

Isolation, characterization, and screening of actinomycetes producing bioactive compounds from Egyptian soil

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Background

Unsuitable prescription and the inappropriate use of antibiotics have led to the development of antibiotic resistance in many pathogenic bacteria. So, there is a need to screen for alternative sources, such as actinomycetes, a potent antibiotic producer for antimicrobial activity, with the hope of discovering novel strains proficient to produce antibiotics against the resistant bacteria.

Materials and methods

In the current work, six actinomycetes were isolated from Egyptian soil, characterized, and then identified by microscopic and macroscopic observations. The bioactive substances were extracted by the solvent extraction method using ethyl acetate. Antimicrobial activity of the obtained extracts was evaluated against some gram-positive and gram-negative bacteria. Fourier-transform infrared spectroscopy analysis and cytotoxic activity of the most active extracts were carried out.

Results and conclusion

Crude extract of A2 showed 30 ± 0.10 -mm mean inhibition zones against gram-positive bacteria *Bacillus megaterium*. Significantly, the isolate A6 showed the highest mean zone of inhibition of 20.0 ± 0.1 , 19.4 ± 0.05 , and 19.0 ± 0.10 mm against *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, and *Escherichia coli*, respectively. The isolate A5 only showed the largest antibacterial activity against *B. megaterium*, with inhibition zone of 69 ± 0.28 mm. Only two isolates (A2 and A6) were chosen for further study based on broad-spectrum activity in comparison with other isolates. The two isolates A2 and A6 were identified as *Streptomyces enissocaesilis* MT658130 and *Streptomyces atrovirens* MT658195, respectively, using 16S rRNA. Fourier-transform infrared analysis of the extract of two strains reported the existence of OH, C=C, C-O, S=O, N=C=S, and C-Br as the most efficient groups. Cytotoxic activity of *S. enissocaesilis* A2 and *S. atrovirens* A6 extracts against hepatocellular carcinoma (HepG2) cell line showed good cytotoxicity, with 2.97 and 1.48 $\mu\text{g/ml}$ IC₅₀ value, respectively. The results evoked that actinomycete isolates under study have potent biological activities.

Keywords:

antibacterial, cytotoxicity and HepG2, Fourier-transform infrared, *Streptomyces*

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Introduction

Actinomycetes are gram-positive, facultative anaerobic bacteria that play a very important role in the production of bioactive compounds of high commercial significance [1]. These compounds can be used for the treatment of infections; they include antitumor, antifungal, and antibacterial agents [2] and enzymes. Actinomycetes have a high content of G+C (>55%) in their DNA [3–6]. The majority of actinomycetes in the soil are neutrophils, growing well between pH 5.0 and 9.0. Moreover, nearly all soil actinomycetes are strict aerobes [7]. Furthermore, they play an important role in soil biodegradation and in humus formation [8] and produce numerous volatile substances such as geosmin, which is responsible for the feature ‘wet earth odor’ [9]. Numerous bioactive metabolites of actinomycetes have shown to possess antimicrobial, antiviral, cytotoxic, antioxidant,

insecticidal, plant growth-promoting, and herbicidal activities [10–12]. Members of the genus *Streptomyces* are common in soil and produce many bioactive compounds. These organisms are potential sources of pharmaceutically and agriculturally vital bioactive compounds. Approximately two-thirds of naturally occurring antibiotics are provided by *Streptomyces* actinomycetes [13,14]. Most pathogenic microbes are becoming multidrug-resistant; therefore, the need for discovery and development of novel antimicrobial compounds with different mechanisms of action was obligatory [15–18]. Bioactive compounds from both culture broth and from solid culture media

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plates can be recovered by organic solvents having different polarities [19,20]. The current study aimed to isolate, screen, and identify antibiotic-producing actinomycetes from Egyptian soil and evaluate the antimicrobial and anticancer activities of their extracts.

Materials and methods

Samples collection

The soil samples were collected from gardens located in Nasr City, Cairo, Egypt. The samples were taken from 5-cm depth and kept in clean plastic bags, then transported to the laboratory and stored at 4°C for further microbiological examination.

Processing of soil samples

The soil samples were put in clean empty Petri dishes for one week and then dried at 55°C for ~15 min to reduce the residents of gram-negative bacteria as recorded by Baskaran *et al.* [21].

Isolation of actinomycetes

Isolation of actinomycetes was carried out according to the procedure described by other studies [22–24], with some modification, where 10 g of soil sample was added in 100-ml of saline solution and serially diluted till 10^{-4} . Then, 100 µl of each soil suspension was pipetted and added to starch casein agar (SCA) plates and allowed to solidify. The incubation of all plates was carried out at 30±2°C for 7 days. The resulting actinomycete colonies were picked up, streaked, recultivated a number of times on SCA plates, and then incubated at 30°C for 7 days to check its purity. The pure actinomycete isolates were maintained on SCA slants at 4°C.

Cultural characteristics

The isolated actinomycetes under study were characterized by morphological, physiological, and biochemical methods. Both macroscopic and microscopic characterizations were used as morphological methods. Concerning the macroscopic one, the isolates under study were differentiated by their colony characteristics, for example, size, shape, color, pigmentation, and consistency. The isolates were grown by the coverslip culture method for the microscopic observations as mentioned by Goodfellow *et al.* [25]. They were then observed for their mycelial structure and spore arrangements by SEM (JSM-5400; JEOL, Jeol 2100 High resolution, Musashino, Akishima, Tokyo, Japan) at National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt. The results were

compared with the morphology of actinomycetes according to Bergey's Manual for the presumptive identification [26]. Various biochemical tests performed were catalase [27], starch hydrolysis, cellulose decomposition [28], gelatin hydrolysis, H₂S production [29], NaCl tolerance, pH range, and temperature tolerance. The ripostes to temperature, pH values, and NaCl tolerance were detected for 7 days.

Production and extraction of bioactive compounds

Each actinomycete isolate was inoculated in 100-ml nutrient broth medium (beef extract, 3.0; peptone, 5.0; sodium chloride, 5.0; and distilled water up to 100 ml). The pH was adjusted at 7.2 before sterilization, autoclaved at 121°C for 20 min, and then incubated in a shaker incubator at 120 rpm, 30°C for 7 days. At the end of the incubation period, the medium was centrifuged for 15 min at 4°C and 10 000 rpm. The recovery of bioactive compounds was carried out by solvent extraction, using ethyl acetate. The solvent was added to the filtrate in the ratio of 1 : 1 (v/v) and then incubated for 60 min on a shaker for complete extraction. After that, the bioactive compounds were separated from the aqueous phase, and the solvents were removed using a vacuum rotary evaporator and were stored at 4°C until further use.

