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Antimicrobial Activities of *Streptomyces* Species Isolated From Various Soil Samples in Federal University of Technology, Akure Environment

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Abstract: *Five (5) different species of Streptomyces were isolated from different soil samples obtained from the Federal University of Technology, Akure, Nigeria and were tested for antagonistic activity against 5 pathogenic bacteria and fungi respectively. During the primary antimicrobial screening, 12% of the strain showed inhibitory potentials against the test microorganisms. The active metabolite was extracted using chloroform. Purification of the extract was performed using column chromatographic technique. Infra-Red spectroscopy carried out on the active fraction revealed four important functional groups which were hydroxyl, carbon-hydrogen, carbonyl and aromatic groups. The nucleotide sequence of the 16S RNA showed 83% identity with Streptomyces albus. From the taxonomic feature, the Streptomyces isolate DSM 40313 matched with S. albus in the morphological, physiological and biochemical characters. Thus, it was assigned the name Streptomyces albusDSM 40313.*

Keywords:*Streptomyces, test microorganisms, soil samples, antagonistic activity.*

I. Introduction

Streptomyces are gram positive, spore-forming bacteria found in soil. They are characterized by their tough, leathery, frequently pigmented colonies and their filamentous growth (Euzéby, 2008). They have genomes with high guanine and cytosine content (Madigan and Martinko 2005). Streptomyces is the largest genus of Actinobacteria and the type genus of the family Streptomycetaceae (Kämpfer, 2006). The production of a number of toxic natural products by Streptomyces isolates was long thought to be a competitive mechanism for the bacteria. Species of this genus are unusually prolific in the production of antibiotics and other types of compounds that are deleterious to competing microorganisms(Laskariset al., 2010).They also have unusually large genome sizes, presumably because of their production of these secondary compounds. Streptomyces griseushas been shown antibioticssuch as streptomycin (Laskariset al. 2010).The resistance of numerous pathogenic bacteria and fungi to commonly used antibiotics require an urgent focus of research and since Streptomyces is the most abundant actinomycete group in soil (Otoguroet al., 2001; Ramakrishnanet al., 2009), Extensive screening of this genus Streptomyces will therefore lead to the discovery of many novel strains that produce useful secondary metabolites necessary to combat these resistant pathogens. This study was therefore investigated to isolate Streptomyces from some soil samples from FUTA environment

II. Materials and Methods

Sample collection and isolation

Soil samples were collected from household waste dumping site, ploughed soil, plantain cultivated soil, vegetative (weed) soil, loamy, sandy and clayey soils. The samples were transferred to the laboratory for further analysis.Plates containing starch casein medium were used for isolation of the Streptomyces. Different species of Streptomyces were isolated using the serial soil dilution technique as described by Olutiolaet al. (2000). Plates were incubated for 5 days at 28^{o}C. Streptomyces colonies were picked from each plate and were streaked onto fresh plates until pure cultures were obtained. The isolates were identified using Gram-staining, catalase production, citrate utilization, starch hydrolysis, and fermentation of sugars according to the protocols of Olutiolaet al. (2000) and Cheesbrough (2006).

Screening of Streptomyces for antimicrobial metabolite production

Fresh culture of Streptomyces was inoculated in starch casein broth and incubated at 28^{0}C for 7 days in water bath with shaking. Growth of the organism in the flask was confirmed by turbidity in the broth. The broth culture was centrifuged at 5000 rpm for 20 minutes and the supernatant was filtered through No 1 Whatman filter paper. The culture filtrate of the Streptomyces species was used for the determination of antimicrobial activity against the test organisms (Singh and Agrawal 2003). Molten agar was aseptically dispensed into Petri dishes containing 1ml of each test organism and was allowed to gel. The seeded plates were allowed to set and wells at equidistant from each other were made in the agar plates with the aid of a cork borer (diameter 10 mm).

Each well was then filled with 0.1ml of the culture filtrate. The plates were allowed to stand for one hour to allow diffusion of the metabolite in the filtrate, then incubated at 37°C for 24 h and observed for zones of inhibition. Three replicates of the experiment were performed and the diameters of the inhibition zones were measured and recorded.

