ISOLATION, CHARACTERIZATION AND ANTIBACTERIAL SCREENING OF ANTIBIOTICS PRODUCED FROM STREPTOMYCES ISOLATED FROM DUMPSITE SOILS IN ILORIN, NORTH CENTRAL NIGERIA

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ABSTRACT Antibiotics are one of the most exploited metabolites produced by soil actinomycetes. This study isolated fifteen actinomycetes (A1 – A15) from dumpsite soils within Ilorin metropolis & screened them for antibacterial activity. Isolates were identified with morphology & biochemical characteristics. Their activity against clinical bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* & *Staphylococcus aureus*) was determined. Their bioactive metabolites after fermentation was assayed for by agar well diffusion &compared with reference antibiotics. Molecular analysis of two most active isolates was done. The most active metabolite was subjected to GCMS. All isolates were identified as *Streptomyces*,7 isolates exhibited activity against 3 selected human pathogen bacteria with highest (10.5mm±0.35) by *Streptomyces griseoplanus* while *S. aureus* demonstrated total resistance to all isolates. Secondary activity revealed 7 of the metabolites as effective against the bacteria with highest activity (28.5±1.04mm) by *S. sparsogenes* showing similar activity with Cefuroxime (28.01±0.01mm). The two most active isolates were Streptomyces *griseoplanus* strain NR725RL-ISP 5009 & *Streptomyces sparsogenes* strain NBRC 1308616S& RNA nucleotide similarity revealed close phylogenic relationship. GC-MS revealed10 compounds in *S. sparsogenes* strain NBRC 1308616S metabolite. *Streptomyces* from dumpsite soils within Ilorin produced bioactive compounds against clinical human pathogen bacteria and thus could be potential sources of antibiotics.

Keywords: *S. sparsogenes* strain NBRC 1308616S, Streptomyces *griseoplanus* strain NR725RL-ISP 5009, Antibiotics, Dumpsite soil, Antibacterial activity, Agar diffusion.

1. INTRODUCTION

In the 20th century, the idea of growth inhibition of one microorganism by another present in the same vicinity became mainstream. It was later discovered that such growth inhibition was mediated by secretion of toxic metabolites later termed antibiotics (Singh *et al*., 2012). Over time, researchers have sought antibiotics from microorganisms isolated from several sources such as plant, soil, marine and freshwater environments. Among these, the soil ecosystem has been a major source of potential antibiotic-producing microorganisms (Simon & Daniel, 2011).

Bacteria genera, *Streptomyces*, *Bacillus* along with the fungal genera *Penicillium* and *Cephalosporium* are commonly found in soil. However, the genus *Streptomyces* is the most prolific antibiotic producers and they are a unique subgroup of bacteria called the actinomycetes (Ohnishi et al., 2008). Actinomycetes are gram positive bacteria consisting of a group of branching unicellular microorganisms of which *Streptomyces* are the most dominant.

Actinomycetes have over the years showed ability to produce a variety of bioactive secondary metabolites and for this reason; the discovery of novel antibiotic from them is becoming increasingly important (Dinos, 2017). Of all bioactive secondary metabolites produced by microorganisms, 45% have been reported to be produced from actinomycetes, thus becoming a significant antibiotic-producing genus exploited by pharmaceutical industries (Sharma et al., 2014). Industrially they have gained importance as antibiotics, anti-parasitic, antifungal, herbicides, pesticides, anticancer or immunosuppressive agents as well as industrially important enzymes (Sikander et al., 2018).

However, in the 21st century, the problem of antibiotic resistance has become one of the leading threats to human existence. Thus, there is an urgent need to discover newer classes of antimicrobial agents effective against resistant pathogenic bacteria and fungi with minimum or no toxicity.

This study was conducted with the aim of isolating actinomycetes from dumpsite soils within Ilorin, north-central, Nigeria and screening them and their bioactive metabolites for antibacterial activity.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 9 waste dumpsite soil samples were randomly collected from three different sites in Ilorin metropolis, North Central Nigeria. Samples were taken at a depth of 10 cm, after clearing away 3 cm of top-soil (Aliero et al., 2017). These samples were air dried for 7 days at room temperature (Njenga et al., 2017).

2.2 Selective Isolation of Actinomycetes

Isolation of actinomycetes was carried out as described by Bizuye et al. (2017) and Chaudhary et al. (2018) using starch casein agar (SCA) supplemented with Rifampicin (2.5 µg/ml) and nystatin (50 µg/ml). One gram of dried soil was suspended in 9 ml sterile water and serially diluted up to 10^{-7}. An aliquot of 0.1 ml of each dilution was spread evenly over the surface of the prepared agar and incubated at 30°C for 7 days. Purified actinomycetes isolates were preserved on starch casein agar slant and incubated at 28°C for growth and preserved at -4°C (Reddy et al., 2011).

2.3 Identification of Actinomycetes

2.3.1 Morphological and Cellular Characterization

The colonies of pure actinomycetes isolates were observed based on; color, aerial and substrate mycelia, surface texture and pigment production as described by Fawole and Oso (2007). The cellular characteristics were studied after Gram's staining techniques and viewing under a light microscope.