Determination of the antimicrobial activity of the obtained extracts

Gram-positive bacteria used were *Bacillus megaterium*, *Bacillus subtilis* SK09, and *Staphylococcus aureus* ATCC 6538, whereas gram-negative bacteria were *Escherichia coli* ATCC 11775, *Klebsiella oxytoca*, and *Pseudomonas aeruginosa* ATCC 27853. The bioactivities of crude ethyl acetate extracts were quantified by the paper disk diffusion method as described by Shieh *et al.* [30]. The bacterial strains were kindly obtained from the Central Water Quality Laboratory of Greater Cairo Water Company, Cairo, Egypt. The antibacterial activity of the isolated actinomycetes extract was separately tested against previously mentioned test organisms. The 24-h cultures of different pathogens were inoculated on nutrient agar plates, then 20 µl of each extract was loaded on each disk, left to stand for 30 min, and finally incubated at 37°C for 24 h. By ending the incubation period, the resulted inhibition zone diameter was measured as mm.

Genomic and phylogenetic characterization

The bioactive-producing strains A2 and A6 were subjected to molecular identification according to 16S rRNA at GIS Company for Genetics, Cairo, Egypt.

Isolation of DNA by using genomic DNA purification (Qiagen, USA)

Overall, 200 µl of the sample (liquid media that contain bacteria: one of the two actinomycetes isolates (A2 and A6)) was added in a microcentrifuge tube, where 95 µl of water, 95 µl of solid tissue buffer (blue), and 10 µl of proteinase K were supplementary; they were mixed well and incubated at 55°C for 2 h. Centrifugation was carried out at 12 000g for 1 min. The aqueous supernatant was transferred to a clean tube (300 µl), and then 600 µl genomic binding buffer was added and mixed thoroughly. The mixture was transferred to a Zymo-Spin IIC-XL column in a collection tube, and centrifuged ($\geq 12\ 000g$) for 1 min. Overall, 400 µl DNA was added with prewash buffer to the column in a new collection tube and centrifuged at 12 000g for 1 min. Overall, 700 µl g-DNA wash buffer was added and centrifuged at 12 000g for 1 min. Empty the collection tube. Then, 200 µl of gDNA wash buffer was supplementary and centrifugation at 12 000g for 1 min was supported then the collection tube was discarded. Thereafter, 0.03 µl of elution buffer was added, incubated for 5 min, and then centrifuged at 12 000g for 1 min.

PCR amplification of 16S rRNA gene

The reaction mixture for amplification the 16S rRNA gene from isolated genomic DNA containing; 10-ng aliquot of DNA; 10 WM of each universal primer to the 16S rRNA of bacteria, 10U buffer; 1mm each dNTPs; 2.0mm MgCl₂; and 1U promegaTaq (Fermentas, Fermentas GmbH Opelstrasse, Baden-Wurttemberg, Germany) up to 50 µl with distilled water. PCR amplification was performed using multi-gene gradient thermocycler (Labnet, headquarters in Edison, New Jersey, USA). Reactions were first incubated at 94°C for 3 min, and then 35 cycles were performed as follows: 94°C for 45 s, 55°C for 1 min, and 72°C for 1 min 30 s. Reactions were incubated at 72°C for another 10 min. The obtained PCR products were visualized on ethidium bromide-stained 1% agarose gel under UV Gel Documentation System with UV transilluminator (Biorad, Germany) to confirm the presence of a full length of 16S rRNA gene of about 1.5-kb band.

XXXX16S rRNA sequencing

Purified PCR product was directly bi-directional sequencing on GATC Company by using ABI 3730xl DNA sequence using forward and reverse primers and Sanger sequences protocol.

Phylogenetic tree construction

The obtained sequencing products were aligned with other similar sequences downloaded from Gene Bank using Cluster X [31] for determining its identical or

unique sequence. The resulted sequences were related to the gathering of the 16S rRNA gene offered in databases NCIB/BLAST [32] to determine the highest similarity.

Fourier-transform infrared spectroscopy analysis of A2 and A6 ethyl acetate extracts

Fourier-transform infrared (FTIR) analysis was performed to determined respective functional groups of A2 and A6 ethyl acetate extracts to specific peaks at the Egyptian Petroleum Research Institute, Central Labs, Cairo, Egypt.

Cytotoxic effect of A2 and A6 ethyl acetate extracts on HepG2 cell

The cytotoxicities of A2 and A6 ethyl acetate extracts were determined *in vitro* against HepG2 cancer cells at the Science Way for scientific researches and consultations laboratory, Cairo, Egypt.

Cell culture

HepG2 cell line was maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic solution. The cells were grown to confluence at 37°C and 5% CO₂ atmosphere. All experiments were performed in tissue culture flasks unless otherwise stated. The cells were seeded onto the plates at a density of 1×10^6 cells per well and incubated for 24 h before the experiments. The cells were incubated in a fresh medium containing different concentrations of A2 and A6 ethyl acetate extracts separately.

In vitro assay of cytotoxic activity (MTT protocol)

The 96-well tissue culture plate was inoculated with 1×10^5 cells/ml (100 µl/well) and incubated at 37°C for 24 h to develop a complete monolayer sheet. The growth medium was decanted from 96-well microtiter plates after the confluent sheet of cells was formed; cell monolayer was washed twice with wash media. Two-fold dilutions of tested sample were made in the RPMI medium with 2% serum (maintenance medium). One-tenth milliliter of each sample dilution was tested in different wells, leaving three wells as control, receiving only maintenance medium. The plate was incubated at 37°C and examined. Any physical signs of cell toxicity like loss of the monolayer, rounding, shrinkage, or cell granulation were detected. MTT solution was prepared (5 mg/ml in PBS) (Bio Basic Canada Inc.). Overall, 20 µl of MTT solution was added to each well. Shaking was carried out at 150 rpm for 5 min, to thoroughly mix the MTT into the media.

Incubation was run at 37°C and 5% CO₂, for 4 h to allow the MTT to be metabolized. The media was dump off (dry plate on paper towels to eradicate residue if needed). The metabolic product (formazan) was resuspended in 200 µl DMSO and shaken at 150 rpm for 5 min, to allow mixing the formazan with the solvent. Optical density was recorded at 560 nm and subtracts background at 620 nm. Optical density must be directly interrelated with quantity of cell. The maximum nontoxic concentration of each extract was also estimated.

Statistical analysis

The values are expressed as mean±SD. All data (three replicates) were subjected to analysis of variance of one-way analysis of variance by using SPSS, version 16.0 (IBM) software. We took the desired level of significance at *P* value of 0.05.

Results

Isolation of actinomycetes

The present study detected a total of six actinomycete isolates that were isolated from two soil samples collected from gardens located in Nasr City, Cairo, Egypt. The Gram reaction of all isolates was gram-positive. Each isolate was characterized according to its morphological characteristics, which included colony color, growth behavior, and diffusible pigmentation, as shown in Table 1.

