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In vitro Antibacterial activity of *Streptomyces* species Isolated from Soils against *Staphylococcus aureus* ATCC 12600 and *Salmonella typhimurium* CCARM 8256

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ABSTRACT

Diseases caused by pathogenic microorganism infections such as diarrhoea, gastrointestinal and urogenital diseases, and wound infections, are on the rise and pose a threat to human health as multidrug-resistant pathogens arise. This study assessed the antibacterial activity of *Streptomyces* species isolated from soil at Rivers State University, Port Harcourt. A total of ten separate surface soil samples were aseptically collected at random from five different locations. Enumeration, isolation and screening of probable *Streptomyces* species for antibacterial activity were conducted using standard microbiological techniques. The mean Total *Streptomyces* count ranged from 0±0.00 x10^3 CFU/g to 3.1±0.14x10^3 CFU/g. Mean range of percentage distribution for the soil samples was 0 to 26.7%. Presumptive characterization and identification revealed that all the 15 distinct isolates exhibited typical features that fitted the genus *Streptomyces*. Primary screening was done using the Perpendicular Cross-streak technique. Secondary screening was performed using the Well in Agar method with Ethyl acetate extracts of *Streptomyces* isolates at a concentration of 50mg/ml. Results shows that majority of the *Streptomyces* extracts exhibited antagonistic activity against both test organisms; *Staphylococcus aureus* ATCC 12600 and *Salmonella typhimurium* CCARM 8256 as evident with moderate zones of inhibition. The partial nucleotide sequences of 16S rRNA gene led to identifying the 5 most potent isolates as various strains of *Streptomyces* species. Overall, the findings of this study has contributed to the discovery that soils at Rivers State University support and provide habitat for potential antibiotic generating *Streptomyces*. To maximise the production of antibiotic chemicals produced by *Streptomyces* isolates, fermentation conditions such as growth media, pH, temperature, and other critical fermentation parameters should be optimized.

Keywords: Soils, *Streptomyces*, screening, ethyl acetate extracts, antibacterial activity, clinical isolates, food sources.

Introduction

Diseases caused by pathogenic microorganism infections, such as diarrhoea, gastrointestinal and urogenital diseases, and wound contamination, are on the rise and pose a threat to human health as multidrug-resistant bacteria arise (Devadass *et al.*, 2016). Multidrug-resistant Gram-positive and Gram-negative bacteria such as *Enterobacteriaceae* (carbapenem) and *Enterococcus* (vancomycin), as well as *Salmonella enterica*, *Staphylococcus aureus* (methicillin), *Pseudomonas aeruginosa*, *Clostridium difficile*, and *Escherichia coli*, are expected to kill up to 10 million people per year by 2050 (Devadass *et al.*, 2016).

Natural products, such as plants, microorganisms, and their compounds, have been used to treat and cure a variety of diseases, including diarrhoea, Cancer, diabetes, Alzheimer's, anti-inflammatory, analgesic, and antipyretic medications, as well as hormone replacement therapy alternatives (Brunel and Guery, 2017). Microorganisms, in particular, are capable of producing a wide range of bioactive metabolites, including antibiotics. Actinomycetes have recently been shown to create over 10,000 bioactive natural antibiotics derived from bacteria, including penicillin, tetracycline, gentamicin, vancomycin, and pimaricin.

These are a large and diversified collection of Gram-positive aerobic bacteria that typically proliferate by filament formation, and they are one of the most abundant microbial populations in soils (Elbendary *et al*, 2018). They are a representative antibiotic-producing prokaryotic group, and their morphological differentiation and genetic features are of basic scientific interest (Ceylan *et al*., 2018).

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Streptomyces species are well recognized as industrially relevant organisms due to their potential to produce a variety of unique secondary metabolites (Cosgrove and Carmeli, 2013). *Streptomyces'* most intriguing trait is its potential to produce bioactive secondary metabolites such as antibacterial, antifungal, antiviral, antitumoral, antihypertensive, immunosuppressive, and, most notably, antibiotics (Ceylan *et al.,* 2018). Streptomycetes are primarily soil-resident mycelial bacteria that produce a large number of secreted proteins and important secondary metabolites, including valuable antibiotics. Several methods have been used to identify *Streptomyces* species, including selective culturing, the development of genetic marker systems, a combination of chemical markers, the presence of LL-diaminopimelic acid, and the absence of characteristic sugars in the cell wall (Arias and Murray, 2015). *Streptomyces* species have been the most abundant and easily recoverable actinomycetes in soil. *Streptomyces* produces secondary metabolites with a wide range of bioactivities, including antibacterial, antifungal, antiviral, anticancer, and enzyme inhibitory chemicals (Bennett, 2018). It is predicted that these thrifty bacteria produce about 7,000 compounds (Castro-Sánchez *et al.,* 2016). *Streptomyces* has produced about 9,000 physiologically active compounds, with more than 60 of them useful in medicine, agriculture, and research (Castro-Sánchez *et al.,* 2016).

