A survey of antibiotic-producing actinomycetes in the soil environment of Keffi metropolis, Nasarawa State, Nigeria

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The soil environment is a vast reservoir that harbours microorganisms that represent virtually all the microbial groups including actinomycetes. An investigation was carried out to determine the species of antibiotic-producing actinomycetes found in the soil environment of Keffi Metropolis, Nasarawa State, Nigeria. Soil samples were obtained from ten different locations of Keffi town for the isolation of actinomycetes. Eight species belonging to two genera were isolated from the soil samples and these include Actinomyces israelii, Actinomyces viscosus, Streptomyces antibioticus, Streptomyces aureofoiens, Streptomyces caespitosus, Streptomyces exfoliatus, Streptomyces griseus and Streptomyces hygroscopicus. All except S. aureofiens were found to have antimicrobial activity against at least one of the four pathogenic organisms tested for sensitivity. The pathogenic organisms tested for sensitivity were Candida albicans, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa. The results of this investigation revealed that species of antibiotic-producing actinomycetes are found in the soil environment of Keffi Metropolis, and these could be harnessed for the industrial production of novel antibiotics.

Key words: Antibiotic-producing actinomycetes, soil, Keffi, Nigeria.

INTRODUCTION

An antibiotic was originally defined as a substance produced by one microorganism, which inhibits the growth of other microorganisms (Berg et al., 2002). However, with the advent of synthetic methods of production, the definition has now been modified and consequently, an antibiotic is currently defined as a substance produced either (partly or wholly) by chemical synthesis which in low concentration inhibit the growth of, or kill other microorganisms (Cidaria et al., 1993; Berg et al., 2002; Dutta, 2005).

Antibiotics are substances produced by the natural metabolic processes of secondary metabolic processes of some microorganisms that can inhibit or even destroy microorganisms completely (Taylor et al., 2003; Denyer et al., 2004). Antibiotics are originally referred to as organic compounds produced by some species of actinomycetes, fungi, and bacteria, which are harmful to other microorganisms (Walsh, 2003).

Alexander Fleming in the year 1928, contributed immensely to the field of antibiotics. He discovered Penicillin accidentally as the first antibiotic which showed its efficacy in laboratory culture against many disease producing bacteria (Denyer et al., 2004).

In recent times, there has been growing interest in enumerating the number and types of microorganisms in the soil. This interest has arose from the desire to use enumeration results to indicate the health and productivity of a soil (Blum et al., 1987).

Soil microorganisms form a robust community capable of surviving and functioning even under extreme environmental conditions of temperature, water accessibility, pH, energy resources, nutrient availability and salt concentration (Butt and Ghaffar, 1972). The soil however, is a favourable habitat for the growth of...
microorganisms and is inhibited by a wide range of microorganisms including bacteria, algae, fungi, viruses and protozoa. Microorganisms are found in large numbers in soil, usually several millions are present per gram of soil with bacteria and fungi being the most prevalent (Desta, 1993). Bacteria are present in large numbers of about \(10^8\) to \(10^9\) organisms per gram of soil (Domsch et al., 1980). Actinomycetes are less common, about \(10^7\) to \(10^8\) organisms per gram of soil (Morell, 1997). For fungi, the figures are between \(10^7\) to \(10^9\) propagules per gram or of soil (Domsch et al., 1980). All these three groups of organisms represent a sizable amount of living matter in the soil (Aizawa et al., 1979).

The fungi are the largest of the group of common soil microorganisms. They are also the most familiar since we can often see colonies of fungi growing on organic matter. The actinomycetes are smaller than fungi but also filamentous and may have branches. They are perhaps best known for production of antibiotics (Dubey and Masheshwari, 2004).

The name actinomycetes stems from a Greek word meaning “ray fungi” (Bacon et al., 1996). Actinomycetes are unicellular organisms that mass together to form filaments called hyphae (Blizzard et al., 1989; Cidaria et al., 1993; Dutta 2005). Colonies of actinomycetes can then form a mass of intertwined hyphae called a mycelium.

