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Production, Purification, Physico-Chemical Characteristics and Biological Activities of Antifungal Antibiotic Produced by *Streptomyces antibioticus*, AZ-Z710

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Abstract: This work was carried out in the course of a screening program for specific actinomycetes bioactive substances that demonstrated inhibitory effects against some pathogenic strains. Thirty-seven actinomycete strains were isolated from soil sample collected from Zagazig districted, El-Sharkia governorate, Egypt. All these strains were screened for their antifungal activity against pathogenic fungi. Among the thirty-seven actinomycetes, the broad spectrum strain *Streptomyces sp.* AZ-Z710 was selected for bioactive compound characterization and purification. The nucleotide sequence of the 16s RNA gene (1.5 Kb) of the most potent strain evidenced an 88% similarity with *Streptomyces antibioticus* and *Streptomyces bungoensis*. From the taxonomic features, the actinomycetes isolate AZ-Z710 matched with *Streptomyces antibioticus* in the morphological, physiological and biochemical characters. Thus, it was given the suggested name *Streptomyces antibioticus,* AZ-Z710. The active metabolite was extracted using ethyl acetate (1:1, v/v) at pH 7.0. The separation of the active ingredient and its purification was performed using both thin layer chromatography (TLC) and column chromatography (CC) techniques. The physico-chemical characteristics of the purified antifungal agent viz. color, melting point, solubility, elemental analysis and spectroscopic characteristics have been investigated. This analysis indicates a suggested empirical formula of $C_{20}H_{24}O_4$. The minimum inhibition concentrations "MICs" of the purified antifungal agent were also determined. In conclusion, the collected data emphasized the fact that the purified antifungal agent was suggestive of being belonging Mycangimycin antibiotic produced by *Streptomyces antibioticus*, AZ-Z710.

Key words: Mycangimycin antibiotic · *Streptomyces antibioticus* · 16s RNA · Production · Extraction · Purification · Physico-chemical characteristics and Biological activities

INTRODUCTION

Among the different types of drugs prevailing in the market, antifungal antibiotics are very small but significant group of drugs have an important role in the control of mycotic diseases [1]. *Streptomyces* is the largest antibiotic producing genus, producing both antibacterials and antifungals and also a wide range of other bioactive compounds such as immunosuppressant [2]. Around 11,900 antibiotics had been discovered by 1994 of which around 6600 (55%) were produced by *Streptomyces*, whereas filamentous fungi produced 2600 (22%), bacteria produced 1400 (12%) and non-*Streptomyces* strains of *Actinomycetes* produced 1300 (11%) [3]. Mycangimycin is conjugated heptaene, 1,2-dioxolane and carboxylic acid is a very sensitive molecule [4]. Mycangimycin has to inhibit *O. minus* more strongly than it inhibits *Entomocorticium* sp., *Candida albicans* wild type, *C. albicans* ATCC10231 and amphotericin resistant *C. albicans* ATCC 200955 and *Saccharomyces cerevisiae* [4, 5]. Mycangimycin's 1,2-dioxolane functionality is similar to pharmacophores with significant antimalarial activity [6], the 1,2,4-trioxane in artimesinin [7] and synthetic 1,2,4-trioxolanes [8]. In the antimalarial assay against Plasmodium falciparum, [9] mycangimycin had an EC_{50} of 17 ng/mL, comparable to clinical antimalarial drugs such as artemisinin, chloroquine, pyrimethamine and mefloquine, which have EC_{50}'s in the 10 ng/mL range in the same assay. Mycangimycin's mode(s) of action as an antifungal or antimalarial agent is not known [10]. Mycangimycin is a yellow powder, which was analyzed for the molecular formula $C_{20}H_{24}O_4$ by high-resolution mass spectrometry at 351.1572 [11]. The ^{1}H NMR spectrum showed 15 olefinic protons from 5.10 to 6.77 ppm, including an unusual terminal olefinic methylene group at 5.23 and 5.10 ppm, two oxygenated methine protons at 4.63 and 4.28 ppm and

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six aliphatic protons between 1.97 and 2.89 ppm. The 15 olefinic carbon resonances were deduced to be seven conjugated double bonds from the UV spectrum (λ_{max} 355, 374 and 395 nm) [4].

The present work described the isolation of a *Streptomyces* strain from Egyptian soil, which generated an antifungal compound. The identification of this strain, based on the cultural, morphology, physiology and biochemical characteristics, as well as 16s rRNA methodology. The purified bioactive substance *Streptomyces* strain was spectroscopic analysis and its biological activities were determined.

MATERIALS AND METHODS

Microorganism: Thirty-seven actinomycete strains were isolated from soil samples collected from Zagazig districted, El-Sharkia governorate, Egypt, using the soil dilution plate technique [12].