2.3.2 Biochemical Characterization

Colonies of pure actinomycetes were selected for biochemical tests such as; indole, catalase, methyl red, citrate utilization, starch hydrolysis, Voges-Proskaeur tests as described by Fay and Ba/rry (1974); Fawole & Oso (2007); Abdulkadir &Waliyu (2012).

2.4 Human Pathogen Bacteria

The human pathogen bacteria strains were obtained from the Teaching Hospital, University of Ilorin, Nigeria. They were; *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.* These were streaked on nutrient agar plates and incubated at 37^{o}C for 24 hours to obtain fresh colonies and subsequently maintained on nutrient agar slants at4^{o}C.

2.5 Primary Antibacterial Screening

Primary screening was performed using cross streak method as described by Bizuye et al. (2013). Metabolite-producing isolates were inoculated as vertical straight lines on Mueller Hinton agar (MHA) and incubated at 28^{o}C for 5-7 days after which human pathogen bacteria were inoculated perpendicularly and incubated at 37^{o}C for 24 hours. Zones of inhibition were measured and recorded.

2.6 Secondary Antibacterial Screening

2.6.1 Fermentation and Extraction of Bioactive metabolite from Streptomyces

Bioactive compounds were produced using actinomycetes isolates in a submerged fermenter. They were separately grown in 100mL starch casein broth and incubated at 30°C for 7 days. Following incubation, 10% of the broth culture was inoculated in an

antibiotic production medium consisting: soluble starch 25 g, glucose 10g, yeast extract 2g, $CaCO_3$ 3g, Trace salts solution 1ml, pH 7.5±0.2 (Trace salt solution– $FeSO_4.7H_2O$ – 0.5g, $CuSO_4.5H_2O$– 0.5 g, $ZnSO_4.7H_2O$ –0.5 g, $MnCl_2.4H_2O$ – 0.5 g in 100 ml of distilled water). The flasks were shaken at 28^0C and 160 rpm for 5-7 days on a rotary shaker after fermentation the broth was centrifuged at 4000rpm for 20 minutes to remove cells and obtain a clear supernatant containing the metabolite (Ashok & Karpagam, 2016). This was mixed with equal volume of 95% ethanol on a rotatory shaker for 24 h. The solvent phase was evaporated in water bath at 40ºC to obtain dried partially purified bioactive metabolite which was redissolved in DMSO to obtain a concentration of 100 µl and stored at 4ºC for further studies (Raja & Prabakarana, 2011; Chaudhary et al., 2018).

2.6.2 Determination of Antibacterial Activity

Antibacterial activity of bioactive metabolite was determined by agar well diffusion method (Ahmed et al., 2015). Cell concentration of all test microorganisms were adjusted to 0.5 McFarland turbidity standards. Muller Hinton agar plates were prepared and spread with some human pathogen bacteria using a sterile cotton swab. Wells were prepared using sterile cork borer. A volume of 100µl of the bioactive metabolite was dispensed into each well and allowed to diffuse for 2 hours and incubated at 37ºC for 24 hours. Following incubation, zone of inhibition around each well was recorded (Kaur et al., 2016) and a comparative study of antibiotic susceptibility was done using Multi-disc (Oxoid) containing standard antibiotics against human pathogen bacteria selected.

2.7 Molecular Identification of Most Potent Actinomycetes

The identities of the two most active isolates were confirmed by the sequence analysis of the small subunit rRNA genes. Following manufacturer's guide, the QIAamp DNA Mini Kit (250) (cat No. 51306) was used for the isolation of the genomic DNA. Sequences of the small subunit 16S rRNA genes were amplified using the standard sets of primers 16SF (GTGCCAGCAGCCGCGCTAA) and 16SR (AGACCCGGGAACGTATTCAC) to obtain an amplicon size of 1500bp. Amplification was carried out in a 25µl total reaction mixture consisting of 10x PCR buffer, 2.5µl; 2.0µl; 2.5 MmDNTPs, 0.1µl; 5.0 u/µlTaq DNA polymerase, 0.2µl; 10ng/µl of each primer, 3.0µl; template DNA, 1.0µl and deionized distilled water 13.4µl in a BioRad (USA) thermal cycler at 94^0C for 5 minutes (Initial denaturation), 94^0C for 30sec (denaturation), 72^0C for 45sec (extension) and 72^0C for 7minutes (final extension). The total number of cycles was 36, with the final extension of 72^0C for 7minutes. To ascertain successful amplification, PCR products (50 µl) were size-separated by electrophoresis on a 1% agarose gel prepared in 1% TAE buffer

containing 0.5µl/ml ethidium bromide and visualized under transilluminator (BioRad, USA) against 1000bp DNA ladder (Genei). The PCR product (1500bp) was purified from contaminating products by electro-elution of the gel slice containing the excised desired fragments with Qiaquick gel extraction kit (Qiagen, USA) in a 300 µl of nuclease free water. Product was diluted in Tris buffer (10 mM, pH 8.5) at 1:1000 to 30ng/µl prior to Sanger sequencing using automated sequencer (ABI PRISM 310, Applied Biosystems, USA). Sequences of each isolates were subjected to similarity check with previously identified species by using BLASTn tool on the NCBI database (www.ncbi.nlm.nih.gov:80/BLASTN/) to identify the isolates up to strain level.

