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Screening for antibacterial producing bacteria isolated from soil in Keffi, Nasarawa, State, Nigeria

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Abstract

Antibiotics are secondary metabolites that inhibit other competing cells to give competitive advantage to the microorganisms that produce them. This study is aimed at screening for antibacterial producing bacteria isolated from soil in Keffi. Standard microbiological methods were used in isolation and screening for antimicrobial of activity of crude extracts. Molecular identification of bacteria isolated were carried out using 16srRNA method. The antibacterial activity was carried out using agar well diffusion methods. The highest bacteria were Isolated from maize rhizosphere (90.0%) and the least was from groundnut rhizosphere (50.0%). Bacterial isolated and identified using 16srRNA were *Pseudomonas fluorescens*, *Pseudomonas antarctica*, *Bacillus megaterium* and *Acetobacter aceti*. The screening for antimicrobial producing bacteria showed that bacterial with laboratory code Gn3 (*Pseudomonas fluorescens*) showed activity against *E. coli* (8.57±1.01 mm), *S. typhi* (5.10±0.11 mm) and *S. aureus* (6.12±0.32 mm). Ma1 had activity against *E. coli* (8.57±1.01 mm), *S. typhi* (5.10±0.11 mm) and *S. aureus* (6.12±0.32 mm). Ma2 had activity against *E. coli* (7.56±1.33 mm) and *S. typhi* (4.54±0.34 mm) and Rc2 had activity only against *S. aureus* (12.12±0.98 mm) The Antibacterial activity of bacteria isolated and grown in complex medium with nitrogen source revealed that *Pseudomonas fluorescens* showed activity against the test organism *E. coli* (14.03±1.06mm), *S. typhi* (9.11±1.11mm) and *S. aureus* (5.12±1.02 mm). It was observed in this study that bacterial isolated from soil in Keffi were able to produce substance that that inhibit other bacterial.

Keywords: Antibacterial activity; Soil; Bacteria isolated; Nitrogen source; Inhibit

1. Introduction

Antibiotics have always been considered as one of the wonderful discoveries of the 20th century [1] Selman Waksman first used the word antibiotic as noun in 1941 to describe any small molecule made by a microbe that antagonizes the growth of other microbes [2]. As currently defined, antibiotics are chemical substances produced by microorganisms which in small amount or low concentration selectively inhibit or kill other microorganisms [3]. They are secondary metabolites that inhibit other competing cells to give competitive advantage to the microorganisms that produce them [4]. According to Sethi, *et al.*, [1] the importance and value of antibiotics cannot be over emphasized; they are used to treat infectious diseases that are caused by microorganisms. Antibiotics are widely distributed in the nature, where they play an important role in regulating the microbial population of soil, water, sewage and compost. Antimicrobial agents are natural products and produced by various types of bacteria and fungi [5].

Antibiotics in one form or the other has been in use for centuries [1]. Hundreds of these natural products have been identified and developed as therapeutic agents against many infectious diseases [5]. Routine screening is ongoing on bacteria for new bioactive compounds because they have provided many important bioactive compounds of high

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commercial value [6]. The isolation of antibiotics from microorganisms has improved the discovery of novel antibiotics that could act as better chemotherapeutic agent [7]. However, Microbial natural metabolites still appear as the most promising sources of antibiotic in future [8]. In addition, due to the fact that *Bacillus* species have produced antibiotics in soluble protein structure and that these antibiotics have been found to be cheaper and more effective in studies conducted to date, these microorganisms are preferable for commercial production [1]. From all the known microbes, bacteria are one of the most important sources of biologically and commercially important antibiotics [9]. This study focus on screening for antimicrobial producing bacteria isolated from soil in Keffi.

2. Material and methods

2.1. Study Area

This study was carried out in Keffi, Nasarawa State, Nigeria. Keffi is approximately 68km away from Federal Capital Territory (FCT), Abuja and 128km away from the State Capital, Lafia and is located at located at longitude 8'5E along the Greenwich meridians and at latitude 7'5N at the equator and is 850m above the sea level [10].