Some scientists considered actinomycetes to be bacteria, while some peg them as fungi, still others think that actinomycetes are the prototype from which both bacteria and fungi are derived (Blum et al., 1987; Morell, 1997). Some believe that actinomycetes should be in a separate group between true bacteria and the filamentous fungi (Dutta, 2005). The soil dwelling actinomycetes that give us a variety of antibiotics are mostly from the genus Streptomyces (Denyer et al., 2004). Actinomycetes are well known as secondary metabolite producers, and hence they are of high pharmacological and commercial interest (Brooks et al., 2004). Waksman (1947) discovered that a soil actinomycete he was studying made actinomycin, a discovery which granted him a Nobel prize. Since then hundreds of naturally occurring antibiotics have been discovered in these terrestrial microorganisms, especially from the genus Streptomyces (Best, 1985). Cheesbrough (2006) outlined four major genera of actinomycetes to include Actinomadura, Actinomyces, Nocardia and Streptomyces. This investigation was aimed at screening for antibiotic-producing species of actinomycetes present in the soil environment of Keffi Metropolis, Nasarawa State, Nigeria.

**MATERIALS AND METHODS**

**Study area**

This study was carried out in Keffi metropolis of Nasarawa state, Nigeria. Keffi is about 58 km from Abuja, the Federal Capital Territory (FCT), and is about 128 km from Lafia, the capital town of Nasarawa state. Keffi is situated on latitude 8°5’N and longitude 7°50’E, and on the altitude of 850 m above the sea level (Akwa et al., 2007).

**Sample collection**

The soil samples were collected from ten different sites which were selected using random sampling technique. The ten sites were Angwan Lambu, Angwan Mada, Angwan Woje, Angwan Tofa, Government Reserve Area (G.R.A), High Court, Emir’s Palace, Karoffi, Nasarawa road and Pyanku campus. All the soil samples were aseptically collected with sterile spatula into newly opened polythene bags and taken to the Laboratory for analysis.

**Determination of physico-chemical properties**

**Soil types**

The soil samples were determined into types by the sieve analysis method. The sieve used had pore diameters of 0.2 to 2.0 mm (Pettijohn, 2000).

**pH**

The pH of the soil samples were determined by digital pH meter using standard methods of Watson and Brown (1998).

**Temperature**

The temperature of the soil samples were determined by the use of field thermometer. The thermometer was inserted 5 cm deep in-situ into each sampling site and this was allowed to stay for 10 min after which the average of three consecutive temperature readings were obtained and recorded for each site (Dix and Webster, 1995).

**Isolation of soil actinomycetes**

The method of Prescott et al. (2006) was adopted for the isolation of the soil actinomycetes with Nutrient Agar (NA). Both the direct soil inoculation and the soil dilution methods were employed using pour plate technique.

**Determination of antimicrobial activity of actinomycetes isolates**

The antimicrobial activity of the actinomycetes isolates was determined by sensitivity assay using pathogenic organisms which included Candida albicans, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa as test organisms. The test organisms were obtained from the Federal Medical Centre, Keffi, where they were isolated from patients. Isolates of these pathogenic organisms were brought to the Microbiology Laboratory of Nasarawa State University, Keffi, where their identities were further confirmed. Culture plates of the test organisms were prepared and standardization of the cultures were done according to the method of Baker and Thomsberry (1983), by suspending five colonies of overnight culture in 5 ml of nutrient broth.

The standardized cultures of the test organisms were inoculated by spread plate method onto Nutrient Agar (Cowen and Steel, 1965) and were allowed to stay for 10 min for the moisture in the inocula to be absorbed by the nutrient agar. Wells were made in
Table 1. Physico-chemical properties of soil samples collected from different locations in Keffi metropolis.