2.8 Gas Chromatography - Mass Spectrometry (GC-MS)

Identification of compounds present in the metabolite using GC-MS were based according to the molecular mass, peak observed, and molecular chemical formula which are directly proportional to the amount of molecules present in the band. Only the most effective metabolite (A10) produced by *S. sparsogens* was further analyzed by GC-MS (GC-MS TQ 8050; Schimadzu, Johannesburg, South Africa) as described by Mangayi et al (2019). The metabolite was passed into the Mass Spectroscopy through Gas Chromatography, and ionized into the gaseous ions state in the ionization source. This was thereafter passed into the mass analyzer. The ions observed with different mass per charge ratios were separated out into the electron multiplier by indicating an electrical signal to determine the identity of compounds (Narendhran et al., 2014). The identities of compounds were determined by searching known molecules in data bases.

2.9 Statistical Analysis of Data

Statistical significance was determined using ordinary one-way analysis of variance (ANOVA), while multiple comparisons between means were determined by Tukey's multiple comparisons test. Analysis was performed using Sigma Plot for Windows version 10.0 (SysStat Softwares Inc.). All data were expressed as means of triplicates ± SE.

3. RESULTS

3.1 Recovery and Identification of *Streptomyces* from Soil Samples

A total of 15 Streptomyces species were isolated from nine (9) soil samples and coded A1 – A15. According to morphological characterization, they exhibited varying elevations, pigments, edge, textures, shape (Table 1). The biochemical characteristics revealed that all isolates were *Streptomyces* species and they were urease positive, indole negative while other biochemical parameters differed with species (Table 2).

Table 1. Colony Morphology of *Streptomyces* Isolates

ISOLATES	ELEVATION	PIGMENT	EDGE	TEXTURE	SURFACE	OPTICAL	SHAPE
A1	Convex	Whitish	Smooth	Powdery	Smooth	Transparent	Circular
A2	Flat	Pink	Smooth	Sticky and Powdery	Smooth	Opaque	Irregular
A3	Raised	Blackish green deep	Rough	Powdery	Smooth	Opaque	Irregular
A4	Convex	Green	Smooth	Powdery	Rough	Opaque	Irregular
A5	Raised	Golden yellow	Smooth	Powdery	Rough	Opaque	Circular
A6	Raised	Yellowish green	Smooth	Powdery	Rough	Transparent	Irregular
A7	Raised	Greenish white	Smooth	Sticky	Smooth	Opaque	Circular
A8	Flat	Orange	Undulating	Smooth	Smooth	Opaque	Round
A9	Raised	Cream	Entire	Smooth	Rough	Opaque	Round
A10	Raised	Green	Undulating	Rough	Rough	Opaque	Irregular
A11	Flat	White	Undulating	Mucoidal	Smooth	Opaque	Round
A12	Raised	Black	Rough	Powdery	Rough	Transparent	Rhizoid
A13	Convex	Brown	Smooth	Smooth	Smooth	Transparent	Irregular
A14	Flat	Grey	Rough	Rough	Smooth	Opaque	Irregular
A15	Raised	Creamy	Rough	Mucoidal	Smooth	Transparent	Irregular

Table 2. Identification of Streptomyces by Biochemical Characterization

ISOLATES	INDOLE	UREASE	OXIDASE	NITRATE REDUCTION	CATALASE	OXYGEN RELATIONSHIP	METHYL RED	VOGES PROSKAUER	STARCH HYDROLYSIS	CITRATE UTILIZATION	TENTATIVE IDENTITY
A1	-	+	+	+	+	A	-	-	+	-	*Streptomyces glaucescens*
A2	-	+	+	+	+	A	-	-	+	-	*Streptomyces sp.*
A3	-	+	+	+	+	A	-	-	+	-	*Streptomyces sp.*
A4	-	+	+	+	+	A	-	-	+	-	*Streptomyces sp.*
A5	-	+	+	+	+	A	-	-	+	-	*Streptomyces sp.*
A6	-	+	+	+	-	A	-	-	+	-	*Streptomyces griseoplanus*
A7	-	+	+	+	+	A	-	-	+	-	*Streptomyces griseoflavus*
A8	_	+	+	+	+	A	_	-	+	+	*Streptomyces cyaneus*
A9	_	+	+	+	+	A	_	-	-	-	*Streptomyces sp.*
A10	_	+	+	+	+	A	_	-	+	-	*Streptomyces sparsogenes*
A11	_	+	+	+	+	A	_	-	-	-	*Streptomyces diastaticus*
A12	-	+	+	+	-	A	-	-	+	-	*Arthrobacter*
A13	-	+	+	+	+	A	+	+	+	-	*Streptomyces atroolivaceus*
A14	-	+	+	+	-	A	-	-	-	-	*Steptomyces flaveolus*
A15	-	+	+	+	+	A	+	-	+	-	*Streptomyces sp.*

Keys: +: Positive reaction; -: Negative reaction; A: Aerobic

3.2 Primary Screening of *Streptomyces* Isolated from Soil

The result of primary screening of fifteen (15) *Streptomyces* species against four (4) human pathogen bacteria revealed that only seven (7) of these (*S. glaucescens, S. griseoplanus, S. griseoflavus, S. cyaneus, S. sparsogenes, S. diastaticus, and S. flaveolus*) exhibited antibacterial activity against three of the human pathogenic bacteria (*E. coli*, *K. pneumonia* & *P. aeruginosa*). All the seven active *Streptomyces* species exhibited inhibitory action against *P. aeruginosa* with the highest activity (10.5mm±0.35) shown by *Streptomyces griseoplanus* while *Staphylococcus aureus* demonstrated total resistant to all seven active isolates without any zones of inhibition (Table 3).