Extraction and bioassay of antimicrobial metabolite

Extraction of crude metabolite

Antimicrobial compound was recovered from the culture filtrate of S. albus (Streptomyces whose filtrate gave the highest zone of inhibition value) by solvent extraction with chloroform. Chloroform was added to the filtrate in the ratio 1:1 (v/v) and shaken vigorously; the organic phase containing the metabolite was separated from the aqueous phase. The extract obtained was evaporated to dryness using rotary evaporator and the crude extract was then used for bio assay (Owolabi and Olarinoye, 2008).

Antimicrobial assay of the crude metabolite

Antimicrobial activity of the crude metabolite was determined by agar well diffusion method as described by Olutiolaet al. (2000). The test organisms were standardized to 0.5 McFarland standards as described by Oyelekeet al. (2008). Sterile Petri dishes were seeded aseptically with 1ml each of the standardized broth cultures and 20ml of sterilized Mueller- Hinton agar was poured aseptically on the seeded plates. The plates were swirled carefully for even distribution and allowed to gel. With the aid of sterile cork borer, wells were created on the solidified agar medium. Exactly 0.5mL of 50mg/mL of the metabolite was then introduced into the wells and approximately labeled. The plates were incubated for 24 h at 37^oC in case of bacterial species and 28^oC for three days in case of fungal species. The plates were observed for zones of inhibition (Atta et al., 2009).

Thin Layer Chromatography (TLC)

The crude extract was subjected to TLC analysis on 2.6 × 8 cm silica gel plate. This was spotted onto a TLC plate 2 cm above the base. After thorough drying, the plate was placed in a solvent system (chloroform: methanol in the ratio 2:1) in a chromatography tank to develop. The plates were removed when the solvent front approached the top of the plates and air-dried. Spots on the plates were visualized in an iodine chamber. The distances travelled by the constituents were measured and used to determine their R_f values(Atta, 2009)

Purification by Column chromatography

The purification of the antimicrobial compound was carried out using silica gel column chromatography as described by Atta (2009) and Ushaet al. (2010). Chloroform and methanol in the ratio 2:1 v/v was used as eluting solvent. The column was packed with silica gel (60-120 mesh). The sample to be separated was then added on the top of the packed column and eluted with the solvent at the flow rate of a drop per 3 sec. A collecting conical flask was placed at the bottom of the column to collect the eluted fractions. The collected elute was distilled, leaving the purified fractions. The fractions obtained were spotted unto TLC plates. Fractions with the same retention factor (R_f) were pooled together.

Antimicrobial Assay of column fractions of crude metabolite

Antimicrobial activity of the column fraction was determined by paper disc diffusion method (Aida et al., 2001). Sterile Petri dishes were seeded aseptically with 0.1ml of the standardized test organisms while about 20ml of sterile Mueller Hinton agar was poured aseptically on the seeded plates. Sterile Whatman filter paper discs (6.00mm in diameter) were impregnated with 30mg/ml of the purified fraction reconstituted with 30% dimethyl sulphonamide (DMSO). The impregnated paper discs were allowed to dry and applied with the aid of sterile forceps on the seeded plates. Filter paper disc dipped into DMSO and allowed to dry served as control. The plates were incubated at 37^oC for 24 h. Antimicrobial activities were determined by the measurement of zone of inhibition around each paper disc.

Determination of Minimum Inhibitory Concentration (MIC) of the metabolite.

The determination of Minimum Inhibitory Concentration (MIC) was carried out using agar well diffusion method as described by Magaldiet al. (2001). Varying concentrations of the metabolite (30mg/mL, 15mg/mL, 7.5mg/mL, 3.75mg/mL and 1.8mg/mL) were reconstituted in 30% DMSO. Sterile Petri dishes were seeded aseptically with 0.1mL of the standardized test organisms while about 20mL of sterile Mueller Hinton agar was poured aseptically on the seeded plates. The plates were swirled carefully for even distribution and allowed to gel. Five wells were created on the agar and the metabolite was introduced at different concentrations into the wells. The plates were incubated and the lowest concentration that inhibited the growth in a well was recorded. A well containing only 30% DMSO served as control

Spectroscopic analysis of the metabolite produced by Streptomycesalbus

Infra red (IR) analysis was performed with the aid of infra red spectrophotometer (Perkin-Elmer spectrum bx). A drop of purified extract was placed on fused sodium chloride (NaCl) cell. It was carefully placed on cell loosely clamped and fixed on the IR beam. The IR data was compared to the table of IR frequencies using the methods of Mayo et al. (2010).