2.2. Sample collection

The soil sample were collected from the rhizosphere of rice, groundnut and maize in farm land within Nasarawa State University main campus using a method described as [11] the soil samples were collected by using spoon from depth of 5cm from the rhizosphere the plants and was transfer to sterile polytene bag and transported to Microbiology Laboratory, Nasarawa State University, Keffi, for analysis.

2.3. Isolation of Bacteria

The bacteria were isolated from soil of rhizosphere of rice, groundnut and maize by modification of the method described [12]. One gram 10 g of rhizosphere soil was suspended in 90 ml of sterile distilled water and 10-fold dilutions was made and 0.5 ml of the aliquot was spread on nutrient and Cetrimide agar and incubated at 30 °C for 24 h. Colonies with light greenish, yellowish colonies were further streaked on King's B agar and incubated at 30 °C for 24 h.

2.4. Test organisms (bacterial)

The test organisms were obtained from Department of Microbiology laboratory Nasarawa State Keffi.

2.5. Identification of Bacteria

2.5.1. Gram Staining

The gram staining of the suspected bacterial isolates was carried out as earlier described [13]. A smear of one (1) pure colonies of suspected organism was made on a drop of water on a slide and allow the slide to air dry. The slide was passed twice the flame to air dry and flood with crystal violet solution for 30 sec. and rinse under running tap water and flood with Lugol's iodine solution for 30 sec. and rinse under running tap water and briefly decolourized with 90% ethanol and immediately rinse under tap water and flood with Safranin for 60sec. and rinse under running tap water and allow to air dry and the slide was examined under x100 oil immersion objective.

2.5.2. Biochemical Test

Biochemical test such as catalase test, indole test, methyl red and Voges-Proskauer test, citrate utilization test, oxidase test were carried out using a described [13].

2.5.3. Molecular Identification of Bacteria Species

DNA was extracted using the following protocol: Single colonies of bacteria grown on medium were transferred to 1.5 mL of nutrient broth and cultures grown on a shaker at 28 °C for 48 hours. After this period, cultures were centrifuged at 4600 xg for 5 minutes. The resulting pellets were re-suspended in 520 µL of Tris Acetate-EDTA buffer (10mM Tris HCl, 1mM EDTA, pH 8.0). Fifteen microliters of 20 % SDS and 3µL of Proteinase K (20 mg/mL) were then added. The mixture was incubated at 37 °C for one hour, then 100 mL of 5M NaCl and 80 µL of a 10% CTAB solution in 0.7 M NaCl were added and mixed. The mixture was incubated at 65 °C for 10 minutes and kept on ice for 15 minutes. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 minutes and centrifugation at 7200 xg for 20 minutes. The aqueous phase was transferred to a new tube, and isopropanol (1: 0.6) was then added and DNA precipitated at –20 °C for 16 hours. The DNA was collected by centrifugation at 7200 xg for 15minutes, washed with 500 µL of 70% ethanol, air-dried at room temperature for three hours and finally dissolved in 50 µL of Tris Acetate-

EDTA buffer [14]. Purity and concentration of DNA solutions were measured using Computerized Nano Drop-spectrophotometer. The Nano Drop spectrophotometer measures DNA purity and concentration according to the following equations: DNA purity = Absorbance at 260 nm / Absorbance at 280 nm DNA yield (µg) = DNA concentration (µg/µl) × total sample volume (ml). The quality of the isolated DNA was also evaluated by 1% Agarose gel electrophoresis. A 100 bp plus DNA ladder (SolGent, Korea) was used as a molecular weight standard to compare the intensity and approximate size of the isolated DNA.

2.5.4. Polymerase Chain Reaction (PCR)

The selected primers in lyophhilized form were re-dissolved with TE(Tris-EDTA) buffer (pH 8) to a final concentration of 100 pmol/µl and stored at -20 °C. The sequence of the primer set Lacto -27 forward - was 5'..AGA GTT TGA TGG CTC CAG CG...3' and the primer set Lacto -1525R reverse - was 5'..AAG GAG GTG ATC AGC GG ..3'. Amplifications were carried out in 30 µl volumes containing (10 pmol/µl) of each primer, 5x Taq colourless reaction and 3µl of $MgCl_{2,.}$ 8µl DNA template and 0.3units of Taq DNA polymerase (Pomega, USA) were mixed to give 42µl. DNA. Amplification was achieved in 30 cycles using a GeneAmp 9700 PCR system (GTC) thermal cycler (Applied Biosystem Inc., USA). Prior to the first cycle, the PCR profile was an initial denaturation of 30 cycles at 94 °C for 5 minutes; 50 °C for 60 seconds, 72 °C for 1 minute 30 seconds and a final extension at 72 °C for 10minutes and was allowed to cool to 4 °C [14].