Table 3. Primary Screening of Isolates against some Human Pathogen Bacteria

	Mean diameter zone of inhibition (mm)														
Test Organism	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**A10**	**A11**	**A12**	**A13**	**A14**	**A15**
E. coli	3.50± 0.35	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	2.50± 0.35	1.50± 0.35	5.50± 0.35	0.00± 0.00	5.50± 0.35	5.50± 1.06	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
S. aureus	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
K. pneumoniae	4.50± 0.35	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	8.50± 0.35	3.50± 0.35	9.50± 0.35	0.00± 0.00	6.00± 0.7	5.50± 0.35	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
P. aeruginosa	7.50± 0.35	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	10.50 ±0.35	2.50± 0.35	6.50± 0.35	0.00± 0.00	6.50± 0.35	6.50± 0.35	0.00± 0.00	0.00± 0.00	3.50± 0.35	0.00± 0.00

Key: Values represented are means of triplicates ±SEM

3.3 Secondary Screening of Active Metabolites from Streptomyces against some human Pathogen Bacteria

The results obtained indicated that seven (7) metabolites produced from the active Streptomyces showed zones of inhibition against human pathogen bacteria with highest antibacterial activity (28.5±3.54mm) by *S. sparsogenes* strain NBRC 1308616S at a concentration of 100µl against *Staphylococcus aureus* (Figure 1) which was the only resistant bacterium in the primary assay. Sensitivity plates of inhibition zones of active metabolites against *P. aeruginosa* is shown below (Plate1).

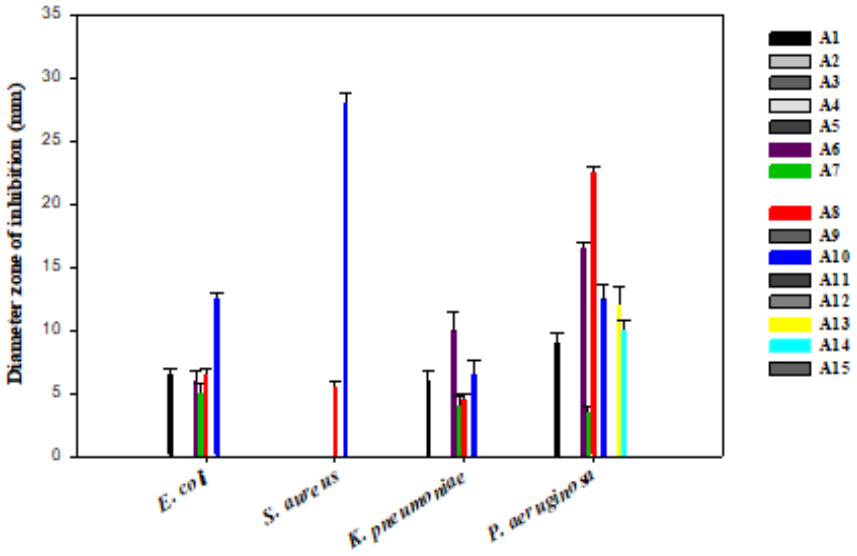


Figure 1. Secondary screening of metabolite from isolates against some selected human pathogen bacteria

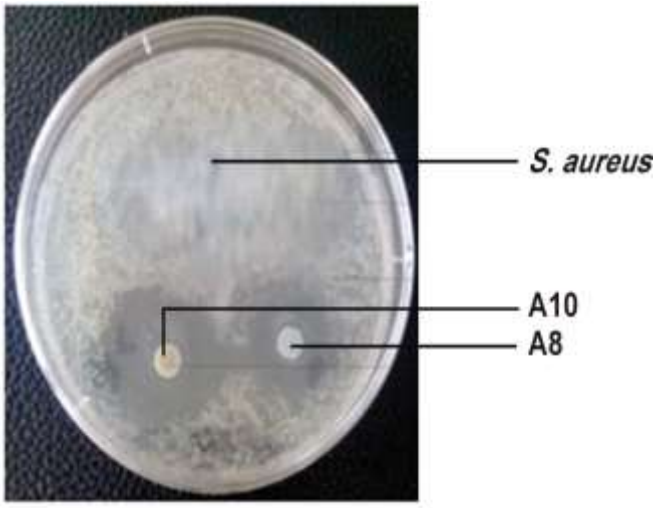


Plate 1. Sensitivity plate showing inhibition of *S. aureus* by metabolites from 2 *Streptomyces* spp.

3.4 Antibacterial Activity of Reference Antibiotics against selected Human Pathogen Bacteria

Generally, the reference antibiotics showed inhibitory activities similar with those from active metabolites against human pathogen bacteria, however, not all the antibiotics exhibited inhibitory activity towards the human pathogen bacteria. ciprofloxacin and augmentin were totally resisted by all four human pathogen bacteria hence showing no zones of inhibition. The highest antibacterial activity (28.01±0.01mm) was observed by Cefuroxime against *Escherichia coli* (Table 4).