Mycelial structure and spore arrangements were studied by SEM, as shown in Fig. 1. The general morphologies of the intact spore chains and mycelia have good resolution both at lower and higher magnifications. The isolates A1, A2, A4, A5, and A6 had spores with smooth surface, whereas those of A3 was warty or rough. As previously stated, the ornamentation of spore surfaces is an important taxonomic characteristic for species of the genus *Streptomyces*.

Physiological characteristics of isolated actinomycetes were studied, and the results indicated that all isolates

grow well at temperature of 20, 30, 40, and 50°C. The results indicated that all isolates could tolerate NaCl concentrations from 1 to 7%. Moreover, A5 isolate could tolerate 9.0% NaCl. All actinomycetes isolates could grow at pH 5–11, except one isolate (A1), which grow at pH 7–10. The capability of isolated actinomycetes to produce enzymes was wide-ranging. The results showed that all isolates were amylase, cellulase, and gelatinase, producers except A1. In addition, all isolates produce catalase except Ap5. All isolates were H₂S producer except isolates (A4 and A6), as shown in Table 2.

In the current study, the actinomycete isolates were tested to evaluate their antimicrobial activity against some pathogenic bacteria. As shown in Table 3, the ethyl acetate extract of A2 showed 30±0.10-mm mean inhibition zones against gram-positive bacteria *B. megaterium*. Significantly, the isolate A6 showed the highest mean zone of inhibition of 20.0±0.1, 19.4±0.05, and 19±0.1 mm against gram-negative bacteria, *P. aeruginosa*, *K. oxytoca*, and *E. coli*, respectively. The isolate AP5 only showed the largest activity against gram-positive bacteria *B. megaterium*, with inhibition zone of 69±0.28 mm (Fig. 2 and Table 3).

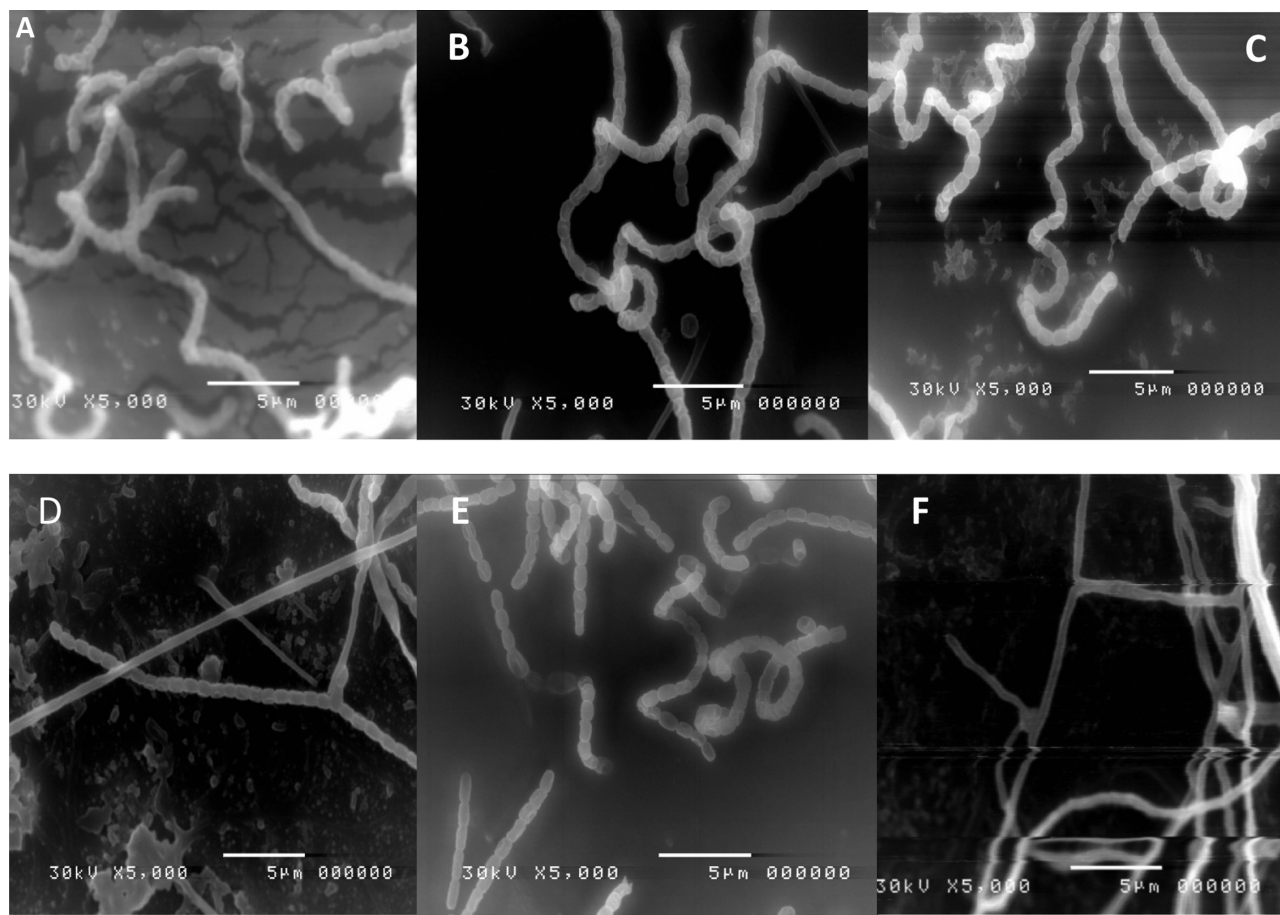
So, based on the previous results, only two isolates (A2 and A6) were chosen for further studies on the basis of broad-spectrum activity and larger zone of inhibition in comparison with other isolates. The NCBI BLAST analysis showed that the isolates A2 showed maximum similarity (93%) with *S. enissocaesilis*, whereas A6 showed maximum similarity (98) with *Streptomyces atrovirens*, and their accession numbers were MT658130 and MT658195, respectively (Figs 3 and 4).

FTIR spectrums of the *S. enissocaesilis* MT658130 and *S. atrovirens* MT658195 ethyl acetate extracts were analyzed as illustrated in Fig. 5a and b. FTIR spectrums of the *S. enissocaesilis* MT658130 showed the occurrence of six bands. The peak at 3445 cm⁻¹ is an implication of strong, broad O-H stretching

Table 1 Morphological and cultural characteristics of the bioactive isolates

Isolate symbol	Morphological and cultural characteristics of the bioactive isolates					
	Morphological characteristics			Cultural characteristics		
	Sporophore	Spore surface	Growth behavior	Substrate mycelia color	Aerial mycelia color	Diffusible pigment
A1	Coiled	Smooth	Abundant	Gray	Gray	yellow
A2	Coiled	Smooth	Abundant	Dust dark gray	Gray	–
A3	Coiled	Warty or rough	Abundant	White	Pale yellow	Yellow
A4	Coiled	Smooth	Good	Gray	Gray	–
A5	Monovercillus	Smooth	Abundant	Gray	Gray	Green
A6	Coiled	Smooth	Good	Pale white	White	Yellow

Figure 1



(a–f) Scanning electron microscopic images showing spore chain morphology of A1, A2, A3, A4, A5, and A6 isolates; samples prepared without using of any chemical adhesive and dehydrating mediator.

