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Antibacterial activity of *Streptomyces* sp. AMM1 Metabolites isolated from Marsa Matrouh soil

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ABSTRACT

Coded Thirty actinobacteria isolates were isolated from the rhizosphere of different plants in the secluded desert to select the promising isolate that has a wide antimicrobial activity against Gram-positive, Gram-negative bacteria and fungi. The most active isolate was coded as AMM1, and it was characterized physically, biochemically, and genetically as *Streptomyces* sp., and showed 99% similarity with *Streptomyces geysiriensis* when compared with the 16S sequences deposited in GenBank. The temperature was 35 °C, the pH was 7, and the best timing was 12 days as the best environmental condition suitable for growth after studying. The agar-well diffusion method was used after the metabolites were mixed with several polar organic solvents (Hexane, chloroform, ethyl acetate, and ethanol) to determine the most effective antibacterial crude extract, and the results found that ethyl acetate crude extract was the most effective one among them, and their minimum inhibition concentration was 2.5 µg/ml recorded against *E. coli ATCC7839* with a value of 9.0 mm, *Candida albicans ATCC10321* with a value of 9.33 mm and 8.33 mm against *Bacillus subtilis ATCC6633*. The same solvent extract produced a mean inhibition zone of 8.33 mm against *P. aeruginosa ATCC9027* and 9.33 mm against *S.* aureus *ATCC6538* at a minimal inhibitory concentration of 5 µg /ml.

Key Words:

Actinobacteria, Streptomyces, antibacterial, antifungal, 2.5 µg/ml Ethyl acetate

1. INTRODUCTION

There are numerous microorganisms have been discovered in soil samples taken from hyper-arid regions, indicating that the desert biome is a unique and understudied source of novel actinobacterial diversity [1]. The actinobacterial communities in the desert environment were rarer and more unusual, and the substantial research required for isolation required creative and efficient taxonomic procedures that could result in the discovery of novel genera and novel species of actinobacteria [2]. which have

received attention recently in an effort to provide a fresh perspective on studying the natural compounds of antimicrobials [3, 4,5]. Based on taxonomic investigations, 129 species in 9 orders, 24 families, and 52 genera were reported from deserts globally between 2000 and 2021. *Streptomyces* and *Geodermatophilus* are the two actinobacteria that are most frequently found in deserts [6]Actinobacteria are known as residents of soil. Rhizospheric streptomyces is one of many groups that are stable in bulk soil and rhizosphere plants. As a result of the metabolic variety of actinobacteria, which is thought to be a good source of lytic enzymes, antibiotics, and other bioactive metabolites [7], researchers need to screen more of these organisms. most notably those that are undiscovered and capable of generating fresh antibiotics effective against bacteria that are currently antibiotic-resistant [8, 9]. Primary screening is typically conducted on desert actinobacteria to explore in vitro their intriguing bioactivity patterns [10] Which can be the most promising solution to the problem of antibiotic-resistant bacteria as well as bacterial pathogens [11]. Depending on these ideas, this study focused especially on the soil of Egypt's deserts for actinobacteria that have bioactive metabolites that have antimicrobial and antifungal activities on tested Gram-positive and Gram-negative bacteria and fungi.

2. STUDY AREA

Site Description: Soil samples were gathered from various locations from wild desert valleys belonging to Marsa Matrouh governorate, Egypt. It was summarized in Table (1)

Table 1: location of sample collections

Locations	Observational sites	Plantation (Orchards) and Number of samples		Coordinates
Al Najila	Zagarat 1	Olive	4	31.472377°N,26.662852°E
	Zagarat 2	Olive	5	31.467241°N,26.664563°E
Matrouh	Waer	Olive	3	31.337237°N,27.123117°E
	Maged	Olive	5	31.223995°N,27.414616°E
Ras El-Hikma	El-Bess	Banat vineyard	4	31.135994°N,27.806372°E
		Olive	5	31.135848°N,27.808494°E
		Banat vineyard	5	31.134238°N,27.81128°E
		Banat vineyard	3	31.135179°N,27.809646°E
	Henash	Olive	5	31.156175°N,27.623831°E