Extraction of the genomic DNA of Streptomycesalbus

Genomic DNA of the bacterial isolate was extracted using CetylTrimethyl Ammonium bromide (CTAB) method as described by Chen et al. (2006) and Sridhar et al. (2010) with little modifications.Broth culture of Streptomyces albus (2.0ml) was centrifuged at 14,000rpm at 25^{o}C for 1 min to pellet the cells. The supernatant was carefully discarded and the pellets were re-suspended in 400µL of pre-warmed CTAB buffer and vortexed gently. A 75µL of 10% SDS was added to the suspension. Thereafter, 12µL of lysozyme solution (400mg/ml) was added to each cell suspension and mixed gently. The suspension was heated in a water bath at 65^{o}C for 30 min. It was then allowed to cool. 10µl of 20mg/mL of proteinase K solution was added to the suspension and incubated at 37^{o}C for 30 min. After incubation, 500µL of chloroform was added and mixed thoroughly. The cell suspension was then centrifuged at 10,000rpm for 10 min and the supernatant was carefully collected into a fresh micro centrifuge tube. 1.0µL of RNAse solution was added to the supernatant and incubated at 37^{o}C for 30 min. After this, 500µL of isopropanol was added to the mixture and kept at -20^{o}C for 1 h. After one hour, the suspension was centrifuged at 10,000rpm for 10 min and the supernatant was carefully discarded. The pellet was rinsed with 500µl of 70% ethanol, mixed thoroughly and then centrifuged at 10,000rpm for 10 min, so as to remove residual contaminants. The supernatant was discarded and the DNA pellet was air- dried for 1h. Finally, the DNA pellet was re-suspended in 200µL of nuclease-free water. The DNA was electrophoresed on 1% agarose gel containing 0.5 µg/ml ethidium bromide. The DNA was visualised by UV transilluminator and photographed.

Preparation of 1% agarose

This was prepared by dissolving 1.0g of agarose in 100mL of 1X Tris Acetate EDTA (TAE). The solution was heated in a microwave for 3 min and allowed to cool. 30µl of ethidium bromide was added and mixed thoroughly. The agarose was then poured into an electrophoresis chamber and allowed to solidify. The samples and DNA ladder were loaded into the wells and the electrophoresis was run at 80V for 1h.

Polymerase Chain Reaction (PCR) analysis using I6S primer

The PCR analysis was run with a universal primer for bacteria called 16S. The PCR mix comprised of 1µl of 10X buffer, 0.4µl of 50mM $MgCl_2$, 0.5µl of 2.5mMdNTPs, 0.5µl 5mM forward primer, 0.5µl of 5mM, reverse primer, 0.05µl of 5units/µl Taq with 2µl of template DNA and 5.05µl of distilled water to make-up 10µl reaction mix.The PCR profile used was initial denaturation temperature of 94^{0}C for 3min, followed by 30 cycles of 94^{o}C for 60sec, 56^{o}C for 60sec, 72^{o}C for 120sec and the final extension temperature of 72^{o}C for 5min and the 10^{o}C hold forever(Chen et al. 2006)

Purification of PCR products

The amplicon was further purified before the sequencing using 2M Sodium Acetate wash techniques. To about 10µl of the PCR product, 1µl 2M NaAct pH 5.2 was added, followed by 20µl Absolute Ethanol, kept at -20^{0}C for 1hr, spinned at 10,000rpm for 10 min, then washed with 70% ethanol and air dried. It was resuspended in 5µl sterile distilled water and kept at 4^{0}C for sequencing(Chen et al. 2006)