<table>
<thead>
<tr>
<th>Location</th>
<th>Soil types</th>
<th>pH</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Loamy</td>
<td>7.2 ± 0.4</td>
<td>25 ± 0.5</td>
</tr>
<tr>
<td>B</td>
<td>Loamy</td>
<td>6.4 ± 0.4</td>
<td>25 ± 0.5</td>
</tr>
<tr>
<td>C</td>
<td>Sandy</td>
<td>5.8 ± 1.0</td>
<td>25 ± 0.5</td>
</tr>
<tr>
<td>D</td>
<td>Sandy</td>
<td>7.4 ± 0.6</td>
<td>26 ± 0.5</td>
</tr>
<tr>
<td>E</td>
<td>Sandy</td>
<td>5.6 ± 0.2</td>
<td>26 ± 0.5</td>
</tr>
<tr>
<td>F</td>
<td>Loamy</td>
<td>6.8 ± 0.0</td>
<td>26 ± 0.5</td>
</tr>
<tr>
<td>G</td>
<td>Sandy</td>
<td>6.0 ± 0.8</td>
<td>26 ± 0.5</td>
</tr>
<tr>
<td>H</td>
<td>Sandy</td>
<td>7.0 ± 0.2</td>
<td>25 ± 0.5</td>
</tr>
<tr>
<td>I</td>
<td>Sandy</td>
<td>7.6 ± 0.8</td>
<td>26 ± 0.5</td>
</tr>
<tr>
<td>M</td>
<td>Sandy</td>
<td>8.0 ± 1.2</td>
<td>26 ± 0.5</td>
</tr>
</tbody>
</table>

A= Angwan Lambu, B= Angwan Mada, C= Angwan Woje, D= Angwan Tofa, E= Government Reserve Area, F= High Court, G= Emir’s Palace, H= Karoffi, I= Nasarawa road, M= Pyanku Campus.

Table 2. Total aerobic plate count of actinomycetes isolates.

<table>
<thead>
<tr>
<th>Location</th>
<th>Total Actinomycetes count (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8.0 × 10^5 ± 2.7</td>
</tr>
<tr>
<td>B</td>
<td>6.0 × 10^5 ± 0.7</td>
</tr>
<tr>
<td>C</td>
<td>4.0 × 10^5 ± 1.3</td>
</tr>
<tr>
<td>D</td>
<td>7.0 × 10^5 ± 1.7</td>
</tr>
<tr>
<td>E</td>
<td>6.0 × 10^5 ± 0.7</td>
</tr>
<tr>
<td>F</td>
<td>6.0 × 10^5 ± 0.7</td>
</tr>
<tr>
<td>G</td>
<td>5.0 × 10^5 ± 0.3</td>
</tr>
<tr>
<td>H</td>
<td>7.0 × 10^5 ± 1.7</td>
</tr>
<tr>
<td>I</td>
<td>4.0 × 10^5 ± 1.3</td>
</tr>
<tr>
<td>M</td>
<td>3.0 × 10^5 ± 2.3</td>
</tr>
</tbody>
</table>

A= Angwan Lambu, B= Angwan Mada, C= Angwan Woje, D= Angwan Tofa, E= Government Reserve Area, F= High Court, G= Emir’s Palace, H= Karoffi, I= Nasarawa road, M= Pyanku Campus.

RESULTS

Table 1 shows the physico-chemical properties of the soil samples collected from different locations in Keffi metropolis, while Table 2 shows the total aerobic plate count of actinomycetes isolated from the ten different soil locations. Table 3 shows the percentage occurrence frequencies of actinomycetes isolates in the soil of the different locations of the Keffi metropolis, and Table 4 shows the diameter (mm) zone of inhibition of sensitive organisms to the supernatant of nutrient culture broth of the actinomycetes isolates.

The result of the physico-chemical properties of soil samples showed that the soil of Angwan Woje, Angwan Tofa, Government Reserve Area, Emir’s Palace, Karoffi, Nasarawa Road and Pyanku Campus are sandy, while the soil of Angwan Lambu, Angwan Mada and High Court are loamy. Soil samples from Angwan Lambu, Angwan Tofa, Nasarawa road and Pyanku Campus had slightly alkaline pH which range from 7.2 to 8.0, while the soil from Angwan Woje and Government Reserve Area had acidic pH which range from 5.6 to 5.8. However, soil samples from Karoffi had a pH of 7.0. The soil temperatures of the different locations in Keffi during this investigation ranged between 25 and 26°C (Table 1).