Table 4. Antibacterial activity of reference antibiotics against human pathogen strains

Reference antibiotic	Diameter of zone of inhibition (mm)			
	SA	**KP**	**PA**	**EC**
CAZ	14.5±0.70	-	-	-
CRX	27.5±4.95	25.0±2.83	-	28.01±0.01
GEN	20.5±2.12	19.0±0.00	-	17.0±1.41
CTR	-	-	27.0±2.83	-
ERY	18.5±4.95	18.5±3.54	-	15.0±1.41
CXC	-	-	-	-
OFL	21.5±0.71	24.5±0.71	25.5±2.12	26.5±0.71
AUG	-	-	-	-

(-) sign indicates no zone formation (no antibiotic activity); all values are mean ± St Dev
Key: CAZ = Ceftazidime, **ERY** = Erythromycin **CRX** = Cefuroxime, **CXC** = Ciprofloxacin, **GEN** = Gentamycin, **OFL** = Ofloxacin, **CTR** = Ceftriaxone, **AUG**= Augmentin **SA** = *Staphylococcus aureus*, **KP** = *Klebsiella pneumoniae*, **PA** = *Pseudomonas aeruginosa*, **EC** = *Escherichia coli*

3.5 Molecular Identification of two most active *Streptomyces*

The molecular characterization of the two most active Streptomyces species during primary and secondary screening assays (A6 and A10) revealed their identity as *S. Streptomyces griseoplanus* strain NR725RL-ISP 5009 and *S. sparsogenes* strain NBRC 1308616S respectively. Their DNA extraction ladder as shown by gel electrophoresis is given in Plate 2 and the phylogenetic tree of their sequences showed that they are closely related (Figure 2).

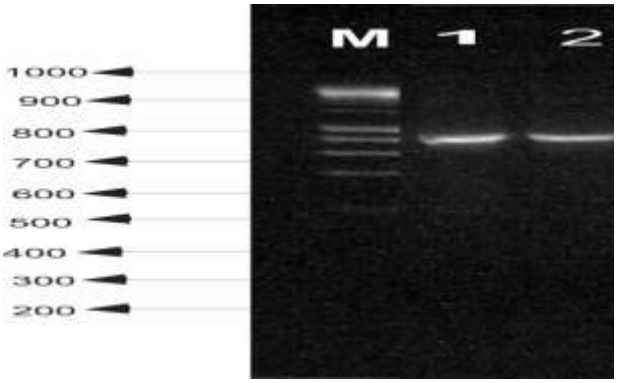


Plate 2. DNA extraction ladder of (1) *Streptomyces griseoplanus* strain NR725RL-ISP 5009(2) *Streptomyces sparsogenes* strain NBRC 1308616S with band size between 200-1000 kda

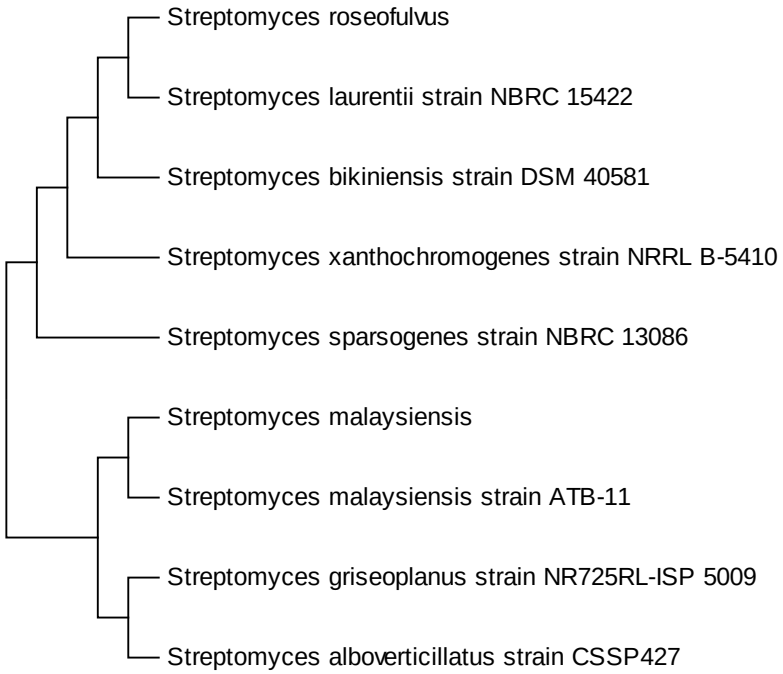


Figure 2. Molecular phylogenetic analysis using the maximum likelihood method

3.6 Gas Chromatography - Mass Spectrometry (GC-MS)

As previously stated, the most effective metabolite (A10) analyzed by GC-MS revealed the presence of 10 compounds out of which fumaric acid was also detected followed by several peaks identified as a cyclic volatile organosilicone compounds (Table 3).