PCR for sequencing

The primer used for the reaction was forward 16S. The PCR mix used included 0.5µl of BigDye Terminator Mix,1µl of 5X sequencing buffer, 1µl of M13 forward primer with 6.5µl Distilled water and 1µl of the PCR product making a total of 10µl. The PCR profile for Sequencing is a Rapid profile, the initial Rapid thermal ramp to 96^{o}C for 1min followed by 25 cycles of Rapid thermal ramp to 96^{o}C for 10 sec Rapid thermal ramp to 50^{o}C for 5 sec and Rapid thermal ramp to 60^{o}C for 4 min, then followed by Rapid thermal ramp to 4^{o}C for 4 min(Chen et al. 2006)

Purification of PCR sequencing products

The PCR sequence product was purified before the sequencing running using 2M Sodium Acetate wash techniques.1µl 2M NaAct pH 5.2 was added to 10µl of the PCR product, then 20µl Absolute Ethanol was added and was kept at -20^{o}C for 1hr, it was then spined at 10,000rpm for 10 min, washed with 70% Ethanol and air-dried. It was re-suspended in 5µl sterile distilled water and kept at 4^{o}C for sequencing running(Chen et al. 2006)

Preparation of sample for Gene Sequencer (ABI 3130xl machine)

The Cocktail mix was a combination of 9µl of Hi Di Formamide with 1µl of Purified sequence making a total of 10µl. The sample was loaded on the machine and the data in form A, C, T, and G was released. The blast program (www.ncbi.nlm.nih.gov/blast) was employed in order to assess the degree of similarity.

Statistical analysis

Data are presented as mean ± standard error (SE). Significance of difference between different treatment groups was tested using one-way analysis of variance (ANOVA) and significant results were compared with Duncan's multiple range tests using SPSS window 7 version 17 software. For all the tests, the significance was determined at the level of $P<0.05$.

III. Results and Discussion

Streptomyces isolated from soil samples

Some of the isolates obtained were hard, chalky while some were leathery, dusty and dry. Microscopic study showed that cell arrangement was either scattered or clustered. All the Streptomyces isolates formed spores ranging from round to oval or chain form. The results of the biochemical tests showed that all the isolates were Gram-positive, urease positive and catalase positive. All except S. flavovirenswere citrate positive. Five species of Streptomyces were isolated; Streptomycesalbus, S. flavovirens, S. antibioticus, S. citreusand S. viridoflavus which grew well on Starch Casein Agar except S. flavovirens which grew moderately.

Antimicrobial activities of culture filtrate of Streptomyces

The culture filtrate ofS. albus was active against all the selected pathogenic bacteria namely Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, Escherichia coli ATCC 25922 and Klebsiella pneumoniae ATCC 3883 while S. antibioticus was active only against B. subtilis. However, S. aureus was more susceptible to the activity of all the Streptomyces species except S. antibioticus.Escherichia coli ATCC 25922 was resistant to filtrate of all the species of Streptomyces except S. albus (Table 1). For the antifungal screening, S. albusexhibited high inhibitory effect against Trichophytonmetagrophyte. Streptomyceantibioticus and S.citreus showed no inhibitory effect on any of test fungi (Table 2).

Table 1: Screening of Streptomyces speciesagainst selected test bacterial isolates

Streptomyces isolates	Bacterial isolates/zones of inhibition (mm)				
	Staphylococcus Aureus	B. subtilis	E.coliATCC25922	K.pneumoniaeATCC3883	Salmonella Typhi
IS 1	14.67±0.58 a	12.33±0.58a	9.67±0.58	11.00±1.00 c	10.67±1.16c
IS 2	10.67±1.16a	0.00±0.00 b	0.00±0.00	5.67±0.58 b	0.00±0.00 b
IS 3	3.33±0.58b	0.00±0.00 b	0.00±0.00	0.00±0.00a	2.67±0.58a
IS 4	0.00±0.00 b	4.00±0.00 b	0.00±0.00	0.00±0.00a	0.00±0.00 a
IS 5	3.00±0.00 b	3.33±1.16 b	0.00±0.00	0.00±0.00a	0.00±0.00 a

Data are presented as mean ± SD of three replicates with significant increases and a,b, and c showed that mean ± SD in the same row with different superscript are significantly different. ($p < 0.05$).