3. METHODS AND MATERIALS

3.1 Sample collection and isolation of actinobateria: The present study was conducted on different sites in Marsa Matrouh governorate (Table 1). Samples were collected in sterile plastic bags from wild desert valleys. Soil samples were collected from a depth of 5–10 cm. By the serial dilution method, 1 gram of soil was diluted with sterile distilled water up to 10^6 dilution, and from the diluted 10^5, one hundred microliters were spread on the surface of the starch nitrate agar media plate. The plates were incubated at 30 °C for a period ranging between 7 and 14 days. Depending on the morphological and further subculture, actinobacteria were selected [12]. The isolates were maintained in slant culture at 4 °C as well as in a 20% (v/v) glycerol stock at -80 °C (nuVE DF590 - Cabinet TK 252).

3.2 Bacterial strains: To study the antimicrobial and antifungal activities, *Staphylococcus aureus ATCC 6538 Bacillus subtilis ATCC6633* as Gram-positive bacterial strains, *P. aeruginosa ATCC9027*and *E. coli ATCC7839* as Gram-negative bacterial strains and *Candida albicans ATCC10231* were selected for this study and obtained from the plant and microbiology department, faculty of science, Al-Azhar University, Cairo, Egypt.

3.3 Primary screening: Thirty actinobacteria isolates were initially screened by growing them on starch nitrate broth media in thirty flasks (250 ml capacity) containing 100 ml broth medium and five discs from each actinobacterial strain were picked up by a 6 mm cork borer. The flasks were then incubated for 14 days at 30 °C and 150 rpm in a shaking incubator. After that, the fermentative media were filtrated with Whatman No1 and tested for antibacterial activity against *B. subtilis ATCC 6633*, *S. aureus ATCC 6538* (as gram-positive), *P. aeruginosa ATCC 9027*, and *E. coli ATCC 7839* (as gram-negative) strains. *C. albicans ATCC 10231* was used as a fungal test.

3.4 Characteristic of the most potent isolate:

3.4.A Morphological characterization: The most promising actinobacteria with high antibacterial and antifungal activities were identified based on morphological structure by using light microscopy to observe spore arrangement in the mycelium with the cover slip method [13], and under a scanning electron microscope, the spore surface morphology was observed. According to Bergey's Manual of Determinative Bacteriology, aerial mass color, reverse side pigments, melanoid pigments, and spore chain shape were studied to determine the genus level [14, 15]

3.4.B Physiological characteristics

Selection of the best incubation Period: The potential isolate was inoculated into 100-ml conical flasks containing 20 ml of basal medium (supplemented with the best carbon source). Each set of the inoculated flasks was incubated at 35 °C and 150 rpm in a rotary shaker for a certain time period. These periods ranged from 7 to 14 days, and the antibacterial activity of cell-free filtrate (CFF) was tested as previously mentioned.

The optimal temperature: Several incubation temperatures, ranging from 20°C to 45°C with 5°C intervals, were used to determine the best temperature affecting the production of bioactive metabolites. In triplicate for each temperature, the actinobacterial isolate was inoculated into 100-ml conical flasks containing 20 ml of basal medium supplemented with the best carbon source and incubated at pH 7 for 12 days in a shaking incubator at 150 rpm after that, the antibacterial activity of CFF was tested as previously mentioned [16]

The optimal pH: One 100-ml conical flask containing 20 ml of basal medium amended with the best carbon source was adjusted to beginning pH values ranging from 5 to 10 using 1N HCl or 1N NaOH to study the effects of the initial pH values of the production medium. The media were inoculated with the isolate and incubated for 12 days at the optimum temperature and shaking rate (150 rpm) for each pH value. After the incubation time, the CFF was prepared and the antibacterial activity was tested as previously mentioned.

The carbon source: By substituting various carbon sources, such as those supplemented with 1% of different carbon sources such as arabinose, cellulose, dextrose, fructose, inositol, mannitol, rhamnose, sucrose, and xylose, for the glucose in the basal medium, the impact of diverse carbon sources on the production of bioactive metabolites produced by the AMM1 isolate was investigated [17]. For each carbon source that was examined, three flasks were set up. After the incubation period, CFF was obtained by centrifugation, followed by filtration through a syringe filter. Then the antibacterial activity of CFF was evaluated against the bacterial model *E. coli ATCC7839* [18].