2.5.5. Integrity Check of the Amplified Gene

The integrity of the amplified gene fragment was checked on a 1% Agrose gel to confirm the amplification. This was carried out by mixing 8 μL of amplified product to 4 μL of loading dye ran on Agrose gel at 110 V for 1 hour. Similarly, the amplified product was checked on a nanodrop of model 2000 from thermo scientific to quantify the concentration of the amplified product.

2.5.6. Purification of Amplified Product

The amplified fragments were purified with ethanol in order to remove the PCR reagents, debris and other reagents as follows: seven point six microliter (7.6 μL) of sodium acetate 3 molar and 240 μL of 95 % ethanol were added to each fragment of the PCR amplified products in a new sterile Eppendorf tube; it was then vortexed for 5 seconds and kept at -50-20 °C for 30 minutes. The mixture was centrifuged at 13000 xg for 10 minutes and 4 °C followed by the removal of the supernatant (by inverting the tube on trash once) after which the pellet was washed by adding 150 μL of 70% ethanol, mixed and then centrifuged for 15 minutes at 7500 rpm and 4 °C. Again the supernatant was decanted and the tube inverted on blotting paper and allowed to dry in the fume hood at room temperature for 15 minutes. It was then suspended in 20 μL of distilled water and kept in a refrigerator at -20 °C prior to sequencing. The purified fragment was checked on a 1.5 % Agarose gel and ran on a voltage of 110 V for about 1 hour, to confirm the presence of the purified product before sequencing [15].

2.5.7. Sequencing and Blasting

The amplified fragments were sequenced using a genetic Analyser 3130xl sequencer from Applied Bio systems. The sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio-Edit software and MEGA 6 were used for all genetic analysis. Blocks of polyacrylamide gels containing selected denaturing gradient gel electrophoresis bands were punched by sterile pipette tips. The blocks were then transferred in 50 µl of sterile water, and the DNA of the bands was left to diffuse overnight at 4 °C. Two microliters of the eluted DNA was used for the reamplification, and the PCR products, generated with the GC (Guanine-Cytosine) clamped primer were checked by denaturing gradient gel electrophoresis with amplified *Lactobacillus* DNA as a control. Only products migrating as a single band and at the same position with respect to the control were amplified with the selected primers without the GC clamp, purified, and sequenced. Identities and accession numbers of the test organisms were determined by BLAST (Basic Local Alignment Search Tool) from the GenBank database of the National Collection for Biotechnological Information [15].

2.6. On spot screening for antibacterial production by bacterial isolated from soil

The spot on lawn technique was used to screen antibacterial activity of the Bacterial isolates. Overnight cultures of each isolate were applied as single plate of Muller-Hinton agar and incubated at 35 °C for 24 hours. After incubation, Muller-Hinton agar plates were covered with 2 mL of Muller-Hinton broth inoculated with one of the test organisms. Separate plates containing one of the Bacterial isolates were overlaid with each of the test organisms and each test was performed in triplicate. After incubation for 24 hours at temperature of 35 °C the test organism, the samples were examined for evidence of inhibition.

2.7. Screening for antibacterial activities of crude cell-free extract of bacteria isolates

The screening of antibacterial activities of the bacterial isolates against clinical isolates obtained from department of microbiology laboratory was carried out using agar well diffusion as described by Makut *et al.* [16]. The wells were punched into the agar medium using 8 mm diameter cap bower and Inoculated with 10^6 cfu/ml of each test bacterial to be tested agar well plates with a sterile swab moistened with the bacterial suspension. Two (2) colonies of bacteria isolate from rhizosphere of rice, groundnut and maize were inoculated into 5 ml of Muller-Hinton broth and incubated at 30 °C for 48 h. The 48h broth were centrifuged at 10000 rpm for 20 mins and 0.1 ml of the cell free extract was introduced in to the agar well made on Muller-Hinton agar plate and the plates were incubated at 30 °C for 24 h and the diameter zone of inhibition was recorded and the experiment was carried out in duplicate.