Screening for Antimicrobial Activity: The anti-microbial activity was determined by cup method assay [13].

Taxonomic Studies of Actinomycete Isolate: Morphological characteristics of the most potent produce strain AZ-Z710 grown on starch nitrate agar medium at 30°C for 5 days were examined. The form of the sporophores was studied with light microscope by directly observation and the morphology of the spores and the spore chains were investigated by electron microscope) (JEOL Technics Ltd.). Physiological and biochemical characteristics were conducted: lecithinase on egg-yolk medium [14]; lipase [15]; Protease [16]; pectinase [17]; α-amylase, hydrogen sulphide production, citrate utilization, urea test, coagulation of milk and oxidase test [18]; catalase test [19]; melanin pigment [20]; degradation of both esculin and xanthine [21] Nitrate reduction [22]. The utilization of different carbon and nitrogen sources [23]. Cell wall was performed by the method of [24, 25]. The general and some other significant properties for the taxonomy of the strain were studied by the methods of the International Streptomycetes Project (ISP) [26]. Colors characteristics were assessed [27].

Dna Isolation and Manipulation: The locally isolated actinomycete strain AZ-Z710 was grown for five days on a starch agar slant at 30°C. Two ml of the spore suspension were inoculated into the starch-nitrate broth and incubated for 3 days on a shaker incubator at 200 rpm and 30°C to form a pellet of vegetative cells (pre-sporulation). Then the genomic DNA was extracted [28].

Amplification and sequencing of the 16S rRNA gene PCR amplification of the 16S rRNA gene of the local actinomycete strain was conducted using two primers, StrepF; 5.-ACGTGTGCAGCCCAAGACA-3 and Strep R; 5.ACAAGCCCTGGAAACGGGGT-3, in accordance with the method described by [29]. (5.-ACGTGTGCAGCCCAAGACA-3 and Strep R; 5.ACAAGCCCTGGAAACGGGGT-3, [29].) The PCR mixture consisted of 30 pmol of each primer, 100 ng of chromosomal DNA, 200 μM dNTPs and 2.5 units of Taq polymerase, in 50 μl of polymerase buffer. Amplification was conducted for 30 cycles of 1 min at 94°C, 1 min of annealing at 53°C and 2 min of extension at 72°C. The PCR reaction mixture was then analyzed via agarose gel electrophoresis and the remaining mixture was purified using QIA quick PCR purification reagents (Qiagen, USA). The 16S rRNA gene was sequenced on both strands via the dideoxy chain termination method [30]. The 16S rRNA gene (1.5 kb) sequence of the PCR product was acquired using a Terminator Cycle Sequencing kit (ABI Prism 310 Genetic Analyzer, Applied Biosystems, USA).

Sequence Similarities and Phylogenetic Analysis: The BLAST program (www.ncbi.nlm.nih.gov/blast) was employed in order to assess the degree of DNA similarity. Multiple sequence alignment and molecular phylogeny were evaluated using BioEdit software [31]. The phylogenetic tree was displayed using the TREE VIEW program.

Preparation of the Crude Extract of *Streptomyces antibioticus*, AZ-Z710

Production: The isolate, AZ-Z710 was inoculated into 250 ml Erlenmeyer flasks containing 75 ml of liquid starch nitrate medium. The flasks were incubated on a rotary shaker (200 rpm) at 30°C for five days. A twenty liter total volume was filtered through Whatman No.1 filter paper and followed by centrifugation at 5000 r.p.m for 20 minutes [32, 33].

Extraction: The clear filtrate was adjusted at different pH values (4 to 9) and extraction process was carried out using different solvents separately at the level of 1:1 (v/v). The organic phase was concentrated to dryness under vacuum using a rotary evaporator.

Precipitation: The precipitation process of the crude compound was carried out using then filtrated and precipitated with petroleum ether (b.p 60-80 °C) and followed by centrifugation at 5000 r.p.m for 15 min.

Purification by TLC: Separation of the antifungal compound into its individual components was conducted by thin layer chromatography using methanol-dichloromethane-water (1:1:1, v/v) [4, 32].

Purification by column chromatography: The purification of the antifungal compound was carried out using silica gel column (2.5 X 50) chromatography. Chloroform-Methanol (9:1 v/v) was used as an eluting solvent [33]. The column was left overnight until the silica gel (Prolabo) was completely settled. One-ml crude extract to be fractionated was added on the silica gel column surface and the extract was adsorbed on top of silica gel. Fifty fractions were collected (each of 5 ml) and tested for their antifungal activities [33].

Physico-Chemical Properties

Elemental Analysis: The elemental analysis C, H, O, N and S was carried out at the microanalytical center, Cairo University, Egypt.