Streptomyces are also well known as biological control agents that inhibit many soil and airborne plant pathogenic fungi and bacteria (Silva *et al.* 2008). *Streptomyces* spp. are a rich source of secondary metabolites, enzymes, and antibiotics (Cosgrove and Carmeli, 2013), owing to their substantially shorter generation period and simplicity of genetic and environmental stage manipulation. Many secondary metabolites, including antibiotics, are produced simultaneously with morphological differentiation (Kieser *et al.,* 2015). Indeed, we see increased production of secondary metabolites during the shift from vegetative to aerial development (Ceylan *et al.,* 2018). Secondary metabolite-producing bacteria produce these beneficial and complex compounds during the lag and stationary phases of their growth. Secondary metabolites, particularly in actinomycetes and *Streptomyces*, can be produced during the exponential, stationary, and death phases (Etebu and Arikekpar, 2016). *Streptomyces* is well-known for producing the vast majority of antibiotics and physiologically active secondary metabolites.

Nearly half of the *Streptomyces* species isolated generate antibiotics (George *et al.,* 2017). Research has also revealed that several variables, such as nutrients, culture, and other factors, may influence how *Streptomyces* develops to produce antibiotics (George *et al.,* 2017). While numerous antibiotics are known to exist, efforts to find novel antibiotics continue. As a result, numerous species, including *Streptomyces*, *Bacillus*, and *Penicillium*, have been extensively researched for their antibiotic-producing abilities. This study aims to evaluate the antibacterial activity of *Streptomyces* spp. isolated from soils within Rivers State University, Port Harcourt.

Materials and Methods

Description and Selection of Study Area

This study was conducted at Rivers State University, Nigeria. The university is located at 4° 48'27.0012" N latitude and 6° 58' 37.7778" E. longitude with an elevation of 46 feet above sea level (Google map).

Collection of soil samples

A total of ten (10) different surface soil samples comprising 1 School farm soil, 3 Rhizosphere soils, 2 Grassland soils, 3 Plantain plantation soils, and 1 Refuse dumpsite soil were randomly collected from five different locations within Rivers State University, Port Harcourt. The surface soil samples were obtained at random depths ranging from 6 to 10 cm with the goal of selectively isolating and screening *Streptomyces* species for antibacterial activity. The samples were aseptically collected into sterilized zip-lock bags with the aid of a sterile hand trowel, labelled properly and brought to the Microbiology laboratory of the Department of Microbiology, Rivers State University (RSU), where they were stored in the refrigerator at 4^{o}C until further processing.

Enumeration, Isolation and Characterization of *Streptomyces* Isolates

Using a sterile 1ml pipette, 0.1 ml of the 10^{-1} and 10^{-3} dilutions from the air dried soil samples were aseptically transferred to already labelled Starch Casein agar (SCA) plates (g/l): (starch: 10, casein: 0.3, $NaNO_3$: 2, K_2HPO_4:2, $MgSO_4.7H_2O$: 0.05, NaCl: 2, $CaCO_3$: 0.02, $FeSO_4.7H_2O$: 0.01, Agar:20, pH:7.2) plates supplemented with 50µg/ml of Nystatin (Afunginal©, ACME Laboratories Ltd, Bangladesh) and 5µg/ml of Ampicillin (Ampicin©,

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Sandoz International GmbH, Germany) to prevent fungal and bacterial contaminants. The aliquot was evenly dispersed with a sterile glass spreader, and the plates were incubated upside down in the dark at ambient temperatures for seven days. Following incubation, the colonies were counted on the 10^{-1} and 10^{-3} dilution plates, and the quantity of *Streptomyces* per gram of soil was calculated by multiplying by the dilution factor. Distinct colonies on the plates were examined microscopically to confirm their identification as typical filamentous Streptomycetes. Isolates from various colonies were subcultured repeatedly on starch casein agar plates until pure cultures were obtained. To guarantee optimum growth, these separate pure cultures were placed in nutrient agar slants and incubated for seven days at ambient temperatures. The bottles were used as stock cultures and kept at 4°C. Presumptive Identification was made based on morphological, physicochemical and biochemical properties, with a strong reference to Bergey's manual of determinative bacteriology (Babadi *et al*., 2020).