3.4.C Biochemical determination: The chosen strain's biochemical activities were used to determine and verify the genus and species levels of the organism. For this purpose, indole, citrate utilization, methyl

red, Voges-Proskauer's, melanin formation, oxidase, catalase, gelatinase, urease, amylase, and lipase were assayed [19].

3.4.D Characterization of molecular taxonomy: Phylogenetic tree construction and 16S rRNA partial gene sequencing were used to identify the potential isolate at the species level. The 16S rRNA gene was amplified after the isolate's genomic DNA was removed. The NCBI GenBank database was searched for sequence similarity using the blast search engine using the 16S rDNA nucleotide sequence of the most active isolate. The obtained aligned sequences were cleaned using Gblocks 0.91b [20]. The phylogenetic tree was created using the neighbor-joining method once the phylogeny study was completed.

3.5 Liquid-liquid extractions: On an incubator shaker for 14 days, the most promising isolate was cultivated on starch-nitrate media and fermentative medium was collected and filtrated through Whatman No. 1 filter paper to be extracted by an organic solvent graduated from a low- to a high-polarity solvent. Hexane, chloroform, ethyl acetate, and methanol were used in a series of solvent extractions on a total volume of 5 liters of culture filtrate. In a separating funnel with a 3000-ml capacity, three times the volume of the solvent was added to the filtrate, which was thoroughly stirred while being let stand for an hour. Utilizing straightforward distillation and a vacuum rotary evaporator at 40°C, the crude solvent extracts were collected until their next usage, and the extracts were kept at 4 °C.

3.6 Screening of different solvent crude extracts: Crude extracts of organic solvents were subjected to primary screening against the aforementioned isolates by an agar-well diffusion assay. the putative isolate's antibacterial properties Whereas for four different solvent extracts, a concentration of 50 mg/ml was prepared in dimethyl sulfoxide (DMSO) and 100 µm were placed in each hole of the agar. Each solvent extract was prepared in three replicates before being incubated for 24 hours at 28 °C. For each extract, DMSO was used as a control. The diameter of the inhibitor was measured in millimeters.

3.7 Determination of minimal inhibition concentrations: The minimum inhibitory concentration (MIC) of the most active antibacterial crude extract was calculated, which meets the requirements of the NCCLS standard [21]. The agar-well diffusion method was used to assess their MIC [22]. Various concentrations of 20, 10, 5, 2.5, and 1.25 ug/ml were obtained using two-fold serial dilution. After adding 100 ul of each prepared inoculum, sterile Petri dishes were pipetted with molten agar. On each plate, four wells were formed, and 100 ul of each extract at concentrations of 10, 5, 2.5, and 1.25 ug/ml were applied to the respective wells. After 30 minutes in the refrigerator, the plates were incubated at 28°C for 24 hours. MIC was once assumed to be the lowest concentration at which the corresponding microorganisms could not grow. Each assay was carried out three times. DMSO was used as a control, while distilled water was used for water extracts.

3.8 Statical analysis: The mean of inhibition zone and standard error were estimated using Microsoft Excel 2010 software (Microsoft Corp., Redmond, WA).

4. RESULTS and DISCUSION

4.1 Screening of active Actinobacterial isolates: Actinobacteria are among the important organisms that have a wide range of biological activities against human pathogenic bacteria [23]. Actinobacteria have recently gained attention because of their isolation from various sources, including desert environments, which are distinguished by their lack of exposure to various environmental pollutants [6]. Actinobacteria are found in the rhizosphere of plants cultivated in valleys and reclaimed land, whose growth depends on rain and groundwater, and the North Coast region of Egypt is one of the major sites since it has a pure and abundant supply of these organisms [24]. Thirty actinobacteria isolates were isolated from soil samples collected from the areas of Al-Najila, Matrouh, and Ras Al-Hikma, which are reclaimed in the desert on the northern coast and rely on irrigation with rainwater that is collected in