2.8. Evaluation of Antibacterial Activities of Bacteria under Complex Carbon and Nitrogen Source.

The antibacterial activities of antibiotic-producing bacteria under different concentration of complex carbon and nitrogen source against clinical isolates was carried out using a method earlier described [16]. 1ml of 24 h nutrient broth culture of antibiotic-producing bacteria isolates were adjusted to 10^7cfu/ml sterile water and inoculated into 49 ml of M1 (0.98 g/L of K_2HPO_4; 0.4 g/L of $MgSO_4$; 0.4 g/L of $CaCO_3$; Banana peels (starch) 20g/l and organic nitrogen sources (soya bean cake); M2 (0.98 g/L of K_2HPO_4; 0.4 g/L of $MgSO_4$; 0.4g/L of $CaCO_3$; 10g/l glucose; 2.0g/l of organic nitrogen sources (soya beans) and adjusted to pH of 7.4 and were incubated at 30°C for 48 h. After incubation, the culture was centrifuge at 10000 rpm for 20 mins and 0.1 ml of the cell free extract was introduced into the prepared agar well made on Muller-Hinton agar plate flooded with adjusted inoculums of the test organisms (10^5 cfu) and the plates was incubated at 30 °C for 24 h and the diameter zone of inhibition was recorded and the experiment was carried out in duplicate.

3. Results

3.1. Occurrence of different bacteria from the rhizosphere of plant

The occurrence of bacterial isolated from rhizosphere is as shown in Table 1. The total occurrence of bacteria isolated was 21(70.0%) where the highest bacteria was isolated from rhizosphere of maize (90.0 %) followed by rice (80.0%) and the least was from groundnut (50.0 %) respectively.

3.2. Identification of Bacteria isolated

The cultural, morphological and biochemical characteristics of bacteria isolates from rhizosphere of rice, maize and ground nut plants from Nasarawa State University Main Campus, Keffi is as shown in Table 2. The agarose gel electrophoresis of the bacteria identified using 16srRNA is as shown in Fig 1. Where the DNA bands of the bacteria and molecular wight is observed at 1200 base pair. The phylogeny tree of evolutionary relatedness of the bacteria is as given in Fig 2- 5.

3.3. Percentage Occurrence of different bacteria from rhizosphere

The percentage occurrence of different bacteria isolates is as given in Table 3. The percentage occurrence of bacteria from rice rhizosphere showed that *Pseudomonas fluorescens* had the highest percentage occurrence (30.0%) followed by *Bacillus* megaterium and *Proteus* sp (20,0%) and the least was *Entrobacter* sp (10.0 %). From Ground nut rhizosphere the highest percentage occurring bacteria was also, *Pseudomonas fluorescens* (30.0 %) followed by *Gluconbacter* sp and *Acetobacter* sp (20.0%) and the lowest was *Bacillus megaterium* (10.0 %) and from Maize rhizosphere *Entrobacter* sp and *Proteus* sp was the highest occurring bacteria (20.0 %) and the least were *Pseudomonas fluorescens* and *Gluconbacter* sp (10.0%) respectively as showed in Table 3.

3.4. Antibacterial Activity of antibiotic producing Bacteria Isolates

The antibacterial activity of cell-free extract of antibiotics producing bacteria isolates against clinical isolates is as shown in Table 4. The antibacterial activity of bacteria isolated showed that Gn3 (*Pseudomonas fluorescens*) had activity against *E. coli* with inhibition zone of 11.2±0.12 mm and *S. aureus* with 9.03±0.03 mm and had no activity against *S. typhi.* Similarly, Ma1 (*Bacillus megaterium*) had activity against all the test organism namely *E. coli* (8.57±1.01mm), *S. typhi* (5.10±0.11 mm) and *S. aureus* (6.12±0.32 mm). Ma2 (*Pseudomonas antarctica*) had activity against *E. coli* (7.56±1.33mm) and *S. typhi* (4.54±0.34 mm) and least Rc2 (*Acetobacter)* aceti had activity only against *S. aureus* (12.12±0.98mm) as shown in Table 4 respectively.