Test Microorganisms

The test organisms used in this study were stock cultures from both clinical (wound swab) and food (tigernut) sources. The clinical isolate was *Staphylococcus aureus* ATCC 12600 collected from the University of Port Harcourt Teaching Hospital (UPTH), while the food pathogen, *Salmonella typhimurium* CCARM 8256 was isolated using the spread plate method. Both organisms were confirmed using conventional and molecular based methods.

Primary Screening of *Streptomyces* Isolates for Antibiosis

The 15 distinct *Streptomyces* isolates were first screened on Mueller-Hinton agar medium using the perpendicular cross-streak method against the selected test organisms. The pure strain of *Streptomyces* was streaked across the plate's diameter in sterile agar medium. The plates were incubated at 28^{o}C for seven days. A pure colony of test bacteria, including *Staphylococcus aureus* ATCC 12600 and *Salmonella typhimurium* CCARM 8256 was inoculated into fresh nutrient broth and cultured at room temperature and conditions until turbidity was observed. After correcting the turbidity to 0.5 McFarland with 1.5 x 10^{8} cells, the test organisms were streaked perpendicular to the isolates. The plates were then incubated at 37^{o}C for 24 hours, and the zone of inhibition was used to evaluate antibacterial activity (Rai *et al*., 2016).

Crude Extraction of Antibacterial Compounds from *Streptomyces* Isolates

Based on the primary screening results, each isolate was treated to a small-scale liquid fermentation in nutrient broth in a shaker incubator at 120rpm for 7 days at 28^{o}C. Following fermentation, each isolate's broth medium was centrifuged for 10 minutes at 10,000 rpm and 40°C. The fermented broth culture containing bioactive metabolites was isolated from the solid residue using Whatman No. 1 filter paper. The filtered broth was mixed in a 1:1 (v/v) ratio with an equal amount of ethyl acetate and allowed to stand for some time. The upper layer, which contains the metabolite solvent, is then separated from the liquid portion using a separatory funnel. To ensure appropriate metabolite extraction, this technique was done three times with the same volume of ethyl acetate. The ethyl acetate fraction was evaporated in a rotary evaporator at 40^{o}C, leaving behind a solid residue. The crude extracts of the individual isolates were made by weighing and dissolving the dried solid residues in 10% dimethyl sulfoxide (DMSO), which were then placed in tiny vials at 4°C to test their antibacterial activity.

Secondary Screening of *Streptomyces* Isolates for Antibiosis

The antibacterial activity of each isolate's crude extract was assessed using the well in agar diffusion method, with minor modifications (Ahmed, 2017). The inoculum generated from each bacterial suspension (1ml) was combined with 9ml of sterile nutritional broth and compared to 0.5 McFarland turbidity standard solutions. The test organisms were swabbed separately and evenly on the surface of the Mueller-Hinton agar plates. Then, 100µl of crude extract of the various *Streptomyces* isolates was poured into each well. Augmentin (30µg/ml) and 10% DMSO solvent served as positive and negative controls, respectively. The plates were allowed to stand for a few minutes before being incubated at 37°C without inversion for 24 hours and examined for zones of inhibition. Duplicate experiments were carried out, and the sizes of the inhibitory zones were measured and reported.

Molecular Identification of most potent *Streptomyces* isolates

The proximity of 16S rRNA gene of selected most potent isolates was compared with similar sequences of National Centre for Biotechnological Information (NCBI) database using BLASTN for genotypic ID.

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Statistical analysis

Statistical analysis was performed by computing the means and standard deviations of the results. One-way Analysis of Variance (ANOVA) was used with the Statistical Package for Social Science (SPSS) software to calculate the difference between means.

Results

Results of the mean Total *Streptomyces* count of the 10 different soil samples from the 5 locations are presented in Table 1. The counts ranged from 0±0.00 x 10^3CFU/g to 3.1±0.14 x 10^3CFU/g and were highly significant (P value < 0.001) for all the soil samples. Refuse dumpsite soil had the highest TSC of 3.10±0.14 × 10^3 CFU/g.