wells. It was planted with different types of perennial trees, such as olives and figs. According to a study conducted by [1], many microorganisms were isolated from soil samples collected from the Atacama Desert (hyperarid areas), showing that the desert ecosystem is a unique and understudied source of novel actinobacterial diversity. The antimicrobial activity of these isolates was tested against two models of Gram-positive bacteria, two of Gram-negative bacteria, and *Candida albicans.* The result of this screening was varied, some of which demonstrated positive results towards all four organisms, as well as the fungus *Candida albicans*, while others demonstrated positive results towards only some of them, as shown in Table (2) Only nine (AMM1, AMM3, AMM4, AMM7, AMM12, AMM15, AMM20, AMM27 and AMM29) isolates had a broad effect against the microbes under test. AMM1 was selected as the most promising among the nine active actinobacterial isolates. These results seem to be consistent with a study by [2] who reported that a total of 134 morphologically unique actinobacterial isolates were isolated from ten different desert soil samples, and preliminary testing showed that only 16 of the isolates had antibacterial activity. One isolate (DA3-7) among them showed broad-spectrum antibacterial action against both gram-positive and gram-negative bacteria, as well as against fungi. In our study, the isolates that exhibited a wide range of antimicrobial activity were selected for further studies, where the most potent isolate coded as AMM1 was chosen because of its excellent activity against tested bacteria and Candida, with a diameter of 24 mm in the inhibition zone on E. coli *ATCC7839* and *C. albicans ATTC10231*, 23mm on *B. subtilis ATCC6633* and finally 21 mm on *P. aeruginosa ATCC9027* and *S. aureus ATCC6538* table (2). In another study, 75 actinomycete strains were isolated from Egyptian desert habitats by [25]. It was found that 15.63% of the 75 isolated organisms are active against Gram-positive bacteria and yeast., while 12.50% are active against Gram-positive, Gram-negative and yeast. The isolated D332 strain was chosen as the most potent actinomycete.

Table 2: Primary Screening of different actinobacteria as wide spectrum of bactericidal activities

	Inhibition zone in mm on Bacterial Isolates				
Isolate code	***P. aeruginosa* ATCC9027**	***E. coli* ATCC7839**	***C. albicans* ATCC10231**	***S. aureus* ATCC6538**	***B. subtilis* ATCC6633**
AMM1	21	24	24	21	23
AMM2	10	10	11	ND	9
AMM3	14	13	12	12	13
AMM4	12	12	13	16	13
AMM5	ND	7	ND	8	ND
AMM6	ND	ND	7	ND	ND
AMM7	8	7	8	8	7
AMM8	ND	ND	ND	ND	ND
AMM9	11	8	8	9	ND
AMM10	ND	ND	ND	ND	ND
AMM11	ND	ND	7	ND	ND
AMM12	8	10	8	8	12
AMM13	ND	ND	ND	ND	ND
AMM14	ND	ND	ND	ND	ND
AMM15	8	11	8	8	9
AMM16	ND	ND	7	ND	9
AMM17	ND	ND	ND	ND	ND
AMM18	9	ND	9	ND	8
AMM19	ND	ND	ND	ND	ND
AMM20	9	9	12	9	7
AMM21	ND	ND	ND	ND	ND
AMM22	10	ND	11	ND	ND
AMM23	9	12	11	11	ND
AMM24	12	ND	ND	ND	9
AMM25	ND	ND	ND	ND	ND
AMM26	ND	ND	7	ND	9
AMM27	10	10	11	12	12
AMM28	ND	ND	ND	ND	ND
AMM29	12	12	13	10	12
AMM30	ND	ND	ND	ND	ND