The antibacterial activity of different antibiotics producing bacteria grown in a complex carbon and nitrogen sources against clinical isolates is as shown in Table 5. *Pseudomonas fluorescens* grown in a complex carbon and nitrogen sources showed activity to the entire test organism where it showed the inhibition zone of 14.03±1.06mm against *E. coli,* 9.11±1.11mm against *S. typhi* and 5.12±1.02mm against *S. aureus* similarly *Pseudomonas antarctica* showed activity of 4.15±0.22mm against *E. coli* and 3.34±0.87mm against *S. aureus. Bacillus megaterium* showed activity of 7.80±0.23mm against *E. coli* and 8.12±0.54mm against *S. aureus. Acetobacter* aceti did not show any activity to any of the test organism.

Table 1 Occurrence of Bacteria Isolate from Rhizosphere

Plants	**No. samples**	**No. (%) isolated**
Rice	10	8(80.0)
ground nut	10	5(50.0)
Maize	10	9(90.0)
Total	30	21 (70.0)

Table 2 Cultural, Morphological and Biochemical Characteristics Bacteria Isolates

Isolates	**Cultural morphology**	**G/ s**	**Biochemical characteristic**							**Inference**
			Cat	**In**	**Ox**	**Nit**	**MR**	**cit**	**Ur**	
Gna	Colonies with greenish on NA	-	+	-	+	+	-	-	+	*Pseudomonas* sp
Gnb	Yellowish smooth edge and large colony	-	-	+	-	+	-	+	-	*Gluconbacter* sp
Gnc	Brown mucoid smooth on Mac Agar	-	+	+	-	+	+	-	+	*Pseudomonas* sp
Gnd	Creamy, bulk dried and nono smooth edge	+	+	-	-	+	-	+	-	*Bacillus* sp
Gne	Creamy cocci, raised colony	+	-	+	+	+	+	-	+	*Gluconbacter* sp
Rca	Greenish on NA	-	+	-	+	+	-	-	+	*Pseudomonas* sp
Rcb	Large, circular, regular and milky white on NA	+								*Acetobacter* sp
Rcc	Brown mucoid smooth on Mac Agar	-	+	+	-	+	+	-	+	*Proteus* sp
Rcd	colonies are Colourless, flat and swarm MaC and NA	-	-	+	-	+	+		+	*Bacillus* sp
Maa	Greenish on NA	-	+	-	+	+	-	-	+	*Pseudomonas* sp

Key: Gna = Groundnut a, Gnb = Groundnut b, Gnc = Groundnut c, Gnd = Groundnut d, Gne = Groundnut e, Rca = Rice a, Rcb = Rice b, Rcc = Rice c, Rcd = Rice d, Maa = Maize a, Mab = Maize b, Mac = Maize c,

Table 3 Percentage occurrence of different species of bacteria

Bacteria species	No. sample	Rice No. (%)	Ground nut No. (%)	Maize No. (%)
Pseudomonas sp	10	3(30.0)	3(30.0)	1(10.0)
Gluconbacter sp	10	0(0.0)	2(20.0)	1(10.0)
Bacillus sp	10	2(20.0)	1(10.0)	0(0.0)
Acetobacter sp	10	0(0.0)	2(20.0)	0(0.0)
Entrobacter sp	10	1(10.0)	0(0.0)	2(20.0)
Proteus sp	10	2(20.0)	0(0.0)	2(20.0)

Table 4 Antibacterial Activities of Cell-free Extract from Bacteria Isolates Against Clinical Isolates

	Inhibition zone (Mean± S.D) in Millimetre (mm)		
Isolates codes	***E. coli***	***S. typhi***	***S. aureus***
Gn1	0.0±0.0	0.0±0.0	0.0±0.0
Gn3	11.2±0.12	0.0±0.0	9.03±0.03
Rc1	0.0±0.0	0.0±0.0	0.0±0.0
Ma1	8.57±1.01	5.10±0.11	6.12±0.32
Gn2	0.0±0.0	0.0±0.0	0.0±0.0
Gn5	0.0±0.0	0.0±0.0	0.0±0.0
Rc4	0.0±0.0	0.0±0.0	0.0±0.0
Gn4	0.0±0.0	0.0±0.0	0.0±0.0
Ma2	7.56±1.33	4.54±0.34	0.0±0.0
Rc2	0.0±0.0	0.0±0.0	12.12±0.98
Rc3	0.0±0.0	0.0±0.0	0.0±0.0
Ma3	0.0±0.0	0.0±0.0	0.0±0.0