Spectroscopic analysis: The Infra-Red (IR), Ultra-Violet (UV), Mass spectrum and Nuclear Magnetic Resonance (NMR) spectrum were determined at the micro analytical center of Cairo University, Egypt.

Biological Activity of the Antifungal Agent: The minimum inhibitory concentration (MIC) has been determined by the paper method assay [13].

Identification of the Antifungal Agent: The antibiotic produced by *Streptomyces antibioticus*, AZ-Z710 was identified according to the recommended international references of [34].

RESULTS

Screening for the Antimicrobial Activities: The active metabolites produced by actinomycete culture, AZ-Z710 exhibited various degrees of activities against unicellular and filamentous fungi (Table 1).

Table 1: Mean diameters of inhibition zones (mm) caused by 100µl of the antimicrobial activities produced by actinomycete isolate AZ-Z710 in the agar plate diffusion assay (The diameter of the used cup assay was 10 mm)

Test organism	Mean diameters of inhibition zone (mm)
A-Bacteria	
1-Gram Positive	
Staphylococcus aureus, NCTC 7447	0.0
Bacillus subtilis, NCTC 1040	15.0
Bacillus pumilus, NCTC 8214	14.0
Sarcina maxima, ATCC 33910	0.0
Micrococcus luteus ATCC 9341	0.0
2-Gram Negative	
Escherichia coli, NCTC10416	0.0
Klebsiella pneumoniae NCIMB 9111	0.0
Pseudomonas aeruginosa, ATCC 10145	0.0
B-Fungi	
1-Unicellular fungi	
Candida albicans, IMRU 3669	27.0
Saccharomyces cerevisiae ATCC 9763	28.0
2-Filamentous fungi	
A. niger IMI 31276	26.0
A. fumigatus ATCC 16424	22.0
A. flavus IMI 111023	25.0
Botrytis fabae	23.0
Penicillium chrysogenum	22.0
Rhizoctonia solani	24.0
Alternaria alternata	23.0

Identification of the Actinomycete Isolate, AZ-Z710: The vegetative mycelia grew abundantly on both synthetic and complex media and showed fragmentation into bacillary elements. The aerial mycelia grew abundantly on starch-nitrate agar medium, oatmeal agar medium (ISP-3) and inorganic salts starch agar medium (ISP-4). The Spore chains were spiral and had a smooth surface (Fig. 1). Whereas, both sclerotic granules and sporangia were not observed. The cell wall hydrolysate contained LL-diaminopimelic acid (LL-DAP) and sugar pattern could not be detected. The physiological and biochemical characteristics of actinomycete isolate AZ-Z710 are summarized in (Table 2).

Color and Culture Characteristics: The isolate AZ-Z710 showed light gray aerial mycelium; substrate mycelium was light yellowish brown and the diffusible pigment could not be detected onto ISP-1,2 3,4and5 (Table 3).

Taxonomy of Actinomycete Isolate: This was performed basically according to the recommended international Key's viz. [35], [36] and [37]. On the basis of the previously collected data and in view of the comparative

Table 2: he morphological, physiological and biochemical characteristics of the actinomycete isolate AZ-Z710

Characteristic	Result
Spore mass	Light Gray
Spore surface	Smooth
Spore chain	Spiral
Color of substrate mycelium	Light yellowish brown
Diffusible pigment	Not produced
Diaminopimelic acid (DAP)	LL-DAP
Sugar Pattern	Not detected
Hydrolysis of: Protein, Starch, Pectin	+
and Egg-yolk (lecithin) Lipid	-
Catalase test	-
Production of melanin pigment on:-	
Peptone yeast-extract iron agar	
and Tyrosine agar medium	+
Tryptone-yeast extract broth	-
Degradation of: Esculin and Xanthine	+
H_2S Production	-
Nitrate reduction	+
Citrate utilization	+
Urea test	-
Coagulation of skim milk	-
Utilization of	
D-Xylose	-
D-Mannose	+
D-Glucose	+
D-Galactose	+
Rhamnose	+
Raffinose	+
D-Mannitol	+++
L-Arabinose	+
meso-Inositol	++
Lactose	-
Maltose	-
Trehalose	+
D-fructose	+
Sucrose	++
Starch	+++
L-Cycteine	+
L-Valine	+
L-Histidine	++
L-Phenylalanine	±
L-Hydroxproline	±
L-Lysine	+
L-Arginine	+
L-Serine	+
L-Tyrosine	+
Growth with	
Thallous acetate (0.001), Sodium azide	
(0.01) and Phenol (0.1)	+
Growth temperature	30 °C (25-50 °C)
Growth at 7% NaCl concentration	-
Resistance to antibiotics	
Ampicillin (10 ug); Ceplalexin (30 ug); olistin	
(10 ug); Erthromycin (15 ug)	
and Rifampicin (5 ug)	+
Antimicrobial activity against	
Bacillus subtilis, NCTC 10400	
Micrococcus luteus, ATCC 9341	+
Saccharomyces cerevisiae, ATCC	
9763 and *Candida*	-
albicans IMRU 3669	+
A. niger IMI 31276	+

=+Positive,= Negative, ++ = moderate growth +++= good growth and ±= doubtful results

study of the recorded properties of AZ-Z710 in relation to the most closest reference strain, viz. *Streptomyces antibioticus*, it could be stated that actinomycetes isolate, AZ-Z710 is suggestive of being likely belonging to *Streptomyces antibioticus,* AZ-Z710.