4.2 Morphological and biochemical characterization

- **cultural, morphological and molecular taxonomical of isolate AMM1.**

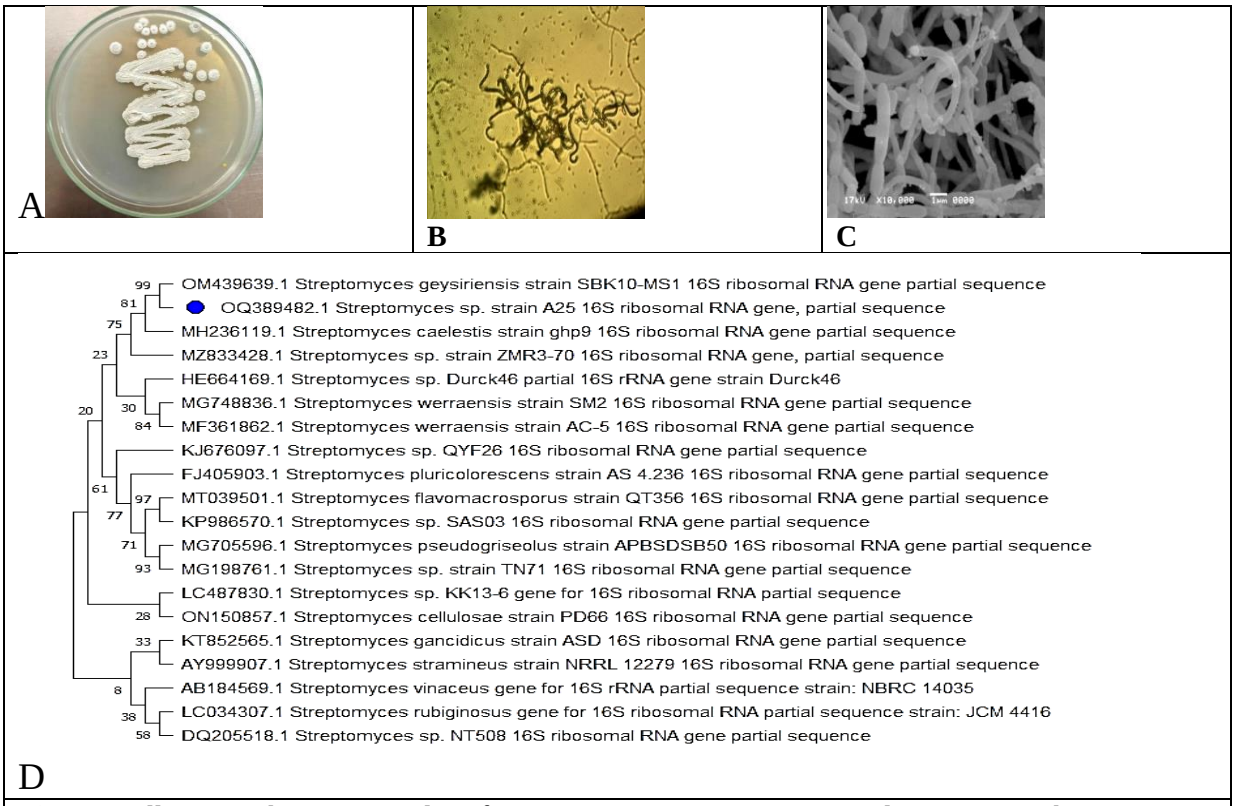

Figure 1: illustrates the creamy color of Streptomyces sp. OQ3894821 colonies on starch nitrate agar media (A). the images of light microscope showed long chain of spores(B). Spore morphology by SEM, which revealed that the aerial hyphae were fractured and that the majority of the spores were rod-shaped with a smooth surface (c). Based on 16S rRNA gene sequences, the evolutionary relationships of Streptomyces sp. OQ3894821 and other members of the genus Streptomyces (D). The tree was built using the Jukes-Cantor model and the neighbor-joining method. Branches connected with partitions that received less than 50% of bootstrap replicates are collapsed. The fraction of duplicate trees in which the related taxa cluster together is given next to the branches in the bootstrap test (1000 repetitions).

On starch nitrate culture media, the isolate AMM1's colonies were medium to large in size, powdery, and had an uneven border. After an incubation period of 7 and 14 days, the aerial mycelium had a creamy color, while the base mycelium had a greyish color (Fig.1, (A)). On the fourteenth day, the isolate's full growth was seen. Using light microscopy, large chains of spores (oblong in shape) were seen on the Gram-positive isolate (Fig.1, (B)) with a scanning electron microscope (1000X magnification)., The hyphal structure has spiral chain-shaped and smooth spore surfaces (Fig.1, (C)). The isolate displayed the normal morphological and structural characteristics of Streptomyces species.

4.3 Optimization of the factors affecting the production of the bioactive metabolites: In order to study the effect of incubation period, pH and temperature on the antibacterial activity of AMM1, each of these factors was examined independently. The impact of the incubation period at a temperature of 30 °C was investigated, and the organism was cultured in a liquid environment containing nitrate starch media for 24 days and collected at intervals for filtering, where it was tested for activity against *E. coli* with the same steps. The two other factors, pH and temperature were studied.