The result of the total actinomycetes count revealed that the soil of Angwan Lambu, Angwan Tofa, Karoffi, and Angwan Mada had higher counts of 8.0 × 10^5, 7.0 × 10^5 and 7.0 × 10^5, respectively (Table 2). The percentage occurrence frequency of actinomycetes isolates were Streptomyces hygroscopicus (50%), Streptomyces griseus (50%), and Streptomyces antibioticus (60%), Streptomyces caesiptosus (30%) Streptomyces exfoliatu (40%), Actinomyces israelii (30%), Actinomyces viscosus (30%), and Streptomyces aureofiens (10%), respectively. The result of the sensitivity test shows that most of the actinomycetes isolates had antimicrobial activities against the test pathogens which demonstrated that the isolates were responsible for producing antimicrobial substance(s) that inhibited the test pathogenic organisms (Table 3).

The diameter zone of inhibition for the supernatant from S. hygroscopicus were C. albicans (21 mm), P. aeruginosa (11 mm), S. aureus (24 mm), and E. coli (11 mm), while the supernatant from S. exfoliatu showed zone of inhibition against only C. albicans (21 mm). The supernatant of A. israelii showed zones of inhibition against C. albicans (17 mm), P. aeruginosa (14 mm) and E. coli (20 mm), but no zone of inhibition against S. aureus. The supernatant of S. antibioticus showed no zone of inhibition against C. albicans but showed zones of inhibition against S. aureus (24 mm) and E. coli (14 mm). The supernatant from S. griseus showed no zone of inhibition against both C. albicans and P. aeruginosa, but showed zones of inhibition against S. aureus (12 mm) and E. coli (10 mm). The supernatant of S. caesiptosus showed no zone of inhibition against C. albicans and P.
Table 3. Percentage occurrence frequency of actinomycetes isolates.

<table>
<thead>
<tr>
<th>Actinomycetes isolates</th>
<th>Locations</th>
<th>Occurrence frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomyces hygroscopicus</td>
<td>A B C D E F G H I M</td>
<td></td>
</tr>
<tr>
<td>Streptomyces exfoliatus</td>
<td>- + + - - + + + + + +</td>
<td>50</td>
</tr>
<tr>
<td>Actinomyces israelii</td>
<td>+ + + + - - - + + + +</td>
<td>40</td>
</tr>
<tr>
<td>Streptomyces caesipitosus</td>
<td>- + - + + - + - - +</td>
<td>30</td>
</tr>
<tr>
<td>Streptomyces griseus</td>
<td>- + - + - - + - + +</td>
<td>30</td>
</tr>
<tr>
<td>Streptomyces antibiotics</td>
<td>+ + + - - - + - + +</td>
<td>30</td>
</tr>
<tr>
<td>Streptomyces aureofiens</td>
<td>- - + + + - + - - -</td>
<td>10</td>
</tr>
<tr>
<td>Actinomyces viscosus</td>
<td>+ + - - - - - + + +</td>
<td>30</td>
</tr>
</tbody>
</table>

+= Positive, - = Negative, A= Angwan Lambu, B= Angwan Mada, C= Angwan Woje, D= Angwan Tofa, E= Government Reserve Area, F= High Court, G= Emir’s Palace, H= Karoffi, I= Nasarawa road, M= Pyanku Campus.

Table 4. Diameter zone of inhibition (mm) of pathogenic organisms to supernatant of nutrient broth of actinomycetes isolates.