Table 1: Mean±SD of Total *Streptomyces* Count and their distribution (%) in the various soil samples

Soil location	Sample code	Total *Streptomyces* Count (10^3CFU/g)	Number of distinct isolates	Distribution (%)
A	School farm soil	1.9±0.14^c	3	20
	Rhizosphere soil A	2.65±0.21^d	3	20
B	Rhizosphere soil B	1.85±0.07^c	3	20
	Grassland soil A	0±0.00^a	0	0
C	Plantain plantation soil A	1.3±0.14^b	2	13.3
	Rhizosphere soil C	0±0.00^a	0	0
D	Grassland soil B	0±0.00a	0	0
	Refuse dumpsite soil	3.1±0.14^e	4	26.7
E	Plantain plantation soil B	0±0.00^a	0	0
	Plantain plantation soil C	0±0.00^a	0	0
	p-value	<0.001	Total =15	Total =100

*Means with different alphabets along the column shows a significant difference (p≤0.001).
*Key: CFU/g: Colony Forming Units per gram.

Results of the biochemical and physiological properties of the 15 *Streptomyces* isolates is presented in Table 2. The 15 unique *Streptomyces* isolates reacted differently when exposed to various carbohydrate sources such as glucose, mannitol, sucrose, lactose, and mannose.

The majority of the isolates exhibited gamma (non-hemolysis). All the isolates were negative for motility and voges-proskauer test.

Plate 1 and 2 represents the micrograph of threadlike filaments of RZSA3 and RDS4 isolates.

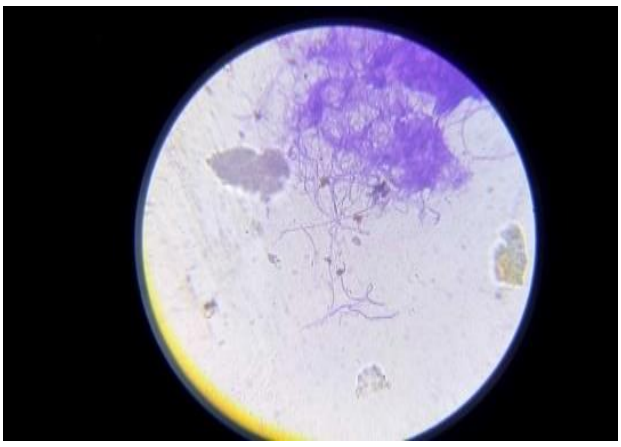

Plate 1: Micrograph of RZSA3

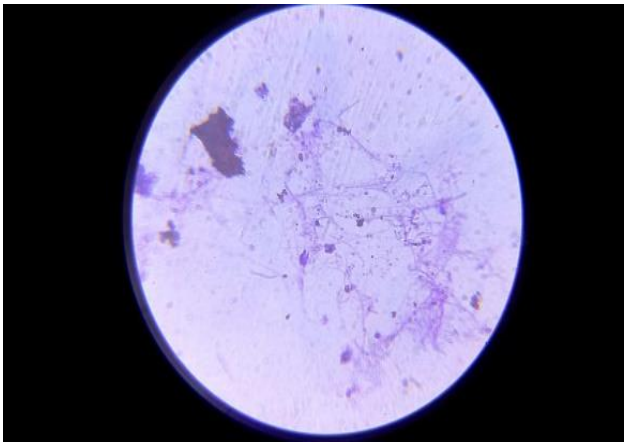

Plate 2: Micrograph of RDS4

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Table 2: Biochemical and Physiological Properties of the *Streptomyces* species Isolated from Rivers State University Soils