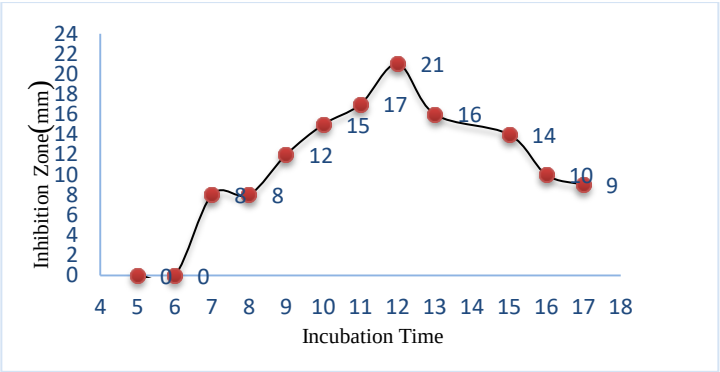

Fig. 2: Effects of incubation time on *Streptomyces* AMM1's ability to produce antibacterial activity

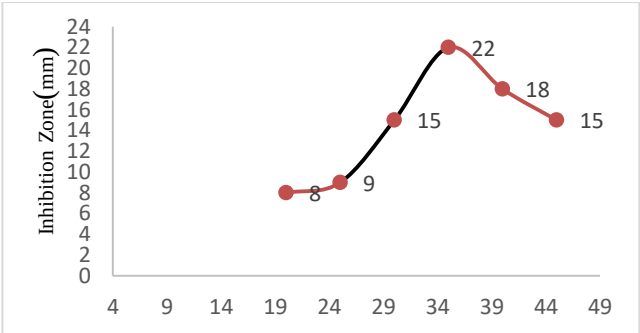

Fig.3: Effects of temperature on *Streptomyces* AMM1's ability to produce antibacterial activity

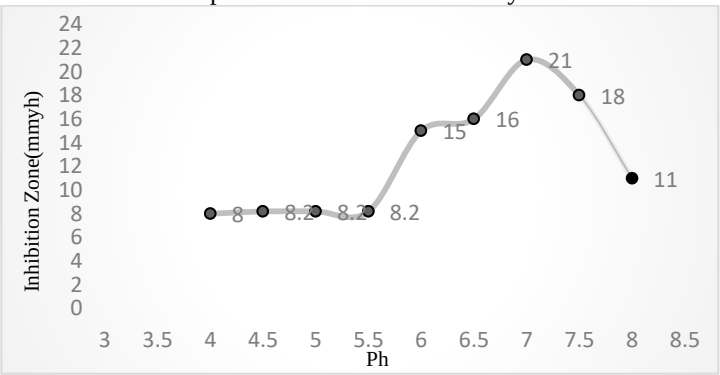

Fig .4: Effects of initial pH on *Streptomyces* AMM1's ability to produce antibacterial activity

The results showed that 12 days were the most suitable to induce the highest inhibition zone, where it recorded 21mm throughout incubation periods (Fig. 2). While the optimum temperature and pH for the production of maximum antibacterial activity were 35 ^{o}C and pH 7 (Fig 3,4) the highest inhibition zones were 21 and 22 mm respectively. This is thought to have happened as a result of strain AMM1's adaptation to the somewhat hot soil from which it was isolated.

4.4 Characterization of Biochemical activities: On all of the evaluated ISP media, the actinobacterial strain AMM1 established an aerial mycelium that was moderate on ISP-2, ISP-3, ISP-5 and ISP-7 media, while on ISP-1 it had excellent growth. The growth of the AMM1 isolate was good on ISP-4 and ISP-6. The aerial mycelium was creamy white on ISP-1, ISP-2, ISP-3, ISP-4 and ISP-5, while the creamy color showed on ISP-6 and ISP-7. Between grey, white and grey colors, substrate mycelium was recoded with different ISP media as summarized in Table (3).