Key:

IS 1: S. albus; IS2: S. viridoflavus; IS3: S. flavovirens; IS4: S.antibioticus; IS5: S. citreus

Table 2: Screening of Streptomyces species against pathogenic test fungal isolates

	Fungal isolates/zones of inhibition (mm)			
Streptomyces isolates	A. niger	C.albicans ATCC 10231	M. canis	T.metagrophyte	A. flavus
IS 1	0.00 ± 0.00	4.00±0.00[b]	0.00±0.00	15.00±0.00[c]	3.33±0.58[b]
IS 2	0.00 ± 0.00	2.67±0.58[a]	0.00±0.00	3.67±0.58[b]	2.67±0.58[b]
IS 3	0.00 ± 0.00	0.00±0.00[c]	0.00±0.00	0.00±0.00[a]	4.67±1.16[a]
IS 4	0.00 ± 0.00	0.00±0.00[c]	0.00±0.00	0.00±0.00[a]	0.00±0.00[c]
IS 5	0.00 ± 0.00	0.00±0.00[c]	0.00±0.00	0.00±0.00[a]	0.00±0.00[c]

Data are presented as mean ± SD of three replicates with significant increases and a,b, and c showed that mean ± SD in the same row with different superscript are significantly different. ($p < 0.05$).

Key:

IS 1: S. albus; IS2: S. viridoflavus; IS3: S. flavovirens; IS4: S.antibioticus; IS5: S. citreus

Bioassay of crude metabolite produced by Streptomyces albus

SinceS.albus showed the highest antimicrobial potency against the test organisms (was selected for extraction and purification for further tests. The result of the antimicrobial assay of the metabolite produced by Streptomyces species is as shown on Table 3. Metabolite from S. albus inhibited S. aureus with zone of inhibition 29.33mm which was recorded as the highest while 4.67mm zone of inhibition was recorded against C. albicans ATCC 10231. The zone of inhibition produced by S. albus was 26.67mm against T. metagrophyte while 9.33mm zone of inhibition was recorded against A. flavus. There was no activity against A. niger and M. canis,

Antimicrobial activities of selected commercial antimicrobial drugs.

Results of antibacterial activities of commercial chloramphenicol, streptomycin and tetracycline showed zones of inhibition which were 24.33mm, 22.33mm, and 26.00mm respectively against S. aureus. The highest zone of inhibition (27.67mm) was recorded for tetracycline against S. typhi while the least zone of inhibition was recorded as 12.00mm for chloramphenicol against E. coli ATCC 25922. The overall best antibiotic was streptomycin which produced 28.00mm zone of inhibition against K. pneumoniaeATCC 3883. Commercially produced Nystatin showed 20.00mm zone of inhibition against A. flavus. Ketoconazole showed no antifungal potency against C. albicans and T. metagrophyte. The overall best antifungal was nystatin which produced 20.00mm zone of inhibition against A. flavus and griseofulvin which also produced 20.00mm zone of inhibition against C. albican ATCC 10231 while ketoconazole has the least antifungal activities (Tables 6).

Bioassay of partially purified metabolite of Streptomyces albus.

Result obtained from the bioassay of the partially purified metabolite of S. albus showed highest zone of inhibition recorded as 29.67mm against Staphylococcus aureus while 5.67mm zone of inhibition was recorded against Candida albicans ATCC 20131. There was no activity against A. niger and M. canis (Table 7). The metabolite exhibited broad spectrum of activity as it was effective against both Gram positive and Gram negative bacteria. Table 8 shows the minimum inhibitory concentration values of the purified metabolite. The highest MIC value recorded was 30mg/mL for C. albicansand A. flavus while the lowest MIC recorded was 3.75mg/mL for S. aureusand B. subtilis. The metabolite was bacteriostatic in its mode of action.

Comparative Antimicrobial activities of purified metabolite of Streptomyces albus and selected commercial antimicrobial drugs.