A. Amplification of the 16s rRNA Gene: The 16s rRNA gene was amplified by polymerase chain reaction (PCR) using the universal primers. The primers that was used to 16S rDNA sequencing were 16F357 of the sequence strepF; 5'-ACGTGTGCAGCCCAAGACA-3' and strpR; 5'-ACAAGCCCTGGAAACGGGGT-3', the product of the PCR was analyzed on 1.5% ethidium bromide gel.

B-molecular Phylogeny of the Selected Isolate: The 16S rDNA sequence of the local isolate was compared to the sequences of *Streptomyces* spp. In order to determine the relatedness of the local isolate to these *Streptomyces* strains. The phylogenetic tree (as displayed by the Tree View program) revealed that the locally isolated strain is closely related to *Streptomyces* sp., the most potent strain evidenced an 88% similarity with *Streptomyces antibioticus* and *Streptomyces bungoensis* (Fig. 2).

Production, Extraction and Purification: The fermentation process was carried out for five days at 30°C using liquid starch nitrate as production medium. Twenty-liter total volume filtered was conducted followed by centrifugation at 5000 r.p.m. for 20 minutes The clear filtrates containing the active metabolite (20 liters), was adjusted to pH 7.0 then extraction process was carried out using Ethyl acetate at the level of 1:1 (v/v). The organic phase was collected and evaporated under reduced pressure using rotary evaporator. The crude extracts were dissolved in a small amount of methanol, then filtrated and precipitated with petroleum ether (b.p 60-80 °C) and followed by centrifuged at 5000 r.p.m for 15 minute. Its color is yellow. Separation of antifungal agent into individual components was carried out by thin-layer chromatography using a solvent system composed of methanol-dichloromethane-water (1:1:1, v/v). Only one band yellowish in color at R_f = 0.55 showed antifungal activity. The purification process through column chromatography packed with silica gel was eluted with a mixture of chloroform-methanol (9:1 v/v), revealed that the most active fractions against the fungal pathogenic range 20 to 27.

Physicochemical Characteristics: The purified antifungal agent of *Streptomyces antibioticus,* AZ-Z710 produced characteristic odour and its melting points was 144°C.

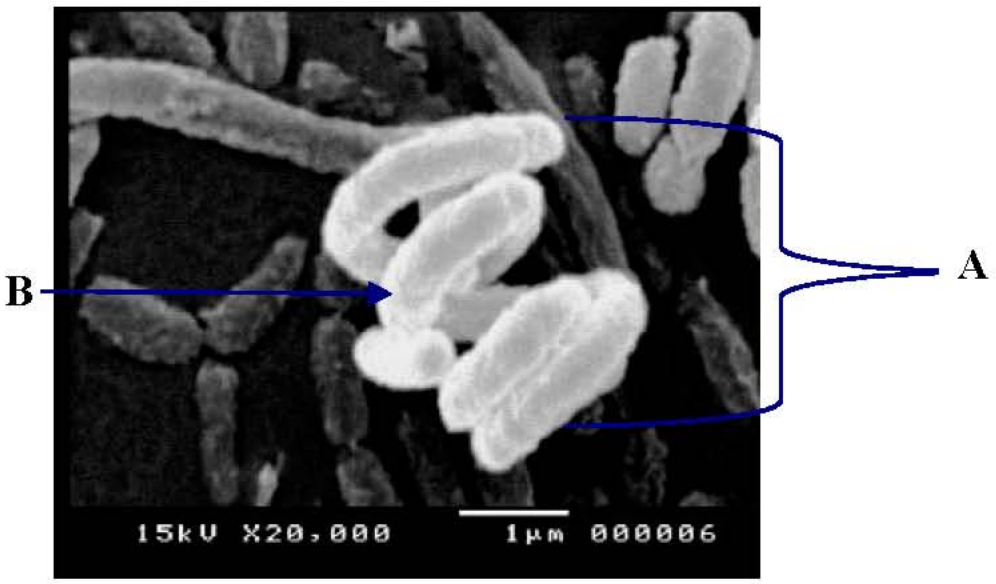

Fig. 1: Scanning electron micrographs of the actinomycete isolate AZ-Z710 growing on starch nitrate agar medium. (A) spore chain spiral.(B) spore surfaces smooth (X20, 000).