Key: 0.0±0.0 = No inhibitionn zone

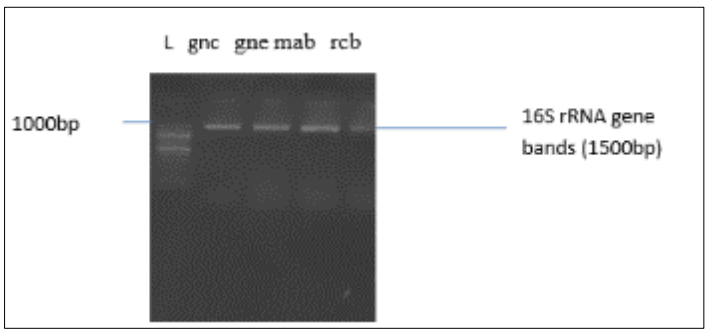

Figure 1 Agarose gel electrophoresis of the 16S rRNA gene of some selected bacterial isolates. Lanes 1= gnc, 2= gne, 3= mab and 4= rcb represent the 16SrRNA gene bands (1500bp), lane L represents the 100bp molecular ladder

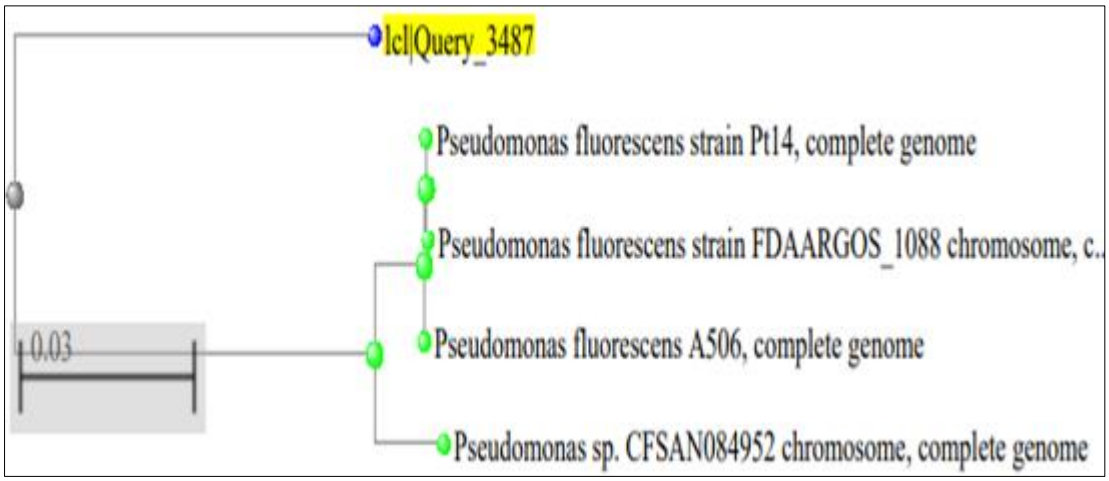

Figure 2 Phylogenic tree showing evolution relatedness of *Pseudomonas fluorescens* isolated