<table>
<thead>
<tr>
<th>Supernatant of nutrient broth of isolates</th>
<th>Candida albicans</th>
<th>Pseudomonas aeruginosa</th>
<th>Staphylococcus aureus</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomyces hygroscopicus</td>
<td>21 ± 3.7</td>
<td>11 ± 1.5</td>
<td>24 ± 8.0</td>
<td>11 ± 1.6</td>
</tr>
<tr>
<td>Streptomyces exfoliatus</td>
<td>21 ± 3.7</td>
<td>00 ± 0.0</td>
<td>00 ± 0.0</td>
<td>00 ± 0.0</td>
</tr>
<tr>
<td>Actinomyces israelii</td>
<td>17 ± 0.3</td>
<td>14 ± 1.5</td>
<td>00 ± 0.0</td>
<td>20 ± 7.4</td>
</tr>
<tr>
<td>Streptomyces caesipitosus</td>
<td>00 ± 0.0</td>
<td>00 ± 0.0</td>
<td>24 ± 8.0</td>
<td>14 ± 2.1</td>
</tr>
<tr>
<td>Streptomyces griseus</td>
<td>00 ± 0.0</td>
<td>00 ± 0.0</td>
<td>12 ± 4.0</td>
<td>10 ± 2.6</td>
</tr>
<tr>
<td>Streptomyces antibiotics</td>
<td>00 ± 0.0</td>
<td>00 ± 0.0</td>
<td>10 ± 6.0</td>
<td>10 ± 2.6</td>
</tr>
<tr>
<td>Streptomyces aureofiens</td>
<td>00 ± 0.0</td>
<td>00 ± 0.0</td>
<td>00 ± 0.0</td>
<td>00 ± 0.0</td>
</tr>
<tr>
<td>Actinomyces viscosus</td>
<td>10 ± 7.3</td>
<td>00 ± 0.0</td>
<td>10 ± 6.0</td>
<td>11 ± 1.6</td>
</tr>
</tbody>
</table>

aeruginosa, but showed zone of inhibition against S. aureus (10 mm) and E. coli (10 mm). The supernatant of S. aureofiens showed no zone of inhibition against any of the test organisms. The supernatant of A. viscosus showed zones of inhibition against C. albicans (10 mm), S. aureus (10 mm), and E. coli (11 mm), but showed no zone of inhibition against P. aeruginosa (Table 4).

**DISCUSSION**

The results of this investigation demonstrate that S. hygroscopicus, S. exfoliatus, S. caesipitosus, S. griseus, S. antibioticus, A. israelii and A. viscosus are antibiotic producing actinomycetes found in the soil environment of Keffi metropolis. These isolates belong to two genera of actinomycetes. Cheesbrough (2006) had earlier reported that there are four genera of actinomycetes (Actinomadura, Actinomyces, Nocardia and Streptomyces). The implication of these findings is that only two out of the four commonly known genera of actinomycetes were present in the soil environment of Keffi metropolis. The continuous threats of drug resistance by some pathogenic strains of microorganisms as reported by several workers (Horvat et al., 2003; Doxboeck et al., 2004; Magee, 2004) has made the search for novel antibiotics a worthwhile venture. Anjea (2003) reported that soil is the best economical source of antibiotic producing microorganisms, and the soils around the world are continuously being screened for novel antibiotics. The soil is thus a veritable source of antibiotic-producing microorganisms from which novel antibiotics can be sought. The results of this investigation has further confirmed that soil dwelling antibiotic producing actinomycetes are mostly in the genus Streptomyces as reported by several workers (Brooks et al., 2004; Denyer et al., 2004; Dubey and Maheshwari, 2004). Therefore the antibiotic producing actinomycetes isolated from the soil environment of Keffi can be harnessed for the production of novel antibiotics. These isolates can be screened to select high yielding strains. The results of this investigation are preliminary, thus the need for further research in order to determine the types and quantities of antibiotics produced by each isolate, and to possibly purify the antibiotics and characterize their chemical structures. Further research into strains of actinomycetes...
that are isolated from local environments would stimulate mass production of novel antibiotics in either synthetic or semi-synthetic forms, and this will go a long way in combating the problem of drug resistance.

The results obtained so far, provides some information that would be useful to pharmaceutical industries that may be interested in the local production of antibiotics using locally isolated strains of actinomycetes. The results of these findings are thus recommended to manufacturers of pharmaceutical products, particularly antibiotics, to fund further research in this area which would eventually lead to development of high yielding strains.

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REFERENCES