Table 3: Culture characteristics of the actinomycete isolate AZ-Z710 after 21 days

Media	Growth	Aerial mycelium (spore mass)	Substrate mycelium	Diffusible pigment
1-Starch nitrate agar medium	Good	264-L. Gray Light gray	57-1.br light brown	58 m-br moderate brown
2-Tryptone yeast extract broth (ISP-1)	No growth	-	-	-
3-Yeast extract malt extract agar medium (ISP-2)	moderate	264-L. Gray Light gray	76-1-y-br Light yellowish brown	-
4-Oat-meal agar medium (ISP-3)	Good	264-L. Gray Light gray	76-1-y-br Light yellowish brown	-
5-Inorganic salts starch agar medium (ISP-4)	Good	264-L. Gray Light gray	76-1-y-br Light yellowish brown	-
6-Glycerol-Asparagine agar medium (ISP-5)	No growth	-	-	-
7-Peptone yeast extract iron agar medium (ISP-6)	moderate	264-L. Gray Light gray	57-1.br light brown	59-d.Br Deep brown
8-Tyrosine agar medium (ISP-7)	moderate	264-L. Gray Light gray	57-1.br light brown	59-d.Br Deep brown

**The color of the organism under investigation was consulted with the ISCC-NBS color-name charts illustrated with centroid color.*

The compound is freely soluble in ethyl acetate, n-butanol, chloroform, acetone, diethyl ether and methanol, but it could not be dissolved in petroleum ether, hexane and benzene.

Elemental Analysis: The elemental analytical data of the antifungal agent produced by *Streptomyces antibioticus,* AZ-Z710 showed the following: C=74.01; H=7.13; N= 0.0.; O= 18.86 and S=0.0. This analysis indicates a suggested empirical formula of $C_{20}H_{24}O_4$.

Spectroscopic Characteristics: The spectroscopic analysis of the purified of antifungal compound produced by *Streptomyces antibioticus,* AZ-Z710, maximal IR spectra showed sixteen absorption peaks in the region of 852, 918, 956, 1067, 1117, 1229, 1306, 1345, 1438, 1641, 1815, 2359, 2884, 2958, 3241 and 3353 cm^{-1} (Fig. 3). The ultraviolet (UV) absorption spectrum are recorded a maximum absorption peak at 355, 374 and 395 nm (Fig. 4). The Mass spectrum showed that the molecular weight at 351.20 (Fig.5).

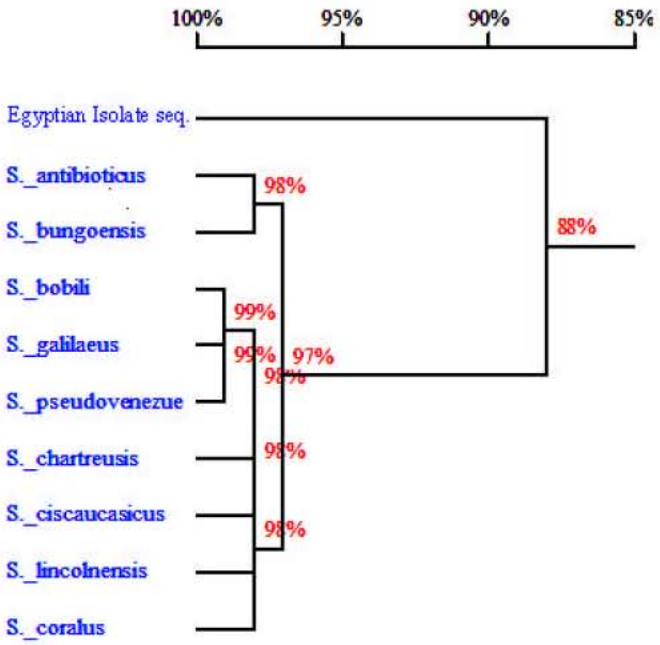

Fig. 2: The phylogenetic position of the local *Streptomyces sp.* strain among neighboring species. The phylogenetic tree was based on the pairwise comparisons of 16S rDNA sequences.