Isolate code	GramReaction	Motility	Citrate	Methyl Red	Voges Proskauer	Indole	Catalase	Oxidase	Starch Hydrolysis	Haemolysis	Gelatin Hydrolysis	Glucose	Lactose	Maltose	Sucrose	Mannitol	Casein Hydrolysis	Spore production	TSI slant	Colour of butt	Hydrogen sulphide (H_2S)	Presumptive identification
RDS1	+	-	+	-	-	+	+	+	+	ɣ	+	AG	-	AG	-	A	+	+	R	Y	-	*Streptomyces* sp
RDS2	+	-	+	+	-	+	+	+	+	A	+	AG	A	A	-	A	+	+	Y	Y	-	*Streptomyces* sp
RDS3	+	-	+	-	-	+	+	+	+	ɣ	-	AG	AG	A	AG	-	+	+	Y	B	+	*Streptomyces* sp
RDS4	+	-	+	+	-	-	+	+	+	ɣ	+	A	-	AG	-	-	-	+	R	B	+	*Streptomyces* sp
SFS1	+	-	+	+	-	-	+	+	+	ɣ	+	AG	-	-	-	A	-	+	R	B	+	*Streptomyces* sp
SFS2	+	-	+	+	-	+	+	+	+	ɣ	+	AG	-	A	-	A	-	+	R	B	+	*Streptomyces* sp
SFS3	+	-	+	+	-	-	+	+	+	ɣ	-	A	-	-	-	AG	+	+	R	B	+	*Streptomyces* sp
RZSA1	+	-	+	+	-	-	+	+	+	ɣ	+	AG	A	A	A	-	-	+	Y	Y	-	*Streptomyces* sp
RZSA2	+	-	+	+	-	-	+	+	+	A	+	A	-	AG	-	A	+	+	R	B	+	*Streptomyces* sp
RZSA3	+	-	+	+	-	+	+	+	+	ɣ	+	A	AG	-	A	AG	+	+	Y	Y	-	*Streptomyces* sp
RZSB1	+	-	+	+	-	-	+	+	+	ɣ	+	AG	A	A	A	-	+	+	Y	Y	-	*Streptomyces* sp
RZSB2	+	-	+	+	-	-	+	+	+	ɣ	+	A	-	-	-	AG	+	+	R	Y	-	*Streptomyces* sp
RZSB3	+	-	+	+	-	-	+	+	+	A	+	A	-	AG	-	A	+	+	R	B	+	*Streptomyces* sp
PTSA1	+	-	+	+	-	-	+	+	+	A	+	AG	AG	-	-	-	+	+	Y	Y	-	*Streptomyces* sp
PTSA2	+	-	+	+	-	-	+	+	+	ɣ	+	AG	-	-	-	AG	-	+	R	B	+	*Streptomyces* sp

***Key:** TSI= Triple Sugar Iron agar, R= Red colour, Y= Yellow colour, B= Blackening (hydrogen sulphide production), (-) = Negative, (+) = Positive, A= Acid, G= gas, α=Alpha or partial haemolysis, ɣ= Gamma or no haemolysis.

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Plate 3 shows zones of inhibition of some *Streptomyces* extracts against *Salmonella typhimurium* CCARM 8256 and *Staphylococcus aureus* ATCC 12600. Results of zones of inhibition of primary screening of the 15 *Streptomyces* isolates against test organisms (*Staphylococcus aureus* ATCC 12600 and *Salmonella typhimurium* CCARM 8256) are presented in Table 3. While the results of zones of inhibition of secondary screening with ethyl acetate crude extracts of the 15 *Streptomyces* species against test organisms (*Staphylococcus aureus* ATCC 12600 and *Salmonella typhimurium* CCARM 8256) are presented in Table 4. Augmentin, Aug (30µg) and 10% dimethyl sulfoxide (DMSO) served as positive and negative control respectively.

Five (5) out of 15 *Streptomyces* extracts with codes SFS3, RDS4, RZSA1, RZSA3, and RDS1 showed positive zones of inhibition against *S. aureus* ATCC 12600 with mean values of 10.5±0.71mm, 8.5±0.71mm, 8.5±0.71mm, 10.5±0.71mm, and 14.5±0.71mm, respectively. RDS4 and RZSA3 demonstrated the largest zones of inhibition (19.5±0.71mm and 19.0±0.71mm, respectively) against *Salmonella typhimurium* CCARM 8256 as compared to the control, augmentin (30µg), having a zone of 18.5±0.71mm. Results of the Agarose Gel Electrophoresis based on the 16S rRNA Gene nucleotide sequences of the most potent *Streptomyces* isolates is presented in Plate 4 below.