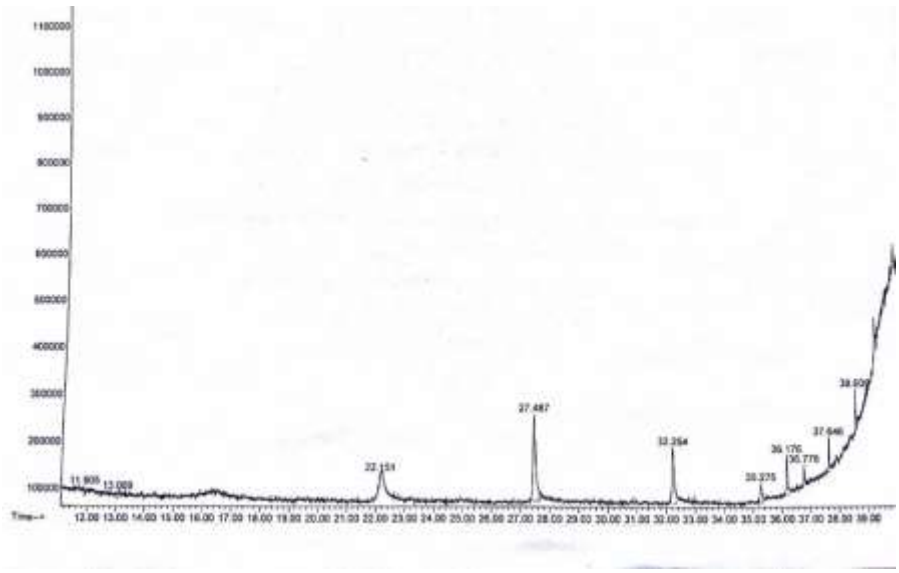
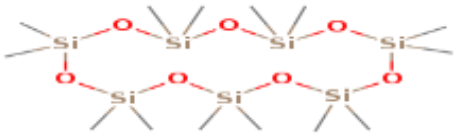
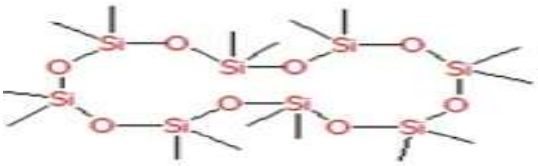
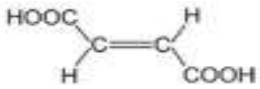
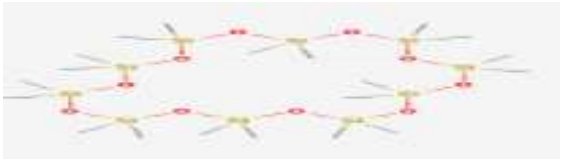
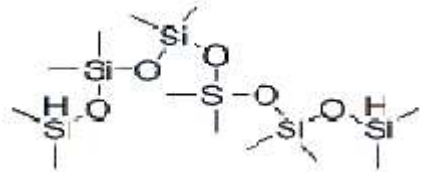


Figure 3. GC-MS spectra of bioactive metabolite from *Streptomyces* <u>*sparsogenes*</u> strain NBRC 1308616S

Table 5. Name and Structure of Compounds from Bioactive metabolite of *Streptomyces sparsogenes* strain NBRC 1308616S Identified by GC-MS

S/N	Name of the compound	Structure
1	(5-Ethoxycarbonylamino-2,6-dimethylpyridin-3-yl	
2	2-p-Tolyl-2,3-dihydro-1H-bezo(1,3,2) diazaborole	
3	Cyclohexasiloxane, dodecamethyl	
4	Cycloheptasiloxane, tetradecamethyl	
5	Cyclooctasiloxane, hexadecamethyl	
6	Fumaric acid, 3-hexyl undecyl este	
7	Cyclononasiloxane, octadecamethyl	

8	Silane, diethylpentadecyloxy(2-phenylethoxy	
9	Cyclodecasiloxane, eicosamethyl	
10	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl	

4. DISCUSSION

Actinomycetes from different sources have been identified as a major producer of a wide range of bioactive substances (Sharma et al., 2014), however, there have been very little or no report regarding screening of extracted bioactive compounds from them in Ilorin, north-central Nigeria.

This present study showed that Streptomyces species were isolated within Ilorin metropolis and their morphological and biochemical characteristics were in line with existing literatures hence confirming their identity. According to a research by Sathi et al. (2001) Streptomyces growth is characterized by small compact and soft colonies tightly attached to the surrounding agar.

The primary screening assay revealed that some of the isolates from this study exhibited antibacterial activity against some selected human pathogen bacteria used except for *S. aureus* while Secondary screening of the product of fermentation of the isolates yielded better activity than did the primary and as a result *S. aureus* which was resistant in the primary assay became susceptible in the secondary screening. Large scale production of antibiotics depends majorly on fermentation because during the process producer organisms multiply rapidly in a free flowing medium with optimized nutrient composition to support and enhance the secretion of copious quantities of the desired bioactive components relative to the quantity produced during primary screening. Further, it has been earlier argued that screening techniques based on the cultivation of live organisms on solid agar substrates is often affected by certain extraneous factors such as other cell lysates there by giving false positive results (Sharma et al., 1986). Hence, the increased concentrations of potent bioactive compounds during fermentation propels it as a reliable antibiotic screening technique for actinomycetes species which was also reflected when compared with the assay of standard reference antibiotics. This may have been responsible for the increased activity demonstrated by the best producer strain. Thus suggesting fermentation as a better

means of obtaining quality antibacterial extract from microorganisms.