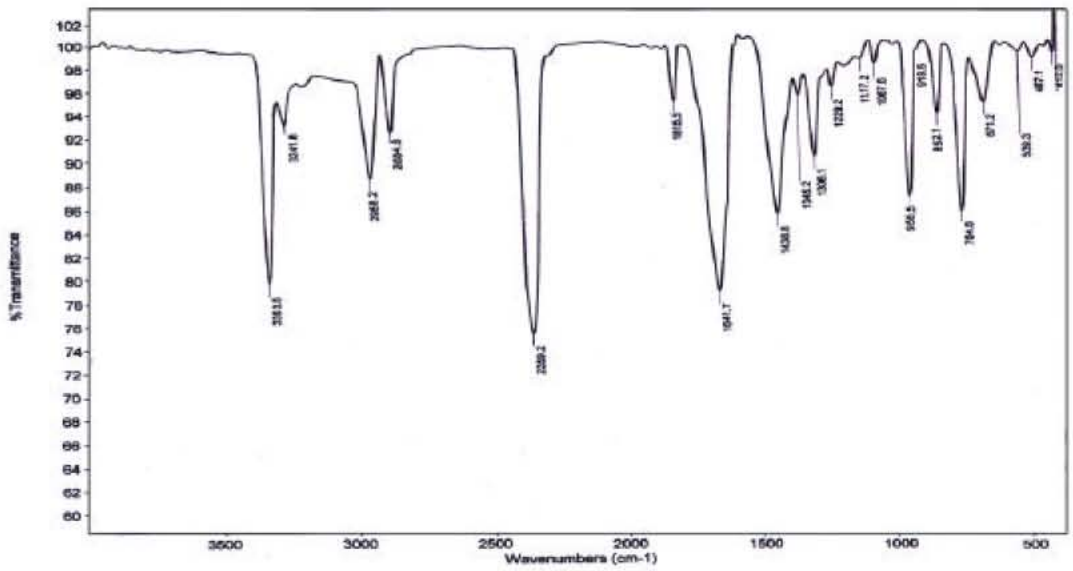

Fig. 3: Infra-Red spectrum of antifungal agent produced by *Streptomyces antibioticus,* AZ-Z710

The NMR spectrum showed those olefinic protons from 5.10 to 6.77 ppm and six aliphatic protons between 1.97 and 2.89 ppm (Fig. 6).

Biological Activities of the Antifungal Agent: Data of the antifungal agent spectrum indicated that the extracted agent is active against unicellular and filamentous fungi strains with MIC ranged from 31.25-93.75 µg/ml. The MIC of *Streptomyces antibioticus,* AZ-Z710 was 31.25 µg/ml for *Saccharomyces cerevisiae* ATCC 9763 and *Candida albicans,* IMRU 3669, 46.9 µg/ml against *Aspergillus niger* IMI 31276, 52.7 µg/ml against *Fusarium oxysporum, Rhizoctonia solani* and *Aspergillus flavus*, 62.5 µg/ml against *Alternaria alternata* and *Botrytis fabae* and 93.75 µg/ml against *Aspergillus fumigatus* ATCC 16424 and *Penicillium chrysogenum* (Table 4).

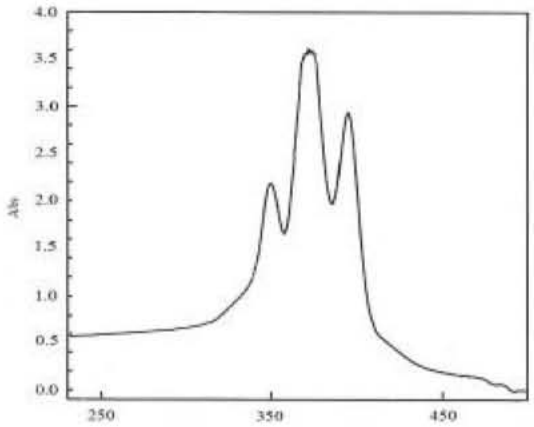

Fig. 4: Ultraviolet absorbance of antifungal agent produced by *Streptomyces antibioticus*, AZ-Z710