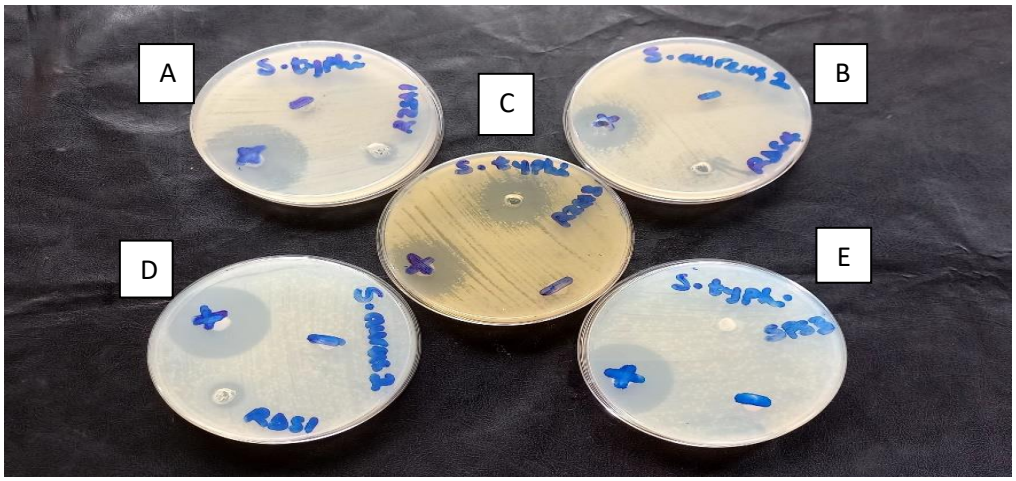

Plate 3: Zones of inhibition of some *Streptomyces* extracts against test organisms
Key: A= RZSA1; B= RDS4; C= RZSA3; D= RDS1; E= SFS3; (+) = augmentin (30µg); (-) = 10% DMSO

Table 3: Zones of inhibition (MN±SD) of primary screening of *Streptomyces* isolates against test organisms

Isolate code	*S. aureus* ATCC 12600	*S. typhimurium* CCARM 8256
RDS1	14.5±0.71	10.5±0.71
RDS1	0±0.00	0±0.00
RDS3	0±0.00	0±0.00
RDS4	0±0.00	15.5±0.71
SFS1	0±0.00	0±0.00
SFS2	0±0.00	0±0.00
SFS3	14±0.00	0±0.00
RZSA1	8.5±0.71	10±0.71
RZSA2	0±0.00	0±0.00
RZSA3	0±0.00	15±0.00
RZSB1	10.5±0.71	0±0.00
RZSB2	0±0.00	0±0.00
RZSB3	0±0.00	0±0.00
PTSA1	8.5±0.71	0±0.00
PTSA2	0±0.00	0±0.00

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Table 4: Zones of inhibition (MN±SD) of secondary screening of *Streptomyces* isolates against test organisms

Extracts (mg/ml)	*S. aureus* ATCC 12600	*S. typhimurium* CCARM 8526
RDS1 (50)	14.5±0.71	10.5±0.71
RDS2 (50)	0±0.00	0±0.00
RDS3 (50)	0±0.00	0±0.00
RDS4 (50)	8.5±0.71	19.5±0.71
SFS1 (50)	0±0.00	0±0.00
SFS2 (50)	0±0.00	0±0.00
SFS3 (50)	10.5±0.71	14.5±0.71
RZSA1 (50)	8.5±0.71	13.5±0.71
RZSA2 (50)	0±0.00	0±0.00
RZSA3 (50)	10.5±0.71	19±0.00
RZSB1 (50)	10±0.00	8.5±0.71
RZSB2 (50)	0±0.00	0±0.00
RZSB3 (50)	0±0.00	0±0.00
PTSA1 (50)	8.5±0.71	0±0.00
PTSA2 (50)	0±0.00	0±0.00
+control (Aug, 30µg)	22.5±0.00	8.5±0.00
-control (10% DMSO)	0±0.00	0±0.00