Table 2 Biochemical characteristics of the isolated actinomycetes

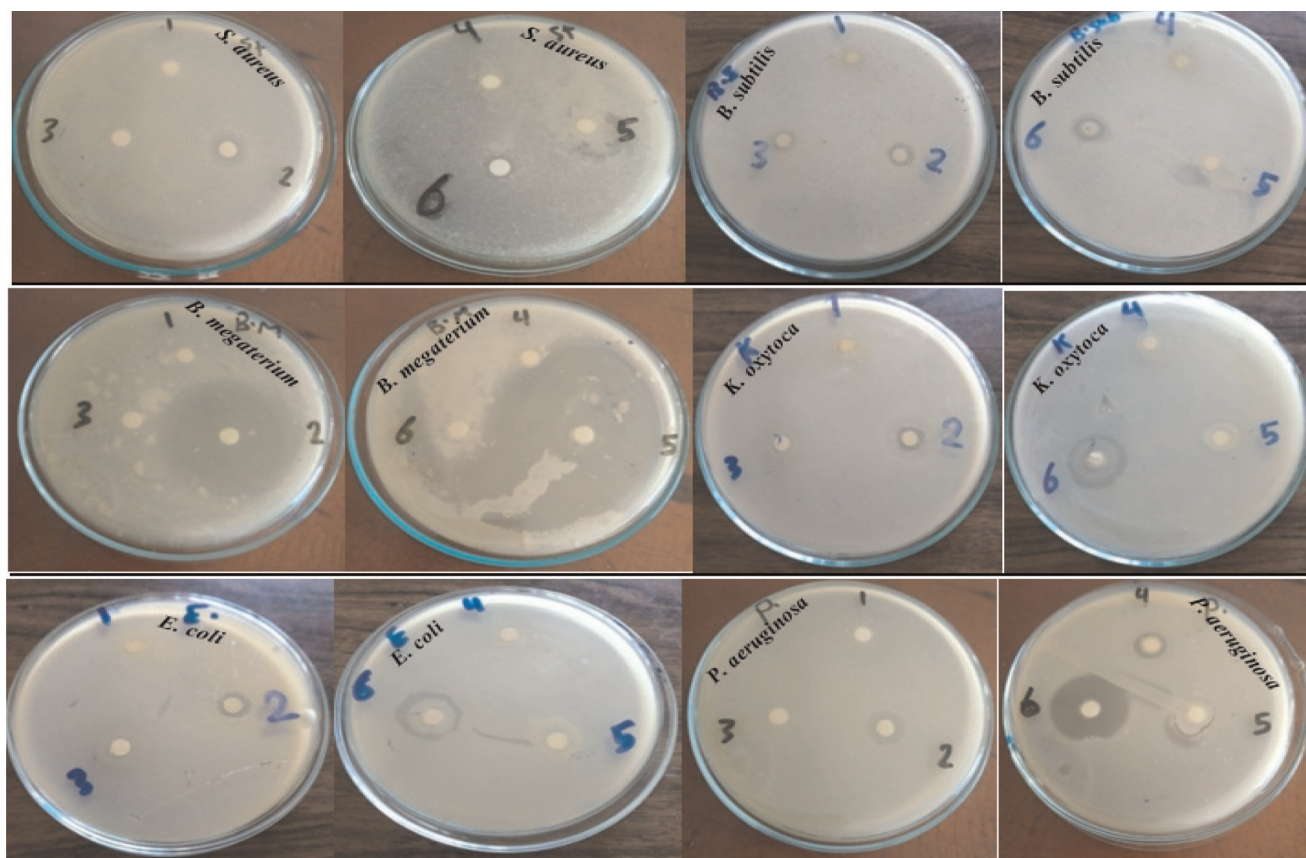
Reaction	Actinomycete isolate symbol					
	A1	A2	A3	A4	A5	A6
Starch hydrolysis	–	+	+	+	+	+
Cellulose decomposition	–	+	+	+	+	+
Gelatin hydrolysis	–	+	+	+	+	+
Catalase	+	+	+	+	–	+
H ₂ S Production	+	+	+	–	+	–
pH tolerance	7–10	5–11	5–11	5–11	5–11	5–11
NaCl tolerance (%)	1–7	1–7	1–7	1–7	1–9	1–7
Growth temperature (°C)						
10	+	+	+	+	+	+
20	+	+	+	+	+	+
30	+	+	+	+	+	+
40	+	+	+	+	+	+
50	+	+	–	+	+	+

alcohol, indicating the presence of OH group. The peak at 2086 cm^{-1} was assigned to the strong $\text{N}=\text{C}=\text{S}$ stretching isothiocyanate. The strong peak observed at 1634 cm^{-1} is owing to the presence of $\text{C}=\text{C}$ stretching conjugated alkene. The peak at 1407 cm^{-1} indicated the presence of $\text{S}=\text{O}$ stretching of sulfonyl chloride. The presence of peak

at 1092 cm^{-1} was an indication of strong C–O stretching secondary alcohol. The peak at 656 cm^{-1} was attributed to strong C–Br stretching halo compound. However, FTIR spectrums of the *S. atrovirens* MT658195 recorded the occurrence of five bands. The peak at 3444 cm^{-1} was characteristic of the strong, broad O–H stretching

Table 3 Mean diameters of inhibition zones (mm) of the investigated bacteria by extract of bioactive actinomycetes isolates

Bioactive actinomycete isolates.	Mean diameters of inhibition zones (mm) of investigated bacteria					
	<i>Bacillus subtilis</i>	<i>Bacillus megaterium</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Klebsiella oxytoca</i>
A1	0±0	0±0	0±0	0±0	0±0	0±0
A2	0.15±4.0	00.1±03	0.10±4.0	0.05±4.0	0.050±5.0	4.0±0.05
A3	0.05±4.0	0±0	0±0	0±0	0±0	0±0
A4	0±0	0±0	0±0	0.05±4.0	0±0	0±0
A5	0±0	0.28±69	0±0	0±0	0±0	0±0
A6	0.1±5.0	0±0	0.11±3.0	0.1±20	0.0.10±19.0	19.4±0.05

Figure 2

Antibacterial activity of bioactive actinomycetes isolate, where 1=extract of A1 isolate, 2=extract of A2 isolate, 3=extract of A3 isolate, 4=extract of A4 isolate, 5=extract of A5 isolate, and 6=extract of A6 isolate.