Molecular characterization serves as a veritable tool in the accurate identification of organisms and in this study it revealed the evolutionary relationship of the identified species showing them to be closely related on the phylogenetic tree. According to separate earlier reports by Ziemert & Jensen (2012) and Ajijolakewu et al. (2016), the molecular phylogeny uses sequenced data to infer evolutionary relationships between organisms and the genes they maintain. In line with this suggestion, Ziemert & Jensen (2012) had earlier opined that phylogenetic relationships among organisms provides unalloyed information which delineate the architecture and function of the genes involved in the biosynthesis of similar secondary metabolite. Thus this may have been responsible for the relative similarities in the activities of the two species of *Streptomyces* that were sequenced.

Streptomyces sparsogenes and *Streptomyces griseoplanus* possessed the higher activity among all other streptomyces species tested, and according to literature, each of these organisms is known to produce highly potent broad spectrum antibiotic substances such as sparsomycine and tubercidin by *Streptomyces sparsogenes* (Zhang et al., 2017), alazopeptin and anticapsin (Boeck & Shah 1971). Erythromycin which is one of the major secondary metabolite secreted by *Streptomyces griseoplanus* is on the World health organization list of essential medicines due to its effectiveness and safety in health care delivery. Its major mechanism of action is its interference with production of functional proteins which form the basis of its antimicrobial action (Dinos, 2017).

GC-MS analysis of metabolite from *Streptomyces sparsogenes* confirmed and identified the presence of 10 compounds as a result of the fragmentation pattern observed which in turn was used to confirm their chemical structures. The high antibacterial activity of the most active metabolite (A10) which outweighed even conventional antibiotics may be attributed to the presence of fumaric acid as one of the compounds present in it. From literature, fumaric acid is used primarily in liquid pharmaceutical preparations as an acidulant and flavoring agent. It has also been established for use as a chelating agent which exhibits synergism when used in combination with other true antioxidants (Das et al., 2016). Fumaric acid has been investigated as bioadhesive microspheres due to its hydrophobic nature, which is a necessary property that aids adherence of the agent to microbial surfaces and thus permeating microbial cell wall and membrane (Tango et al., 2015). It has also been used in film-coated pellet formulations as an acidifying agent and also to increase drug solubility. Furthermore, the presence of several organosilicone compounds present in the metabolite may have added to the antibacterial activity recorded. From the GC-MS analysis it was evident that seven organosilicone compounds were present at varying peaks of the spectra. According to Mary and Giri (2017) organosilicone compounds possess antimicrobial and antiseptic properties.

This study has established that Streptomyces isolated from dumpsite soils within Ilorin metropolis possessed the potential to produce antibacterial metabolites with activity against pathogenic bacteria. It has also demonstrated that the fermentation carried out on isolates produced bioactive metabolites with enhanced antibacterial activity. The different species of *Streptomyces* exhibited varying antibacterial abilities indicating that they may have produced different antibacterial compounds

with varying mechanisms and modes of action responsible for their respective activities.

5. CONCLUSION

This study has demonstrated that metabolite produced from *S. sparsogenes* possessed highest antibacterial activity which inhibited clinical pathogens better than conventional antibiotics suggesting it as a viable source of antibiotics which can be harnessed in treatment of microbial infections. Furthermore, the GC-MS analysis revealed the presence of active compounds such as fumaric acid which is a chelating agent that can be harnessed for pharmaceuticals.

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7. REFERENCES

Abbas KH., Al-Snaf AE. &Bander KI. (2009). Isolation and Identification of Antibiotics Produced by *Penicillium brasilium* Batista Isolated from Salahaddin Province Soils. Thi-Qar Medical Journal (TQMJ) 3: 71-87.

Abdulkadir M. & Waliyu S. (2012). Screening and isolation of the soil bacteria for ability to produce antibiotics. European Journal of Applied Science 4(5): 211-215.

Adegboye MF. & Babalola OO. (2013). Isolation, characterization and antibacterial activity of Streptomycetes from rhizosphere soils in North West Province, South Africa. The Asian International Journal of Life Sciences 9: 403-421.

Ahmed RN., Abdullahi MA., Zakariyah RF., Gambari-Ambali RO., Adeyemi SB., Afonja AI. & Adebisi OO. (2015). Antibacterial Action of Silver Nano Particles of Extract of *Thevetia nerifolia*. International Journal of Phytofuels and Allied Sciences4(1): 31-55.

Ajijolakewu K., Leh C., Wan AbduLLah W. & Lee C. (2016). Assessment of the Effect of Easily-metabolised Carbon Supplements on Xylanase Production by Newly Isolated *Trichoderma asperellum* USM SD4 Cultivated on Oil Palm Empty Fruit Bunches. BioResources11(4): 9611-9627. doi:10.15376/biores.11.4.9611-9627

Aliero AA., Emmanuel E., Josephat MN., Sambo HA., Matilda AO. & John O. (2017). Antibacterial Activity of Actinomycetes Isolated from Waste Dump Soil from Western Uganda. Microbiology Research Journal International 21(5): 1-14.

Ashok KP. & Karpagam P. (2016). Screening of Antibiotic Producing Actinomycetes from the Sediments of Undisturbed Forest Areas and Its Hyper Activity after Mutation. International Journal of Advances in Chemical Engineering and Biological Sciences 3(2**)**: 46-43.