The zones of inhibition recorded for purified metabolite of S. albus against S. aureus,B. subtilis and E. coli ATCC 25922 (29.67mm, 28.00mm and 27.00mm respectively) were higher compared to tetracycline, chloramphenicol and streptomycin which produced 26.00mm, 24.33mm and 22.33mm zones of inhibition respectively. Lower zones of inhibition were produced by the metabolite against K. pneumoniae ATCC 3883 and S. typhi (19.67mm and 23.33mm respectively) in comparison with the commercial antibacterial drugs. Commercially produced antifungal agent, nystatin showed potent antifungal activities against C. albicans ATCC 10231 and A. flavus in comparison with the metabolite produced by S. albus.

Infra-red spectrum

The infrared spectrum of the antimicrobial agent showed bands corresponding to 19 peaks, with four important functional groups revealing the hydroxyl group at 3342. The peak at 2926 showed the existence of C-H, carbonyl group at peak 1667 and the aromatic group at peak 577 (Table 9; figure 10).

Table 3: Antimicrobial activities of crude metabolite produced by Streptomyces albus

Test organisms	zones of inhibition (mm)
Staphylococcus aureus	29.33±0.58
Bacillus subtilis	27.67±1.15
Escherichia coli (ATCC 25922)	26.33±0.58
Klebsiellapneumoniae(ATCC3883)	19.33±0.58
Salmonella typhi	22.67±1.15
Candida albicans	4.67±0.58
Aspergillusflavus	9.33±0.58
Trichophytonmetagrophyte	26.67±0.58
Aspergillusniger	0.00±0.00
Microsporum canis	0.00±0.00

Table 4: Antimicrobial activities of selected commercial antimicrobial drugs

Test organisms	Antimicrobials (zones of inhibition (mm))					
	TET	CHL	STR	NYS	KET	GRS
S. aureus	26.00±0.00^{c}	24.33±0.58^{b}	22.33±0.00^{a}	ND	ND	ND
B. subtilis	18.00±0.00^{c}	26.00±0.00^{b}	27.67±0.00^{a}	ND	ND	ND
E. coli ATCC 25922	22.00±0.00^{c}	12.00±0.00^{b}	14.00±0.00^{a}	ND	ND	ND
K. pneumoniae ATCC 3883	26.00±0.00^{c}	24.00±0.00^{b}	28.00±0.00^{a}	ND	ND	ND
S. typhi	27.67±0.57^{a}	24.00±0.00^{b}	24.33±0.58^{b}	ND	ND	ND
C. albicans ATCC 10231	ND	ND	ND	6.00±0.00^{a}	0.00±0.00^{a}	20.00±0.00^{b}
A. Flav	ND	ND	ND	20.00±0.00^{a}	18.67±0.58^{b}	18.00±0.00^{b}
T. metagrophyte	ND	ND	ND	12.33±0.58^{b}	0.00±0.00^{a}	19.67±0.58^{c}
M. canis	ND	ND	ND	16.67±1.53^{d}	12.00±1.00^{c}	13.00±1.00^{d}
A. niger	ND	ND	ND	18.33±1.53^{d}	8.67±0.58^{b}	9.33±0.58^{c}

Data are presented as mean ± SD of three replicates with significant increases and a,b, c and d showed that mean ± SD in the same row with different superscript are significantly different. ($p < 0.05$).

Key

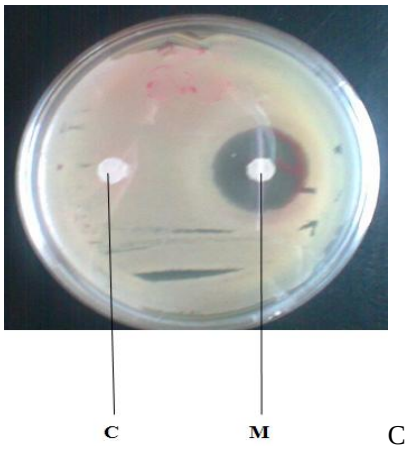
TET: Tetracycline; CHL = Chloramphenicol; KET = Ketoconazole; NYS: Nystatin

STR = Streptomycin GRS = Griseofulvin

ND = Not Determined

Table 5: Antimicrobial activities of partially purified metabolites of S. albus against selected organisms.