Table 3: Morphological characteristics of *Streptomyces* AMM1 isolate on ISP media

Media	**Growth**	**Aerial mycelium**	**Substrate mycelium**
ISP-1	Excellent	Creamy white	Grey White
ISP-2	Moderate	Creamy white	Grey White
ISP-3	Moderate	Creamy white	Grey
ISP-4	Good	Creamy White	Grey
ISP-5	Moderate	Creamy white	Grey
ISP-6	Good	Creamy	Grey
ISP-7	Moderate	Creamy	Grey

In addition to the physical traits, the physiological and biochemical traits are thought to be the most crucial for identifying a particular microbial isolate. Selected isolates' capacity to use various carbon and nitrogen sources available to promote their growth is included in the biochemical and physiological

investigations table (3). The actinobacteria strain AMM1 established an aerial mycelium with excellent sporulation. *Streptomyces sp*. AMM1's growth, color of the aerial mycelium, color of the substrate mycelium, and pigmentation were all summarized in Table (3).

Table 4: General characterization of *Streptomyces* MM1			
Sporophore morphology	Spiral	Spore surface	Smooth
Shape of spores	oblong		
Biochemical tests			
Melanin pigment	-	Catalase	+
Indole production	-	Oxidase	-
Methyl red test	+	Urease	+
Citrate utilization	+	H2S production	-
Gelatin hydrolysis	-	Starch hydrolysis	+
Nitrate reduction	-		
Utilization of Carbone sources			
Arabinose	+	Glucose	++
Dextrose	+	Mannitol	+
Fructose	-	Cellulose	-
Xylose	+	Inositol	+
Sucrose	++	Raminose	-
Chemotaxonomic characters			
1. Cell wall amino acid analysis		L-DAP	
2. Gram staining		+	

To identify the isolate, various biochemical tests were performed and results are summarized in Table (4). The isolate tested positive for the methyl red test and the citrate utilization test but was determined to be indole-negative. It did not create nitrate reduction, gelatinase or lipase, but it did produce amylase, catalase, oxidase, and urease. *Streptomyces* sp. AMM1 could utilize Arabinose, dextrose, glucose, Inositol, mannitol, sucrose and xylose as sole carbon sources; but cellulose, fructose, and raminose were not consumed by the isolate. The chemotaxonomic characteristics demonstrated that L-DAP was positive for Gram stain and had a cell wall amino acid analysis Table (4).

4.5 Molecular characteristics taxonomy: The selected isolate was identified as *Streptomyces* based on morphological, physiological, and culture properties, as well as biochemical tests [26]. By using a blast search, the isolate's 16S rDNA nucleotide sequence (856 bp) was compared to the NCBI GenBank database, which confirmed 99 percent identity with *Streptomyces geysiriensis*. The isolate's 16S rDNA nucleotide sequence was deposited into GenBank (NCBI) under accession number OQ389482. The isolate's membership in the genus *Streptomyces* was established through molecular characterization and phylogeny, and it was given the name *Streptomyces sp*. A25 (Fig. 1, (D) The phylogenetic tree of *Streptomyces sp*. A25

4.6 Antibacterial activity of different solvent crude extracts: According to numerous other studies [27, 4, 28], ethyl acetate is a useful solvent for extracting antimicrobial chemicals from actinobacteria, notably from the species *Streptomyces*. This is what the current investigation found, in which the ethyl acetate solvent was shown to be the most suitable of the solvents tested. The extracts were tested against the organisms under experimentation. It was found that the ethyl acetate extract is the most effective of them, with mean inhibition zones of 21.22, 19, 18.67, 17.67 and 17 mm against *P. aeruginosa ATCC9027*, *E. coli ATCC7839, B. subtilis ATCC6633, S. aureus ATCC 6538* and *C. albicans ATCC 10231*. Then, in regards to effectiveness, the ethanol extract comes in as the second-best extract, while the least effective of the three extracts was the hexane extract Table (5). Thus, we can say that the ethyl acetate extract of *streptomyces* AMM1 had the highest biocidal activity on Gram-negative and Gram-positive bacteria, as well as Candida albicans, of the gradient polar solvent extracts tested. These outcomes were consistent

with an investigation carried out by [29]when found that ethyl acetate of *Streptomyces* sp. SML10 a contains the extract's active ingredients and inhibits both Gram-positive and Gram-negative bacteria and fungi.