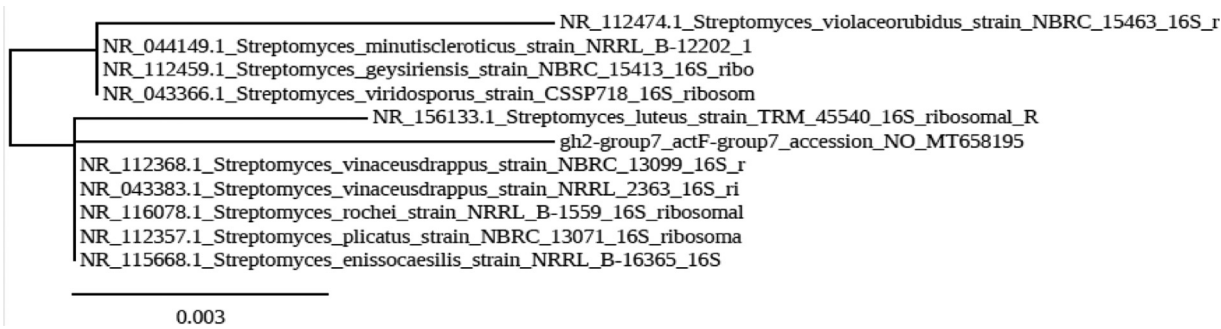
alcohol. The stretching band at 2079 cm^{-1} was assigned to strong $\text{N}=\text{C}=\text{S}$ stretching isothiocyanate. The peak at 1634 cm^{-1} indicated the presence of $\text{C}=\text{C}$ stretching conjugated alkene. The peak at 1384 cm^{-1} was attributed to $\text{C}-\text{H}$ bending alkane. The strong peak at 645 cm^{-1} is owing to the incidence of strong $\text{C}-\text{Br}$ stretching halo compound.

Cytotoxic effect of *Streptomyces enissocaesilis* MT658130 and *Streptomyces atrovirens* MT658195 extracts

Cytotoxic effect of *S. enissocaesilis* MT658130 and *S. atrovirens* MT658195 extracts against human

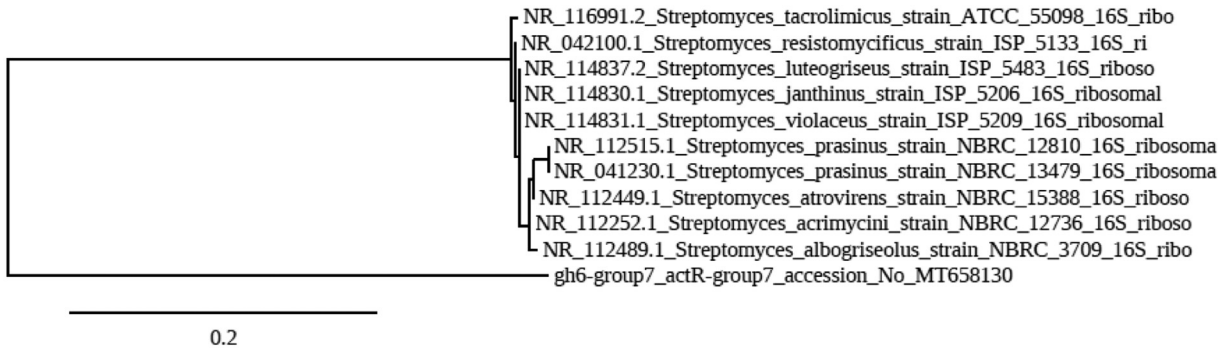
hepatocellular carcinoma (HepG2) cell line was carried out. The liver cancer (HepG2) cells were treated with different concentrations of *S. enissocaesilis* MT658130 and *S. atrovirens* MT658195 extracts for 24 h. The results indicated that *S. enissocaesilis* MT658130 extract was potent in inducing cell death of HepG2 and showing 94.64, 93.708, and 52.30% toxicity at 250, 125, and $62.5\text{ }\mu\text{g/ml}$, respectively. *S. enissocaesilis* MT658130 extract was found to be morphologically cytotoxic to the cancer cells, which appeared as morphological changes viz., loss of membrane integrity and shrinkage of cell and reduced cell density (Fig. 6a).

Figure 3



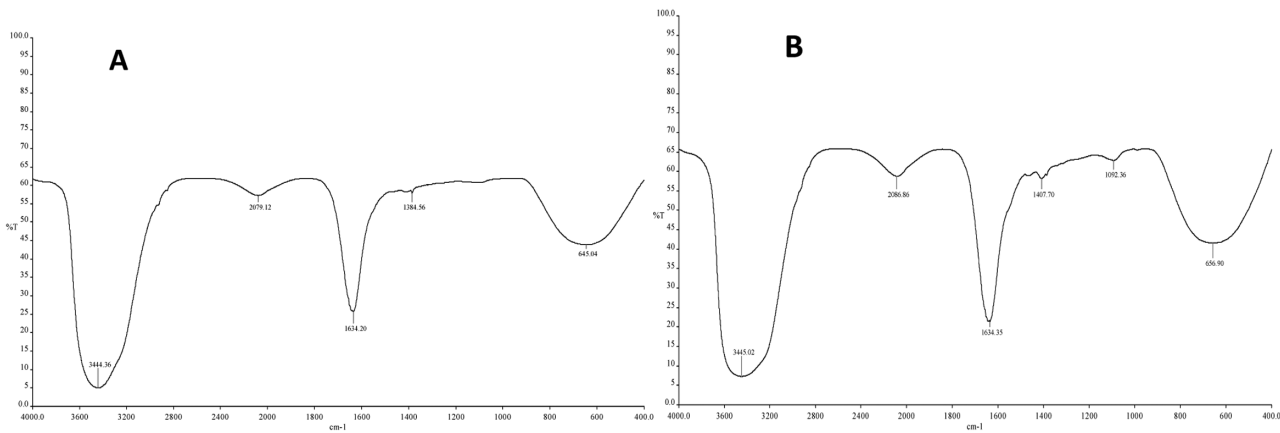
Phylogenetic tree (dendrogram) showing the sequence relationships between *Streptomyces enissocaesilis* MT658130 and other *Streptomyces* strains.

Figure 4



Phylogenetic tree (dendrogram) showing the sequence relationships between *Streptomyces atrovirens* MT658195 and other *Streptomyces* strains.

Figure 5



FTIR analysis of (a) *Streptomyces enissocaesilis* ethyl acetate extracts, (b) *Streptomyces atrovirens* ethyl acetate extracts. FTIR, Fourier-transform infrared.

IC₅₀ value of *S. enissocaesilis* MT658130 extract was 2.97 µg/ml.