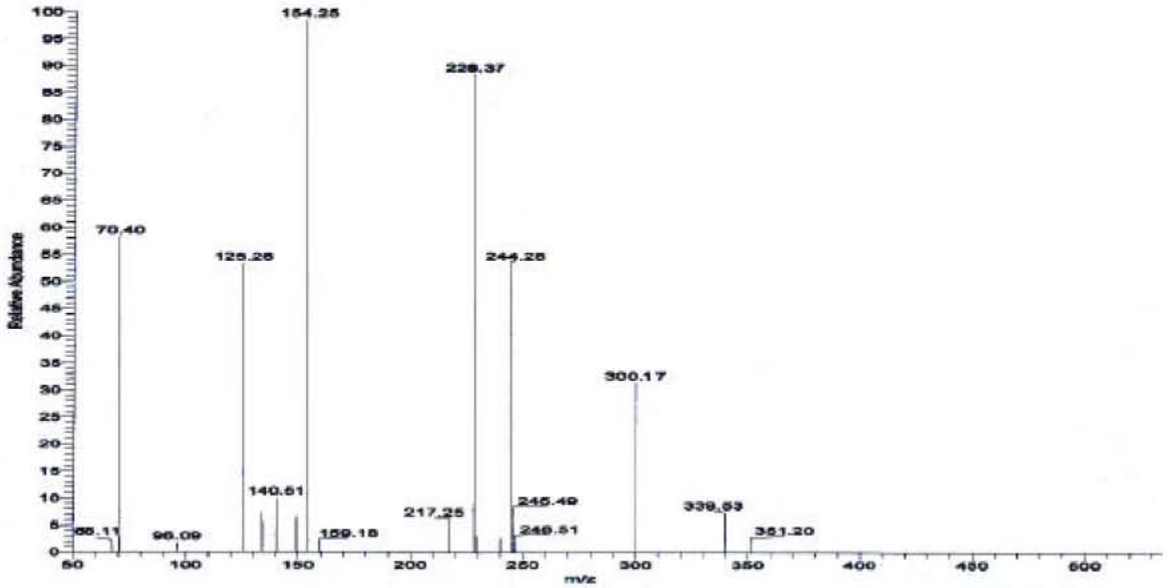

Fig. 5: Mass spectrum of antifungal agent produced by *Streptomyces antibioticus*, AZ-Z710

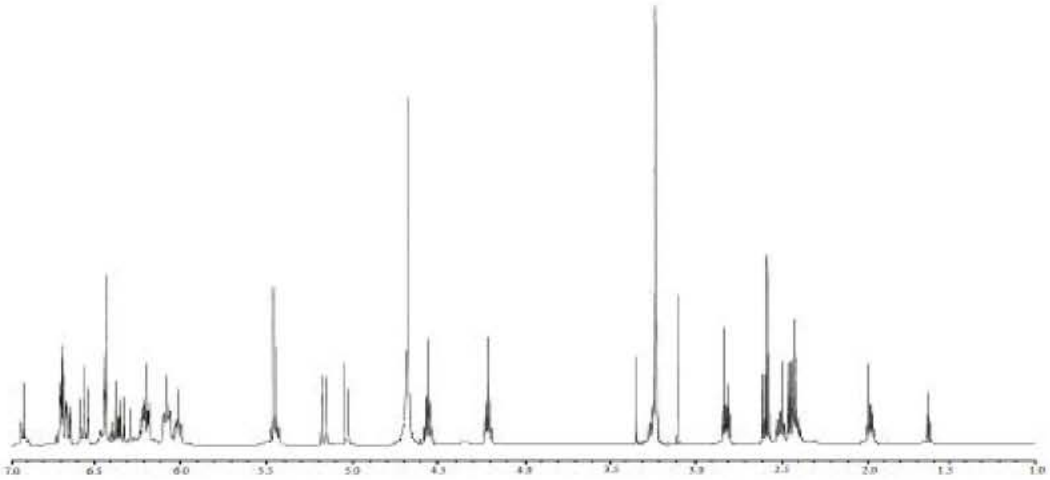

Fig. 6: NMR spectrum of antifungal agent produced by *Streptomyces antibioticus*, AZ-Z710

Table 4: Biological activities (MIC) of the antifungal agent by paper method assay

Tested organisms	MIC (mg/ml) concentration
1-Unicellular fungi:	
Candida albicans, IMRU 3669	31.25
Saccharomyces cervisiae ATCC 9763	31.25
2-Filamentous fungi:	
Aspergillus niger IMI 31276	46.90
Aspergillus fumigatus ATCC 16424	93.75
Aspergillus flavus IMI 111023	52.70
Fusarium oxysporum	52.70
Rhizoctonia solani.	52.70
Alternaria alternata	62.50
Botrytis fabae	62.50
Penicillium chrysogenum	93.75

Table 5: A comparative study of the characteristic properties of the antifungal agent produced by *Streptomyces antibioticus,* AZ-Z710 in relation to Reference antibiotic Mycangimycin

Characteristic	Purified Antifungal agent	Mycangimycin
Melting point	144°C	ND
Molecular weight	351. 20	351.1572
Chemical analysis:		
C	74.01	ND
H	7.13	ND
N	0.0	ND
O	18.86	ND
S	0.0	ND
4-Ultra violet	355, 374 and 395 nm	355, 374 and 395 nm
5-Formula	$C_{20}H_{24}O_4$	$C_{20}H_{24}O_4$
6-Active against	Active against unicellular and filamentous fungi.	Active against unicellular and filamentous fungi.

ND= no data

Identification of the Antifungal Agent: On the basis of the recommended keys for the identification of antibiotics and in view of the comparative study of the recorded properties of the antifungal agent, it could be stated that the antifungal compound is suggestive of being belonging to Mycangimycin antibiotic (Table 5).