Table5: Mean ±stander error effect of different polar solvent extracts on tested Gram positive and negative bacteria and fungi

Solvent	*P. aeruginosa* *ATCC9027*	*E. coli* *ATCC7839*	*C. albicans* *ATCC10231*	*S. aureus* *ATCC6538*	*B. subtilis* *ATCC6633*
Hexane	ND	ND	7.67±0.27	ND	ND
Chloroform	9.67±0.54	9.33±1.19	9.67±0.54	10.67±0.72	11.33±0.54
E. acetate	21.33±0.72	19.00±0.94	17.00±0.82	17.67±0.72	18.67±0.27
Ethanol	10.67±0.72	14.33±0.93	13.67±0.54	9.67±0.54	13.33±0.72

4.7 Minimal inhibition concentrations determination: Minimal inhibitory concentrations According to our data in Table (6), the lowest inhibitory concentration was recorded at 2.5 µg/ml against E. coli *ATCC7839, B. subtilis ATCC6633* and *C. albicans ATCC10231*, while it was 5 µg/ml against *P. aeruginosa ATCC9027* and *S. aureus* ATCC6538. The crude ethyl acetate extract of *Streptomyces* sp. PJ85 for MIC was evaluated by [30] with the two-fold macro-dilution method and the results of the MIC value were 2, 2, 16, 2, and 1 µg/mL against *Staphylococcus aureus ATCC29213*, *Staphylococcus epidermidis TISTR518*, *Bacillus subtilis TISTR008*, and *B. cereus TISTR687* respectively. Based on the results listed above, the study was completed on ethyl acetate extract to determine the lowest inhibitory concentration.

Table6: Mean ±stander error Effect of different concentrations of E. acetate solvent extracts on tested (Gram positive and negative) bacteria and fungi

Con.	*Minimal inhibition concentrations (mm) of E. acetate extract of Streptomyces AMM1*				
µg/ml	***P. aeruginosa*** ***ATCC9027***	***E. coli*** ***ATCC7839***	***C. albicans*** ***ATCC10231***	***S.*** *aureus* ***ATCC6538***	***B. subtilis*** ***ATCC6633***
1.25	ND	ND	ND	ND	ND
2.5	ND	9.00±0.47	9.33±0.27	ND	8.33±0.27
5	8.33±0.27	14.33±0.27	14.67±0.72	9.33±0.27	14.67±0.72
10	14.00±0.47	16.33±0.54	17.00±0.47	14.67±0.72	17.67±0.72
20	17.33±0.72	21.33±1.19	20.67±0.72	19.00±0.47	23.33±0.27

5. DISCUSSION

Thirty isolates were taken from desert areas that had been recovered for agricultural use and initially evaluated to see if they had any antibacterial action. A study of its morphological, biochemical, and molecular taxonomic properties was conducted on one of them after it was chosen as a promising isolation. *Streptomyces* sp. A25 with the accession number OQ389482 has been identified as *Streptomyces geysiriensis*. It was cultivated, and several organic solvents were combined with the filtrate. The results confirmed that E. acetate was the most active organic solvent and had the most promising compounds.

Recommendations: We recommend in order to conduct a more in-depth study and arrive at the characterization and description of the active ingredients.

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Conflict of interest: The authors declare no conflict of interest.

6. CONCLUSION

In conclusion, tyrosinase was purified successfully from *Aspergillus nidulans*. The results illustrated that ethanolic extracts from *Morus alba* fruits, *Chenopodium album* leaves, *Rumex dentatus* leaves, *Eruca sativa* leaves and *Urtica urens* leaves have considerable tyrosinase inhibitory activity. This effect is more or less similar to the standard inhibitors of tyrosinase. Moreover, the ethanolic extract of the tested plants exhibited appreciable scavenging activity due to presence of phenols as well as flavonoids as pointed by the results. These results suggest that the tested plant extracts could be applied as supplier for bioactive phytochemicals to control hyperpigmentation and for whitening of skin.

Recommendations:

The results recommend to use the plant extract as inhibitors for fungal tyrosinase to avoid pigment formation.

Conflict of interest:

The authors declare that there is no conflict of interest.

Fund: No fund

Data availability: Available under request

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