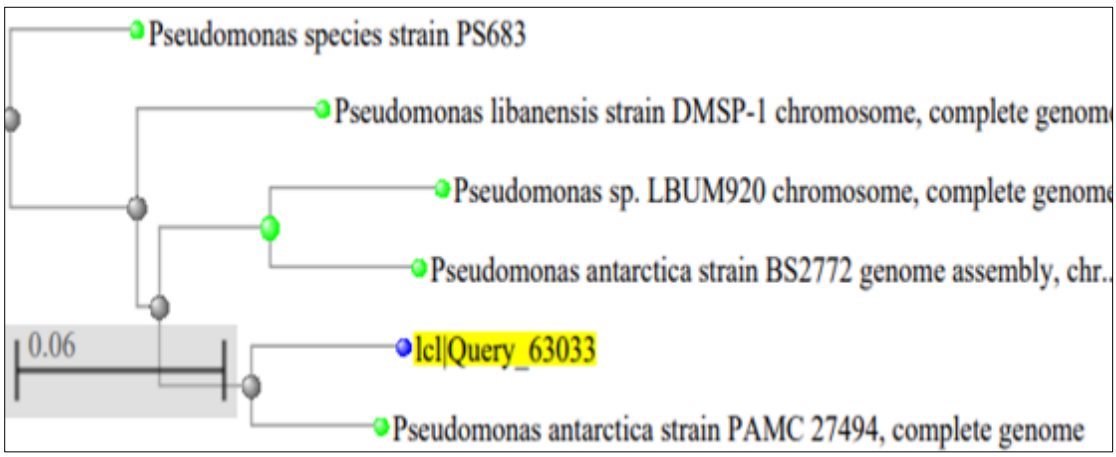

Figure 3 Phylogenic tree showing evolution relatedness of *Pseudomonas antarctica* isolated

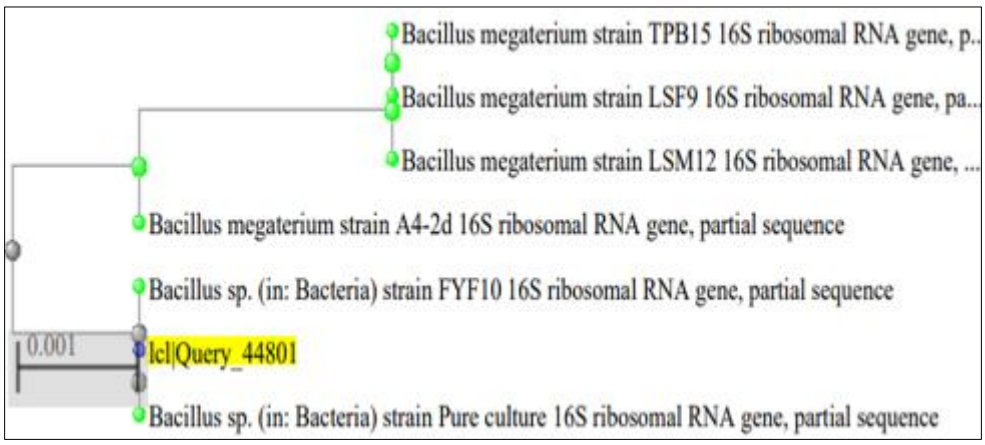

Figure 4 Phylogenic tree showing evolution relatedness of *Bacillus* species *i*solated

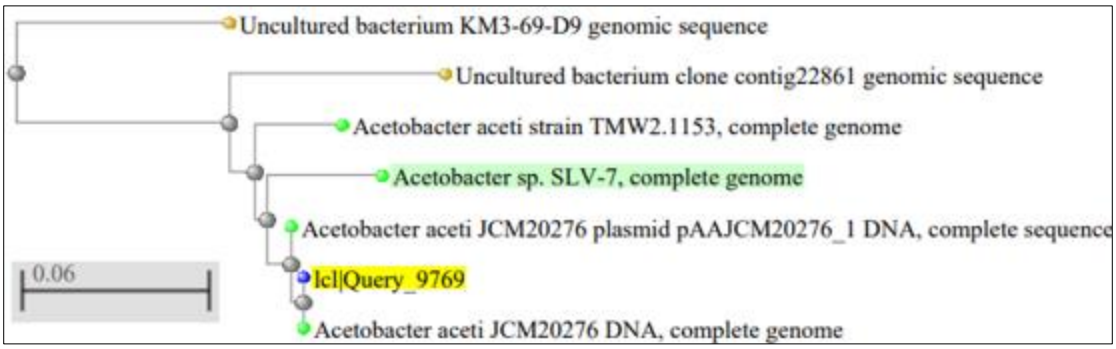

Figure 5 Phylogenic tree showing evolution relatedness of *Acetobacter* species isolated

Table 5 Antibacterial Activities of different bacteria against clinical isolates of complex carbon and nitrogen sources