DISCUSSION

The actinomycete isolate, AZ-Z710 was isolated from soil sample collected from zagazig districted, El-Sharkia governorate, Egypt. The isolate was growing on starch nitrate agar medium for investigating its potency to produce antimicrobial agents. The actinomycete isolate, AZ-Z710 exhibited a wide spectrum antifungal agent [13]. Identification process has been carried out according to [35, 36] and Numerical taxonomy of *Streptomyces* species program [37]. For the purpose of identification of actinomycete isolate, the morphological characteristics and microscopic examination emphasized that the spore chain is spiral. Spore mass is light gray; while spore surface is smooth, substrate mycelium is light yellowish brown and no diffusible pigment was produced on ISP-media No. 3, 4 and 5. The results of physiological, biochemical characteristics and cell wall hydrolysate of actinomycetes isolate, exhibited that the cell wall containing LL-diaminopimelic acid (DAP) and sugar pattern of cell wall hydrolysate could not detected. These results emphasized that the actinomycetes isolate related to a group of *Streptomyces* [35, 36]. In view of all the previously recorded data, the identification of actinomycete isolate AZ-Z710 was suggestive of being belonging to *Streptomyces antibioticus,* AZ-Z710. The resulted sequence was aligned with available almost compete sequence of type strains of family streptomycetaeae. The phylogenetic tree (diagram) revealed that the local isolate is closely related *Streptomyces antibioticus* and *Streptomyces bungeonis* (similarity matrix is 88%). The active metabolites were extracted by Ethyl acetate at pH 7 at the level of 1:1 (v/v) [38]. The organic phase was collected and evaporated under reduced pressure using rotary evaporator [33].

The crude extracts were dissolved in a small amount of methanol, then filtrated and precipitated with petroleum ether (b.p 60-80°C) and followed by centrifuged at 5000 r.p.m for 15 minute. Separation of antifungal agent into individual components was carried out by thin-layer chromatography using a solvent system composed of methanol-dichloromethane-water (1:1:1, v/v) [4]. Only one band yellowish in color at R_f = 0.55 showed antifungal activity. The purification process through column chromatography packed with silica gel was eluted with a mixture chloroform-methanol (9:1 v/v), revealed that the most active fractions against the fungal pathogenic range 20 to 27 [39]. The purified antifungal agent produced by *Streptomyces antibioticus,* AZ-Z710 are produces characteristic odour, their melting points are 144°C. The compound is freely soluble in ethyl acetate, n-butanol chloroform, acetone, diethyl ether and methanol, but it could not be dissolved in petroleum ether, hexan and benzene [33]. The elemental analytical data of the antifungal agent produced by *Streptomyces antibioticus,* AZ-Z710 showed the following: C=74.01; H=7.13; N= 0.0.; O= 18.86 and S=0.0. This analysis indicates a suggested empirical formula of $C_{20}H_{24}O_4$ [4]. The spectroscopic analysis of the purified of antifungal compound produced by *Streptomyces antibioticus,* AZ-Z710 maximal IR spectra showed sixteen absorption peaks in the region of 852, 918, 956, 1067, 1117, 1229, 1306,1345, 1438, 1641, 1815, 2359, 2884, 2958, 3241 and 3353 cm^{-1}. The ultraviolet (UV) absorption spectrum are recorded a maximum absorption peak at 355, 374 and 395 nm. The Mass spectrum showed that the molecular weight at 351.20. The NMR spectrum showed those olefinic protons from 5.10 to 6.77 ppm and six aliphatic protons between 1.97 and 2.89 ppm [4]. The biological activities (MIC) of the antifungal agent emphasized that the antibiotic are active against unicellular and filamentous fungi (MIC ranged from 31.25 to 93.75 µg/ml). The antifungal activity produced by *S. antibioticus,* AZ-Z710 showed maximum inhibitory activity against unicellular fungi *Saccharomyces cervisiae* ATCC 9763 (31.25) and *Candida albicans,* IMRU 3669 (31.25) and maximum inhibitory activity was observed against filamentous fungi *Aspergillus niger* IMI 31276 (46.9) *Fusarium oxysporum* (52.7) *Rhizoctonia solani* (52.7), *Aspergillus flavus* (52.7), *Alternaria alternate* (62.5), *Botrytis fabae* (62.5) *Aspergillus fumigatus* ATCC 16424 (93.75) and *Penicillium chrysogenium* (93.75) [40-42]. Identification of antifungal agent according to recommended international keys indicated that the antifungal antibiotic is suggestive of being belonging to Mycangimycin antibiotic [4]. It could be concluded that: Mycangimycin antibiotic produced by *S. antibioticus,* AZ-Z710 that demonstrated inhibitory affects against fungal pathogenic.

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