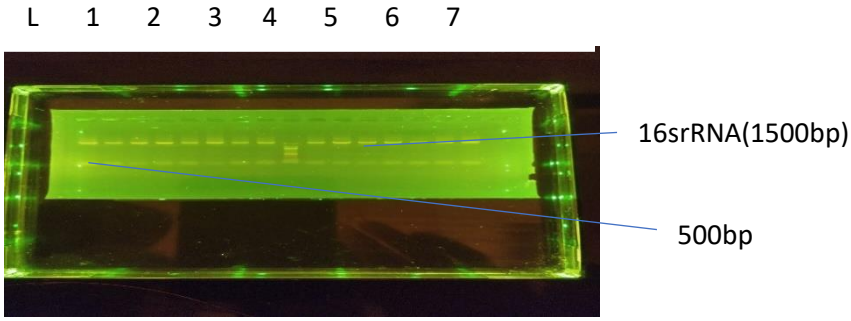

Plate 4: Agarose Gel Electrophoresis of 16S rRNA Gene of *Streptomyces* Isolates

Discussion

In this work, surface soil samples (6-10) cm were obtained for the selective isolation of *Streptomyces*. Also, previous studies suggest that a depth range of 5-10 cm is ideal (Kim *et al.,* 2004). The results for the percentage (%) distribution of the 15 unique *Streptomyces* isolates revealed that the Refuse dumpsite soil had the greatest percentage (26.7%), while Plantain plantation soil had 13.3%. A 20% dispersion was observed for school farm soil and rhizosphere soil (A and B). Rhizosphere soil C, grassland soil (A, B), and plantain plantation soil (B, C) all had a zero % distribution. According to (Shahidi, 2004), the percentage of *Streptomyces* spp in the total microbial population is similarly impacted by soil depth, and can be acquired from soil horizon C.

A variety of biochemical and physiological tests were conducted on the 15 different *Streptomyces* isolates. To determine the overall activity of all strains, various tests such as hemolysis, starch hydrolysis, gelatin hydrolysis, and hydrogen sulphide tests were performed on the various isolates, in addition to specific biochemical tests such as methyl red-voges proskauer, citrate, catalase, oxidase, and some carbohydrate utilisation tests.

Most *Streptomyces* isolates demonstrated varying responses to casein, gelatin, and hydrogen sulphide. These reactions were consistent with the results reported in earlier studies (Kokare *et al.,* 2004; Sripreechasak *et al.,* 2014).

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Based on experiments carried out in this current study, results showed that some of the *Streptomyces* isolates showed promising antagonistic activity as evidenced by moderate zones of inhibition against gram positive and gram negative test organisms. Several studies have demonstrated the antibacterial properties of *Streptomyces* spp using the cross-streak method. For example, a study by (Velho-Pereira and Nandkumar, 2012) found that several *Streptomyces* isolates exhibited strong antibacterial activity against a range of pathogenic bacteria, including *Staphylococcus aureus* and *Escherichia coli*. Another study by Jain *et al*. (2011) showed that *Streptomyces* spp isolated from soil samples were able to inhibit the growth of multidrug-resistant bacteria.

The results from the secondary screening shows that some of the *Streptomyces* extracts had zones of inhibition (MN±SD) mm against some of the bacterial pathogens, while some had no inhibition. These zones of inhibition could be attributed to the production of secondary metabolites produced by these *Streptomyces* isolates in the fermentation broth. This assumption was also supported by recent research by Liu *et al*. (2021) that identified several bioactive compounds with strong antibacterial properties using crude extracts of soil *Streptomyces*. Furthermore, a study by (Sharma and Thakur, 2020) evaluated the antibacterial activity of *Streptomyces* against clinical isolates of multi-drug resistant bacteria.

The partial nucleotide sequences of 16S rRNA gene led to identifying the 5 most potent isolates as various strains of *Streptomyces* species. Also, study by Labeda *et al*. (2012) demonstrated the utility of 16S rRNA gene sequencing for the identification of *Streptomyces* species. Isolates with codes RDS4, RZSA3, RZSA1, SFS3 and RDS1 that exhibited good antagonistic activity against *Salmonella typhimurium* CCARM 8256 and *Staphylococcus aureus* ATCC 12600 were identified genotypically as *Streptomyces griseus ATCC* 10137, *Streptomyces orientalis* IIRRACT9, *Streptomyces orientalis* IMSNU 20058T *Streptomyces venezuelae* JCM 4526 *and Streptomyces griseus* KACC 20084 respectively.

In conclusion, this current study revealed that some of the *Streptomyces* species isolated from soils at Rivers State University showed potential for antibiosis, as evidenced by moderate zones of inhibition against clinical and foodborne pathogens such as *Staphylococcus aureus* ATCC 12600 and *Salmonella typhimurium* CCARM 8256 during primary and secondary screening. As a result, these isolates can be viewed as promising candidates for the development of new antibacterial agents aimed at avoiding infectious diseases and maintaining human and environmental health.

To maximise the production of antibiotic chemicals produced by *Streptomyces* isolates, fermentation conditions such as growth media, pH, temperature, and other critical fermentation parameters should be optimised. In future research, purification procedures such as thin-layer and column chromatography should be used to properly extract cell-free bioactive components from these putative antibiotic producing *Streptomyces* isolates.

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