Bizuye A., Moges F. & Andualem B. (2013). Isolation and screening of antibiotic producing actinomycetes from soils in Gondar town, North West Ethiopia.

Asian Pacific Journal of Tropical Diseases 3(5): 375-381.

Bizuye A., Bii C., Erastus G. & Maina N. (2017). Antibacterial metabolite prospecting from actinomycetes isolated from damped soils from Thika central part of kenya. Asian Pacific Journal of Tropical Diseases 7(2): 757-764.

Boeck LD., Christy KL. & Shah R. (1971). Production of anticapsin by Streptomyces griseoplanus. Applied microbiology 21(6): 1075–1079.

Chaudhary HS., Yadav J., Shrivastava AR., Singh S., Singh AK. & Gopalan N. (2018). Antibacterial activity of actinomycetes isolated from different soil samples of Sheopur (A city of central India). Journal of Advanced Pharmaceutical Technology and Research 4(2): 118-123.

Das RK., Brar SK. & Verma M. (2016). Fumaric Acid: Production and Application Aspects. Platform Chemical Biorefinery 8: 133-157.

Dinos PG. (2017). The Macrolide Antibiotics Resistsnce. British Journal of Pharmacology 174: 2967-2983.

Emami E., Issazadeh K., Akhavan SA. & Tajehmiri A. (2017). Isolation and Characterization of Aerobic Actinomycetes from Soil in Northern Iran and Evaluation of their Antimicrobial Potential J Med Bacteriol 6(3 &4): 14-22.

Fawole MO. & Oso BA. (2007). Laboratory manual of microbiology, 5th edition spectrum book limited, Ibadan, Nigeria, pp. 16-33.

Fay GD. & Barry AL. (1974). Methods for detecting indole production by gram-negativenon-spore forming anaerobes. Applied Microbiology 27(3): 562-565.

Kaur S., Kaur HP. & Kaur G. (2016). Isolation and characterization of antibiotic producing actinomycetes from agriculture soil. World Journal of Pharmacy and Pharmaceutical Sciences 5(6):1109-1117.

Njenga WP., Mwaura FB., Wagacha JM. & Gathuru EM. (2017). Methods of isolating actinomycetes from the soils of Menengai crater in Kenya. Archives of Clinical Microbiology 8: 3.

Manganyi MC., Tchatchouang CK., Regnier T., Bezuidenhout CC. & Ateba CN. (2019) Bioactive Compound Produced by Endophytic Fungi Isolated from Pelargonium sidoides Against Selected Bacteria of Clinical Importance. Mycobiology 47(3): 335-339.

Mary PA. & Giri RS. (2017). GC-MS Analysis of Bioactive Compounds of *Acryranthes aspera*. World Journal of Pharmaceutical Research 7(1): 1015-1056.

Ohnishi Y., Ishikawa J., Hara H., Suzuki H., Ikenoya M., Ikeda H., Yamashit A.,

Hattori M. & Horinouchi S. (2008). Genome sequence of the streptomycin- producing microorganism *Streptomyces griseus* IFO 13350. Journal of Bacteriology 190(11): 4050-4060.

Raja A. & Prabakarana P. (2011). Actinomycetes and Drug-An Overview. American Journal of Drug Discovery and Development 1: 75-84.

Sathi ZS., Rahman MAA. & Gafur MA. (2001). Identification and *in vitro* antimicrobial activity of compound isolated from *Streptomyces* species. Pakistanian Journal of Biological Science 4: 1523-1525

Sharma P., Pajni S., Dhillon N., Vadehra D. & Dube D. (1986). Limitations of the Congo-Red staining techniques for the detection of cellulolytic activities. Biotechnology letters 8(8): 579-580.

Sharma M., Dangi P. & Choudhary M. (2014). Actinomycetes: Source, Identification, and Their Applications. International Journal of Current Microbiology and Applied Sciences 3(2**)**: 801-832.

Sikander A., Maria N., Aiman TL., Maryam S. & Attia M. (2018). Production of Microbial Metabolites and Optimization of Key Factors Involving their Hyperproduction in Batch Culture. European Journal of Pharmaceutical and Medical Research 5(5**)**: 80-88.

Simon C. & Daniel R. (2011). Metagenomic analyses: past and future trends. Applied and Environmental Microbiology 77(4**)**: 1153-1161.

Singh AP., Singh RB. &Mishra S. (2012). Studies on isolation and characterization of antibiotic producing microorganism from industrial waste soil sample. The Open Nutraceutical Journal 5: 169-172.

Tango CN., Mansur RA. & Oh DH. (2015). Fumaric Acid and Slightly Acidic Electrolyzed Water Inactivate Gram Positive and Gram Negative Foodborne Pathogens. Microorganisms 3(1): 34-46

Zhang Hongyu., Qiuling Zhou., Tingting Lou., Suying Wang. & Haihua Ruan. (2017). Draft genome sequence of broad-spectrum antibiotic sparsomycin-producing Streptomyces sparsogenes ATCC 25498 from the American Type Culture Collection. Journal of Global Antimicrobial Resistance 11: 159-160.

Ziemert N. & Jensen PR. (2012). Phylogenetic approaches to natural product structure prediction. Methods in enzymology517, 161–182.