	Amount of crude cell-free extract (5ml) Inhibition zone (Mean± S.D) in Millimetre (mm)		
Isolates	*E. coli*	*S. typhi*	*S. aureus*
Pseudomonas fluorescens	14.03±1.06	9.11±1.11	5.12±1.02
Pseudomonas Antarctica	4.15±0.22	0.0±0.0	3.34±0.87
Bacillus megaterium	7.80±0.23	0.0±0.0	8.12±0.54
Acetobacter aceti	0.0±0.0	0.0±0.0	0.0±0.0

Key: 0.0±0.0 = No inhibition zone

4. Discussion

Antibiotic is one of the most important commercially exploited secondary metabolites produced by bacteria and employed in a wide range. Most of the antibiotics used today are from the microbes. The high isolation of bacteria from rhizosphere of plants shows that bacteria are highly predominantly isolated in soil and they carried out different functions ranging from fixing chemicals in the soil and acting as antagonise to pathogenic bacteria that infect plants. The total bacteria isolated were high from rhizosphere of maize and the least was from groundnut. The bacterial isolated were namely *Pseudomonas* fluorescens, *Pseudomonas antarctica, Bacillus* megaterium, *Gluconbacter* sp and *Acetobacter* aceti. These bacteria are known to be fixing nitrogen in the soil and producing bioactive agent that antagonize other bacteria. This is similar to work reported by Arifuzzaman *et al.* [17]. With the increasing demand for new antibiotics and novel bioactive compounds, it is necessary to find new strategies that can increase the effectiveness of the search. This present study was carried out to study the production of antibiotic from bacteria isolated soil, out of twenty-one (21) isolated and screen for antibiotics production it was observed that five of the bacterial were able to inhibit test organism used, the that showed producing substance that inhibit the test organisms are *Pseudomonas* fluorescens, *Pseudomonas antarctica, Bacillus* megaterium. These bacteria have been reported by different author of their active in screening for bacterial capable of been to produce new generation of antibiotics. The other bacterial that was not able to show any active against test organisms were *Proteus* sp, *Entrobacter* sp, *Gluconbacter* sp and *Acetobacter* aceti this may be these bacterial do not have the ability to produce any bioactive substance. This similar to study reported by Yunus *et al*. [18]. From this study it was observed that the different bacterial that have the ability to produce substance or compounds that inhibit the active of other bacterial or test organism when grow in simple medium did not show high activity against the test organisms but when grown in a complex medium isolates like *Pseudomonas fluorescens* was able to produced compound that inhibit the three test organism showing large inhibition zone than the other bacterial such as *Pseudomonas antarctica* and *Bacillus megaterium* which showed activity on two organism namely *E. coli* and *S. aureus* but did not show any activity against *S. typhi* while *Acetobacter* aceti that was able to inhibit the growth of the test organism when grown in simple medium was not able to produce any substance that inhibit the test organism when grown iin a complex medium. This maybe the inability of the isolate (*Acetobacter* aceti) to either breakdown the complex medium to produce the substances that can inhibit the test organism or it does not produced substance or compound in high quantity because of the nature of the complex medium. This findings is similar to the studies reported [19,20],

they report different *Pseudomonas species* and *Bacillus* species have the ability to produce substance that inhibit other bacterial but bacterial like *Proteus* species*, Micrococcus* species were found to be incapable of exhibiting antibiotic activity against the various test organisms.

The findings of this study suggested that the problem of antibiotics resistance by pathogenic bacterial can be solve by screening and developing other strains of bacterial isolated from soil for the purpose of antibiotics production.

5. Conclusion

The conclusion of this study is that antibiotic producing bacteria were isolated from soil the highest bacteria isolated from soil was. The antibiotic producing bacteria are *Bacillus* megaterium, *Pseudomonas fluorescens, Pseudomonas antarctica and Acetobacter aceti***.** These bacteria produced antibiotic agents that are capable to inhibit other bacteria both when grown in non-complex medium and complex medium. These bacteria produced substance that inhibits other bacteria more when grown in non-complex medium.

Compliance with ethical standards

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Disclosure of conflict of interest

There was no conflict of interest all through the period of this research work or during the time of drafting of this paper

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