.	*Penicillium sp*
P14	Colour: White Texture: Cottony Elevation: Raised Reverse: Yellowish brown	Hyphae slender and tapering with no chlamydospore	*Fusarium sp*
P15	Colour: Red Texture: Cottony Elevation: Raised Reverse: Red brown	Hyphae slender, longer and tapering (septate) with swollen, thick-walled chlamydospore	*Fusarium sp*

Table 3. *Occurrence* of fungal isolates gotten from each soil samples

	DAC	DANG	DANF	AKEN	SASS	OCCURRENCE (%)
Aspergillus niger	+	+	_	+	+	80
Aspergillus fumigatus	_	+	+	+	+	80
Aspergillus sp	+	_	_	+	_	40
Penicillium sp	+	_	+	_	_	40
Fusarium sp	+	+	+	_	+	80
Fusarium sp	+	_	_	_	_	20

KEY: - = Absent; + = Present; DAC= Division of Agricultural College, DANG= Dangote, DANF= Danfodio, AKEN= Akenzua, SASS= Sassakawa

Table 4. Antibacterial activity of the fungal isolates against the test bacteria

		Zones of Inhibition (mm)		
Sample code	**Isolate Identity**	***S. aureus***	***E. coli***	***K. pneumoniae***
P10	*Aspergillus niger*	9	5	6
P11	*Aspergillus fumigates*	NA	5	5
P12	*Aspergillus* sp	6	6	4
P13	*Penicillium* sp	10	7	6
P14	*Fusarium* sp	NA	NA	NA
P15	*Fusarium* sp	NA	3	NA

KEY: NA= No Activity

3.8.2.4 Citrate utilization test

A simmon citrate agar was inoculated with the suspected isolates and incubated at 37c for 24 hours after which the medium was observed for color change. Citrate positive organisms, growth will be visible on the slant surface and the medium will be an intense prussian blue. The alkaline carbonates produced as by-product of citrate catabolism raises the pH of the medium to above 7.6, causing a colour change from the original green color to blue. *Klebsiella pneumoniae is* citrate utilization positive while *Escherichiacoli* are citrate utilization negative [37].

3.9 Determination of Antibiotic Production by the Fungal Isolates

The antibiotic generation capability of fungal isolates in vitro against common human diseases was determined using methods developed by Williams and Cross [43]. The human pathogens used for this study were: *Escherichia coli, Klebsiella pneumonia* and *Staphyllococcus aureus.* Each fungal isolate was streaked on Nutrient Agar as a straight line and incubated at 30°C. After two days of incubation, the human pathogenic test organism *Escherichia coli*, *Klebsiella pneumonia* and *Staphylloccus aureus* were streaked perpendicular to the streaked line of the growing fungus. After a 24-hour incubation period at 30°C, the Zone of Inhibition of each test organism from the streaking line of the developing fungus was determined.

4. RESULTS AND FINDINGS

4.1 Results

An investigation was carried out in this study to determine the presence of antibiotic- producing fungi in the soil samples collected from five different sites within Ahmadu Bello University Main Campus Samaru, Zaria. The experimental result obtained in this study were presented in a tabular form. The list of tables is shown below:

Table 1. Show results of the physico-chemical Properties of soil samples gotten from the soil environment of Danfodio, Division of Agricultural College (DAC), Dangote, Akenzua and Sassakawa. The soil pH varies from 5.92 to 8.03 and the temperature range is between 26^{o}C to 32^{o}C respectively.

Table 2. Shows the macroscopic and microscopic characteristics of the fungal isolates. A total of six species of fungi were isolated. The macroscopic characteristics were based on colony colour, texture, elevation and reverse and also microscopic characteristics was based on their conidia, stipes, and vesicles.

Table 3. shows the occurrence of the fungal isolates from each soil samples. *Aspergillus niger*, *Aspergillus fumigatus* and *Fusarium* sp had occurence of 80% each *Penicillium* sp and *Aspergillus* sp had 40%

Table 4. Show the results of the antimicrobial activities of the fungal isolates metabolites against *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumonia.* The zone of inhibition produced against the test pathogens ranges from 3mm to 10mm respectively.

5. DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

The soil pH, is therefore, described as the "master soil variables" that influences myriads of soil biological, chemical and physical properties and processes that affect plant growth and biomass yield (Minasny et al., 2016).

In this study, the pH values of the soil samples ranges from 5.92 to 8.03. Danfodio had a pH of 5.92 and Sassakawa had pH of 8.03.

The variation in soil pH might be due to the activities carried out by microorganisms in the soil. This is in agreement with recent studies done by [44].

The temperature of the soil as at the time of collection (rainy season) revealed that the soil environment had a temperature range of 26□C to 32□C.

Aspergillus niger, *Aspergillus fumigatus* and *Fusarium* sp (P14) had the highest occurrence of 80% each. *Fusarium* sp (P15) had the least occurrence of 20%.

The high occurrence might be because the soil condition was favourable for the fungal growth and also because hyphae forming microorganisms (fungi) are best adapted to benefit from increased nutrient concentration in soil. The hyphae of fungi are able to colonize discrete pieces of organic matter very rapidly and are able to form bridges between nutrient rich areas [2].

Penicillium sp and *Aspergillus niger* produce the highest zone of Inhibition of 10mm and 9mm and *Fusarium* sp (P15) produced the least zone of Inhibition of 3mm.
The zone of inhibition produced might be as a result of some bio-active compounds or metabolites produced by the fungal isolates due to limiting nutrients. The antibiotic production is of importance in enhancing the ability to compete for nutrient [2].

5.2 Conclusion

The soil sample from each sites was found to be vary from slightly acidic to slightly alkaline with pH ranging from 5.92 to 8.03. The soil temperature ranges from 26□ to 32□.

A total of six fungi were isolated from the soil and they were identified based on their macroscopic and microscopic characteristics. They were *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus* sp, *Penicillium* sp, *Fusarium* sp (P14) and *Fusarium* sp (P15).

Aspergillus niger, *Aspergillus fumigatus* and *Fusarium* sp (P14) had the highest occurrence of 80% and *Fusarium* sp (P15) had the least occurrence of 20%.

All the fungal isolates metabolites inhibited at least one of the test organisms except *Fusarium* sp (P14). *Penicillium* sp and *Aspergillus niger* metabolites produced the highest zone of inhibition of 10mm and 9mm against *Staphylococcus aureus*.

This investigation reveals that some Fungal species isolated from the soil environment do produce some form of antimicrobials.

5.3 Recommendation

1. The soil harbors numerous kinds of microorganisms such as fungi and so activities involving the use of soil should be done appropriately or carefully.
2. Fungi are said to be both beneficial and harmful and so care should be taken while culturing them in the laboratory as some are said to be sporogenous (spore formers) which could be inhaled.
3. In the determination of antibiotic producing fungi, further methods should be used such as aqueous extract of the fungal isolates.
4. This investigation is a primary study; further investigation needs to be embarked upon to determine the type of antimicrobial substance(s) produced or the type of effect they cause on the pathogens whether static or cidal.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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