Test organisms	Mean zones of inhibition (mm)
Staphylococcus aureus	29.67±0.58
Bacillus subtilis	28.00±1.00
Escherichia coli (ATCC 25922)	27.00±1.00
Klebsiellapneumoniae(ATCC3883)	19.67±1.15
Salmonella typhi	23.33±0.58
Candida albicans	5.67±1.15
Aspergillusflavus	11.00±1.00
Trichophytonmetagrophyte	27.00±0.58
Aspergillusniger	0.00±0.00
Microsporum canis	0.00±0.00



C = Control, M= Metabolite

Plate 1: Antimicrobial activities of partially purified metaboliteof S. albus against S. aureus

Table 6: Minimum inhibitory concentration (MIC) values of the metabolite against selected test organisms

Test organisms	MIC value (mg/mL)
Staphylococcus aureus	3.75
Bacillus subtilis	3.75
Escherichia coli (ATCC 25922)	7.5
Klebsiella pneumoniae (ATCC 13883)	15
Salmonella typhi	15
Candida albicans ATCC 10231	30
Trichophyton metagrophyte	7.5
Aspergillus flavus	30

Table 7: Characteristics Infra-Red frequencies for metabolite produced by Streptomyces albus

Wave number	Absorbance
3421.83	O-H, N-H
3394.83	O-H
2926.11	C-H
2354.71	C-H
2362.88	C-H
2088.98	C-H
1666.55	C-O
1649.19	C-O
1384.94	C-O
1281.49	C-O
1143.83	C-O
1074.93	C-O
1035.81	C-O
931.65	Aromatic C-H
827.49	Aromatic C-H
771.55	Aromatic C-H
576.74	Aromatic C-H
551.66	Aromatic C-H
513.08	Aromatic C-H

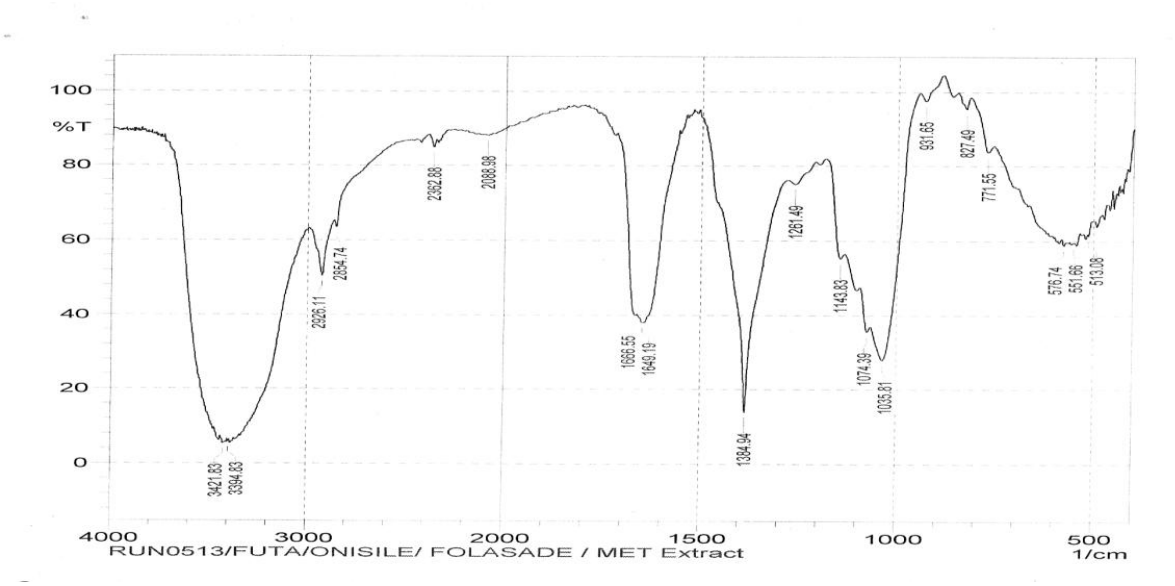


Figure 1: Infra-red spectrum showing different peaks

TCGGATATTGGGCGTAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAAC
CGGGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTA
GCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTG
ACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAAC
GATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGC
CTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTA
GAGATAGGGCTTTCCCTTCGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGT
GAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTTAGTTGGGC
ACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCC
TTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCTGCGAGACCGCAAGGTTTA
GCCAATCCCATA