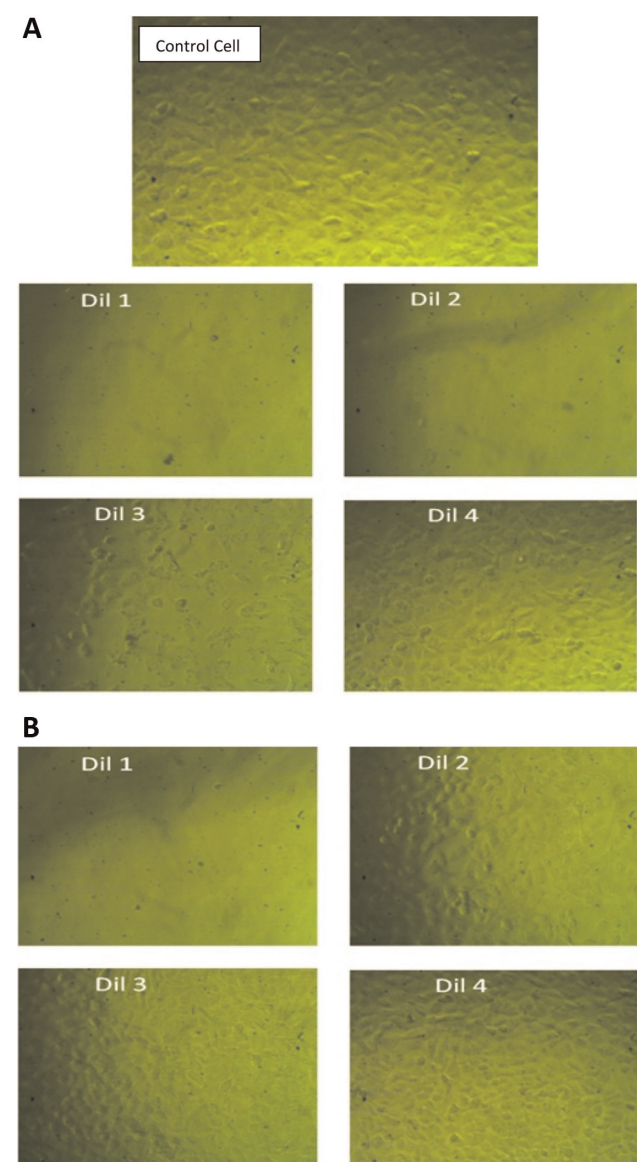
On the contrary, extract of *S. atrovirens* MT658195 showed 87.79 and 9.67% toxicity toward HepG2 at 250 and 125 µg/ml, respectively (Fig. 6b). At concentrations less than these, it had no cytotoxic effect on HepG2 cell line. This finding showed that

the cytotoxicity was dose dependent. IC₅₀ value for *S. atrovirens* MT658195 extract was 1.4897 µg/ml (Table 4).

Discussion

Actinomycetes forms a large part of soil microbiota. Commonly, *Streptomyces* species are saprophytic

Figure 6



(a) Morphological changes of HepG2 cell line exposed to different concentrations of *Streptomyces enissocaesilis* MT658130 extract.
 (b) Morphological change of HepG2 cell line exposed to different concentrations of *Streptomyces atrovirens* MT658195 extract.

microorganisms associated with soil decomposition of complex compounds such as cellulose and lignin and antibiotics formation [33]. Approximately 90% actinomycetes in soil are reported to be *Streptomyces* species [34].

In our screening program of isolation and characterization of some actinomycetes, the isolates were carefully chosen according to their antimicrobial and broad-spectra antagonistic activities. Identification of actinomycetes isolates had been approved according to the Key's assumed in Bergey's Manual of Determinative Bacteriology 8th edition [35], Bergey's Manual of Determinative Bacteriology, Vol. 4 [36], and International Journal of Systematic Bacteriology [37,38].

Morphology plays a vital part in characterization of *Streptomyces* from other spore-forming actinomycetes and also in the classification of *Streptomyces* species [39,40]. Three features for microscopic characterization of *Streptomyces* life cycle were provided, viz., substrate mycelium, aerial mycelium, and the characteristics of spores. Aerial mycelium and the characteristics of spores construct the majority of diagnostic information. Knowing cultural characteristic and arrangement of spores along with biochemical properties provides classification of actinomycetes as members of the genus *Streptomyces*.

Actinomycetes are known to produce an assortment of metabolites that are active against antibiotic-resistant bacteria [41]. Previous studies have shown that actinomycetes produce higher antibacterial activity toward gram-negative bacteria when compared with gram-positive bacteria, and this is owing to the cell wall for the gram-negative bacteria is greatly easier to crack than those of the gram-positive bacteria [42]. Additionally, one similar study done in Punjab, India, reported higher inhibitory effect on gram-negative than gram-positive bacteria, which is consistent with our study [43]. Conversely, this proposition does not agree with the findings of many researchers, where they recorded that antagonistic action against the gram-positive bacteria was greatly superior to the gram-negative ones [44–47]. Additionally, FTIR spectrum of the two selected isolates exhibited prominent peaks which assigned to OH, C=C, C-O, S=O, N=C=S, and C-Br as the most efficient groups. Groups present in the bioactive compounds were determined by FTIR analysis. The functional groups were easily identified through the standard IR functional data report [23,48,49].

Significantly, molecular level characterization was notorious by 16S rRNA sequencing with the aid of sequence results [50]. The active strains are taxonomically very close to *S. enissocaesilis* (A2) and *S. atrovirens* (A6). Moreover, this was confirmed by BLAST and phylogenetic analysis. Notably, 16S rRNA sequences of active strains deposited in NCBI and acknowledged the accession numbers MT658130 and MT658195.

Cancer is a worldwide scourge, which affects people of all ages, and is rapidly becoming a global pandemic [51]. The superlative chemotherapy drug should be more specific and able to differentiate between normal and cancer cells. Several studies conducted by Chabner and Roberts [52] and Chari [53] reported that most

Table 4 Cytotoxic effect of *Streptomyces enissocaesilis* MT658130 and *Streptomyces atrovirens* MT658195 extracts against human hepatocellular carcinoma (HepG2) cell line

ID	Conc. (µg/ml)		OD		Mean OD	SE	Viability %	Toxicity %	IC ₅₀ (µg/ml)
HepG2	1:2	0.356	0.341	0.368	0.355	0.00781	100	0	dil
	1	0.02	0.018	0.019	0.019	0.000577	5.352112676	94.64788732	
A2	2	0.023	0.024	0.02	0.022333	0.001202	6.291079812	93.70892019	2.97
	3	0.152	0.169	0.187	0.169333	0.010105	47.69953052	52.30046948	
	4	0.351	0.346	0.366	0.354333	0.006009	99.81220657	0.187793427	
	5	0.358	0.35	0.364	0.357333	0.004055	100.657277	0	
	6	0.362	0.36	0.348	0.356667	0.004372	100.4694836	0	
	7	0.349	0.37	0.35	0.356333	0.006839	100.3755869	0	
	8	0.351	0.359	0.355	0.355	0.002309	100	0	
	1	0.036	0.052	0.042	0.043333	0.004667	12.20657277	87.79342723	
A6	2	0.296	0.324	0.342	0.320667	0.013383	90.3286385	9.671361502	1.48
	3	0.353	0.367	0.35	0.356667	0.005239	100.4694836	0	
	4	0.348	0.359	0.356	0.354333	0.003283	99.81220657	0.187793427	
	5	0.35	0.364	0.362	0.358667	0.004372	101.0328638	0	
	6	0.351	0.361	0.358	0.356667	0.002963	100.4694836	0	
	7	0.37	0.352	0.351	0.357667	0.006173	100.7511737	0	
	8	0.357	0.348	0.363	0.356	0.004359	100.2816901	0	

