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Isolation, Characterization and Biological Activities of Antibacterial Antibiotics Produced by Streptomycesalbus Dsm 40313

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Abstract: *This research was carried out in the search for bioactive substances from Streptomyces that demonstrated inhibitory effect against some pathogenic bacteria. Twelve Streptomyces strains were isolated from soil samples collected from different soil samples in Federal University of Technology environment. All the Streptomyces isolated were screened for characterization and purification. The active metabolite was extracted using chloroform and methanol. The separation of active compound was performed using thin layer chromatographic and column chromatographic techniques. The broad spectrum strain, Streptomyces albus DSM 40313 was selected for characterization and purification. the nucleotide sequence of the 16sRNA gene of the most potent strain evidenced on 83% similarity with Streptomyces albus in the morphological and biochemical characters and as a result, it was given the name Streptomyces albus DSM 40313.*
Keywords: *Streptomyces albus, Extraction, Purification, 16sRNA, Antibacterial activities.*

I. Introduction

Streptomyces is the largest antibiotic producing genus that producesantibacterial and antifungals. They are found in dusts, soil, grain and straws. Most Streptomyces produce spores, and are noted for their distinct "earthy" odour that results from production of a volatile metabolite, geosmin[8]

They produce over two-thirds of the clinically useful antibiotics of natural origin (e.g., neomycin and chloramphenicol) [9]Recognition of the value of *Streptomyces* species and their relatives as versatile producers of secondary metabolites began with the discovery of actinomycin in 1940, followed by streptomycin in 1943, and currently two-thirds of marketed antimicrobial drugs are produced by actinomycetes[7]

Various antimicrobial substances from *Streptomyces* sp. have been isolated and characterized including aminoglycosides, anthracyclins, glycopeptides, β-lactams, macrolides, nucleosides, peptides, polyenes, polyester, polyketides, actinomycins and tetracyclines[2].

II. Materials and Methods

Sample Collection
Soil samples were collected and transferred to the laboratory for further analysis.

Isolation
*Streptomyces*species were isolated from soil using serial dilution techniques. Starch Casein agar was used for the isolation[11].

Biochemical Tests
For identification of the bacterial isolates, biochemical tests were performed. These included Gram-staining, catalase production, citrate utilization, starch hydrolysis, and fermentation of sugars[3, 11].

Screening for Antibacterial Activities
The antibacterial activity was determined by using agar well diffusion method. Fresh and pure culture of *Streptomyces* was inoculated in starch casein broth and incubated at 28^0C 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 standard test organisms[14].

Morphological and physiological characteristics of *Streptomyces* isolates
The morphological and physiological study of the isolate was compared withBergy's manual of determinative bacteriology.

Purification by column chromatography

The purification of the antimicrobial compound was carried out using silica gel column chromatography[12]. Chloroform and methanol in the ratio 2:1 v/v was used as eluting solvent.

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 [10].

Extraction of the genomic DNA of *Streptomycesalbus*

Genomic DNA of the bacterial isolates was extracted using CetylTrimethyl Ammonium bromide (CTAB) method[4,15].

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. 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[4, 15].

PCR analysis using I6S primer

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^{0}C for 60sec, 56^{0}C for 60sec, 72^{0}C for 120sec and the final extension temperature of 72^{0}C for 5min[4, 15].

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.

PCR for sequencing

The primer used for the reaction was forward I6S. 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^{0}C for 1min followed by 25 cycles of Rapid thermal ramp to 96^{0}C for 10 sec Rapid thermal ramp to 50^{0}C for 5 sec and Rapid thermal ramp to 60^{0}C for 4 min, then followed by Rapid thermal ramp to 4^{0}C for 4 min.

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^{0}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^{0}C for sequencing running.

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.

Blasting of the sequence

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

Table 1 Screening of *Streptomycesalbus*DSM 40313against bacterial isolates

Test organisms	Mean zones of inhibition (mm)
Staphylococcus aureus	29.33±0.58
Bacillus subtilis	27.67±1.15
Escherichia coli (ATCC 25922)	26.33±0.58
Klebsiellapneumonia(ATCC3883)	19.33±0.58
Salmonella typhi	22.67±1.15

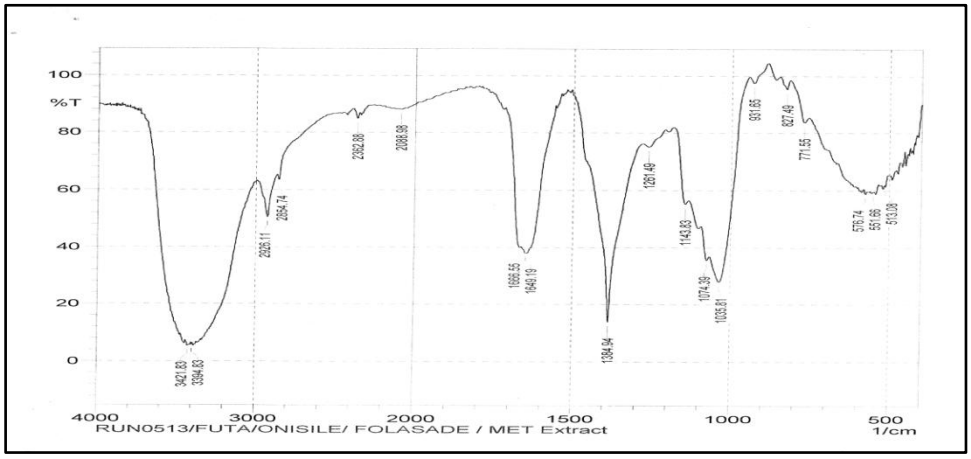

Figure 1: Infra-red spectrum showing different peaks

Table 2: Blasting of sequence producing significant alignments

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

IV. Discussion

The metabolite produced by *Streptomycesalbus* exhibited broad spectrum of activity as it showed active potency against both Gram positive and Gram negative bacteria.

Gram positive bacteria were inhibited better than Gram negative bacteria. This could be attributed to the differences in the sensitivities of Gram-positive and Gram-negative. 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. Gram-positive bacteria only possess the peptidoglycan layer which is not a very effective permeability barrier[1].

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.Phenolic compounds are also known to act as natural antioxidants and antinitrosating agents. A regular intake of (poly)phenolic compounds widely found in fruits, vegetables, tea, and red wine is believed to decrease the incidence of certain forms of cancer, and for that reason they are commonly regarded as chemopreventive agents[5].

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 albus*DSM 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[6].

V. Conclusion

*Streptomyces albus*DSM 40313 produces metabolites with active potency against both Gram positive and Gram negative bacteria. It also contains bioactive compounds from *Streptomycesalbus* which are the phenolic, carbonyl and aromatic compounds.

The cooperation of scientists from various relevant scientific disciplines is required for discovery, preparation and production of new biologically active compounds and their introduction into clinical practice.

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