Figure 2: Gene Sequence of Streptomyces albus

Table 8: Blasting of sequence producing significant alignments

	Description	Max score	Total score	Query cover	E value	Degree of relatedness	Accession
NR_025615	Streptomyces albus subsp. albus strain DSM 40313 16S ribosomal RNA, partial sequence	619	619	95%	1e-178	83%	NR_025615
NR_043342	Streptomyces albospinusstrain JCM 3399 16S ribosomal RNA, partial sequence	623	623	98%	1e-179	82%	NR_043342
NR_024723	Streptomyces albulus strain IMC S-0802 16S ribosomal RNA, partial sequence	623	623	98%	1e-179	82%	NR_024723

IV. Discussion

Screening for antimicrobial activity of microbes isolated from FUTA environment has not been reported yet. Results from this study have however shown the presence of Streptomyces capable of producing antimicrobial metabolites in these habitats. The metabolite produced inhibited gram-positive bacteria better than the gram-negative bacteria and this agrees with the work of Ama (2011) where the active metabolites produced from the isolates under study inhibited gram-positive bacteria better than the gram-negative bacteria. This could be attributed to the differences in the sensitivities of gram-positive and gram-negative bacteria due to the differences in the structure and composition of their cell walls (Ama, 2011). Gram-negative bacteria possess a thin peptidoglycan layer and a unique outer membrane which consists of lipopolysaccharide (LPS) components. This outer membrane makes the cell wall impermeable to lipophilic solutes thus blocking certain antibiotics such as penicillin, dyes, and detergents from penetrating the cell.

Although five (5) out of the 12 Streptomyces species (26%) showed antimicrobial activity in this study, some inhibitory screening investigations have recorded values closer to what was obtained in this study. For example, a study carried out by Ivanova et al. (1998) reported that out of the 491 bacteria isolated from different marine sources, 26% of the isolates were active. Zheng et al. (2005) also reported that eight out of twenty-nine (29) strains, representing 28 % of the microbes considered in their study were able to inhibit the growth of at least one of the target microorganisms.

The purification process through column chromatography packed with silica gel and an eluting solvent composed of chloroform-methanol (2:1) showed fractions having the same R_f Similar results were obtained by Hitchens and Kells (2003), Naggar (2007) and Atta et al. (2009).The metabolite appeared to be bacteriostatic in its mode of action. Bacteriostatic agents (e.g. β-lactams, chloramphenicol, tetracycline, clindamycin and macrolides) have been effectively used for treatment of a range of bacterial infections, including endocarditis, meningitis, and osteomyelitis (Pankey and Sabath, 2004).

The spectroscopic characteristics of the antimicrobial agent under study revealed the IR spectrum with peaks at 3421 - 3394, thereby suggesting an hydroxyl group. The peak at 2926-2088 showed the existence of C-H while 1667-1035 peak indicated that there is a carbonyl group and the 931-513 peaks showed the existence of the aromatic groups. The hydroxyl group is suggesting that a phenolic compound is present in the metabolite produced by the organism. Phenolic-type antimicrobial agents have long been known for their antiseptic, disinfectant, or preservative properties, depending on the compound (Hugo, 2002).

The nucleotide sequence of the 16S RNA showed 83% identity with Streptomyces albus. From the taxonomic feature, the Streptomyces isolate DSM 40313 matched with S. albus in the morphological, physiological and biochemical characters. Thus, it was assigned the name Streptomyces albusDSM 40313. Analyses based on RNA polymerase β−subunit (rpoB) genesequences have provided valuable data in polyphasic studies designed to clarifyrelationships within and between genera of Actinomycetes (Goodfellowet al., 2007).

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