OD, optical density.

anticancer drugs are still missing the drug specificity, for example, they kill cancer cells and normal cells together. Marvelous efforts are invested to go looking for new chemotherapy drugs with high potency and specificity. This study showed the specificity of *S. enissocaesilis* MT658130 and *S. atrovirens* MT658195 extracts, indicating that these extracts were less toxic against normal cell lines compared with HepG2 cell lines. Suffness and Pezzuto [54] declared that the IC₅₀ values fewer than 30 µg/ml in cancerous cell lines are well thought-out as hopeful for anticancer drug progress. These vital results provide new approaches into the cytotoxic potential of *S. enissocaesilis* MT658130 and *S. atrovirens* MT658195 against (HepG2) cell lines with high specificity.

Conclusion

An attempt to isolate some actinomycetes strains having antimicrobial activity from Egyptian soil was carried out. The bioactive-producer actinomycete isolates that have been identified in this work were members of the genus *Streptomyces*. The promising cytotoxicity of the extracts also suggests that they have the potential to exhibit anticancer activities. Additional studies are essential to elucidate the structure of the compounds. *In vivo* experiments should be carried out to confirm the pharmacological activities of the extracts.

Authors contributions

Author contributions: all authors designed the experiments, analyzed experimental data, performed the experiments, provide the chemicals, and prepared the figures and tables. Amira Y. Mahfouz

wrote the manuscript text and Ghadir E. Daigham reviewed the manuscript.

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Conflicts of interest

There are no conflicts of interest.

References

- Sapkota A, Thapa A, Budhathoki A, Sainju M, Shrestha P, Aryal S. Isolation, characterization, and screening of antimicrobial-producing actinomycetes from soil samples. *Int J Microbiol* 2020; 2020:2716584.
- Bizuye A, Moges F, Andualem B. Isolation and screening of antibiotic producing actinomycetes from soils in Gondar town, North West Ethiopia. *Asian Pac J Trop Dis* 2013; 3:375–381.
- Ghosh M, Chattopadhyay GN, Baral K. Transformation of phosphorus during vermicomposting, *Bioresour Technol* 1999; 69:149–154.
- Ndonge MJM, Semu E. Preliminary characterization of some *Streptomyces* species from four Tanzanian soils and their antimicrobial potential against selected plant and animal pathogenic bacteria. *World J Microbiol Biotechnol* 2000; 16:595–599.
- Lam KS. Discovery of novel metabolites from marine actinomycetes. *Curr Opin* 2006; 9:245–251.
- Ningthoujam DS, Sanasam S, Nimaich S. Screening of actinomycete isolates from niche habitats in Manipur for antibiotic activity. *Am J Biochem Biotechnol* 2009; 5:221.
- Goodfellow M, Williams S. Ecology of actinomycetes. *Annu Rev Microbiol* 1983; 37:186–216.
- Stach J, Bull A. Estimating and comparing the diversity of marine actinobacteria. *Antonie Van Leeuwenhoek* 2005; 87:3–9.
- Wilkins K. Volatile metabolites from actinomycetes. *Chemosphere* 1996; 32:1427–1434.
- Ruanpanum P, Laatsch H, Tangchitsomkid N, Lumyong S. Nematocidal activity of fervenulin isolated from a nematocidal actinomycete, *Streptomyces* sp.CMU-MH21, on *Meloidogyne incognita*. *World J Microbiol Biotechnol* 2011; 27:1373–1380.
- Alam M, Dharni S, Abdul-Khaliq Srivastava SK, Samad A, Gupta MK. A promising strain of *Streptomyces* sp. with agricultural traits for growth promotion and disease management. *Ind J Exp Biol* 2012; 50:559–568.

- 12 Kumar S, Krishnan K. Cytotoxicity and antioxidant activity of 5-(2, 4-dimethyl benzyl) pyrrolidin-2-one extracted from marine *Streptomyces* VITSVK5 spp. Saudi J Biol Sci 2012; 19:81–86.
- 13 Narayana KJP, Prabhakar P, Vijayalakshmi M, Venkateswarlu Y, Krishna PSJ. Biological activity of phenyl propionic acid isolated from a terrestrial *Streptomyces*. Polish J Microbiol 2007; 56:191–197.
- 14 Rahman MA, Islam MAU. Molecular characterization of actinomycin D producing *Streptomyces* strain isolated from soil samples. Bangl Pharma J 2012; 15:113–117.
- 15 Miller WR, Munita JM, Arias CA. Mechanisms of antibiotic resistance in *enterococci*. Expert Rev Anti-infect Ther 2014; 12:1221–1236.
- 16 Nguyen L. Antibiotic resistance mechanisms in *M. tuberculosis*: an update. Arch Toxicol 2016; 90:1585–1604.
- 17 Peng Z, Jin D, Kim HB, Stratton CW, Wu B, Tang Y, Sun X. Update on antimicrobial resistance in *Clostridium difficile*: resistance mechanisms and antimicrobial susceptibility testing. J Clin Microbiol 2017; 55:1998–2008.
- 18 Costa-Lourenco APR, Santos KTB, Moreira BM, Fracalanza SEL, Bonelli RR. Antimicrobial resistance in *Neisseria gonorrhoeae*: history, molecular mechanisms and epidemiological aspects of an emerging global threat. Braz J Microbiol 2017; 48:617–628.
- 19 Selvin J, Shanmugapriya S, Gandhimathi R, Seghal KG, Rajeetha RT, Natarajaseenivasan K, Hema TA. Optimization and production of novel antimicrobial agents from sponge associated marine actinomycetes *Nocardopsis dassonvillei* MAD08. Appl Microbiol Biotechnol 2009; 83:435–445.
- 20 Chaudhary HS, Yadav J, Shrivastava AR, Singh S, Singh AK, Gopalan N. Antibacterial activity of actinomycetes isolated from different samples of Sheopur (a city of central India). J Adv Pharm Technol Res 2013; 4:118–123.
- 21 Baskaran R, Vijayakumar R, Mohan P. Enrichment method for the isolation of bioactive actinomycetes from mangrove sediments of Andaman Islands, India. Malays. J Microbiol 2011; 7:26–32.
- 22 Rahman MA, Islam MZ, Islam MAU. Antibacterial activities of Actinomycete isolates collected from soils of Rajshahi, Bangladesh. Biotechnol Res Int 2011; 2011:857925.
- 23 Balachandar R, Karmegam N, Saravanan M, Subbaiya R, Gurumoorthy P. Synthesis of bioactive compounds from vermicast isolated actinomycetes species and its antimicrobial activity against human pathogenic bacteria. Microb Pathog 2018; 121:155–165.
- 24 El-Hadi AA, Ahmed HM, Hamzawy RA. Optimization and characterization of L-asparaginase production by a novel isolated streptomyces spp. strain. Egypt Pharma J 2019; 18:111–122.
- 25 Goodfellow M, Kampfer P, Dusse HJ, Trujillo M, Ludwig W, Suzuki K. Bergey's manual of systematic bacteriology. 2nd ed. New York, NY, USA: Springer; 2012.
- 26 Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergey's manual of determinative bacteriology. 9th edn. Maryland, Baltimore, USA: Williams and Wilkins; 1994. 518–537.
- 27 Schumann P, Prauser H, Rainey F, Stackebrandt E, Hirsch P. *Friedmanniella antarctica* gen. nov., sp. nov., an LL-diaminopimelic acid-containing actinomycete from Antarctic sandstone. Int J Syst Bacteriol 1997; 47:278–283.
- 28 Bradner J, Gillings M, Nevalainen K. Qualitative assessment of hydrolytic activities in Antarctic micro fungi grown at different temperatures on solid media. World J Microbiol Biotechnol 1999; 15:131–132.
- 29 Clarke P. Hydrogen sulphide production by bacteria. J Gen Microbiol 1953; 8:397–407.
- 30 Shieh WY, Chen YW, Chaw SM, Chiu HH. *Vibrio ruber* sp. nov. Facultatively anaerobic, marine bacterium isolated from sea water. Int J Syst Evol Microbiol 2003; 53:479–484.
- 31 Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acid Res 1997; 25:4876–4882.
- 32 Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acid Res 1997; 25:3389–3402.
- 33 Islam MR, Jeong YT, Ryu YJ, Song CH, Lee YS. Isolation, identification and optimal culture conditions of *Streptomyces albidoflavus* C247 producing antifungal agents against *Rhizoctonia solani* AG2-2. Mycobiology 2009; 37:114–120.
- 34 Smaoui S, Mathieu F, Ben-Fguira LF, Merlina G, Mellouli L. Taxonomy and antimicrobial activities of a new *Streptomyces* sp. TN17 isolated in the soil from an oasis in Tunis. Arch Biol Sci 2011; 63:1047–1056.
- 35 Buchanan RE, Gibbons NE. Berge's manual of determinative bacteriology. 8th ed. Baltimore: The Williams and Wilkins Company; 1974. pp. 747–829.
- 36 Locci R. Streptomyces and related genera. In: Williams ST, Sharpe ME, Holt JG, editors. Bergey's manual of systematic bacteriology. Baltimore: Williams and Wilkins 1989. pp. 2451–2493.
- 37 Shirling EB, Gottlieb D. Cooperative description of type cultures of streptomyces II. Species descriptions from first and second studies. Int J Syst Bacteriol 1968; 18:69–189.
- 38 Shirling EB, Gottlieb D. Cooperative description of type cultures of streptomyces III. Additional species descriptions from first and second studies. Int J Syst Bacteriol 1968; 18:279–392.
- 39 Anderson AS, Wellington EMH. The taxonomy of *Streptomyces* and related genera. Int J Syst Evol Microbiol 2001; 51:797–814.
- 40 Taddei A, Rodriguez MJ, Marquez-Vilchez E, Castelli C. Isolation and identification of *Streptomyces* spp. from Venezuelan soils: morphological and biochemical studies. I. Microbiol Res 2006; 161:222–231.
- 41 Zhang X, Ren K, Zhang L. Screening and preliminary identification of medicinal plants endophytic actinomycetes used for inhibiting penicillin-resistant *Staphylococcus aureus*. Int J Biol 2012; 4:119–124.
- 42 Jeffrey LSH. Isolation, characterization and identification of actinomycetes from agriculture soils at Semongok, Sarawak. Afr J Biotechnol 2008; 7:3697–3702.
- 43 Rana S, Salam MD. Antimicrobial potential of actinomycetes isolated from soil samples of Punjab, India J Microbiol Exp 2014; 1:00010.
- 44 Basilio A, González I, Vicente MF, Gorrochategui J, Cabello A, González A, Genilloud O. Patterns of antimicrobial activities from soil actinomycetes isolated under different conditions of pH and salinity. J Appl Microbiol 2003; 95:814–823.
- 45 Sacramento DR, Coelho RRR, Wigg MD, Linhares LFTL, Santos MGM, Semêdo LTAS, Silva AJR. Antimicrobial and antiviral activities of an actinomycetes (*Streptomyces* sp.) isolated from a Brazilian tropical forest soil. World J Microbiol Biotechnol 2004; 20:225–229.
- 46 Parungao MM, Maceda EBG, Villano MAF. Screening of antibiotic-producing actinomycetes from marine, brackish and terrestrial sediments of Samal Island, Philippines. J Res Sci Comput Eng 2007; 4:29–38.
- 47 Das A, Bhattacharya S, Mohammed AYH, Rajan SS. In vitro antimicrobial activity and characterization of mangrove isolates of streptomyces effective against bacteria and fungi of nosocomial origin. Braz Arch Biol Technol 2014; 57:349–356.
- 48 Gopinath V, Velusamy P. Extracellular biosynthesis of silver nanoparticles using *Bacillus* sp. GP-23 and evaluation of their antifungal activity towards *Fusarium oxysporum*. Spectrochim. Acta Mol Biomol Spectrosc 2013; 106:170–174.
- 49 Saravanan M, Arokiyaraj S, Lakshmi T, Pugazhendhi A. Synthesis of silver nanoparticles from *Phenerochaete chrysosporium* (MTCC-787) and their antibacterial activity against human pathogenic bacteria. Microb Pathog 2018; 117:68–72.
- 50 Farris MH, Olsson JB. Detection of actinobacteria cultivated from environmental samples reveals bias in universal primers. Lett Appl Microbiol 2007; 45:373–381.
- 51 El-Ansari MA, Ibrahim LF, Sharaf M. Natural phenolics: a source of anticancer agents. Egypt Pharmaceut J 2019; 18:1–7.
- 52 Chabner BA, Roberts TG Jr. Timeline: chemotherapy and the war on cancer. Nat Rev Cancer 2005; 5:65–72.
- 53 Chari ST. Detecting early pancreatic cancer: problems and prospects. Semin Oncol 2007; 34:284–294.
- 54 Suffness M, Pezzuto JM. Assay related to cancer drug discovery. In: Hostettman K, editor. Methods in plant biochemistry: assay for bioactivity. London: Academic Press 2009. 71–133